Insect herbivory alters impact of atmospheric change on northern temperate forests

J. J. Couture[1][†][*], T. D. Meehan[1], E. L. Kruger[2] and R. L. Lindroth[1]

Stimulation of forest productivity by elevated concentrations of CO_2 is expected to partially offset continued increases in anthropogenic CO_2 emissions. However, multiple factors can impair the capacity of forests to act as carbon sinks; prominent among these are tropospheric O_3 and nutrient limitations[1,2]. Herbivorous insects also influence carbon and nutrient dynamics in forest ecosystems, yet are often ignored in ecosystem models of forest productivity. Here we assess the effects of elevated levels of CO_2 and O_3 on insect-mediated canopy damage and organic matter deposition in aspen and birch stands at the Aspen FACE facility in northern Wisconsin, United States. Canopy damage was markedly higher in the elevated CO_2 stands, as was the deposition of organic substrates and nitrogen. The opposite trends were apparent in the elevated O_3 stands. Using a light-use efficiency model, we show that the negative impacts of herbivorous insects on net primary production more than doubled under elevated concentrations of CO_2, but decreased under elevated concentrations of O_3. We conclude that herbivorous insects may limit the capacity of forests to function as sinks for anthropogenic carbon emissions in a high CO_2 world.

Forest ecosystems are experiencing an array of environmental stressors of a magnitude, scale and complexity unparalleled in human history[3,4]. Prominent among these are increased concentrations of greenhouse gases, principally CO_2 and tropospheric O_3. These gases strongly influence tree physiology and growth[4–7], ultimately affecting forest structure, function and sustainability[4,7,8]. Because forest ecosystems play prominent roles in global carbon cycling and climate change, and constitute Earth's largest terrestrial carbon sink[3], improved understanding of their responses to the independent and interactive effects of atmospheric change and other stressors is critically important.

Although knowledge regarding the effects of atmospheric change on forest structure and function has grown considerably, the influence of these gases on ecosystem processes mediated by herbivorous insects, the major consumers in forests, remains largely unknown. To date, no studies have examined how elevated levels of CO_2 and O_3 influence insect-mediated ecosystem processes at the level of forest stands. Moreover, ecosystem productivity models generally characterize the impact of insects on forests only as periodic disturbance events by outbreak populations, and fail to account for potential shifts in background activity by insect herbivores at endemic population levels. Understanding the relationships between atmospheric change and insect-mediated ecosystem processes is particularly important considering that herbivorous insects influence multiple facets of forest structure and function (for example, species composition, productivity, nutrient cycling)[9–12].

Here, we present findings from a 3-year study examining the effects of enriched atmospheric CO_2 and O_3 on insect-mediated ecosystem processes. The study was conducted at the Aspen FACE facility, the largest FACE facility to date and the only one to examine the effects of both CO_2 and O_3 on forest ecosystems. In aggrading stands of trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*) we tracked rates of leaf damage, measured as per cent area damaged and amount of biomass removed. We also followed organic substrate (frass (insect faecal material) and greenfall (leaf fragments dropped by herbivorous insects)) and nitrogen deposition (the nitrogen fraction of organic substrates) by herbivorous insects. We then examined the effects of insect herbivory on aboveground net primary production (ANPP) by modelling altered light absorption and light use efficiency (ε, the efficiency by which plants utilize light to produce biomass) in the presence or absence of damage. Finally, we examined relationships between (1) canopy damage and the relative growth of stands exposed to elevated CO_2 and O_3 and (2) stand growth potential and the ANPP lost due to herbivorous insects.

