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## Supplementary information

# Microbial competition for phosphorus limits the $CO_2$ response of a mature forest

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# Supplementary Information

## Microbial competition for phosphorus limits $CO_2$ response of a mature forest

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**Supplementary Table List**

## 1. Additional data to place EucFACE in a global context

The size of phosphorus (P) pool in stored in plants at EucFACE is relatively low, but it is broadly comparable to those estimated for broadleaf forests in the temperate and tropical or subtropical regions, in that it is above the 25th percentile of a regional dataset [1] (n=1969; Extended Data Figure 1d). Similarly, the amount of P stored in plant relative to soil at EucFACE (i.e., plant over soil P pool ratio) is broadly comparable to those estimated for tropical or subtropical broadleaf forest (Extended Data Figure 1e). This result suggests that the size of P pools estimated for EucFACE is representative of a large variety of forests.

In terms of the amount of P stored in soil microbes relative to the size of soil organic P pool, it is observed that EucFACE is on the relatively high end of the global estimate [2] (n=144; Extended Data Figure 1f). Soil microbes at EucFACE stored about 6.0 ± 1.43 g P $m^{-2}$ over the top 60 cm of the soil, which accounts for 24% of the soil organic P pool. The global dataset indicates a mean fraction of 11.6% and a median fraction of 7.2%, significantly lower than the fraction estimated for EucFACE. This result suggests that microbes at EucFACE may have contained a disproportionally large amount of P in their biomass.

Plant P resorption played an important role to meet the annual plant nutrient demand. At EucFACE, canopy leaves on average retranslocated 55% of P from its tissue before leaf senescence (Supplementary Table 1). This canopy P resorption fraction is above the global average of 48% reported for evergreen broadleaf forests [3]. In comparison, understorey leaves are less efficient in resorbing P (43%) than the overstorey tree leaves. Sapwood is the most efficient plant organ in resorbing P, in that about 71% of P is retranslocated each year.

**Supplementary Table 1. Phosphorus (P)-resorption coefficient calculated for canopy leaf, understorey shoot, and sapwood, under ambient and elevated $CO_2$.** Canopy and understorey P resorption was calculated based on the difference between P concentrations in mature green leaves and leaf litter. Sapwood P resorption was calculated as the difference between sapwood P concentration and heartwood P concentration. Values are time-averaged means ± standard deviations (n=3).

| Treatment | Canopy | Understorey shoot | Sapwood |
|---|---|---|---|
| $aCO_2$ | 0.546 ± 0.058 | 0.423 ± 0.110 | 0.710 ± 0.061 |
| $eCO_2$ | 0.552 ± 0.060 | 0.417 ± 0.188 | 0.673 ± 0.055 |

## 2. Uncertainties associated with temporal fluctuations in data

### 2.1. Linear mixed effect model to quantify $CO_2$ by time interaction

In this study, we used data collected over a 6-year period (i.e., 2013-2018) to understand how atmospheric $CO_2$ enrichment ($eCO_2$) affects ecosystem P-cycle processes at EucFACE. There are three treatment plots for each $CO_2$ treatment (n=3), and data were collected in sub-replicates within each treatment plot at each sampling date. We calculated the means for each treatment plot to ensure the true replicate number is three for each $CO_2$ treatment at each sampling time point. In the overall budget reported in the main text, we reported the time-averaged values for each $CO_2$ treatment. To understand how temporal fluctuation may have affected the main $CO_2$ effect, we built a linear mixed-effects model, with $CO_2$ treatment and Date considered as fixed factors, and Plot considered as a random factor. We used R package "lme4" [4] to perform this analysis. The statistical results are summarized in Supplementary Tables 2, 3 and 4, for major P concentration, pool and flux variables, respectively.

