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Understorey productivity in temperate grassy woodland responds to soil water availability but not to elevated [CO_2]

Running head: Understorey productivity in elevated [CO_2]

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Abstract

Rising atmospheric [CO_2] and associated climate change are expected to modify primary productivity across a range of ecosystems globally. Increasing aridity is predicted to reduce grassland productivity, though rising [CO_2] and associated increases in plant water use efficiency may partially offset the effect of drying on growth. Difficulties arise in predicting the direction and magnitude of future changes in ecosystem productivity, due to limited field experimentation investigating climate and CO_2 interactions. We use repeat near-surface digital photography to quantify the effects of water availability and experimentally manipulated elevated [CO_2] (eCO_2) on understorey live foliage cover and biomass over three growing seasons in a temperate grassy woodland in south-eastern Australia. We hypothesised that (i) understorey herbaceous productivity is dependent upon soil water availability, and (ii) that eCO_2 will increase productivity, with greatest stimulation occurring under conditions of low water availability. Soil volumetric water content (VWC) determined foliage cover and growth rates over the length of the growing season (August - March), with low VWC (< 0.1 m^3 m^{-3}) reducing productivity. However, eCO_2 did not increase herbaceous cover and biomass over the duration of the experiment, or mitigate the effects of low water availability on understorey growth rates and cover. Our findings suggest that projected increases in aridity in temperate woodlands are likely to lead to reduced understorey productivity, with little scope for eCO_2 to offset these changes.

Introduction

Concentrations of atmospheric CO_2 have risen at unprecedented rates since the industrial revolution, resulting in increased global temperatures and reduced precipitation across many biomes (IPCC, 2014). Global climate change and elevated atmospheric [CO_2] (eCO_2) can modify ecosystem primary productivity via changes to plant growth, mortality and phenology, influencing carbon fluxes between the atmosphere and biosphere (Hufkens *et al.*,

2016, Ma *et al.*, 2015, Reyes-Fox *et al.*, 2014, Zhu *et al.*, 2016b). Modelling, satellite and field studies have found evidence that eCO_2 can increase ecosystem productivity and carbon sequestration across many terrestrial biomes (Donohue *et al.*, 2013, Kolby Smith *et al.*, 2016, Morgan *et al.*, 2004), partially offsetting global emissions (Ballantyne *et al.*, 2012). Earth system models used to set greenhouse gas emission targets have been shown to over-predict the enhancement of productivity associated with increasing [CO_2], partially owing to poor representation of climate feedbacks within temperate and arid ecosystems (Kolby Smith *et al.*, 2016). Experimental research aimed at understanding the interactive effects of CO_2 and climate on ecosystem productivity will be instrumental in facilitating improved predictions of ecosystem change under future climates (Hufkens *et al.*, 2016, Kolby Smith *et al.*, 2016).

Increased atmospheric [CO_2] can stimulate plant growth and ecosystem productivity directly by increasing rates of CO_2 assimilation in C3 species, and indirectly by reducing stomatal conductance in C3 and C4 species, potentially resulting in greater soil water availability (Ainsworth & Long, 2005, Morgan *et al.*, 2004). In periodically water limited ecosystems (e.g. semi-arid and temperate grasslands), water savings are the primary mechanism through which eCO_2 may increase ecosystem productivity and growing season length (Morgan *et al.*, 2004), though increased CO_2 assimilation may also be important (Pathare *et al.*, 2017). Field based CO_2 enrichment studies have shown that eCO_2 typically increases productivity during periods of rainfall deficit, with eCO_2-induced water savings increasing water availability and consequently growth (Hovenden *et al.*, 2014, Morgan *et al.*, 2011, Morgan *et al.*, 2004). Increased net primary productivity under eCO_2 may arise from both increased growth rates and an extension of the growing season, by prolonging the final days of growth or reducing the number of periodic drought days throughout the growing season (Morgan *et al.*, 2004). Water savings may also enhance productivity under a warming climate by counteracting increased evaporative demand (Morgan *et al.*, 2011), which may be particularly evident during periods of low moisture availability.

Grass dominated ecosystems, including woodlands and savannas that consist of a mixture of grasses and trees, occupy a large portion of the terrestrial biome (> 30%) and account for ~30 % of global aboveground net primary production (NPP) (Asner *et al.*, 2004, Del Grosso *et al.*, 2008). These ecosystems characteristically experience periods of water limitation, due to highly seasonal rainfall (e.g. Mediterranean grasslands, tropical savannas) or unpredictable wet/dry periods (e.g. temperate woodlands, semi-arid grasslands) (Lehmann *et al.*, 2011, Lunt

et al., 2012, Morgan *et al.*, 2004). Ecosystem productivity, community composition and grass-tree ratios are strongly mediated by water availability, though temperature, edaphic factors and disturbances such as fire and herbivory also exert considerable influence (Del Grosso *et al.*, 2008, Lehmann *et al.*, 2014, Lunt *et al.*, 2012, Sala *et al.*, 2012). Due to the importance of water availability for grassland growth, grass productivity is likely to be highly sensitive to increasing [CO_2] and climate change (e.g. Morgan *et al.*, 2011, Reyes-Fox *et al.*, 2014). Increasing atmospheric [CO_2] has been proposed as a major driver of recent broadscale increases in ecosystem productivity, including grasslands (Kolby Smith *et al.*, 2016), and changes to grass-tree ratios in savannas (Bond & Midgley, 2012). Field experimentation examining the sensitivity of grass productivity to eCO_2 and climatic patterns is limited and has predominantly occurred in semi-arid and temperate grasslands (e.g. Hovenden *et al.*, 2014, Morgan *et al.*, 2004, Reich *et al.*, 2014), with few studies taking place in woodlands and savannas, where the response of the herbaceous understorey to eCO_2 may have potentially far-reaching implications for ecosystem function and structure (e.g. through effects on water balance, fuel properties and fire regimes; Bond & Midgley, 2012).

Studies examining the effect of eCO_2 and climate on grassland productivity have largely used periodic (i.e. seasonal - annual) destructive harvesting, which only allows for the assessment of aggregated climate effects on biomass production (e.g. Hovenden *et al.*, 2014, Reich *et al.*, 2014). However, grassland productivity is dynamic and responds to climatic events (e.g. rainfall, drying, heatwaves) over short time frames (e.g. weeks) (Migliavacca *et al.*, 2011, Morgan *et al.*, 2004, Pathare *et al.*, 2017). Consequently, high frequency measures of ecosystem productivity are required to adequately capture climate and eCO_2 effects on grassland productivity (Migliavacca *et al.*, 2011, Zelikova *et al.*, 2015), and will provide greater value for the development and testing of process-based ecosystem models (Hufkens *et al.*, 2016). Near-surface remote sensing using timelapse digital photography provides an ideal tool for the quantification of trends in grassland growth at fine temporal scales (Hufkens *et al.*, 2016, Migliavacca *et al.*, 2011, Zelikova *et al.*, 2015). Greenness indices derived from digital photographs are highly correlated with grassland projected foliage cover and gross primary productivity (Hufkens *et al.*, 2016, Migliavacca *et al.*, 2011, Moore *et al.*, 2016, Toomey *et al.*, 2015, Zelikova *et al.*, 2015). Stereo imaging techniques are also effective at characterising vegetation structure and can provide estimates of vegetation biomass (Yoon & Thai, 2010).