We found that insect-mediated forest processes were substantially altered in elevated CO_2 and O_3 environments (Table 1 and Fig. 1). On average, CO_2 enrichment led to a striking (88%) increase in canopy damage rates (Fig. 1a) and this response was larger in aspen (110%) than in aspen–birch (70%), stands. Although damage consistently increased under elevated CO_2, the magnitude of the increase varied among years (aspen, 20–325%; aspen–birch, 10–160%). Elevated O_3 elicited a modest (16%) decrease in canopy damage (Fig. 1a) that was generally consistent among community types and years. Organic deposition, as well as associated nitrogen flux, increased (36% and 38%, respectively, Fig. 1b,c) under elevated CO_2, and this response was consistent among communities and years. Although organic deposition by insects was similar in ambient and enriched O_3 environments, and varied little among community types and years, the flux of nitrogen from the canopy to the soil decreased by 19% in elevated O_3 (Fig. 1c), ultimately due to an increase in the ratio of foliar carbon to nitrogen[14]. On average, elevated CO_2 increased the sum of actual and potential productivity (ANPP) lost due to herbivory by 167%; the response was larger in aspen (180%) than in aspen–birch (110%) stands. Although the loss of ANPP was consistently greater under elevated CO_2, the magnitude of loss varied among years (aspen, 105–220%; aspen–birch, 60–170%). Elevated O_3 decreased the negative effect of herbivory on ANPP by 23% (Fig. 1d) and this pattern was generally consistent among communities and years. These results demonstrate that atmospheric change can strongly influence insect-mediated carbon and nutrient fluxes and is likely to alter the impacts of insects on forest productivity.

Examination of stand ANPP enhancement under elevated, compared with ambient, CO_2 against canopy damage revealed a pronounced negative relationship (Fig. 2a); in years of high insect

[1]Department of Entomology, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA. [2]Department of Forest and Wildlife Ecology, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA. [†]Present address: Department of Forest and Wildlife Ecology, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA. [*]e-mail: jjcouture@wisc.edu

Table 1 | Summary of analysis of variance statistics (F and P values) describing the influence of CO_2, O_3, community, time, and their interactions on forest processes.

Treatment combinations	d.f.	Canopy damage: per cent leaf area		Organic substrate deposition		Nitrogen deposition		ANPP lost	
		F	P	F	P	F	P	F	P
CO_2	1, 6	253.0	<0.001	16.0	0.007	19.6	0.004	103.2	<0.001
O_3	1, 6	18.2	0.005	3.1	0.127	7.9	0.031	9.3	0.022
$CO_2 \times O_3$	1, 6	0.8	0.399	0.5	0.490	0.2	0.689	2.0	0.210
Community	1, 8	1.8	0.188	32.9	<0.001	17.2	<0.001	4.2	0.046
$CO_2 \times$ community	1, 8	9.8	0.003	0.5	0.490	0.1	0.785	7.6	0.009
$O_3 \times$ community	1, 8	0.1	0.737	2.2	0.148	1.0	0.323	2.4	0.127
$CO_2 \times O_3 \times$ community	1, 8	1.0	0.319	1.2	0.274	0.2	0.646	2.6	0.116
Year	2, 32	39.8	0.001	43.9	<0.001	2.8	0.073	4.3	0.021
$CO_2 \times$ year	2, 32	4.0	0.027	5.4	0.008	4.7	0.015	3.5	0.038
$O_3 \times$ year	2, 32	43.1	<0.001	1.2	0.312	2.7	0.082	6.4	0.004
$CO_2 \times O_3 \times$ year	2, 32	4.2	0.022	1.1	0.347	0.8	0.450	0.4	0.648
Community $\times$ year	2, 32	2.7	0.079	0.1	0.873	0.7	0.490	0.1	0.992
$CO_2 \times$ community $\times$ year	2, 32	3.2	0.051	0.2	0.798	1.0	0.387	0.1	0.968
$O_3 \times$ community $\times$ year	2, 32	1.8	0.182	3.5	0.041	4.2	0.022	0.7	0.495
$CO_2 \times O_3 \times$ community $\times$ year	2, 32	0.4	0.692	0.7	0.524	1.0	0.395	0.1	0.931