As the statistics show, the Date factor is typically a strong predictor of the response variables (i.e., $P < 0.05$), but there is a general lack of main $CO_2$ effect, and Date typically does not interact with the Treatment factor (i.e., $P > 0.05$). This result indicates that the main $CO_2$ effect is consistent regardless of the temporal fluctuation of the data. The two exceptions are canopy P pool and fine root P concentration variables, both showing a statistically significant ($p < 0.05$) Treatment by Date interaction (Supplementary Table 2 and 3). More specifically, canopy P pool is higher (but statistically non-significant) in the $eCO_2$ treatment in all years except the later half of 2016 (Supplementary Figure 1a). In comparison, fine root P concentration is consistently higher (non-significant) in the $eCO_2$ treatment across all sampling dates except June and December in 2014 (Supplementary Figure 2a). However, when averaging these two pools at the annual timestep (Supplementary Figures 1b and 2b), none of the $CO_2$ comparisons show a significant treatment effect. Taken together, our statistical analysis suggests that the reported main $CO_2$ effect is generally consistent at different temporal scales.

**Supplementary Table 2. Statistical summary of the linear mixed-effects model on major phosphorus (P) concentration variables.** Year indicates the main effect of year, Treatment indicates the main effect of $CO_2$ treatment, whereas the Year: Treatment indicates the $CO_2$ by year interaction. Df and Df.res indicate the numerator and denominator degree of freedom, respectively.

| Canopy P concentration | F | Df | Df.res | P-value |
|---|---|---|---|---|
| Year | 11.09 | 5 | 67.55 | <0.0001 |
| Treatment | 0.20 | 1 | 3.66 | 0.683 |
| Year: Treatment | 0.75 | 5 | 67.53 | 0.587 |
| **Fine root P concentration** | **F** | **Df** | **Df.res** | **P-value** |
| Year | 9.50 | 6 | 24 | <0.0001 |
| Treatment | 4.15 | 1 | 4 | 0.111 |
| Year: Treatment | 3.20 | 6 | 24 | 0.019 |
| **Leaf litter P concentration** | **F** | **Df** | **Df.res** | **P-value** |
| Year | 4.20 | 5 | 20 | 0.009 |
| Treatment | 1.14 | 1 | 4 | 0.345 |
| Year: Treatment | 1.44 | 5 | 20 | 0.252 |
| **Understorey P concentration** | **F** | **Df** | **Df.res** | **P-value** |
| Year | 3.06 | 6 | 5.2 | 0.117 |
| Treatment | 0.28 | 1 | 3.3 | 0.632 |
| Year: Treatment | 0.02 | 2 | 4.7 | 0.985 |
| **Frass P concentration** | **F** | **Df** | **Df.res** | **P-value** |
| Year | 19.39 | 23 | 92 | <0.0001 |
| Treatment | 0.17 | 1 | 4 | 0.700 |
| Year: Treatment | 0.96 | 23 | 92 | 0.771 |
| **Microbial P concentration** | **F** | **Df** | **Df.res** | **P-value** |
| Year | 71.45 | 6 | 24 | <0.0001 |
| Treatment | 0.42 | 1 | 4 | 0.553 |
| Year: Treatment | 1.74 | 6 | 24 | 0.154 |
| **Soil P concentration** | **F** | **Df** | **Df.res** | **P-value** |
| Year | 2.76 | 5 | 20 | 0.047 |
| Treatment | 0.06 | 1 | 4 | 0.815 |
| Year: Treatment | 0.67 | 5 | 20 | 0.651 |
| **Soil labile P concentration** | **F** | **Df** | **Df.res** | **P-value** |
| Year | 46.58 | 15 | 60 | <0.0001 |

| | | | | |
|---|---|---|---|---|
| **Treatment** | 0.10 | 1 | 4 | 0.765 |
| **Year: Treatment** | 1.20 | 15 | 60 | 0.298 |

**Supplementary Table 3. Statistical summary of the linear mixed-effects model on major phosphorus (P) pool variables.** Year indicates the main effect of year, Treatment indicates the main effect of $CO_2$ treatment, whereas the Year: Treatment indicates the $CO_2$ by year interaction.