We used near-surface digital photography and stereo imaging to measure and monitor the response of understorey live foliage cover (3 day intervals) and aboveground biomass (~30 – 60 day intervals) to experimentally manipulated eCO_2, water availability and biotic factors in a temperate grassy woodland over a ~2.5 year period comprising 3 full growing seasons. We targeted the interactive effects of eCO_2 and water availability on understorey productivity, as eCO_2 is predicted to mitigate the effect of increased aridity under future climate change (Morgan *et al.*, 2011). We hypothesise that (i) understorey herbaceous productivity depends on soil water availability, and (ii) there will be periodic increases in understorey productivity under eCO_2, which will be greatest when soil moisture is limiting.

Materials and Methods

Site description and study design

The EucFACE Free Air CO_2 Enrichment experimental facility is located in a remnant temperate Eucalypt woodland at Western Sydney University, Sydney, Australia (EucFACE; 33° 36' 58" S, 150° 44' 22.1" E). The tree canopy is dominated by *Eucalyptus tereticornis* (~25 m tall) and is characterised by a relatively low foliage cover (low leaf area index 1.1 – 2.2 m^2 m^{-2}) (Duursma *et al.*, 2016). The understorey is largely comprised of a mixture of C3 and C4 grasses and C3 forbs, with the native C3 grass *Microlaena stipoides* accounting for ~70% of the herbaceous biomass (Pathare *et al.*, 2017). The C3 herbs *Commelina cyanea* and *Pratia purpurascens*, the introduced C3 grass *Cynodon dactylon* and the C4 grass *Cymbopogon refractus* are also common across the study site (Drake *et al.*, 2016). Shrub cover is sparse and dominant species include *Melaleuca decora*, *Acacia parramattensis*, *Breynia oblongifolia*, *Hakea sericea* and *Bursaria spinosa* (Drake *et al.*, 2016, Pathare *et al.*, 2017).

The EucFACE consists of six 25 m diameter circular plots (designated hereafter as 'rings') each consisting of 32 vent pipes that extend above the tree canopy (~28 m tall). Prediluted CO_2 is released through adjustable ports located at 50 cm intervals along the length of the vent pipes and facing toward the centre of the ring. Three rings were randomly assigned to an ambient CO_2 treatment and three assigned to the elevated treatment (ambient + 150 ppm CO_2). The rings were constructed in 2010 and fumigation commenced in September 2012 (Drake *et al.*, 2016). The mean CO_2 concentrations at 20 cm above the ground for the three

147 eCO_2 rings over the first four years of fumigation (February 2013 – February 2017) ranged
148 between 565 ppm to 589 ppm, exceeding the target of ~550 ppm.

149 Four 2 m x 2 m understorey monitoring plots were randomly located in each ring prior to the
150 commencement of fumigation in September 2012. A Wingscapes timelapse camera (model
151 WTC-00122, Wingscapes, Alabaster, AL) was installed at each understorey plot in July 2014.
152 Cameras were attached to a horizontal crossbar extending from a vertical pole, so that they
153 were ~ 2.7 m above the ground and facing vertically downwards towards the centre of the
154 understorey plot (Fig. 1a). The support poles were located on the southern edge of the
155 understorey plot. The cameras used an automatic exposure and a fixed focus (2 m to ∞) and
156 photos were captured at the finest resolution possible (8 megapixels). Photos were captured at
157 10 minute intervals for at least one hour pre- and post-sunrise. Images captured shortly after
158 sunrise were targeted because they did not contain shadows from surrounding trees and site
159 infrastructure. Photos were captured between the 1st of August 2014 and the 31st of March
160 2017.

161 **Image greenness and live foliage cover**

162 Image analysis focused on a 1.5 m^2 (1.23 m x 1.23 m) region of interest at the centre of each
163 plot (Fig. 1b). The green chromatic coordinate index (GCC) was used in our study to quantify
164 the greenness of the live vegetation component, calculated using the following equation
165 (Sonnentag *et al.*, 2012):

166 $$GCC = \frac{G_{DN}}{R_{DN}+B_{DN}+G_{DN}} \quad \text{Eqn 1.}$$

167 Where R_{DN}, B_{DN} and G_{DN} are the red, blue and green digital numbers of the image
168 respectively. Previous work has found a strong correlation between GCC and grassland
169 phenology including live biomass, leaf area index and aboveground gross primary production
170 (Migliavacca *et al.*, 2011, Toomey *et al.*, 2015). The GCC was calculated for each image
171 using R code available from https://www.bitbucket.org/remkoduursma/phenora.

172 A number of image quality checks were undertaken to exclude unsuitable images from the
173 analysis. Heavy rainfall occasionally flooded the rings, which obscured live vegetation and
174 increased the reflectance of light from the ground surface. We manually inspected images
175 taken during and after periods of heavy rainfall (i.e. > ~40 mm of precipitation over a 3-day

window) when flooding may have occurred. Images with evidence of flooding were subsequently excluded from the analysis. A subset of photos from two plots within an eCO_2 treatment ring and one plot within an aCO_2 treatment ring were excluded due to the encroachment of shrubs. Photos were excluded when shrub foliage cover exceeded ~20% of the image region of interest, resulting in the removal of images beyond August 2015 and November 2015 for the two respective eCO_2 plots and March 2017 for the aCO_2 plot.

Illumination and shadow affect the red-green-blue (RGB) brightness levels of an image, and can consequently add unwanted variability in time series analysis of photos (Sonnentag *et al.*, 2012). Although GCC has been shown to be less sensitive to the effects of scene illumination than other indices (Sonnentag *et al.*, 2012), additional controls were put in place to minimise the effect of scene illumination and shadow. Images captured 10 minutes to 40 minutes following sunrise were used for data analysis, as preliminary examination of data revealed that images captured in this period provide a reliable estimate of GCC and are not impacted by shadows (S1). Images with very low illumination were filtered out by excluding images with a total RGB value less than 250. The 90^{th} percentile value for each camera was then calculated over a three day moving window (GCC_{90th}) in order to further minimise the effect of daily scene illumination (Sonnentag *et al.*, 2012).