CO_2 and O_3 were analysed as whole-plot effects, while community was a sub-plot effect and year was a sub-sub-plot effect. Forest functional variables included canopy damage, organic substrate and nitrogen deposition, and aboveground net primary production (ANPP) lost due to herbivory.
Numerator and denominator degrees of freedom (d.f.: numerator, denominator) were calculated using the Satterthwaite approximation.

damage, CO_2-mediated enhancement of ANPP was reduced. We also found that the negative effects of insects on ANPP were strongly and positively related to a key determinant of potential stand productivity (modelled ε in the absence of herbivory; Fig. 2b). These results suggest that stands with greater growth potential will be subjected to greater levels of insect-related reduction in ANPP. In contrast to forest stands under enriched CO_2, stands exposed to enriched O_3 alone exhibited no relationships between productivity and damage.

The patterns we observed are likely to be caused by the influence of elevated CO_2 and O_3 across multiple trophic levels in this system. For example, during the course of this study elevated CO_2 and O_3 concentrations altered foliar characteristics (for example, nutrients,

structural compounds, secondary metabolites), which in turn influenced the feeding rates, growth and reproduction of herbivorous insects[13,14]. Examination of the herbivorous insect communities coinciding with our collections revealed that elevated CO_2 increased, whereas elevated O_3 decreased, total abundance of herbivorous insects at the canopy level[15]. Furthermore, increases in both gases, singly and in combination, modified insect community composition, especially affecting parasitoids, and thereby are likely to alter predator–prey interactions[16]. Known effects of atmospheric change on insect populations and communities provide several explanatory mechanisms for our results: altered rates of herbivore consumption and growth, changes in herbivore population dynamics and variation in the strength of predation on herbivore
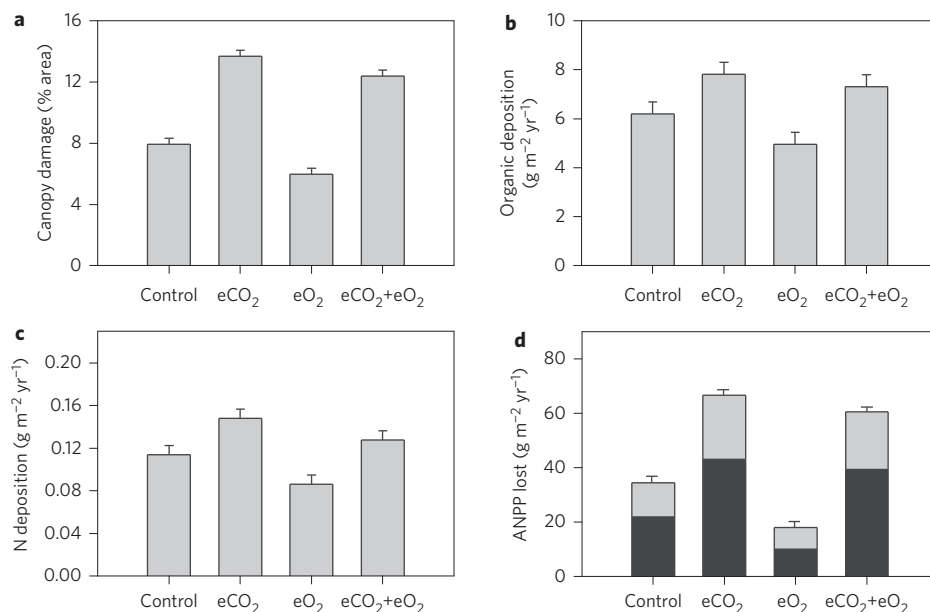

Figure 1 | Independent and interactive effects of elevated CO_2 and O_3 on multiple ecosystem processes directly and indirectly mediated by insects. a, Per cent of canopy area damaged; **b**, organic substrate (frass and greenfall) flux; **c**, nitrogen (N) deposition resulting from frass and greenfall; **d**, aboveground net primary productivity (ANPP) removed either directly, by biomass removal (grey), or indirectly, via reduction in potential growth (black). Error bars represent ±1 s.e., pooled across communities and years. Control represents ambient CO_2 and O_3; eCO_2 is elevated CO_2, ambient O_3; eO_3 is ambient CO_2, elevated O_3; eCO_2+eO_3 is elevated CO_2 and elevated O_3.

