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Predator-induced reduction of freshwater carbon dioxide emissions

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Predators can influence the exchange of carbon dioxide between ecosystems and the atmosphere by altering ecosystem processes such as decomposition and primary production, according to food web theory[1,2]. Empirical knowledge of such an effect in freshwater systems is limited, but it has been suggested that predators in odd-numbered food chains suppress freshwater carbon dioxide emissions, and predators in even-numbered food chains enhance emissions[2,3]. Here, we report experiments in three-tier food chains in experimental ponds, streams and bromeliads in Canada and Costa Rica in the presence or absence of fish (*Gasterosteus aculeatus*) and invertebrate (*Hesperoperla pacifica* and *Mecistogaster modesta*) predators. We monitored carbon dioxide fluxes along with prey and primary producer biomass. We found substantially reduced carbon dioxide emissions in the presence of predators in all systems, despite differences in predator type, hydrology, climatic region, ecological zone and level of *in situ* primary production. We also observed lower amounts of prey biomass and higher amounts of algal and detrital biomass in the presence of predators. We conclude that predators have the potential to markedly influence carbon dioxide dynamics in freshwater systems.

The Earth is experiencing its sixth mass species extinction, which like those before it, is markedly altering the abundance and diversity of predator species[1,4]. The loss and global homogenization of predators due to extinctions and introductions is expected to have far-reaching effects on biogeochemical cycling and the functioning of ecosystems[1,5]. Predators play a potentially important, but unclear role in local and global carbon cycling. The removal or introduction of predators can trigger alternating changes in the relative populations of lower trophic levels, a phenomenon called a trophic cascade. Trophic cascades can have striking effects on the abundance or biomass of both heterotrophs and autotrophs within virtually every type of ecosystem[6,7]. Changes in the abundance or biomass of heterotrophs and autotrophs can alter the rates of photosynthesis and community respiration, two biologically driven processes that underpin global carbon cycling[8] (Fig. 1).

Studies investigating the impact of changes in predator abundance on carbon cycling have largely been conducted in terrestrial ecosystems[9–11], despite the fact that freshwater ecosystems often experience stronger top-down control than terrestrial ones[12] and are estimated to emit as much CO_2 gas (up to 1.65 Pg C yr^{-1}) as emissions due to land-use change[13,14]. However, evidence for top-down effects on CO_2 dynamics of freshwater ecosystems comes from only two studies conducted in experimental lentic

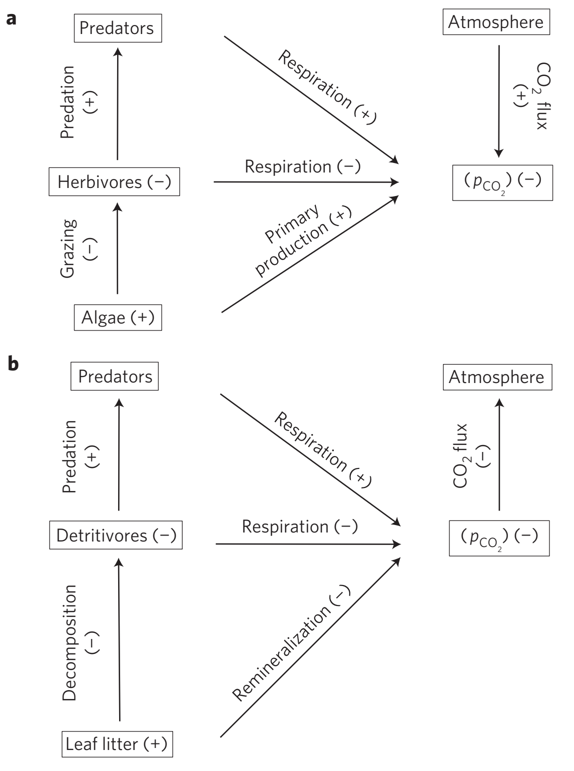

Figure 1 | Predicted effects (depicted by + or −) of predators on community composition, ecosystem processes and carbon flux to the atmosphere. a, Predators in algal-based freshwater ecosystems can negatively influence *in situ* CO_2 concentrations ([p_{CO_2}]) and positively influence the uptake of CO_2 from the atmosphere by creating trophic cascades that increase primary production and alter community respiration. **b**, Predators in detrital-based freshwater ecosystems can negatively influence [p_{CO_2}] and CO_2 efflux to the atmosphere by creating trophic cascades that reduce remineralization of leaf litter and alter community respiration. Predator effects depicted are representative of odd-numbered food chains; opposite effects are predicted for even-numbered food chains.