| **Canopy P pool** | **F** | **Df** | **Df.res** | **P-value** |
|---|---|---|---|---|
| **Year** | 22.90 | 399 | 1596 | <0.0001 |
| **Treatment** | 0.08 | 1 | 4 | 0.786 |
| **Year: Treatment** | 1.54 | 399 | 1596 | <0.0001 |
| **Wood P pool** | **F** | **Df** | **Df.res** | **P-value** |
| **Year** | 19.15 | 4 | 16 | <0.0001 |
| **Treatment** | 0.04 | 1 | 4 | 0.858 |
| **Year: Treatment** | 0.28 | 4 | 16 | 0.888 |
| **Fine root P pool** | **F** | **Df** | **Df.res** | **P-value** |
| **Year** | 6.14 | 6 | 24 | 0.0005 |
| **Treatment** | 0.15 | 1 | 4 | 0.715 |
| **Year: Treatment** | 0.89 | 6 | 24 | 0.519 |
| **Leaf litter P pool** | **F** | **Df** | **Df.res** | **P-value** |
| **Year** | 4.43 | 45 | 180 | <0.0001 |
| **Treatment** | 6.30 | 1 | 4 | 0.066 |
| **Year: Treatment** | 0.74 | 45 | 180 | 0.879 |
| **Understorey P pool** | **F** | **Df** | **Df.res** | **P-value** |
| **Year** | 8.54 | 17 | 68 | <0.0001 |
| **Treatment** | 1.95 | 1 | 4 | 0.235 |
| **Year: Treatment** | 0.85 | 17 | 68 | 0.635 |
| **Coarse root P pool** | **F** | **Df** | **Df.res** | **P-value** |
| **Year** | 19.88 | 4 | 16 | <0.0001 |
| **Treatment** | 0.0002 | 1 | 4 | 0.990 |

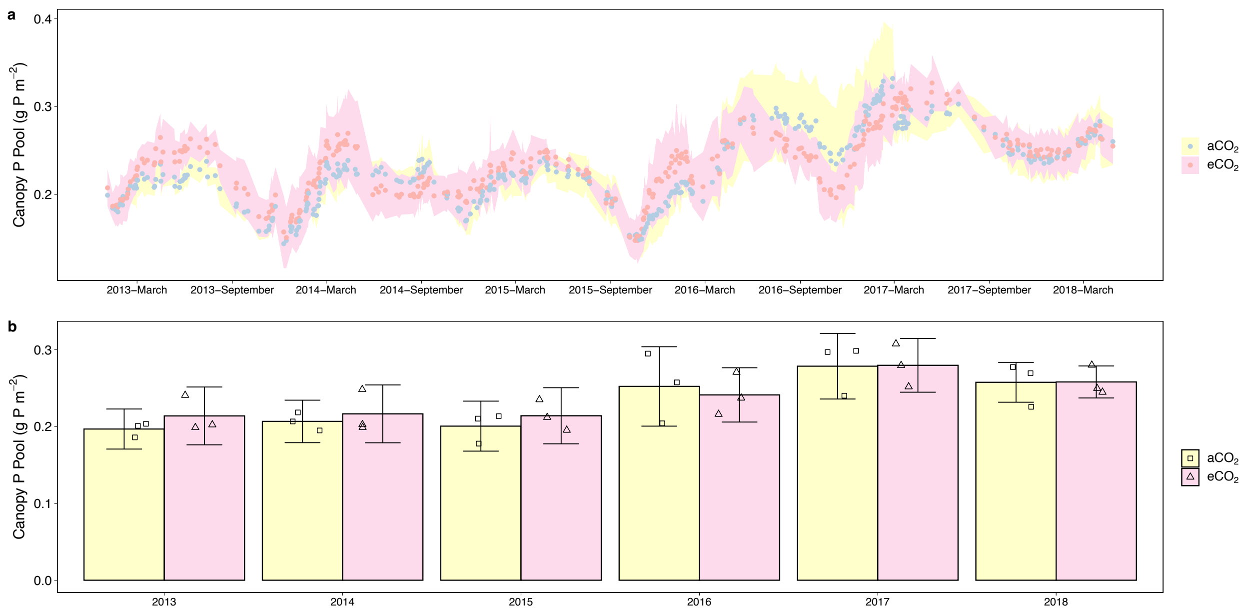
| | | | | |
|---|---|---|---|---|
| **Year: Treatment** | 0.29 | 4 | 16 | 0.882 |
| **Microbial P pool** | **F** | **Df** | **Df.res** | **P-value** |
| **Year** | 77.77 | 6 | 24 | <0.0001 |
| **Treatment** | 1.17 | 1 | 4 | 0.341 |
| **Year: Treatment** | 2.21 | 6 | 24 | 0.078 |
| **Soil P pool** | **F** | **Df** | **Df.res** | **P-value** |
| **Year** | 2.78 | 5 | 20 | 0.046 |
| **Treatment** | 0.001 | 1 | 4 | 0.974 |
| **Year: Treatment** | 0.63 | 5 | 20 | 0.681 |
| **Soil labile P pool** | **F** | **Df** | **Df.res** | **P-value** |
| **Year** | 46.28 | 15 | 60 | <0.0001 |
| **Treatment** | 0.006 | 1 | 4 | 0.944 |
| **Year: Treatment** | 1.45 | 15 | 60 | 0.154 |