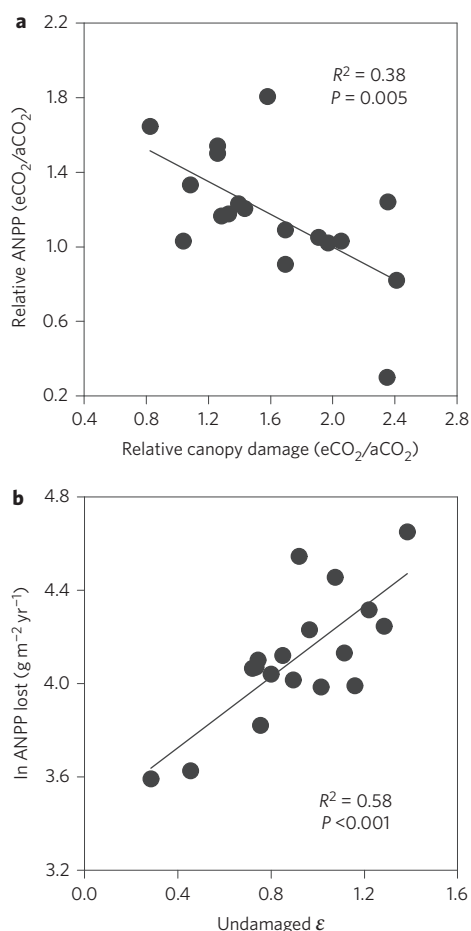**Figure 2 | Relationships between relative influence of CO_2 on ANPP and canopy damage and ANPP lost via herbivory and undamaged ε in elevated CO_2. a,** Relative ANPP enhancement and relative canopy damage were calculated by comparing values averaged by FACE block ($n = 3$) from elevated (eCO_2) and ambient (aCO_2) CO_2 levels (averaged across O_3 levels) for each forest community (aspen and aspen-birch) and year (2006–2008; $n = 18$). **b,** ANPP lost to herbivory and undamaged ε were averaged similarly to the values described above, and the figure depicts information from only elevated CO_2 environments.

populations all influence the impact of herbivores on forest productivity. The recognized impacts of environmental changes, individually or interactively, on these processes support the notion that linkages from individuals to communities will temper the influence of global change on forest ecosystem functioning[17].

The effects of atmospheric change on the direction of the responses we report were consistent across stand communities and years, although the response magnitudes varied moderately among stands and considerably across years. These variations are not surprising, given the potential interactions of atmospheric change with abiotic factors that influence forest functioning. For example, changes in temperature are likely to alter the impacts of herbivorous insects on forest systems in future environments. Inter-annual temperature variation (in the form of estimated herbivore growing-degree days), however, had no relationship with damage rates (Supplementary Fig. 1), suggesting that in the current study changes in foliar traits[13,14] and herbivore abundances[15] had relatively more influence on canopy damage than inter-annual variation in temperature. These results suggest that the effects of CO_2 and O_3 on insect herbivory within the next four decades are likely to exceed the impacts of small increases in temperature predicted for the same period.

Few studies to date have examined the effects of predicted levels of CO_2 and O_3 on patterns of herbivory in stands of plants in open-air systems. In a loblolly pine forest, broadleaf understory tree species tended to have reduced damage rates under elevated CO_2 (ref. 18), in contrast to the increases observed in our study. In that study, CO_2 had no effect on leaf quality or insect abundance. In soybean, elevated CO_2 increased, whereas elevated O_3 had no influence, on canopy damage rates with the increase in damage under enhanced CO_2 levels largely being resolved by changes in leaf quality[19]. These findings, combined with our own, indicate that atmospheric change is likely to influence rates of herbivory across both managed and natural plant systems, but highlight that responses may be context specific.