Manual classification of live foliage cover was undertaken on a set of images taken from each camera at four points in time (August 2014, February 2015, August 2015 and February 2016). Images captured towards the start and finish of the southern hemisphere growing season were targeted as they coincide with the seasonal minima and maxima in understorey cover. A 12 x 12 (n = 144) grid of regularly spaced survey points was generated across the image region of interest (Fig. 1b) in ArcGIS (Esri, Redlands, CA). The substrate intersected by each point was inspected on the screen and classified as (i) live foliage, (ii) dead foliage, (iii) leaf litter, (iv) branches, twigs or bark, or (v) soil, by one operator (SV) to minimise bias. The percentage of points classified as live vegetation was calculated and used as an estimate of live foliage cover (%). GCC_{90th} was calculated for the region of interest in each photo within three days of the photo capture. The relationship between live foliage cover and GCC_{90th} was modelled using a linear mixed effect model, with camera and ring specified as random effects. GCC_{90th} was found to be a good predictor of live foliage cover ($R^2 = 0.84$, $F_{1;66} = 693.85$, $P < 0.001$, Fig. 2). The modelled relationship was used to estimate live foliage cover for the GCC_{90th} time series (Live cover = $612.97 GCC_{90th} - 189.01$).

Understorey biomass

Non-destructive estimates of understorey biomass were undertaken at the understorey plots using plant height measurements obtained from stereo-photography. Stereo-photography has been found to be a cost efficient and effective means to derive structural information for plants, which can be utilised to estimate a range of plant attributes including vegetative biomass (Yoon & Thai, 2010). A Bumblebee XB3 stereo video camera (Point Grey Research) was used in our study, with details of camera specifications, settings and calibration provided in S2. Plots were surveyed at one to three month intervals between February 2015 and March 2017. Measurements were taken at dusk under diffuse light conditions to avoid measurement errors related to shadows from trees and EucFACE infrastructure on the understorey vegetation canopy. On each sampling date, three consecutive measurements were taken over the centre of each understorey plot, from which mean plant height (H_{mean}, in m) was calculated for each plot. Aboveground biomass (B, in Kg m^{-2}) in each plot was estimated from mean plant height using an empirical model developed for the grassy understorey vegetation at the EucFACE ($B = 1.72H_{mean} - 0.05$, see S2); estimated aboveground biomass for each ring was calculated by averaging the four plot estimates obtained for each ring.

Statistical analysis

Generalised Additive Mixed Models (GAMMs) were used to assess temporal patterns in live foliage cover and biomass and the effect of CO_2 treatments and environmental parameters. GAMMs utilise smoothing functions to link response and predictor variables, allowing for estimation of non-linear relationships (Zuur *et al.*, 2009). Cubic regression splines were used in all fitted GAMMs. The smoother term degrees of freedom were set at values that provide biologically realistic representation of changes in live foliage cover and biomass (i.e. the number of increasing and decreasing trends) over the study period.

Temporal patterns in understorey growth were initially plotted and visually compared with daily trends in precipitation, volumetric water content (VWC) of soil and temperature. A GAMM was fitted to the time series of live foliage cover, with the smoothed term in the model (Time) allowed to have up to 25 degrees of freedom. Numerical derivatives of live foliage cover (dC/dT) and confidence intervals were estimated from the GAMM and used to identify any significant increase or decrease in live foliage cover (see Duursma *et al.*, 2016),

239 which represent periods of growth and senescence respectively. Daily precipitation was
240 obtained from the Australian Bureau of Meteorology (BOM) rain gauge at the EucFACE
241 facility (Station 67021, www.bom.gov.au, accessed 19th July 2017). Average daily VWC to
242 30 cm depth was calculated using data collected at 15 minute intervals from 48 soil moisture
243 probes (CS650-L; Campbell Scientific, Logan, UT, USA) located within the six experimental
244 rings (8 probes per ring). Daily temperature data was obtained from a BOM station located
245 ~5 km from EucFACE (Station 67105, www.bom.gov.au, accessed 19th July 2017).

246 GAMMs were fitted to the aCO_2 and eCO_2 subsets of the live foliage cover and biomass
247 datasets separately, with time since the commencement of the photo capture (Time) specified
248 as a smoother term and Ring as a random effect to account for repeated sampling within
249 Rings. The smoothed term (Time) was allowed to have up to 25 and 12 degrees of freedom
250 for live cover and biomass respectively, reflecting differences in the sampling frequency.
251 Model predictions and 95% confidence intervals were plotted to determine if CO_2 treatment
252 was leading to differences in live foliage cover and biomass over time. A linear mixed effect
253 model (LMM) was used to assess the statistical significance of the interaction between CO_2
254 treatment and Time, with Time and CO_2 being treated as fixed factors and Ring as a random
255 effect. Fitting of the LMM was restricted to dates when there was data available for all six
256 rings, which included 67% of the live foliage cover data and all of the biomass data. Residual
257 and quantile plots were visually assessed to determine if the assumptions of homogeneity of
258 variance and normality were met. Variance showed a fanning pattern as fitted values
259 increased for live foliage cover, so a square root transformation was applied and a power of
260 the covariate variance structure was added to allow variance to change with Time, to satisfy
261 the assumption of homogeneity of variance (Zuur *et al.*, 2009). A square root transformation
262 was applied to biomass and a constant variance function was included to allow different
263 variance for CO_2 treatment levels (Zuur *et al.*, 2009).

264 The effect of eCO_2 on vegetation cover and growth are often dependent upon water
265 availability across semi-arid and temperate systems. There was a strong spatial gradient in
266 soil VWC across EucFACE, which may result in spatial and temporal variability in CO_2
267 effects on vegetation growth that would be undetected in time series analysis. We tested
268 whether the relationship between soil VWC and live foliage cover and growth was effected
269 by eCO_2 by comparing GAMMs fitted with (i) a single smoother for VWC and (ii) smoothers
270 for VWC that were allowed to vary depending on CO_2 treatment. Data from the growing

season (August to March, Fig. 3a & b) was used for this analysis. The number of days since the first of August (i.e. growing season start date, GSS) and tree canopy leaf area index (LAI) were included as covariates in the GAMM. We favoured using GSS as a covariate over temperature, because GSS will incorporate the effects of both temperature and photoperiod. Overstorey LAI was estimated from measurements of below- and above-canopy PAR on cloudy days, following Duursma *et al.* (2016). To generate daily values of overstorey LAI, the data were interpolated using a smoother, as described by Duursma *et al.* (2016).

Vegetation and climatic time series data will have strong temporal auto-correlation (Zuur *et al.*, 2009). In order to minimise the effect of autocorrelation in our analysis of growth we sampled live foliage cover at 15-day intervals. A 15-day window was selected as it reflected a time scale at which meaningful changes in foliage growth rates and VWC occurred (Fig. 3) and it provided a fine enough sample frequency to capture a large range of environmental conditions associated with GSS and LAI. Average values of VWC and LAI were calculated for the 15 days preceding the live foliage cover measurement. Growth rate was calculated as the change in live foliage cover between the start and end of the 15-day window. A GAMM was initially fitted with smoothers for VWC, GSS and LAI and Ring as a random effect. Smoothers were allowed to have up to 5 degrees of freedom. A semi-variogram was visually assessed for evidence of temporal autocorrelation of residuals. There was evidence of temporal auto-correlation of the residuals for live foliage cover, so data were resampled at 30 day intervals and a correlation structure (Spherical Correlation Structure) was added to the GAMM in order to reduce auto-correlation of the residuals. Dependent variables that were not statistically significant ($P > 0.05$) were dropped from the model to derive the most parsimonious model containing significant effects. A fixed CO_2 term and separate VWC smoothers for aCO_2 and eCO_2 were then added to the model and a likelihood ratio test was used to compare the fit of the two models.