ecosystems[2,3], one of which was unreplicated[2]. Although the results of those studies suggest that predators can indirectly influence CO_2 dynamics of more complex ecosystems, they provide only an inductive generalization from a single ecosystem type. To predict

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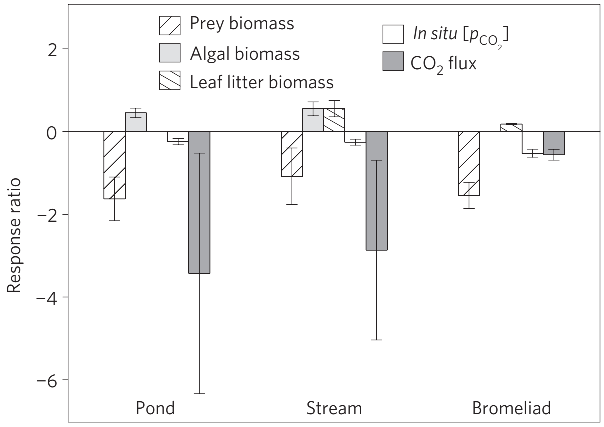

Figure 2 | Demonstrated effect sizes of predators on prey, primary producers and CO_2 dynamics of ponds, streams and bromeliads. Results are shown as log-ratios ±95% confidence intervals. Predator effects on prey biomass, algal biomass, leaf litter biomass, *in situ* CO_2 concentrations and CO_2 flux to the atmosphere were significant (MANOVA, $F_{1,34} = 32.97$, $P < 0.001$).

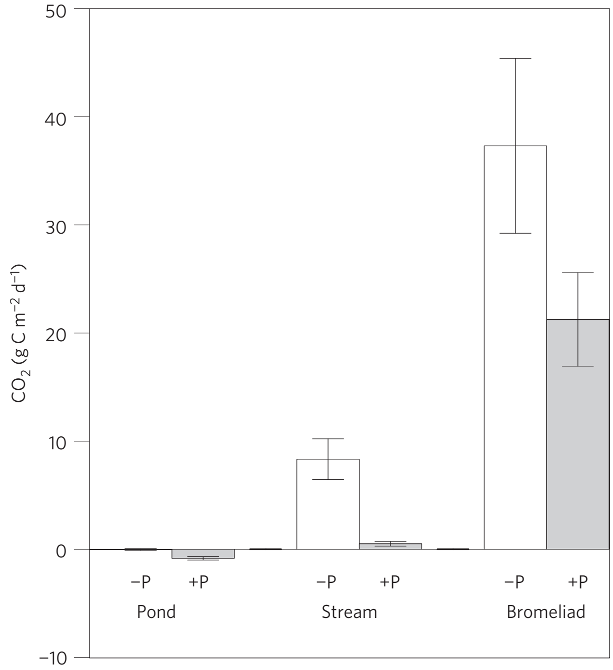

Figure 3 | Effects of predator manipulations on mean (±95% confidence intervals) CO_2 flux of ponds, streams and bromeliads. Predator-absent (−P) treatments significantly differed from predator-present (+P) treatments for all three ecosystems (analysis of variance, $F_{1,34} = 27.25, P < 0.001$). Ponds exposed to no-predator treatments were at equilibrium with the atmosphere.

how changes to predator abundance may influence carbon cycling more generally, broader experimental testing is needed.