Elevated concentrations of CO_2 generally increase tree growth. Nutrient availability, however, often constrains forest productivity[20], and progressive nitrogen limitation could ultimately limit enhanced growth[21]. Nevertheless, sustained enhancement of NPP under elevated CO_2 has been observed in cases where increased carbon fluxes to the soil microbial community occur, facilitating increased nitrogen uptake via microbial stimulation and enhanced root production[22–24]. We found that elevated CO_2 increased organic substrate deposition by herbivorous insects, ultimately elevating the flux of labile forms of carbon and nitrogen to the forest floor during the growing season. Although the proportion of nitrogen fluxed via frass is small compared with that from litter inputs, and, at the levels of herbivory we report, unlikely to affect forest productivity, the increased nitrogen flux is comparable to 10–70% of the level of atmospheric nitrogen deposition predicted for this region by the year 2050[12]. Although elevated O_3 independently reduced insect-related deposition, O_3 levels had little influence on the enhanced deposition observed in CO_2-enriched environments when the two gases co-occurred. Thus, regardless of O_3 levels, increased organic deposition by herbivores under elevated CO_2 levels altered stand nutrient dynamics and is likely to contribute to the stimulation of microbial communities, helping to sustain tree growth reported in this system[23].

Although elevated CO_2 concentrations generally stimulate ecosystem productivity, uncertainty remains in model predictions about the magnitude of productivity in future environments. This uncertainty stems primarily from the various potential interactions and feedbacks imposed by abiotic and biotic factors on ecosystem productivity[25,26]. With the exception of infrequent disturbance events, insect-related canopy damage is typically not included in ecosystem models. Although insect outbreaks can dramatically perturb ecosystem processes, evidence suggests that chronic, endemic levels of canopy damage can have a more long-term, negative impact on tree growth and productivity than outbreak defoliation events[27]. We found that increased canopy damage substantially reduced potential ANPP enhancement under elevated CO_2, whereas damage, and its negative impacts on potential productivity, decreased under elevated O_3. We estimate that, on average, nearly 70 g of biomass m^{-2} year^{-1} was lost to insect herbivory under elevated CO_2, regardless of O_3 level. These values suggest that approximately 35% (and up to 50%) of the potential ANPP enhancement under elevated CO_2 at this site was not realized due to changes in endemic herbivory levels in elevated CO_2 environments. Our findings suggest that changes in endemic levels of insect damage during the peak of the growing season will operate independently of, and in addition to, multiple environmental factors (that is, tropospheric O_3, nutrient limitation) to further constrain carbon sequestration by forests under enriched CO_2 environments.

We detected a negative relationship between relative ANPP and canopy damage. This finding is especially interesting because it suggests that levels of canopy damage will temper the effects of CO_2 on forest productivity. We also observed a positive relationship between light-use efficiency (ε; a principal determinant of potential

stand productivity) and biomass removed due to herbivory, indicating that forest stands capable of growing more rapidly under elevated CO_2 may suffer greater losses of productivity. Taken together, these two relationships suggest that herbivorous insects have the potential to 'level the playing field' for forest stands under future atmospheric CO_2 conditions, disproportionately limiting stands that would grow rapidly under high CO_2 environments. Our findings also suggest that herbivorous insects may have less of an impact on the diminished stand productivity predicted to result from elevated O_3 levels in future environments.