Analyses were undertaken using R version 3.4.1 (R Development Core Team, 2016). GAMMs were fitted using the 'mgcv' package (Wood, 2006). LMMs were fitted using the 'nlme' package (Pinheiro *et al.*, 2017).

Results

Environmental conditions and phenology

Environmental conditions experienced at EucFACE varied considerably during the course of the experiment (Fig. 3c & d). Total precipitation ranged from 587 mm to 631 mm across the three growing seasons (August - March). However, precipitation events were sporadic, resulting in extended dry periods during which soil VWC fell below 0.07 m^3 m^{-3} (Fig. 3c), which have been shown to reduce CO_2 assimilation of the dominant C3 grasses by ~30% - 50% at EucFACE (Pathare *et al.*, 2017). These dry periods were generally associated with declining live foliage cover during our study (e.g. Nov 2014, Mar 2015, Apr – Jun 2016, Nov – Dec 2016; Fig. 3a & b). Periods of high water availability (VWC > 0.2 m^3 m^{-3}) occurred periodically during both the warm and cool months (i.e. Apr – Sept 2015, Jan – Feb 2016, Jun – Sep 2016, Mar 2017; Fig. 3c & d). Maximum daily temperature ranged between 11.8°C and 47.0°C (Fig. 3d), with a mean maximum temperature of 30.8°C during summer and 18.5°C during winter. Maximum daily temperature exceeded 33.9°C on 25% of days during summer and heatwaves with several days exceeding 40.0°C were not uncommon (e.g. Nov 2015, Jan2017; Fig. 3d). Minimum daily temperatures ranged between -2.5°C and 14.3°C (Fig. 3d), with a mean minimum temperature of 5.3°C.

Patterns of understorey growth varied across growing seasons (Fig. 3a & b), reflecting patterns in both temperature and water availability. The onset of growth typically commenced in August, as indicated by the increasing trend in live foliage cover (Fig. 3a) and the significant positive response in the first derivative (dC/dt) of the GAMM (Fig. 3b). The end of the growing season was not as distinct, with live foliage cover declining gradually over the Autumn and Winter months in both years (Fig. 3a & b), though periods of growth were uncommon after the end of March (Fig. 3b). Multimodal growth patterns occurred over each growing season (August – March) which was associated with soil VWC (Fig. 3). Despite different patterns in the timing and amount of rainfall in each growing season, maximum foliage cover always reached ~40%, with the exception of a spike in March 2017 which was driven by the growth of annual forbs (Fig. 3a).

Rapid understorey senescence was observed during two heatwaves that occurred under conditions of water limitation (VWC <0.1 m^3 m^{-3}; Inset 1 and 4, Fig. 3a). Both heatwaves were followed by rainfall events that facilitated rapid re-growth (Fig. 3), highlighting the

capacity for rapid recovery of understorey biomass in these ecosystems. Psyllid infestations resulted in periodic defoliation of tree canopies throughout the study (Fig. 3a) (Gherlenda *et al.*, 2016), leading to increased photosynthetically active radiation (PAR) reaching the understorey (Duursma *et al.*, 2016). This led to exceptionally high live understorey foliage cover values recorded during January – February 2015 (Inset 2, Fig. 3a) and may have contributed to rapid growth in Spring 2015 (See arrow in Fig. 3a).

Effects of eCO_2 on live foliage cover and biomass

There was no effect of CO_2 treatment on either live foliage cover or biomass over the duration of the experiment, as evidenced by a consistent overlap of the estimated 95% confidence interval from the fitted GAMMs (i.e. $P > 0.05$ at any given time, Fig. 4). A LMM found no significant interaction between date of measurement (Time) and CO_2 treatment for both cover and biomass (Table 1), providing support for this observation. However, we do note that mean biomass (± 95% confidence interval) in the aCO_2 plots (0.345 ± 0.028 Kg m^{-2}) was generally greater than in the eCO_2 plots (0.312 ± 0.037 Kg m^{-2}), with a difference of 0.032 ± 0.019 Kg m^{-2} across the measurement periods. There was considerable variability in both cover and biomass within CO_2 treatments at any given point in time, which likely reflects gradients in environmental conditions (e.g. soil VWC) across the EucFACE site.

Effects of climate and eCO_2 on live foliage cover

Soil VWC was the only significant predictor of growth rate ($F_{2, 224} = 8.2349$, $P = 0.0004$, $R^2 = 0.14$), whereby growth rate showed a hump-shaped relationship with soil VWC, peaking at ~0.15 m^3 m^{-3} (Fig. 5). When VWC fell below ~0.06 m^3 m^{-3} declines in live foliage cover were recorded (Fig. 5), indicating periods of senescence. Patterns in live foliage cover were significantly affected by soil VWC and time since the growing season start date (GSS) ($F_{3, 123} = 109.7660$, $P < 0.0001$, $R^2 = 0.40$; S3). Live foliage cover showed an asymptotic relationship with water availability late in the growing season, increasing with soil VWC up to ~0.1 m^3 m^{-3} then levelling off (Fig. 6). A similar relationship was evident for GSS when soil VWC exceeded ~0.15 m^3 m^{-3}, whereby foliage cover increased up to 150 days from the growing season start date and levelled off thereafter (Fig. 6). Model predictions indicate greatest live foliage cover occurs late in the growing season when soil VWC exceeds ~0.10 m^3 m^{-3} (Fig. 6).

Comparison of models with and without unique VWC smoothers for the CO_2 treatments showed that allowing smoothers to vary did not improve the model goodness of fit for live foliage cover (L = 2.5457, df = 3, P = 0.4671) or growth rate (L = 0.4988, df = 3, P = 0.9192). These analyses indicate that eCO_2 did not modify the effect of VWC on growth and cover, hence there is no evidence that eCO_2 increased live foliage cover or growth rates during periods of low soil moisture.