**Supplementary Table 4. Statistical summary of the linear mixed-effects model on major phosphorus (P) flux variables.** Year indicates the main effect of year, Treatment indicates the main effect of $CO_2$ treatment, whereas the Year: Treatment indicates the $CO_2$ by year interaction.

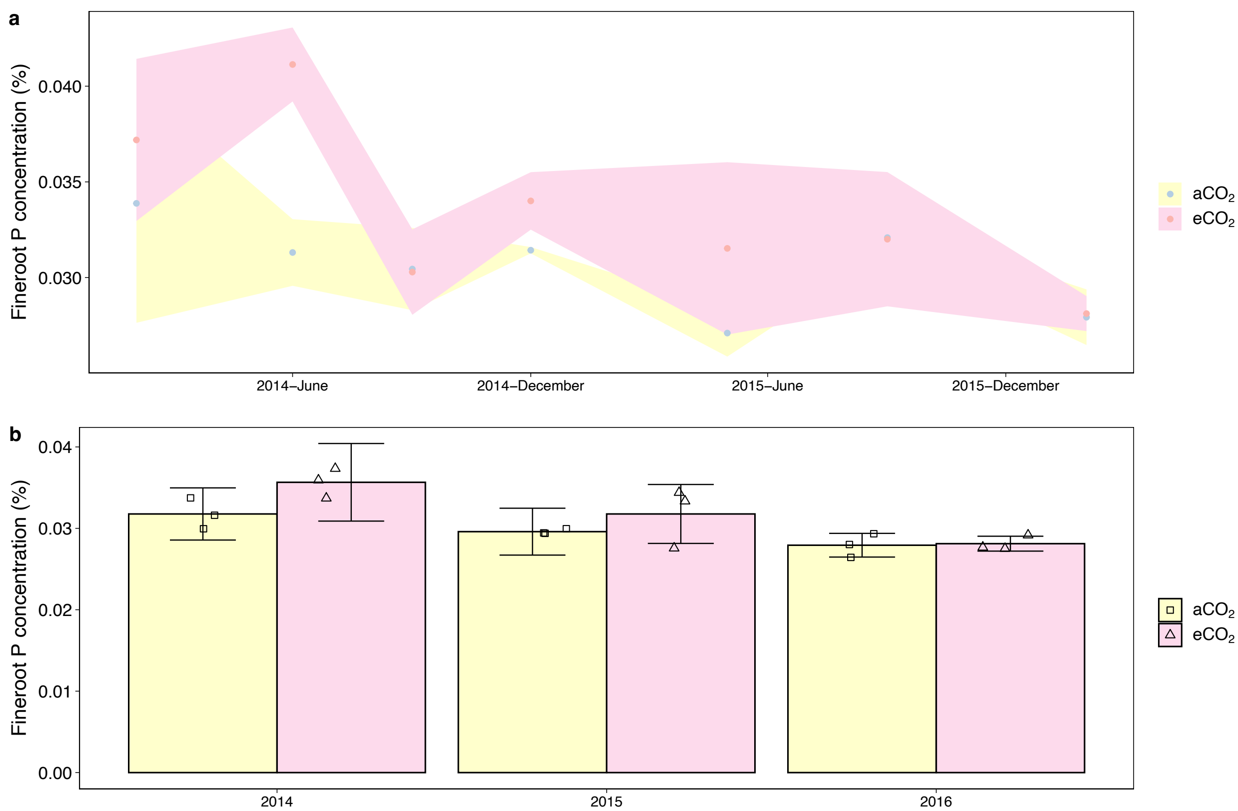
| **Canopy P flux** | **F** | **Df** | **Df.res** | **P-value** |
|---|---|---|---|---|
| **Year** | 18.82 | 48 | 192 | <0.0001 |
| **Treatment** | 0.04 | 1 | 4 | 0.858 |
| **Year: Treatment** | 1.22 | 48 | 192 | 0.173 |
| **Wood P flux** | **F** | **Df** | **Df.res** | **P-value** |
| **Year** | 3.40 | 3 | 12 | 0.054 |
| **Treatment** | 0.41 | 1 | 4 | 0.558 |
| **Year: Treatment** | 0.71 | 3 | 12 | 0.562 |
| **Fine root P flux** | **F** | **Df** | **Df.res** | **P-value** |
| **Year** | 20.72 | 2 | 8 | 0.001 |
| **Treatment** | 0.26 | 1 | 4 | 0.637 |
| **Year: Treatment** | 0.46 | 2 | 8 | 0.646 |