Enhanced productivity under elevated CO_2 environments indicates that forests will increase carbon storage in future environments[28]. The strength of that carbon sink, however, will be affected by numerous environmental factors, including ozone levels, nutrient availability and insect pests. Although it is uncertain whether other forest ecosystems (for example, tropical) will respond similarly, disregard for the factors that may interact with elevated CO_2 to influence forest growth is likely to lead to overestimation of the carbon storage potential of forests in future environments. Results from this study highlight the benefits gained by incorporating endemic insect-related damage into models estimating ecosystem responses to environmental change. Further, they reveal that herbivorous insects have the potential to shape the influence of global change on forest ecosystem functioning both indirectly, by influencing substrate fluxes and nutrient cycling, and directly, by limiting enhanced productivity in elevated CO_2 environments.

Methods

This study was conducted at the Aspen FACE research facility in northern Wisconsin, United States, where early-successional forest trees were exposed to all combinations of ambient and elevated CO_2 (~560 ppm) and O_3 (1.5× ambient) in a full-factorial, randomized complete-block design that included three blocks of the four treatments. We measured canopy damage, organic substrate fluxes and the associated nitrogen deposition, and modelled the effects of damage on stand productivity. We quantified canopy damage as both per cent area and biomass removed by analysing approximately 42,000 leaves collected over 3 years of the study. Leaf mass per unit area (M_{area}) was calculated as the quotient of dry leaf mass and fresh leaf area, and foliar nitrogen concentration was determined using near-infrared reflectance spectroscopy (NIRS)[16]. Leaves were collected from the lower and upper thirds of the canopies of 16 trees from each of the 12 rings, in June, July and August of 2006, 2007 and 2008. Frass and greenfall were collected using 57 × 41 cm plastic baskets lined with muslin sheets in five or six 10-day increments[14]. Frass nitrogen concentrations were determined via combustion analysis and greenfall nitrogen was determined by NIRS (ref. 16). We used a light-use efficiency (ε) model to estimate the effects of canopy damage on ANPP. We calculated ε empirically and used an index[29] highly correlated with ε to adjust the empirical ε based on canopy damage. We then recalculated potential ANPP in the absence of herbivory. Analysis of variance was used to determine the statistical significance of treatment effects on canopy damage, organic deposition and ANPP lost via herbivory; regression analysis was used to relate canopy damage and productivity. Statistical analyses were performed using JMP v.10 statistical software.

Research site. Aspen FACE was a 32 ha research facility located in northern Wisconsin, United States (W 89.5°, N 45.7°). The site contained 12, 30-m-diameter experimental rings, with three blocks of four treatment combinations. The full factorial, randomized complete-block design provided all four combinations of ambient and elevated (~560 ppm) CO_2 and ambient and elevated (~1.5 × ambient) O_3 levels. One-half of each experimental ring contained multiple genotypes of aspen and one-quarter of each ring contained a mixture of aspen and paper birch. One-year-old seedlings were planted in the rings in 1997 and fumigation treatments began in 1998. Trees were 10 years old when our study began in 2006.

Leaf collections, damage quantification and chemical analysis. Leaves used for quantifying canopy damage were collected in June, July and August of each year from 2006 to 2008. Leaves were collected from 16 trees per ring, four trees each of two aspen genotypes '42' and '271' (constituting the aspen-only community) and four trees each of aspen genotype '216' and paper birch (constituting the aspen–birch community). Briefly, we randomly selected multiple short-shoots from each of two canopy positions (lower one-third and upper one-third) of individual trees. We harvested short-shoots using a methodology demonstrated to preclude or limit phytochemical induction (R.L.L., personal observation). We then selected every second or third leaf from each short-shoot for a total of 10–12 aspen and 8–12 birch leaves per canopy position per tree. Approximately 42,000 leaves were collected and

analysed for damage, representing on average <0.5% total canopy biomass removed per year.

