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Effects of Elevated Atmospheric CO_2 Concentration on Insect Herbivory and Nutrient Fluxes in a Mature Temperate Forest

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Abstract: Insect herbivory is one of the most important ecological processes affecting plant–soil feedbacks and overall forest ecosystem health. In this study, we assess how elevated carbon dioxide (eCO_2) impacts (i) leaf level insect herbivory and (ii) the stand-level herbivore-mediated transfer of carbon (C) and nitrogen (N) from the canopy to the ground in a natural mature oak temperate forest community in central England at the Birmingham Institute of Forest Research Free Air CO_2 Enrichment (BIFoR FACE) site. Recently abscised leaves were collected every two weeks through the growing season in August to December from 2017–2019, with the identification of four dominant species: *Quercus robur* (pedunculate oak), *Acer pseudoplatanus* (sycamore), *Crataegus monogyna* (common hawthorn) and *Corylus avellana* (hazel). The selected leaves were scanned and visually analyzed to quantify the leaf area loss from folivory monthly. Additionally, the herbivore-mediated transfer of C and N fluxes from the dominant tree species *Q. robur* was calculated from these leaf-level folivory estimates, the total foliar production and the foliar C and N contents. This study finds that the leaf-level herbivory at the BIFoR FACE has not changed significantly across the first 3 years of eCO_2 treatment when assessed across all dominant tree species, although we detected significant changes under the eCO_2 treatment for individual tree species and years. Despite the lack of any strong leaf-level herbivory response, the estimated stand-level foliar C and N transferred to the ground via herbivory was substantially higher under eCO_2, mainly because there was a ~50% increase in the foliar production of *Q. robur* under eCO_2. This result cautions against concluding much from either the presence or absence of leaf-level herbivory responses to any environmental effect, because their actual ecosystem effects are filtered through so many (usually unmeasured) factors.

Keywords: carbon; free-air CO_2 enrichment (FACE); leaf area loss; nitrogen; nutrient transfer

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1. Introduction

1.1. Forests, Climate and CO_2

Atmospheric carbon dioxide (CO_2) concentrations are controlled by the exchange of CO_2 between the atmosphere, terrestrial biosphere and oceans, which impact the global climate and overall carbon (C) budget [1]. Increasing atmospheric CO_2 levels will likely alter multiple important system properties through higher temperatures [2], precipitation acidity [3], altered organic matter turnover [4], reduced soil moisture [5] and an overall influence on plant and animal biodiversity, composition and competition [6,7]. Plants

grown under elevated CO_2 have enhanced global photosynthesis by 11.85–13.98 PgC of carbon and are thus contributing a large proportion to the current terrestrial carbon sink [8]. Currently, CO_2 fertilization offsets 20%–30% of the CO_2 released by human activities [9], predominantly due to carbon sequestration in mature forest ecosystems [10,11]. Both experimental data and global climate models, however, predict that the CO_2 fertilization effect may subside over time, but at varying magnitudes, which could accelerate the rate of climate change [12,13]. In order to moderate the progression of climate change, it is important, therefore, to understand the uncertainty, pressures and responses of increasing atmospheric CO_2 concentrations on plants [14] and the associated impacts of this on the terrestrial and C cycle. Temperate forests are particularly important, covering just 8% of the global land area but accounting for 40% of the total C stored terrestrially [15]. These forests are not only important for sequestering C from the atmosphere [16], but temperate forest canopies promote insect species' richness and abundance [17].

1.2. FACE Experiments

Forest-based free-air CO_2 enrichment (FACE) experiments were established to help understand forest ecosystem responses to a future atmosphere with CO_2 enrichment. Extensive knowledge has already been gained with a decade of FACE studies showing an increase in net primary productivity, leaf area index (LAI), nutrient cycling and soil microbial activity [18]. However, because most of these historic FACE experiments (AspenFACE and DukeFACE) were conducted on young mono-species forest plantations and concentrated around North American and European ecosystems [19], the conclusions from these experiments may not easily translate to diverse and mature ecosystems which are more representative of the natural forests around the world [20]. Currently, there are only two ongoing forest FACE experiments in natural, mature forest ecosystems: EucFACE in the eucalyptus forests of Australia and BIFoR in a mature northern temperate forest in the United Kingdom [20].