| Leaf litter P flux | F | Df | Df.res | P-value |
|---|---|---|---|---|
| Year | 17.83 | 48 | 192 | <0.0001 |
| Treatment | 0.03 | 1 | 4 | 0.871 |
| Year: Treatment | 1.10 | 48 | 192 | 0.322 |
| **Understorey P flux** | **F** | **Df** | **Df.res** | **P-value** |
| Year | 9.81 | 3 | 12 | 0.001 |
| Treatment | 4.36 | 1 | 4 | 0.105 |
| Year: Treatment | 1.19 | 3 | 12 | 0.355 |
| **Coarse root P flux** | **F** | **Df** | **Df.res** | **P-value** |
| Year | 3.30 | 3 | 12 | 0.058 |
| Treatment | 0.18 | 1 | 4 | 0.692 |
| Year: Treatment | 0.75 | 3 | 12 | 0.544 |
| **Fine root litter P flux** | **F** | **Df** | **Df.res** | **P-value** |
| Year | 20.72 | 2 | 8 | 0.001 |
| Treatment | 0.26 | 1 | 4 | 0.637 |
| Year: Treatment | 0.46 | 2 | 8 | 0.646 |
| **Frass P flux** | **F** | **Df** | **Df.res** | **P-value** |
| Year | 16.91 | 21 | 78 | <0.0001 |
| Treatment | 0.08 | 1 | 4 | 0.796 |
| Year: Treatment | 0.59 | 21 | 78 | 0.911 |
| **Understorey litter P flux** | **F** | **Df** | **Df.res** | **P-value** |
| Year | 9.58 | 3 | 12 | 0.002 |
| Treatment | 1.63 | 1 | 4 | 0.271 |
| Year: Treatment | 1.22 | 3 | 12 | 0.344 |
| **Canopy resorption P flux** | **F** | **Df** | **Df.res** | **P-value** |
| Year | 19.71 | 48 | 192 | <0.0001 |
| Treatment | 1.27 | 1 | 4 | 0.323 |
| Year: Treatment | 1.27 | 48 | 192 | 0.135 |
| **Sapwood resorption P flux** | **F** | **Df** | **Df.res** | **P-value** |
| Year | 3.30 | 3 | 12 | 0.058 |