Collected leaves were first scanned on an Epson 1680 flatbed scanner and then flash-frozen in liquid nitrogen, lyophilized and weighed. Scanned leaf images were analysed using WinFOLIA PRO v2006a software, to which the scanner was calibrated. Per cent canopy damage was measured by reconstructing the missing area. Canopy damage continually increased as the growing season progressed; thus we used August damage levels for presentation and analysis of per cent foliar area damaged because they were the best representation of cumulative herbivory experienced by the stands. Canopy damage was also calculated as biomass removed, determined by subtracting the dry mass of the collected (that is, damaged) leaves from the dry mass of the estimated area from recreated (that is, undamaged) leaf images, where the dry mass of the reconstituted leaves was calculated using a ratio-based equation relating leaf area and mass. Biomass removed due to herbivory was then scaled to the canopy using mean annual biomass removed multiplied times biomass estimates from litter collections. Leaf mass per unit area (M_{area}) was determined as the quotient of dry leaf mass and scanned leaf area, while foliar nitrogen concentration was determined using NIRS (ref. 16).

Collection and chemical analysis of frass and greenfall. Insect frass and greenfall were collected from the mixed-aspen and aspen-birch communities during five or six 9- or 10-day, sampling campaigns conducted between late-May and mid-September of 2006 to 2008. Frass and greenfall were collected using 57 × 41 cm plastic baskets lined with muslin sheets[14]. Briefly, during each campaign four baskets were placed on the floor in each of the two forest community types in each ring. Samples were air dried and sorted into frass and greenfall. Frass deposits were easily identifiable by their shape and texture. To ensure that the substrates deposited were insect-mediated and not caused by factors other than herbivory, we defined greenfall as green leaf material that did not have an intact petiole. These substrates included both smaller leaf fragments and larger fragments with partial petioles dropped by caterpillars during feeding. Annual deposition of frass and greenfall was determined for each sampling location by (1) calculating daily deposition rate for each 9- or 10-day campaign, (2) setting input rates to zero on 15 May and 15 September of each year, (3) using linear interpolation to estimate daily input rates during periods that were not sampled and (4) calculating the area under the daily input curve over the entire season. Sorted samples were freeze-dried, weighed and stored frozen until greenfall nitrogen concentrations were determined by NIRS (ref. 16) and frass nitrogen concentrations were determined via a Thermo Finnigan Flash 1112 elemental analyser.

Modelling the effect of canopy damage on forest productivity. We determined the effect of canopy damage on ANPP using a model coupling canopy absorption of photosynthetically active radiation (APAR) with light-use efficiency (ε). ANPP was determined as

$$\text{ANPP} = \text{APAR} \times \varepsilon$$

where growing season APAR (APAR$_{GS}$) was calculated as the sum of the daily incident PAR from 15 May to 15 September, multiplied by the fraction of incident PAR absorbed by the canopy (f_{APAR}):

$$\text{APAR}_{GS} = \Sigma \text{PAR} \times f_{APAR}$$

Daily incident PAR was obtained from the WLEF-TV tower near Park Falls, Wisconsin (ChEAS (Chequamegon Ecosystem Atmosphere Study) FTP Data Access, http://cheas.psu.edu). f_{APAR} was calculated as $1 - e^{-kL}$, where k is a light extinction coefficient of 0.5 (ref. 30) and L is leaf area index. We calculated ε (empirical ε) for each year in 2006–2008 by dividing the annual, empirically determined ANPP (ref. 23) by APAR$_{GS}$ specific to each year.

To determine the effects of herbivorous insects on stand ANPP, we used an index of ε (ref. 29) that incorporates foliar morphology and canopy nitrogen content, and adjusted the index to account for variation in canopy damage. The ε index, which has been shown to correlate strongly and positively with ε across a broad array of plant taxa and within plant taxa across environmental gradients[29], was calculated as

$$\varepsilon\,\text{index} = (N_{canopy}/f_{APAR})/M_{area}$$

where N_{canopy} is canopy nitrogen content (kg ha^{-1}) and M_{area} is foliar mass per unit area. The index also performs well using the data from this study (Supplementary Fig. 2).