Discussion

Our experiment provides the first field based assessment of the interactive effect of eCO_2 and water availability on understorey productivity in a grassy woodland ecosystem. Through the use of repeat near-surface digital photography we were able to quantify short term responses (weekly – monthly) of understorey cover and biomass, and assess the effect of eCO_2 during periods of varying water availability. Climatic conditions experienced during the ~2.5 year study included growing seasons with extended periods of water limitation (VWC < 0.07 m^3 m^{-3}), sufficient to reduce the rate of photosynthesis of the dominant herbaceous species at the site (Pathare *et al.*, 2017). Water availability was the dominant driver of understorey productivity, with low understorey growth rates and cover being recorded under conditions of low soil water availability, as has been found in grasslands globally (Del Grosso *et al.*, 2008, Hovenden *et al.*, 2014, Morgan *et al.*, 2004, Reich *et al.*, 2014). Contrary to expectations, eCO_2 did not increase understorey productivity during periods of water limitation. This is surprising as water savings associated with eCO_2 have consistently been found to increase grassland productivity by alleviating water stress in semi-arid and temperate environments (Hovenden *et al.*, 2014, Morgan *et al.*, 2011, Morgan *et al.*, 2004, Reyes-Fox *et al.*, 2014, Smith *et al.*, 2000, Zhu *et al.*, 2016a). Our findings suggest that any effect of eCO_2 on herbaceous understorey productivity was much smaller than the effect of climate variability. Consequently, future increases in [CO_2] may not offset the effect of future drying on understorey productivity in temperate grassy woodlands of Australia.

Although canopy trees at EucFACE have exhibited reduced stomatal conductance in response to eCO_2 (Drake *et al.*, 2016, Gimeno *et al.*, 2016), with no concurrent effect of eCO_2 on canopy LAI (Duursma *et al.*, 2016), there has been no eCO_2 treatment effect on stomatal conductance for the dominant C3 herbaceous understorey plants (Pathare *et al.*, 2017). VWC in the upper 30 cm of the soil profile at EucFACE has not increased in response to eCO_2 (Pathare *et al.*, 2017), which is one likely explanation for the absence of eCO_2 stimulation of

understorey productivity during periods of water limitation. However, carbon assimilation (A_{net}) in the C3 herbaceous understorey plants at EucFACE did increase by ~30% under eCO_2, with the greatest stimulation occurring during periods of low water availability (Pathare *et al.*, 2017). It is possible that extra assimilated carbon is being invested into below ground growth of grasses (Suter *et al.*, 2002), which cannot be resolved using the remote sensing techniques employed in our study. Increased allocation of carbon to roots has been observed for grasses grown under eCO_2 in the absence of nutrient addition (e.g. N, Suter *et al.*, 2002). Previous studies have documented that N availability decreases in eCO_2 (e.g. Reich *et al.*, 2006) but the lack of response in our site may have been, at least partially, driven by a putative P-deficiency (see Ellsworth *et al.*, 2017), although detailed discussions are beyond scope. Alternatively, the absence of growth response to eCO_2 may reflect light limitations in the understorey. Gas exchange measurements conducted by Pathare *et al.* (2017) used saturating light levels (1800 μmol m^{-2} s^{-1}), in order to simulate maximum C assimilation during periodic (30 mins/day) sun flecks in Spring and Summer. Consequently, competition for light with trees may potentially explain the different growth response between the woodland understorey examined in our study and temperate grasslands elsewhere (e.g. Hovenden *et al.*, 2014, Morgan *et al.*, 2004, Reyes-Fox *et al.*, 2014).

A key advantage of the application of near-surface remote sensing is the ability to detect short-term responses in above ground productivity in response to environmental change, which cannot be characterised by annual measures of growth (Hufkens *et al.*, 2016, Zelikova *et al.*, 2015). Using this approach we were able to quantify temporal patterns in understorey productivity and detect thresholds in VWC that were associated with understorey mortality (<0.06 m^3 m^{-3}) and peak growth rates (~0.1 – 0.2 m^3 m^{-3}), which correspond to periods of minimum and maximum CO_2 assimilation for the dominant herbaceous plants in the study area (Pathare *et al.*, 2017). The hump shaped response of understorey growth to soil water availability was not entirely unexpected, reflecting the effects of water limitations during periods of low VWC and changes to environmental conditions associated with high VWC (Zhu *et al.*, 2016a). Substantial volumes of rainfall (>120 mm in 7 days) are needed to increase surface VWC beyond 0.2 m^3 m^{-3} at EucFACE, with periods of consistent rainfall (i.e. weeks) required to sustain high surface VWC (Fig. 3c). Consequently, periods of high VWC are typically associated with cloudy conditions, which lead to lower growth rates due to light limitation. Nutrient leaching may have also contributed to low growth rates under very wet conditions, though such effects may vary seasonally (Hovenden *et al.*, 2014).

Productivity of the understorey community was resilient to the effects of environmental variability, displaying the ability for rapid recovery following periodic drought and heatwaves (Inset 1 and 4, Fig. 3a). Rapid recovery of cover and biomass in native Australian temperate grasslands following drought has been demonstrated in a number of experiments (Mitchell *et al.*, 2016, Power *et al.*, 2016), facilitated by the ability for vegetative regrowth of dominant native grasses, such as *M. stipoides* (Mitchell *et al.*, 2016). Total growing season precipitation was relatively consistent across years, though the timing of wet and dry cycles within growing seasons varied. The maximum cover and biomass recorded within each year was reasonably consistent, suggesting that above ground productivity may be insensitive to the timing of water limitation during the growing season, unlike some temperate grasslands (Craine *et al.*, 2012, Hovenden *et al.*, 2014). This observation is supported by experimental manipulations using herbaceous species common to Australian temperate grasslands and woodlands, which show that aboveground productivity of the understorey responds to the amount of precipitation over the growing season, not the timing of rainfall events within the growing season (Gibson-Forty *et al.*, 2016, Power *et al.*, 2016). Continued monitoring at EucFACE will provide further insight into the effects of rainfall variability on the dynamics of understorey growth and the potential for temporal lags in growth response to water availability (i.e. legacy effects; Sala *et al.*, 2012).

Productivity has been observed to increase in response to climate change in some grasslands, with higher temperatures leading to earlier Spring growth and eCO_2 effectively extending the growing season by increasing plant water use efficiency (Barichivich *et al.*, 2013, Hufkens *et al.*, 2016, Reyes-Fox *et al.*, 2014). However, our results suggest that trends of declining grass productivity may occur in Australian temperate grassy woodlands under future climate. A drying trend is projected for temperate regions of Australia, with declines (~20 - 30%) in winter and spring precipitation and increases in the number of days spent in drought expected by the end of the century (CSIRO and Bureau of Meteorology, 2015). Although rising temperatures will result in earlier onset of Spring growth, lower Spring precipitation and increased frequency of Summer droughts will likely result in reduced understorey productivity. Consequently, the understorey growth response of grassy woodland ecosystems to future climate across temperate regions in Australia may not contribute towards the mitigation of rising atmospheric [CO_2], contrary to predictions for many other ecosystems (e.g. Hufkens *et al.*, 2016).