1.3. Herbivory and CO_2

One potentially critical process which remains understudied in FACE experiments is the impacts of eCO_2 on herbivory and the consequences for herbivore-mediated ecosystem processes [21]. Insect herbivory is one of the most important ecological processes affecting plant–soil feedbacks [22,23], forest ecosystem health [24,25], resource availability [26], function and structure and the composition and productivity of plant communities [27]. Research has been conducted on the effects of eCO_2 treatments on insect herbivory under controlled situations [28], but studies based in mature and diverse forest ecosystems are still rare [21,29]. Interactions between eCO_2 and insect herbivory appear to be predominantly regulated by plant chemistry and metabolism [30,31]. The responses of plants to eCO_2 show that foliar nitrogen (N) decreases and leaf toughness, leaf biomass and the carbon-to-nitrogen ratio increase [32–34]. This altered foliar chemistry then negatively impacts herbivore performance by significantly decreasing the relative growth rate and pupal weight [35]. It can also result in compensatory feeding due to a reduction in food quality, causing increased defoliation, but still with a cost to the early stages of larval development and the overall herbivore abundance [31]. However, the limited evidence for eCO_2 impacts on herbivore leaf damage from FACE studies suggests mixed, relatively weak responses. Some studies show a decline in herbivory under eCO_2, apparently related to leaf chemistry, but this varies among species and years of measurement [36,37]. Another study found no clear effect [29], while others found an increase in insect herbivory under eCO_2 dependent on the feeding guild and host plant type [21,38].

In this study, we assess how eCO_2 impacts insect herbivory in a natural mature oak temperate forest community [39]. Further, for the dominant oak species, we estimate the net impact of CO_2-induced shifts in canopy productivity, foliar chemistry and leaf-level herbivory on ecosystem-level herbivore-mediated C and N fluxes from the canopy to the ground. Specifically, we ask three questions:

1. How does eCO_2 affect leaf-level insect herbivory?
2. Do eCO_2 effects on leaf-level herbivory vary among tree species and years?
3. How and why does eCO_2 affect the herbivore-mediated transfer of C and N from the forest canopy to the ground?

2. Materials and Methods

2.1. Study Site

The BIFoR FACE experiment is located in a mature, temperate oak forest on Orthic Luvisol dominant soil [40] with a mul-moder humus classification in central England (52.801° N, 2.301° W), United Kingdom. The forest is characterized as a deciduous forest with a broadleaf tree species, with a dominant old oak (*Quercus robur*) overstory (~25 m tall, 175 years old) and a mid-understory of sycamore (*Acer pseudoplatanus*) and hazel (*Corylus avellana*).

The BIFoR FACE site consists of six structural arrays (eCO_2, n = 3; Control, n = 3) and three non-infrastructural arrays (Figure 1). Their location and pairings were determined by testing key forest characteristics such as soil analysis, species distribution and biomass densities [41]. Each array consists of 16 peripheral towers and one central tower ~1 m above the canopy with a 15 m radius ground space for research. A highly consistent +150 ppm above ambient eCO_2 treatment has been applied within FACE 'treatment' arrays since April 2017, running in parallel with 'control' arrays supplying ambient air via an identical infrastructure [39]. The plot tree species composition and basal area were recorded for all trees greater than 10 cm at diameter breast height (dbh) in all the arrays.