We manipulated the presence of predators within small-scale experimental ponds, streams and bromeliad phytotelmata to determine the effects of predators on prey biomass, decomposition rates, algal biomass, *in situ* CO_2 concentrations and CO_2 flux to the atmosphere. Experimental food chains used contained three trophic levels and predator types consisted of both vertebrate (*G. aculeatus* in ponds) and invertebrate (*H. pacifica* in streams and *M. modesta* in bromeliads) primary predators that largely feed on invertebrate herbivores (mainly zooplankton), grazers and detritivores. We focused our study on pond, stream and bromeliad freshwater ecosystems for three reasons. First, despite their small global surface area, ponds, streams and bromeliads have been shown to be large sources of CO_2 and methane, and thus, represent an integral part of regional carbon cycles[13,15–17]. Second, these ecosystems allowed us to test our hypothesis that predators influence the CO_2 dynamics of freshwater ecosystems, regardless of differences with respect to predator type (invertebrate or vertebrate), hydrology (lentic or lotic), climatic region (temperate or tropical), ecological zone (pelagic or benthic) and level of *in situ* primary production (autochthonous, allochthonous or mixed). Finally, these systems can be easily replicated using mesocosms that support naturally complex food webs, but control for physical characteristics within ecosystem types that may influence CO_2 flux (for example, flow rate, depth, surface area, wind speed). Thus, indirect predator effects on CO_2 flux generated through trophic cascades can be more easily isolated, providing a mechanistic understanding of how predators influence CO_2 dynamics of freshwater ecosystems. We used a multivariate analysis of variance (MANOVA) test to demonstrate differences between predator treatments (predator present or predator absent) for all response variables tested. Univariate analyses were then performed on individual response variables to determine where significant differences occurred.

We found strong effects of predators on prey biomass, plant biomass, *in situ* CO_2 concentrations and CO_2 flux across all three ecosystems (MANOVA, $F_{1,34} = 32.97, P < 0.001$; Fig. 2 and Supplementary Table S1). Predators in each system significantly reduced prey biomass by $\sim 75 \pm 67\%$ (mean ± s.d.; $F_{1,34} = 50.96, P < 0.001$; Fig. 2), and cascading indirect effects led to $\sim 47 \pm 10\%$ lower detrital loss ($F_{1,34} = 38.49, P < 0.001$; Fig. 2) and $65 \pm 15\%$ higher algal biomass ($F_{1,34} = 14.19, P < 0.001$; Fig. 2). Furthermore, predators significantly decreased *in situ* CO_2 concentrations by $\sim 42 \pm 23\%$ (Fig. 2). These effects were also manifested in the CO_2 flux, where predators negatively influenced CO_2 emissions ($F_{1,34} = 27.25, P < 0.001$; Fig. 2). Here, predator treatments emitted $\sim 93 \pm 44\%$ less CO_2 gas to the atmosphere per day compared with non-predator treatments (Fig. 3). These results provide experimental evidence that predators can alter CO_2 emissions to the atmosphere in freshwater ecosystems, and suggest that predators have the potential to play a key role in local and global C cycles.

The magnitude of the indirect effect of predators on CO_2 emissions is dependent on the strength of the trophic cascade. The use of experimental ecosystems with low diversity and simplified physical structure can result in stronger top-down effects of predators on communities and ecosystem processes. However, a graphical comparison of the trophic cascade strengths for our three experimental ecosystems with averages of natural partner ecosystems calculated in a meta-analysis[12] showed that top-down control of plant biomass in natural ecosystems was, if anything, greater compared with our experimental ones (Fig. 4). This shows that predators in complex ecosystems are capable of generating trophic cascades of magnitudes equal to or greater than those demonstrated in this study, and suggests that trophic cascades could have a greater influence on CO_2 dynamics in natural ecosystems. In addition, the effects of predators on communities and CO_2 dynamics in natural ecosystems may be further exaggerated by other anthropogenic influences, such as climate warming[18] and increased nutrient loading to freshwater ecosystems[2,3]. Despite broad differences in predator type, all predators in our study were capable of creating trophic cascades that influenced CO_2 dynamics of their ecosystems. However, the magnitude of trophic cascades can be influenced by the biological characteristics of the predator, and thus effects on CO_2 dynamics may also be influenced by predator identity[19,20].