| | | | | |
|---|---|---|---|---|
| **Treatment** | 0.53 | 1 | 4 | 0.507 |
| **Year: Treatment** | 0.65 | 3 | 12 | 0.596 |
| **Fine root resorption P flux** | **F** | **Df** | **Df.res** | **P-value** |
| **Year** | 20.72 | 2 | 8 | 0.001 |
| **Treatment** | 0.26 | 1 | 4 | 0.637 |
| **Year: Treatment** | 0.46 | 2 | 8 | 0.646 |
| **Coarse root resorption P flux** | **F** | **Df** | **Df.res** | **P-value** |
| **Year** | 3.19 | 3 | 12 | 0.063 |
| **Treatment** | 0.27 | 1 | 4 | 0.631 |
| **Year: Treatment** | 0.69 | 3 | 12 | 0.575 |
| **Understorey resorption P flux** | **F** | **Df** | **Df.res** | **P-value** |
| **Year** | 7.26 | 3 | 12 | 0.005 |
| **Treatment** | 0.30 | 1 | 4 | 0.615 |
| **Year: Treatment** | 0.94 | 3 | 12 | 0.451 |
| **Net P mineralization flux** | **F** | **Df** | **Df.res** | **P-value** |
| **Year** | 8.55 | 13 | 52 | <0.0001 |
| **Treatment** | 0.02 | 1 | 4 | 0.893 |
| **Year: Treatment** | 0.82 | 13 | 52 | 0.635 |
| **Soil P leaching flux** | **F** | **Df** | **Df.res** | **P-value** |
| **Year** | 14.69 | 11 | 34.4 | <0.0001 |
| **Treatment** | 0.74 | 1 | 3.78 | 0.441 |
| **Year: Treatment** | 01.48 | 11 | 34.4 | 0.184 |




**Supplementary Figure 1. Temporal fluctuations in the canopy P pool under ambient and elevated $CO_2$ treatment.** Panel (a) shows the temporal fluctuations at each date, whereas panel (b) shows the annual-averaged P pools. The points and shaded areas in panel (a) indicates the mean ± standard deviation range, with dots indicating the treatment mean (n=3). Error Bars in panel (b) indicates the  mean ± standard deviation range (n=3), and open squared and triangle dots denote plot-level data under ambient and elevated $CO_2$ treatment, respectively. Linear mixed-effects model indicates a lack of main $CO_2$ effect ($P < 0.05$) at the annual timestep.




**Supplementary Figure 2. Temporal fluctuations in fineroot P concentration under ambient and elevated $CO_2$ treatment.** Panel (a) shows the temporal fluctuations at each date, whereas panel (b) shows the annual-averaged P pools. The points and shaded areas in panel (a) indicates the mean ± standard deviation range, with dots indicating the treatment mean (n=3). Error Bars in panel (b) indicates the mean ± standard deviation range (n=3), and open squared and triangle dots denote plot-level data under ambient and elevated $CO_2$ treatment, respectively. Linear mixed-effects model indicates a lack of main $CO_2$ effect ($P < 0.05$) at the annual timestep.

### 2.2. Bootstrapping approach to systematically quantify the uncertainties

We further developed a bootstrapping approach to quantify the uncertainty associated with the temporal fluctuation in data. For each variable that has a temporal data coverage, we calculated the plot-level mean at each sampling date, and then randomly selected three data points per treatment from the pool of sampling dates to calculate the treatment mean. We repeated this process by 1000 times to obtain the bootstrapped mean and the associated confidence intervals. Essentially, this bootstrapping approach allows us to use different combinations of values to estimate the overall $CO_2$ effect and the uncertainties associated with the mean effect size. We used the R package "boot" [5,6] to perform this analysis. We then compared the estimated $CO_2$ effect size and the confidence intervals against those obtained from the original approach as reported in the main text.

Our results show that the $CO_2$ effect sizes and the associated confidence intervals reported in the main text are similar to those estimated based on the bootstrapping method (Extended Data Figures 6 – 8). For P concentration variables, the two approaches result in similar $CO_2$ effect sizes and similar confidence intervals (Extended Data Figure 6). For P pool variables, the bootstrapping method result in comparable confidence intervals relative to the original approached reported in the main text (Extended Data Figure 7). Similarly, for P flux variables, the bootstrapping method result in generally similar confidence intervals as compared to the original approached reported in the main text (Extended Data Figure 8). Notably, the sign of the $CO_2$ responses and the magnitude of the estimated effect sizes are comparable by the two approaches (Extended Data Figures 6 – 8). Therefore, we report the results based on the simple approach in the main text.