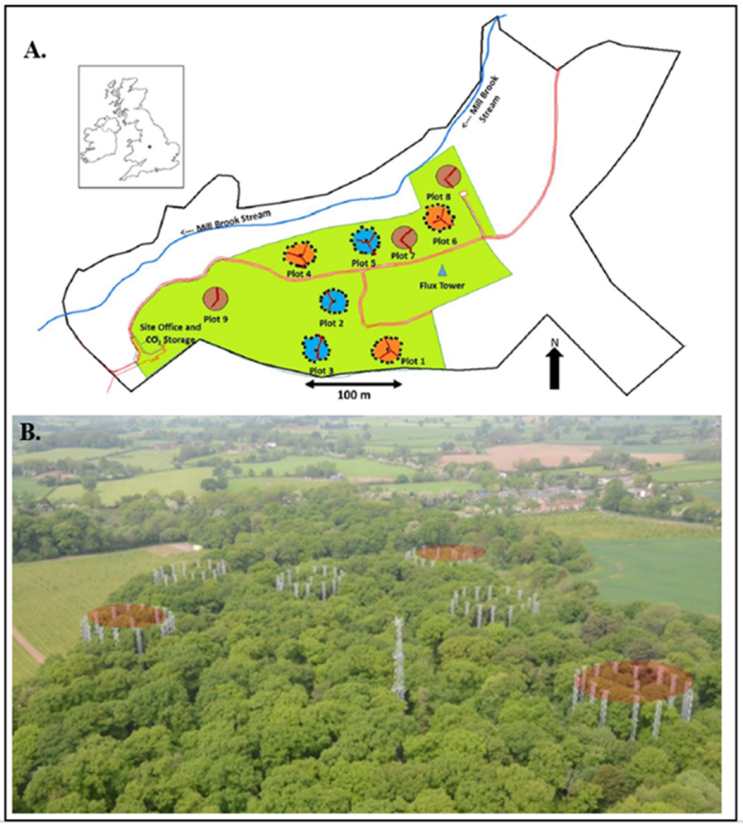


Figure 1. (**A**). Map of the 19.1ha BIFoR FACE facility. Three enriched CO_2 arrays are shown in orange; three experimental control arrays are shown in blue. The red line indicates elevated walkways to access plots, reducing forest floor disturbance. The 40 m high flux tower (blue triangle) collects environmental conditions at the site. (**B**). An aerial view of the experimental design, illustrating the treatment rings in relation to the ecosystem.

2.2. Leaf Herbivory

Two 1 m^2 leaf litter traps have been in place within each of the three control and treatment arrays since 2017. Leaf litter was collected every two weeks through the growing season in August to December from 2017–2019, with the identification of four dominant species (S1): *Quercus robur* (pedunculate oak), *Acer pseudoplatanus* (sycamore), *Crataegus monogyna* (common hawthorn) and *Corylus avellana* (hazel).

After sorting by species, 10 leaves per species per month from each trap were randomly selected for scanning (with no overlap) and scanned at a 300 dpi tiff. format. The insect herbivory was visually quantified as the proportion of the leaf area missing (H) from the scanned images using a six-class leaf area loss system for calibration [42] (Figure 2). Additionally, Digimizer v.5.4.7 ® (MedCalc Software Ltd., Ostend, Belgium, 2020) was used to measure the individual leaf area for all leaves collected only in 2019 (N = 435 leaves). Skeletonizing, leaf mining and leaf rolling were negligible for the leaf tissue lost in the leaves sampled by this study and therefore were not included in the analysis. All the material collected in each trap was then dried at 60 °C for 24 h and weighed.

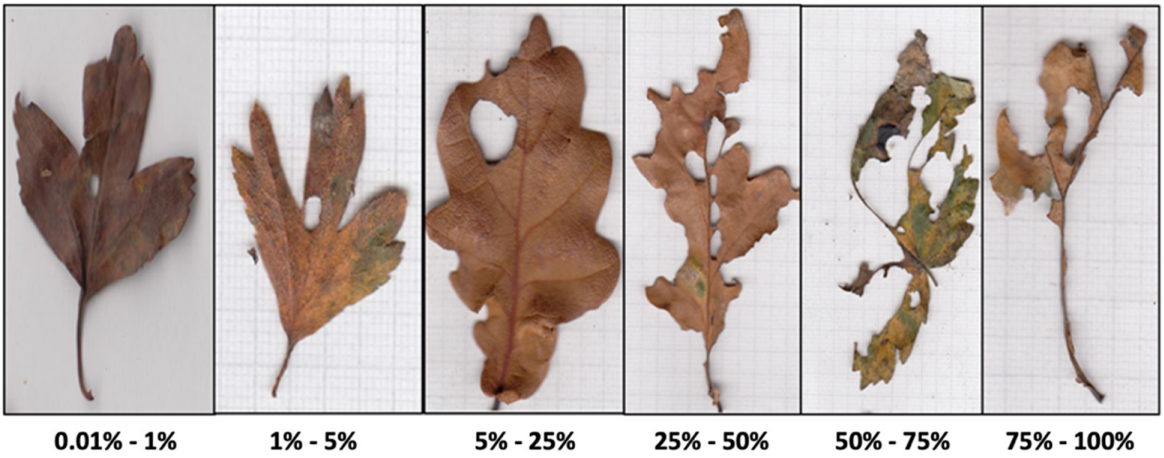


Figure 2. Exemplar images for the visual quantification of herbivory and subsequent categorization of samples into six leaf area loss classes [42].