The importance of indirect effects of invertebrate herbivores on canopy – understorey interactions was evident in our study. Psyllids are common agents of periodic canopy senescence within eucalypts across Australian forests and woodlands (Gherlenda *et al.*, 2016). Two major psyllid overstorey defoliation events occurred during the study period, driven by the leaf senescence-inducing *Cardiaspina fiscella* (Gherlenda *et al.*, 2016), which subsequently resulted in increased productivity of understorey vegetation (Fig. 3a). Defoliation of trees in Ring 6 during November and December 2014 resulted in increased photosynthetically active radiation (PAR) reaching the understorey (Duursma *et al.*, 2016), which along with sufficient soil water availability, contributed to the exceptionally high understorey live foliage cover recorded in the understorey during January – February 2015 (Inset 2, Fig. 3a). A subsequent Psyllid outbreak affecting the entire study area occurred at the onset of the 2015/2016 growing season (See arrow in Fig. 3a), though it is difficult to discern to what degree the rapid growth was due to suitable climatic conditions, increased PAR or the combination of both. These observations suggest that climate-herbivore interactions will be influential in determining ecosystem productivity and ecosystem carbon exchange, as has been observed in other ecosystems (Allen *et al.*, 2010, Keith *et al.*, 2012).

Predicted shifts towards greater aridity and more frequent drought in semi-arid, temperate and tropical regions of the globe (CSIRO and Bureau of Meteorology, 2015, IPCC, 2014) are likely to modify herbaceous productivity across large areas of the grassland, woodland and savanna biomes (Hufkens *et al.*, 2016, Lehmann *et al.*, 2014, Zhu *et al.*, 2016a). While water savings associated with increasing [CO_2] have the potential to offset the effects of increasing aridity on grass productivity (Reyes-Fox *et al.*, 2014), these effects are not ubiquitous (Morgan *et al.*, 2004, Reich *et al.*, 2014, Zelikova *et al.*, 2015), as further demonstrated in our study. Future climate change could lead to lower understorey productivity in Australian temperate grassy woodlands, via reductions to Spring rainfall and increasing drought. However, the sensitivity of these systems to total precipitation suggest large inter-annual variability in grass productivity is likely, as has been suggested for other ecosystems globally (e.g. Zhu *et al.*, 2016a). Climate change and biotic interactions such as herbivory are likely to exert much greater influence on understorey productivity than increased atmospheric [CO_2] in temperate grassy woodlands.

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Tables

Table 1 Summary of linear mixed effect models testing the effects of [CO_2] and Time on the cover and biomass of understorey vegetation. Cover and biomass were square root transformed to satisfy the assumption of homogeneity of variance.

Response variable	Predictor variable	*F*	DF	DFres	*P*-value
Cover	Intercept	760.64	1	848	**<0.0001**
	Time	36.56	212	848	**<0.0001**
	CO_2	0.30	1	4	0. 6144
	Time x CO_2	0.82	212	848	0.9650
Biomass	Intercept	660.42	1	68	**<0.0001**

Time	7.61	17	68	**<0.0001**
CO_2	0.54	1	4	0.5040
Time x CO_2	0.70	17	68	0.7943

Figures

Fig. 1 (a) Camera set up and (b) 1.5 m^2 (1.23 m x 1.23 m) region of interest (shaded red) used for analysis across all photos

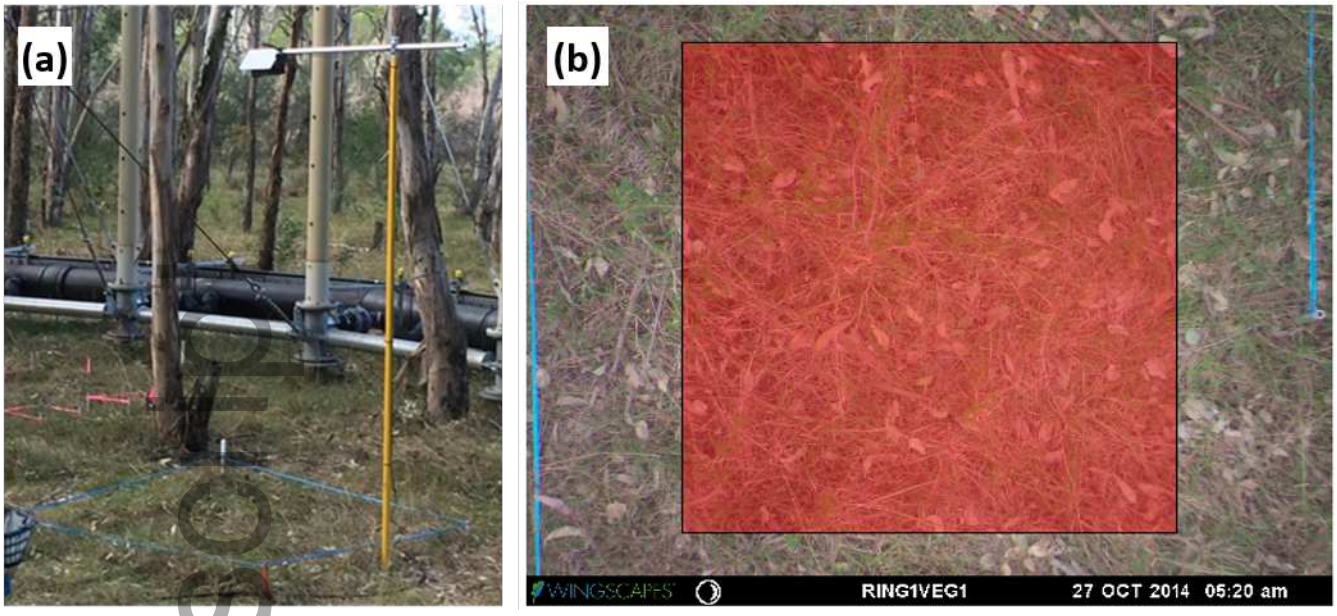
Fig. 2 The fitted relationship (± 95% confidence interval) between GCC_{90th} and understorey live foliage cover (%). The open grey circles and grey crosses are the observations used to fit the LMM from winter and summer respectively. The shaded area is the 95% confidence interval.

Fig. 3 (a) Smoothed time series of understorey live foliage cover (%) averaged across all six treatment rings. Box 1 and Box 4 depict episodes of grass curing triggered by extreme temperatures and water deficit. Box 2 depicts the effects of overstorey canopy defoliation in Ring 6 (ambient CO_2) due to Psyllids. Arrow 3 identifies the onset of Psyllid defoliation of overstorey trees across all Rings. (b) Change in understorey live foliage cover (dC/dt), including the 95% confidence intervals (shaded grey polygon). (c) Time series of daily precipitation (black bars) and site level averages of soil volumetric water content (VWC) (grey line). (d) Time series of daily maximum temperature (black points) and minimum temperature (grey points).

Fig. 4 (a) Mean understorey live foliage cover (%) and (b) biomass (Kg m^{-2}) for the fitted GAMMs for aCO_2 (blue line) and eCO_2 (red line). Points are the observed data for aCO_2 (blue) and eCO_2 (red). Shaded areas are the 95% confidence intervals.

Fig. 5 Smooth regression models (black lines) for trends in understorey growth rate as a function of mean soil VWC. The grey polygon represents the 95% confidence interval. Mean values for VWC were calculated for the 15 days prior to the growth rate measurement date.