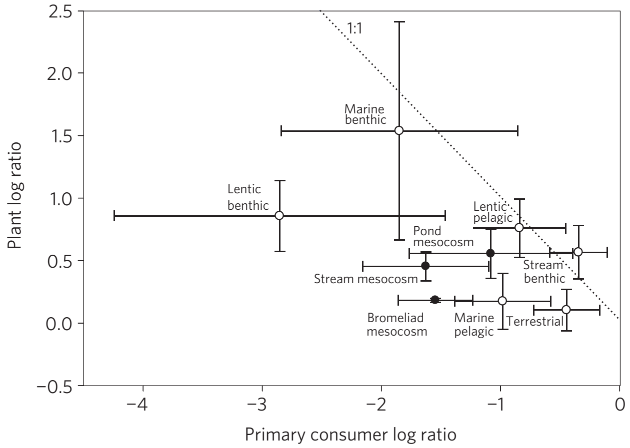

Figure 4 | Comparison of trophic cascade strength from the present study with natural ecosystems. Effect sizes (log ratio ± 95% confidence intervals) of predators on primary producers versus primary consumers from our experimental ponds, streams and bromeliads (filled circles), and those calculated from natural ecosystems (open circles)[12]. Primary producer data for stream mesocosms are representative of the effect size of predators on leaf litter biomass; however, predator effect size on algal biomass was similar in magnitude. The dotted line shows the 1:1 relationship. Data for lentic benthic, lentic pelagic, stream benthic, marine pelagic, marine benthic and terrestrial ecosystems were replotted from ref. 12.

We showed that predators decreased CO_2 emissions to the atmosphere in predominantly three-tier food chains consisting of predators, primary consumers and primary producers. However, the direction of the indirect effect of predators on CO_2 emissions is dependent on food chain length[2]. In odd-number trophic-level systems, such as the systems presented in our study, predators are predicted to decrease CO_2 emissions. Conversely, the indirect effect of predators in even-number trophic-level systems is predicted to cause an increase in CO_2 emissions.

The consistency in the effect of predators on CO_2 emissions in our study was remarkable, given the substantial differences among our experimental systems. Perhaps most surprisingly, predators had similar indirect effects on CO_2 flux for both detrital-based (bromeliads and streams) and algal-based (ponds and streams) food webs. This suggests that although predators may affect different underlying processes (photosynthesis or community respiration) behind the changes in CO_2 concentrations of the ecosystem, their effects on carbon storage generate a similar ecosystem response. Together, the consistency of our results and the comparison of our trophic cascade strengths with those of natural ecosystems provide evidence that predators have the potential to markedly influence CO_2 dynamics of freshwater ecosystems, and further supports evidence that predators can have strong effects on biogeochemical processes[20,21]. The marked influence of predators on CO_2 emissions from our freshwater ecosystems also indicates that human-induced removal of predators, or introduction of non-native predators, may have complex consequences for regional and global C cycles. Although predators are well known to shape ecological communities, our multisystem approach provides evidence that changes to predator abundance can extend beyond the biotic realm of an ecosystem and may fundamentally alter biogeochemical cycling and greenhouse-gas dynamics.

Methods

Ponds. We manipulated the presence of planktivorous fish, *G. aculeatus* (Linnaeus, 1758), in ten freshwater experimental ponds (surface area = 2.16 m^2) located in Vancouver, Canada. One month before the start of the experiment, mesocosms received nutrients (nitrogen and phosphorus) and an inoculum of phytoplankton, zooplankton and benthic invertebrates from a nearby pond[18,22]. Following inoculations, five fish per pond were introduced to five ponds. After 530 d, which allowed sufficient time for the communities to colonize, primary producer biomass, pelagic and benthic consumer biomass, and p_{CO_2} concentrations were measured in each pond. Detailed methods for control and predator treatment set-up, and sampling of benthic and pelagic organisms are described in refs 18,22.

Streams. We manipulated the presence of a predatory larval stonefly, *H. pacifica* (Banks, 1900), in six flow-through experimental streams (surface area = 7.52 m^2) located in Maple Ridge, Canada. One month before the start of the experiment, freshly cleaned channels were connected to a continuous flow of natural stream water. *H. pacifica* were added to three channels at densities similar to nearby streams (2.66 individuals per square metre; ref. 23). Before the start of the study and during the study, *H. pacifica* densities were maintained by passing water through a 4-mm-mesh filter before entering the channels. Every third day, *H. pacifica* were removed from the invertebrate community caught in filters, and the remaining organisms were emptied into their respective stream channels. Three leaf packs of ∼2 g of dried, senesced *Alnus rubra* (Bong) leaves and three unglazed ceramic tiles were placed randomly within each stream.