2.3. Leaf nutrients and Production

Oak leaves were collected from the top of the canopy in each month from 2017–2019 and stored immediately at −25 °C. Two upper canopy leaves from one tree per plot were selected for elemental analyses. Dried leaves were ground and analyzed for total C and N using an elemental analyzer interfaced with an isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK; [43]).

2.4. Calculations of Herbivore-Mediated Nutrient Fluxes

To estimate the canopy biomass production per plot, all oak leaves collected within the litter traps from our sampling years of 2017–2019 were dried at 60 °C for 2–3 days until constant mass and weighed. We calculated the mean oak basal area (BA, m^2) across all six plots; then, we divided the individual plot BA by the mean BA. This value (the fraction of individual plot BA: mean plot BA) was then multiplied by the individual plot oak raw dry litterfall mass per unit ground area (L_{raw}, g m^{-2} $year^{-1}$) to derive the abundance-corrected dry litterfall mass (L_{corr} g m^{-2} $year^{-1}$). The total peak oak live dry foliage mass per unit ground area (Q, g m^{-2}) was calculated as $Q = L_{corr} + (L_{corr} \times H)$. The herbivore-mediated transfer of C and N fluxes per unit ground area from oak (Fx, g m^{-2} $year^{-1}$) at the study site was then calculated following the basic approach in [44], as follows:

$$F_x = Q \times H \times N_x \quad (1)$$

where subscript x is substituted with C or N for reference F_x and for leaf C and N content expressed as a proportion of dry biomass (N_x).

2.5. Statistical Analysis

The response variable of the leaf-level insect herbivory was evaluated using repeated measured MANOVA with fixed effects of the eCO_2 treatment and tree species. All statistical analyses were performed using R Statistical Software 1.1442 [45,46]. Before analysis, data normality was checked and the data were transformed where necessary, with a Box Cox transformation of the suggested 0.501 lambda. The values are reported as the mean ± SE or the percentage (%).

3. Results

3.1. Leaf-Level Insect Herbivory

Overall, the leaf-level herbivory showed no significant response to the eCO_2 treatment across sampling years and tree species (Table 1), though there were some apparent effects within species and years (Table 1, Figure 3). Specifically, there was no significant difference in herbivory between the eCO_2 treatment and control plots across all years for oak, while hawthorn and hazel experienced a significant reduction in leaf-level herbivory under eCO_2 compared to the control in 2019 and 2018, respectively (Table 1, Figure 3). Sycamore experienced a significant increase in leaf-level herbivory for both treatments in 2019 (Figure 3).

Table 1. F Ratio for MANOVA Wilk's Lambda test of insect herbivory responses across different dominant tree species (oak, sycamore, hawthorn, hazel) and between eCO_2 and controlled treatments measured over 2017, 2018 and 2019. Significant main effects and interactions are indicated with asterisks: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

Parameter	Wilk's Lambda	*p*-Value	DF
All Between	4.35 ***	0.0009	7
All Within	2.71 **	0.0022	14
Year	12.5 ***	<0.0001	2
Treatment	0.11	0.7385	1
Species	8.4 ***	0.0001	3
Treatment × Species	1.3	0.2924	3
Year × Treatment	0.09	0.9129	2
Year × Species	3.31 **	0.0053	6
Year × Treatment × Species	2.2 *	0.0500	6

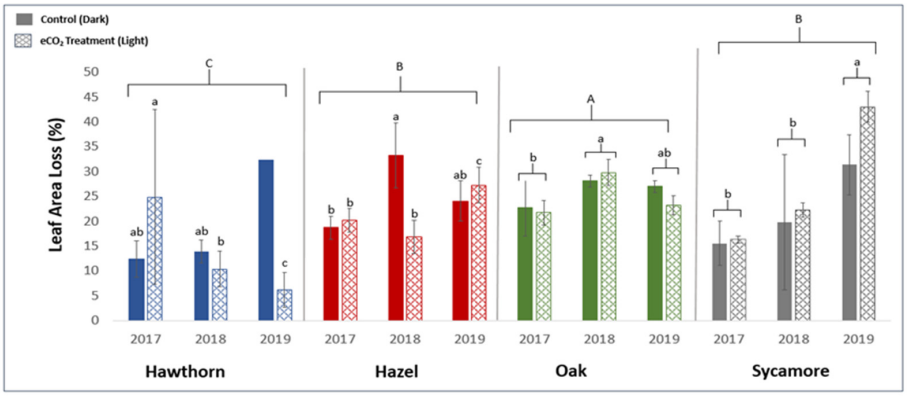


Figure 3. The mean ± S.E. leaf area lost by insect herbivory per leaf for three years on hawthorn, hazel, oak and sycamore tree leaves. n = 3. Significant main effects and interactions ($p \leq 0.05$) are indicated with letters between species, years and treatments (ABC, abc). For the 2019 control hawthorn, there was only one litter trap of material, and, thus, no S.E could be calculated.