3. **The $CO_2$ effect on the relative abundance of saprotrophic and mycorrhizal fungi in soils at EucFACE**

**Supplementary Table 5. Relative abundances of ectomycorrhizal and saprotrophic fungi in soils at EucFACE during the months prior to $CO_2$ fertilization (sampling dates in 2012) and the first five years of $CO_2$ fertilization.** Estimated means represent log10-transformed response ratios (ectomycorrhizal: saprotrophic) of DNA sequence read counts following Illumina sequencing of the fungal ITS2 region; see Ref 7 for details of DNA extraction, sequencing and data processing methods. The soil samples were collected on each of the 17 dates indicated and, within 24 hours, passed through a 2mm sieve and stored at -80 °C until DNA extraction. Contrasts between ambient and elevated $CO_2$ conditions were estimated for each sampling date after fitting a linear mixed effects model that included nested random effects ('ring' [three per $CO_2$ treatment] and 'plot' [four per ring]; N=23-24 per sampling date, one sample from the first sampling date was removed due to a sequencing failure) and using Kenward-Roger degrees of freedom. The model indicated a non-significant effect of $CO_2$ treatment overall ($F = 0.78$; df = 1,4; $P = 0.43$), the only significant contrast was observed during the pre-treatment phase, and this contrast was only marginally significant ($P = 0.045$; consistent with the finding of a non-significant $CO_2$ treatment by date interaction: $F = 0.84$; df = 1,351.03; $P = 0.64$).

| Date | Treatment | emmean | Contrast | | |
|---|---|---|---|---|---|
| | | | Estimate | SE | P-value |
| 2012-05-21 | ambient | 2.67 | 0.07 | 0.56 | 0.90 |
| 2012-05-21 | elevated | 2.60 | | | |
| 2012-09-03 | ambient | 2.47 | 1.15 | 0.55 | 0.04 |
| 2012-09-03 | elevated | 1.32 | | | |
| 2012-12-11 | ambient | 1.75 | 0.73 | 0.55 | 0.19 |
| 2012-12-11 | elevated | 1.02 | | | |
| 2013-03-11 | ambient | 2.17 | 0.70 | 0.55 | 0.21 |
| 2013-03-11 | elevated | 1.47 | | | |
| 2013-06-11 | ambient | 2.36 | 0.59 | 0.55 | 0.29 |
| 2013-06-11 | elevated | 1.77 | | | |
| 2013-09-03 | ambient | 2.63 | 0.63 | 0.55 | 0.26 |
| 2013-09-03 | elevated | 2.00 | | | |
| 2013-12-10 | ambient | 2.25 | 0.45 | 0.55 | 0.42 |
| 2013-12-10 | elevated | 1.79 | | | |
| 2014-03-10 | ambient | 1.92 | -0.12 | 0.55 | 0.83 |
| 2014-03-10 | elevated | 2.04 | | | |
| 2014-06-17 | ambient | 0.77 | 0.09 | 0.55 | 0.87 |
| 2014-06-17 | elevated | 0.68 | | | |
| 2014-09-09 | ambient | 2.46 | 0.44 | 0.55 | 0.43 |
| 2014-09-09 | elevated | 2.02 | | | |

| | | | | | |
|---|---|---|---|---|---|
| 2014-11-17 | ambient | 2.65 | 0.08 | 0.55 | 0.89 |
| 2014-11-17 | elevated | 2.57 | | | |
| 2015-03-10 | ambient | 2.57 | -0.51 | 0.55 | 0.36 |
| 2015-03-10 | elevated | 3.08 | | | |
| 2015-06-09 | ambient | 2.86 | 0.57 | 0.55 | 0.31 |
| 2015-06-09 | elevated | 2.29 | | | |
| 2015-09-07 | ambient | 2.78 | 0.18 | 0.55 | 0.74 |
| 2015-09-07 | elevated | 2.60 | | | |
| 2015-11-30 | ambient | 2.49 | -0.24 | 0.55 | 0.66 |
| 2015-11-30 | elevated | 2.74 | | | |
| 2016-03-10 | ambient | 3.76 | 0.04 | 0.55 | 0.95 |
| 2016-03-10 | elevated | 3.72 | | | |
| 2017-08-21 | ambient | 2.95 | -0.00 | 0.55 | 1.00 |
| 2017-08-21 | elevated | 2.95 | | | |

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