664 **Fig. 6** A contour plot showing the effect of days since the start of the growing season and
665 mean soil VWC (m^3 m^{-3}) on understorey live foliage cover (%). Values are based on
666 predictions from a GAMM. Mean values for VWC were made over a 15 day period prior to
667 the live foliage cover measurement.



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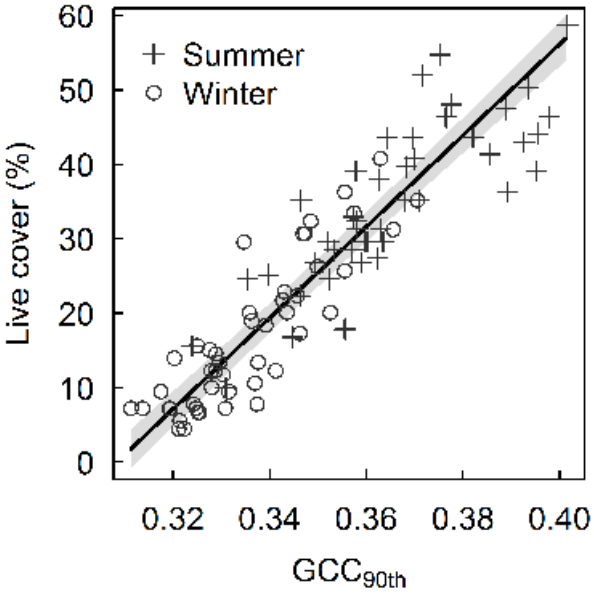

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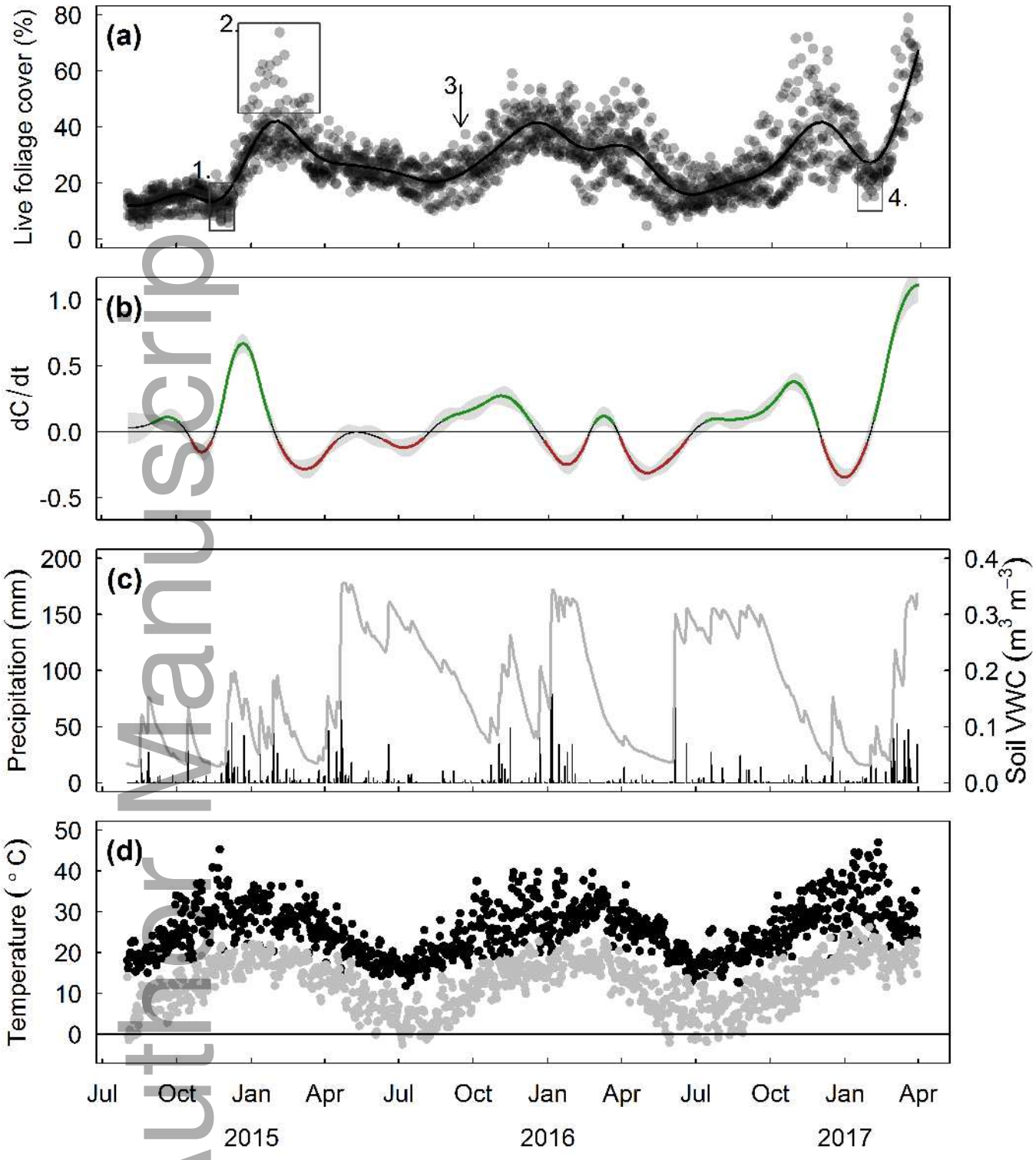