3.2. Estimated Carbon and Nitrogen Fluxes from Insect Herbivory

Herbivore-induced C and N fluxes from oak to the ground were the net product of the leaf-level herbivory rate, the litterfall rate and the leaf carbon and nitrogen contents, all of which were influenced in different ways by the CO_2 treatment. Across all scaled-up years, eCO_2 was associated with a slight decrease in leaf-level insect herbivory and slightly increased foliar C and N but substantially increased tree abundance-corrected litter fall (Figure 4). Thus, even though leaf-level insect herbivory on oak was apparently slightly suppressed by eCO_2, the estimated C and N nutrient fluxes from the canopy to the ground via herbivory were ~60–70% greater on the eCO_2 treatment compared to those on the control.

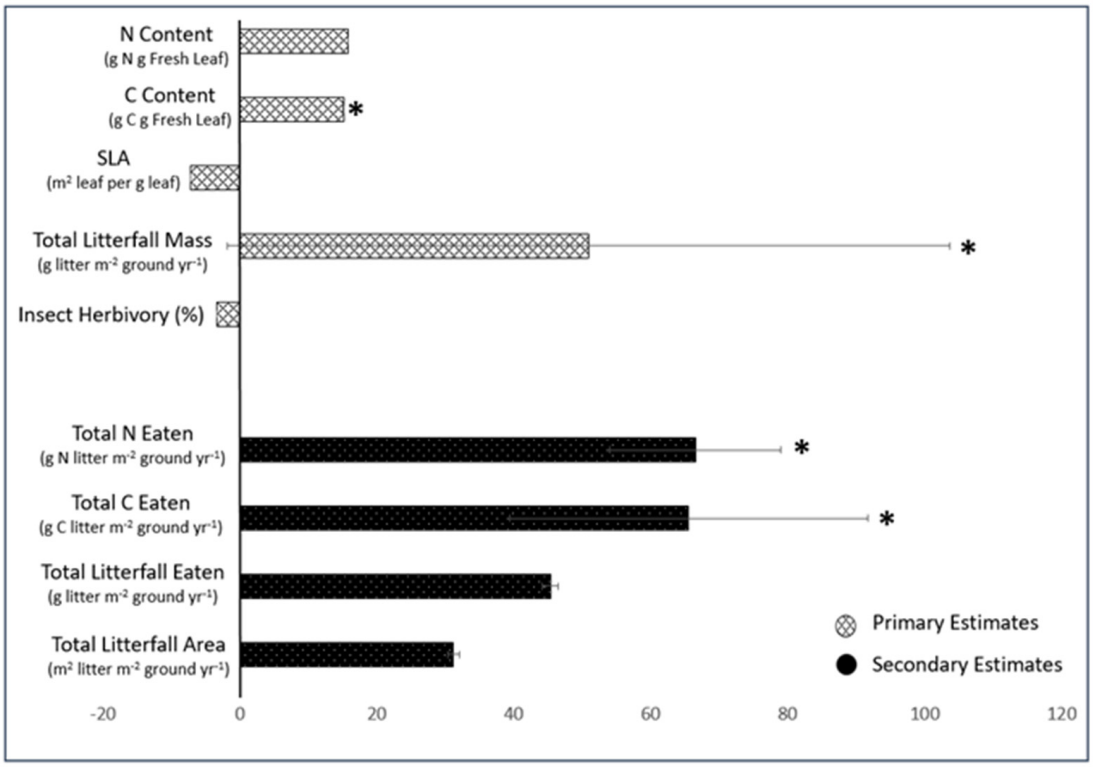


Figure 4. Percent change from control treatment compared to that from eCO_2 for total N content, C content, Specific Leaf Area and Total Litterfall Mass and Insect Herbivory for oak leaves over three sampling years (2017–2019). Bars represent mean total % change on the eCO_2 plots relative to the control ± standard error, n = 3. Primary estimates are defined as parameters that were all directly measured in the field/lab; secondary estimates are parameters calculated from primary parameters. Significant percent change from control treatment compared to that from eCO_2 are indicated with an asterisks *, $p \leq 0.05$.