We adjusted the model parameters determining the index for loss of leaf area and biomass removed due to canopy damage, affecting L and N_{canopy}, respectively, to determine the effect of canopy damage on forest productivity. In the aspen community, L, M_{area} and N_{canopy} were calculated using simple averages of foliar traits across genotypes, by treatment within each year, whereas in the aspen–birch community, these averages were based on the relative proportions of foliar biomass for each species[28]. Leaf expansion at this site generally ended in late May, so L had stabilized by the onset of our damage and substrate collections (June of each year). Thus, in our models, base levels of L and f_{APAR} remained constant during a given

growing season. L was adjusted to account for leaf area removed (where $L_{undamaged} = L/(1 - \text{proportion damage})$), thus also altering f_{APAR} and N_{canopy}. The 'damaged' and 'undamaged' indices were then converted back into their respective ε values. Next, we related undamaged and damaged ε (undamaged ε/damaged ε) to produce a multiplier that was used to express the effect of herbivory on the empirically determined ε. Finally, we used the empirical ε, adjusted to remove damage, along with an estimate of undamaged f_{APAR}, to estimate potential forest productivity (ANPP) in the absence of herbivory. The difference between empirical ANPP (in the presence of herbivory) and potential ANPP (in the absence of herbivory) was then designated as the indirect effect of herbivory on forest productivity.

Next, we evaluated relationships between stand productivity and canopy damage. We analysed relationships between (1) relative stand growth under elevated CO_2 and O_3 and relative canopy damage in elevated CO_2 and O_3, and (2) potential for greater forest growth (modelled ε in the absence of herbivory) and the total ANPP lost to herbivory. Relative ANPP enhancement and relative canopy damage were calculated by comparing yearly values averaged by FACE block ($n = 3$) from elevated and ambient CO_2, averaged across O_3 levels, for each of the forest communities (aspen and aspen-birch) and year (2006–2008; $n = 18$). The same analysis was conducted comparing ambient with elevated O_3 treatments.

Statistical analyses. Statistical significance of treatment differences in canopy damage, organic substrate and nitrogen deposition, and the effects of damage on ANPP were determined by analysis of variance, with a split-plot design, using the model $Y_{ijklm} = b_i + C_j + O_k + CO_{jk} + e_{ijk} + S_l + CS_{jl} + OS_{kl} + COS_{jkl} + \varepsilon_{ijkl} + T_m + CT_{jm} + OT_{km} + COT_{jkm} + ST_{lm} + CST_{jlm} + OST_{klm} + COST_{jklm} + \epsilon_{ijklm}$. In this model, b represents block i, C represents CO_2 level j, O represents O_3 level k, e_{ijk} represents the whole-plot error, S represents stand community type l, ε represents the sub-plot error, T represents year m, and ϵ represents the sub-sub-plot error. Y_{ijklm} represents the average response of block i, CO_2 level j and O_3 level k, community type l and year m. The main effects of CO_2 represent differences between plots having elevated or ambient CO_2, regardless of O_3 level. Similarly, the main effects of O_3 represent differences between plots having elevated or ambient O_3, regardless of CO_2 level. For analysis of variance, all variables met assumptions of normality and homogeneity of variance. Regression analysis was used to determine relationships between relative stand growth and relative canopy damage and between potential stand growth and total biomass lost. After examination of residuals, total biomass lost was natural-log transformed to meet assumptions of normality. Statistical analyses were performed using JMP v.10 statistical software.

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Author contributions

R.L.L. designed the experiment and secured funding for the project; J.J.C. and T.D.M. collected field and laboratory data; J.J.C. and E.L.K. designed and performed the modelling exercise relating canopy damage to forest productivity; J.J.C. analysed the data and wrote the manuscript with the participation of R.L.L., T.D.M. and E.L.K.

Additional information

Supplementary information is available online. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to J.J.C.

Competing interests

The authors declare no competing financial interests.