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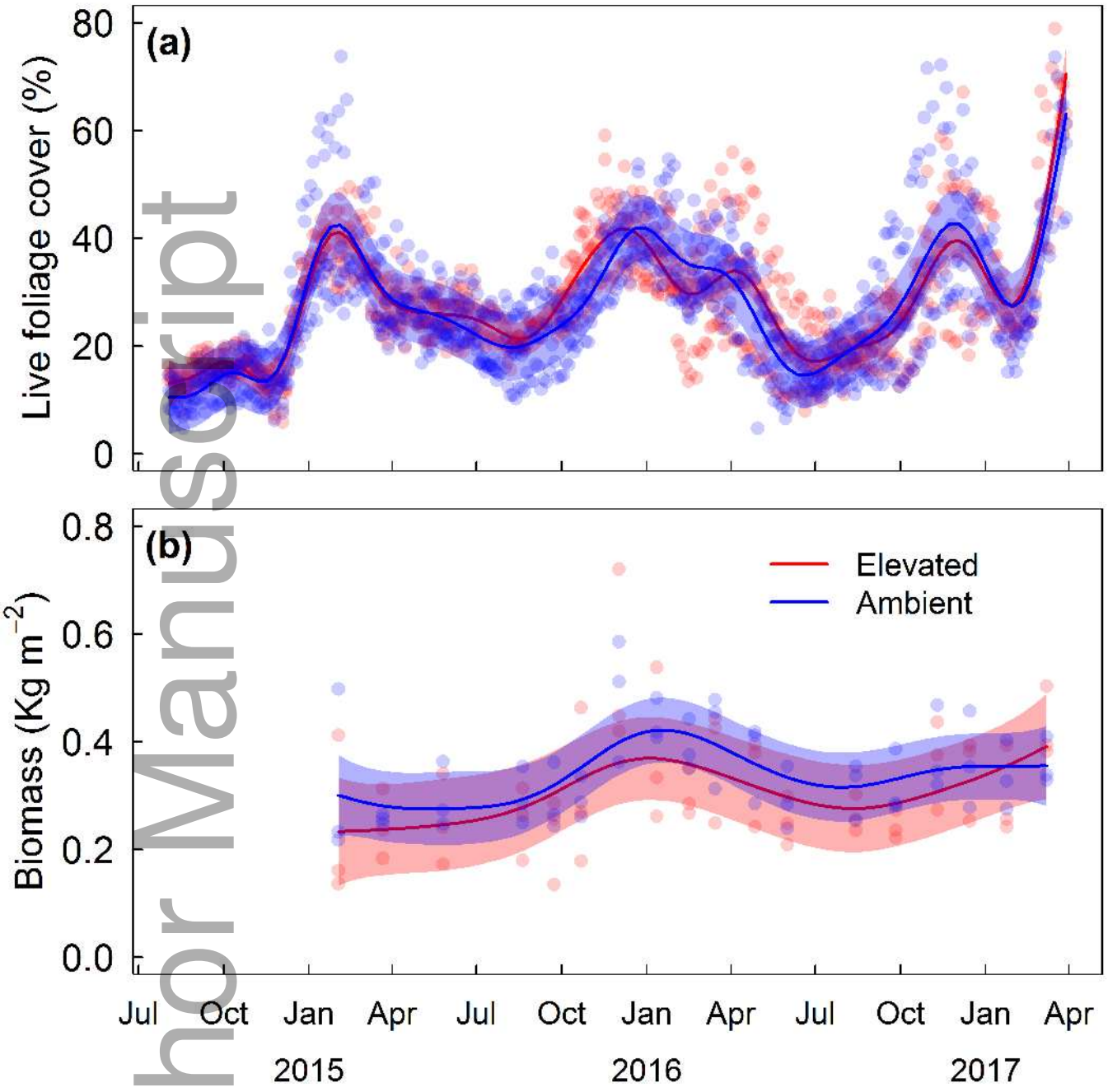

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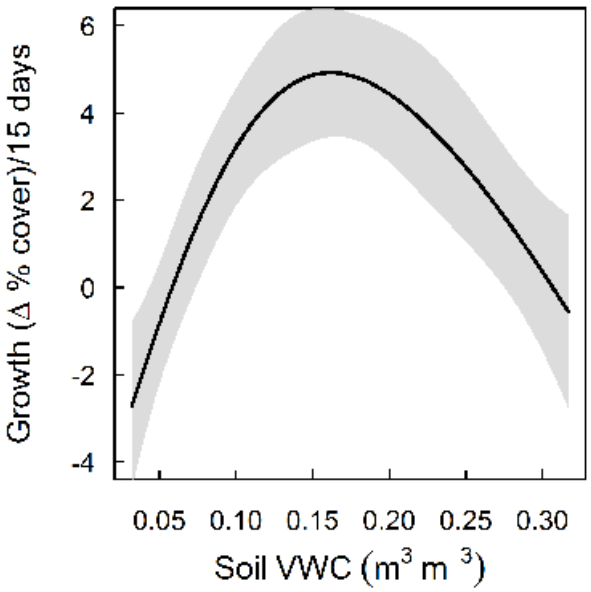

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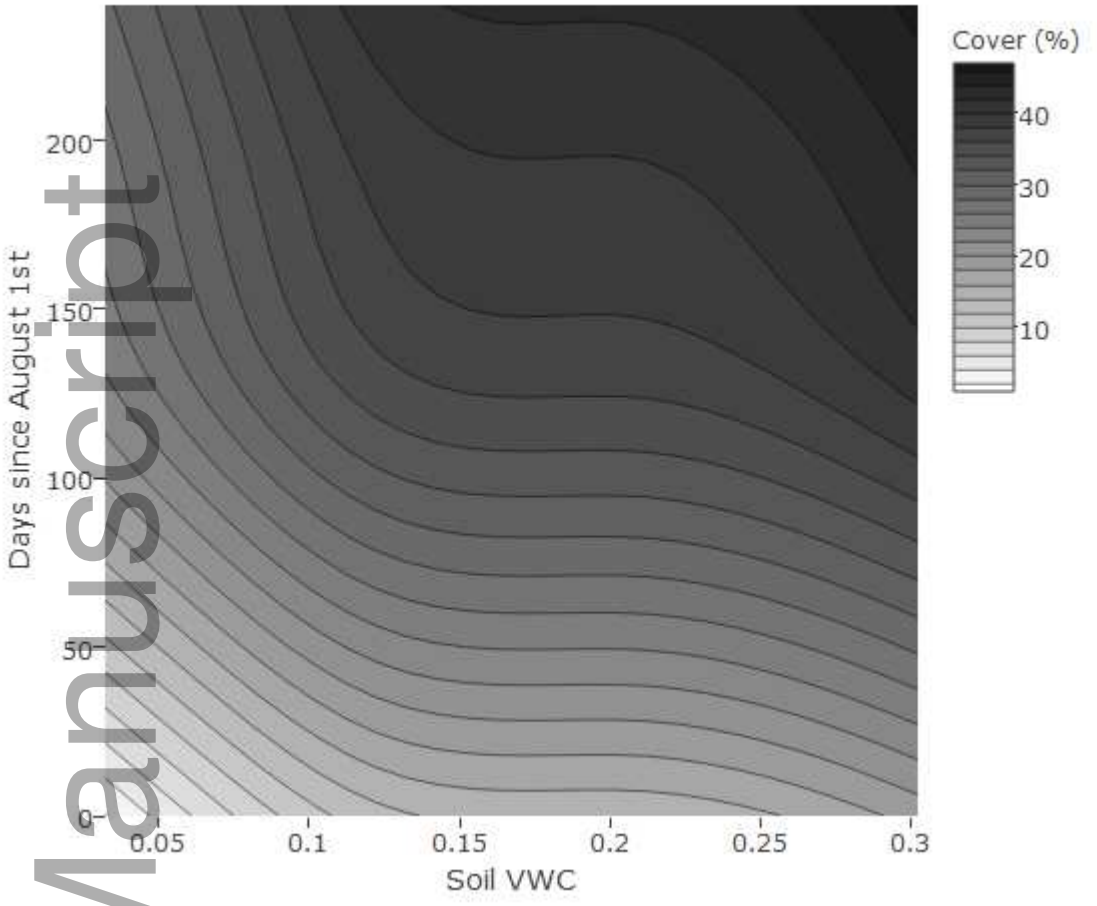

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