4. Discussion

4.1. Leaf-Level Insect Herbivory

The current study has shown that the total leaf level herbivory at the BIFoR FACE has not changed significantly over the first 3 years of eCO_2 treatment (2017–2019) when assessed across all dominant tree species combined (Table 1; Figure 3). This agrees with the results from the EucFACE experiment in Australia, where the leaf-level herbivory observed on living leaves in the upper canopy was not affected by CO_2 enrichment for the first two years since the initiation of the experiment [29]. There were, however, significant increases in herbivory noted in our study for hazel and sycamore in 2019, suggesting a potential rise in herbivory for certain tree species under eCO_2. The overall weak herbivory response to eCO_2 at the BIFoR may be due to the limited biochemical response of dominant and more mature oak trees, with no significant change in the foliar N of oak from 2015–2019 [43].

While our own calculations indicate an increase in the oak foliar N concentrations within eCO_2 plots between 2017–2019 (Figure 4), no significant change in oak herbivory was noted for any year.

4.2. Plant Nutrients and Insect Herbivory

Field studies on the plant-mediated effects of eCO_2 on insects are rare, but the consequences are potentially substantial at both an individual and ecosystem level [47]. Negative consequences on plant chemistry, such as reduced N and increased defensive compounds, e.g., tannins, can affect overall herbivore development [48] and potentially impact herbivore abundance and biodiversity. It is worth noting, however, that no significant changes in insect community composition were recorded between 2017 and 2019 at the BIFoR [38]. In line with our findings, varying plant species responses to eCO_2 are common [47]. The current study reinforces the need to investigate biochemical changes in multiple tree species, as well as the impacts on several different insect herbivores. For example, studying the effects of leaf miners provides a localized perspective on the exposure to eCO_2 or controlled conditions, as they typically spend their life on a single leaf from egg to adulthood. A detailed study of several leaf miner species has been undertaken at the BIFoR on both oak and hazel, which indicate differing responses across different tree and miner species combinations [38]. Our study observed a decrease in insect herbivory for oak, a canopy dominating species under eCO_2 conditions [49].

4.3. Ecosystem Responses to Insect Herbivory

Beyond just understanding the direct interactions between herbivores and plants, it is also important to quantify whole ecosystem responses to insect herbivory, particularly the herbivore-mediated transfer of carbon and nutrients to the soil [50]. Inferring the ecosystem impacts of insect herbivory from leaf-level observations is not straightforward [51,52] because the leaf-level impacts are filtered through a range of other factors which are also affected by the environment such as the light intensity, nutrient availability and leaf composition [53]. For example, in our study, while there is lower leaf-level herbivory under elevated CO_2, this is offset by the large increase in litterfall [54] on the eCO_2 plots compared to the control, even after correcting for pre-existing plot differences in oak abundance and size. Increased litterfall from our diverse temperate species system tends to follow the deceleration model in response to herbivory [54] by inducing defenses, decreasing litter quality and increasing the C/N ratio [36,55]. In other words, herbivores on the eCO_2 plots are able to maintain roughly similar levels of leaf-level damage despite a ~60% increase in foliar resources, indicating that the activity and/or abundance of the herbivore population is in fact higher on the eCO_2 plots despite the lack of any strong leaf-level herbivory treatment difference. This result cautions against concluding much from either the presence or absence of leaf-level herbivory responses to any environmental effect, because their actual ecosystem effects are filtered through so many (usually unmeasured) factors.

5. Conclusions

We present a three-year study from a mature temperate forest, experimentally measuring the effects of eCO_2 on insect herbivory rates and the stand-level herbivore-mediated transfer of C and N from the canopy to the ground. We found no overall effect of eCO_2 on leaf-level herbivory within the first 3 years of the BIFoR FACE experiment, though some significant effects among individual tree species and measurement years were found, along with potential trajectories of increasing herbivory on some species in 2019. We showed that the herbivore-mediated transfer of C and N fluxes from the dominant tree species oak was 60%–70% higher under eCO_2 even though the leaf level consumption was lower. This was because the total oak foliage mass was ~50% higher in the eCO_2 treatments, even after correcting for the pre-existing plot variation in oak abundance. As such, our findings suggest that herbivores' activity and/or abundance in eCO_2 plots was enhanced.

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