After 70 d, p_{CO_2} concentrations were measured and leaf packs and tiles were removed. The percentage of leaf biomass remaining was calculated following procedures in ref. 23. Periphyton biomass was determined fluorometrically following acetone extraction of chlorophyll-*a* pigments from tile scrapings. Benthic invertebrate communities were sampled from three sections of the streams using a Surber sampler (sampling area = 402 cm^2, 102 μm mesh) and biomass was measured as wet mass. The duration (70 d) of this experiment was chosen because it allowed sufficient time for communities to colonize, while reducing the risk of an early winter freeze.

Bromeliads. We manipulated the presence of a predatory damselfly, *Mecistogaster modesta* (Selys, 1860), in 20 bromeliad phytotelmata mesocosms (surface area = 0.02 m^2) located in the Área de Conservacion Guanacaste, Costa Rica[24]. On day 1, detritus (∼2 g dried *Conostegia xalapensis* Bonpl. leaf litter) and detritivores (larvae of chironomids, scirtid beetles and tipulids) were added at natural densities[24]. A single damselfly larva was added to each of ten bromeliad mesocosms. After initial communities were assembled, mesocosms were covered with 2 mm mesh to prevent insects from ovipositing and placed outside under a rain shelter. After 40 d, water samples for p_{CO_2} concentrations and remaining detritivores and leaf litter were collected. Detritivore biomass was quantified using length–mass regressions and leaf litter biomass was quantified as dry mass. The duration of this experiment (40 d) was chosen because it allowed for measurable detrital loss, while minimizing the loss of detritivores through pupation and predation[24].

CO_2 collection and flux calculations. Water samples for dissolved CO_2 concentrations were extracted at dusk using 50-ml Pressure-Lok syringes (VICI Precision Sampling) and stored in vacutainers (Labco Limited High). Sample CO_2 concentrations were analysed on a 5890 Series II gas chromatograph within 24 h for ponds and streams or 72 h for bromeliads using headspace equilibrium analysis[25].

CO_2 flux (g C m^{-2} d^{-1}) to the atmosphere was calculated as follows:

$$CO_{2\text{flux}} = (p_{CO_{2\text{water}}} - p_{CO_{2\text{air}}})^* k$$

Here, $p_{CO_{2\text{water}}}$ is the temperature-corrected partial pressure of CO_2 measured in the water, $p_{CO_{2\text{air}}}$ is the partial pressure of CO_2 in the overlying atmosphere (390 ppm) and k is the CO_2 exchange velocity coefficient (m d^{-1}). Stream k values (4 m d^{-1}) were estimated using the equation from ref. 16. Bromeliad and pond k values were estimated using literature values for no (bromeliads; $k = 0.48$ m d^{-1}) and low (ponds; $k = 0.63$ m d^{-1}) wind speeds[26].

Statistical analyses. We contrasted predator versus non-predator treatments for all response variables using a MANOVA (test = Pillai trace). To compare the predator effects across different ecosystems using a single MANOVA analysis, ecosystem response variables (prey biomass, percentage leaf litter remaining, algal biomass and CO_2 flux) for each ecosystem type (pond, streams and bromeliads) were converted into z-scores. As *in situ* CO_2 concentrations and CO_2 flux were co-linear factors, only CO_2 flux was added to the MANOVA model. We found no significant differences among ecosystem types ($F_{2,32} = 0.00, P = 1.00$) and so removed this factor from subsequent analyses. To determine where significant differences occurred in our model, subsequent univariate analyses were performed on the individual response variables.

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Author contributions

All authors contributed to the design of the study and to the writing of the manuscript. Data were collected in the field by T.B.A., E.H., H.S.G. and P.K.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to T.B.A.

Competing financial interests

The authors declare no competing financial interests.