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Rising atmospheric CO_2 is reducing the protein concentration of a floral pollen source essential for North American bees

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At present, there is substantive evidence that the nutritional content of agriculturally important food crops will decrease in response to rising levels of atmospheric carbon dioxide, C_a. However, whether C_a-induced declines in nutritional quality are also occurring for pollinator food sources is unknown. Flowering late in the season, goldenrod (*Solidago* spp.) pollen is a widely available autumnal food source commonly acknowledged by apiarists to be essential to native bee (e.g. *Bombus* spp.) and honeybee (*Apis mellifera*) health and winter survival. Using floral collections obtained from the Smithsonian Natural History Museum, we quantified C_a-induced temporal changes in pollen protein concentration of Canada goldenrod (*Solidago canadensis*), the most widespread *Solidago* taxon, from hundreds of samples collected throughout the USA and southern Canada over the period 1842–2014 (i.e. a C_a from approx. 280 to 398 ppm). In addition, we conducted a 2 year *in situ* trial of *S. canadensis* populations grown along a continuous C_a gradient from approximately 280 to 500 ppm. The historical data indicated a strong significant correlation between recent increases in C_a and reductions in pollen protein concentration ($r^2 = 0.81$). Experimental data confirmed this decrease in pollen protein concentration, and indicated that it would be ongoing as C_a continues to rise in the near term, i.e. to 500 ppm ($r^2 = 0.88$). While additional data are needed to quantify the subsequent effects of reduced protein concentration for Canada goldenrod on bee health and population stability, these results are the first to indicate that increasing C_a can reduce protein content of a floral pollen source widely used by North American bees.

1. Introduction

As has been observed in nearly a 100 individual studies and several meta-analyses, as atmospheric carbon dioxide (C_a) increases, nitrogen (protein) concentration declines in a wide range of plant species [1–5]. This decline can be associated with dilution resulting from increased carbohydrate production [4,6]; reduced transpiration and a reduction in mass flow of N and other mobile elements [4,7,8] and/or the need for less Rubisco with a subsequent lowering of plant demand for N, particularly in photosynthetic tissues [9]. The C_a-caused reduction in protein has been observed in a wide range of plant tissues, including leaves, stems, roots, tubers, seeds and grains [1–3,5] and has been correlated with negative effects on human nutrition on a global scale [3,10]. However, whether such reductions also occur for pollen, with subsequent effects on pollinator nutrition, are unknown.

At present, there is considerable concern regarding the widespread decline in native bee and honeybee colony numbers and increasing annual colony losses, particularly in the US and Europe. Drivers that have been associated with these declines include, but are not limited to, socioeconomic concerns (e.g. agricultural intensification), invasive pests and/or pathogens (e.g. Varroa, Nosema), agrochemicals (e.g. neonicotinoids) and a decline in genetic diversity of bee populations and food sources (e.g. over-reliance on one floral source) [11–17]. These factors may act singly or in combination to influence bee health [14,15].

Vulnerability to these reported environmental stressors is, in part, linked to pollinator nutrition [13,18]. For example, nutrition/diet can affect bee immunocompetence and the response to parasites (e.g. *Varroa destructor* or *Nosema ceranae*), which, in turn, can feedback to exacerbate nutritional stress [19–21]. Good nutrition can also aid in pesticide detoxification [22–24].

As all bees, wild or domesticated, obtain their energy and nutrition from flowering plants, any stressor that affects floral physiology on a panoptic scale could, potentially, alter long-term bee health. Nectar is the primary source of energy for the colony; however, pollen is the sole source of protein for all bees, wild and domesticated, and fulfils dietary requirements for the lipids, sterols, vitamins and minerals needed for larvae development [25–27]. In contrast to nectar, only a small amount of pollen is stored in the colony at any given time, making bee colonies susceptible to sudden fluctuations in pollen quantity or quality [28–30]. Lower pollen quality has been shown to negatively impact adult longevity in bees in a number of studies [31–34].

In northern latitudes in North America and Europe where little pollen is available during winter, plants that serve as late-season pollen sources are especially important for winter survival [35]. Among such sources, goldenrod (*Solidago* spp.) is a late-season perennial with a long bloom period from late July through to October. Although national estimates of pollen sources for bee diets do not exist; goldenrod has been recognized as a primary autumn pollen source for many pollinators in North America, including wild and domesticated bees, by apiarists and extension agents throughout Canada [36,37], New York [38], Ohio [39], Wisconsin [40], Michigan [41] and Minnesota [42], *inter alia*. The USDA Handbook of Agriculture lists goldenrod as an important nectar and pollen plant for all regions of the USA except the West [43].

Although there are numerous *Solidago* species, *Solidago canadensis* (Canada goldenrod) is the widest spread taxon, and includes several taxa which are frequently granted species status including *Solidago altissima* L., lepida DC (*sensu* Fernald), *Solidago gilvocanescens* (Rydb.) Smyth, *Solidago scabra* Muhl., *Solidago elongate* Nutt., *Solidago salebrosa* (Piper) Rydb. and *Solidago pruinosa* Greene [36]. *Solidago canadensis* is found in almost every state in the USA and throughout Canada (http://www.fs.fed.us/database/feis/plants/forb/solcan/all.html).

Whether recent or projected increases in C_a can induce changes in pollen protein concentration that could, potentially, also impact pollinator health, has not been established. Yet, such information may be particularly relevant for bees and other pollinators, given their role in global food production; hence, we wished to determine whether C_a has, or could, affect pollen protein levels using *S. canadensis* as a test case.

2. Material and methods

(a) Anatomical considerations

To determine variation between floral parts with respect to nitrogen and carbon, pollen was collected *in situ* for tall or Canada goldenrod at three locations within Indiana and two locations in Maryland in 2012. Pure pollen was compared to pollen/anther composition with respect to carbon and nitrogen concentration using carbon, hydrogen, nitrogen (CHN) analysis (see CHN analysis section). Pollen and anther C and N were similar in concentration and highly correlated ($r^2 > 0.96$). This allowed a relative comparison between pollen *per se* and the anther/pollen samples taken from the historical and experimental studies.

(b) Historical

Canada goldenrod (*S. canadensis*) is the largest goldenrod taxon and includes a number of taxa that have species status such as *S. altissima* (http://plants.usda.gov/core/profile?symbol=soal6). Taxonomically identified specimens of *S. canadensis* were obtained from the Smithsonian Institution's Museum of Natural History's collection. Pressed plants contained both vegetative and floral tissue, as well as date and location of the collected specimen. All samples contained fully developed flowers. Because these flowers were dry and subject to protein degradation, elemental analysis (C, H and N) was used to estimate protein concentration.

The collected plants from the USA were from Arizona, California, Colorado, Indiana, Maryland, Texas and the District of Columbia. Canadian samples, all from Ontario province, were also examined. Floral branches, usually 4–6 per plant and 4–10 cm in length were excised from each sample. Each branch was placed in a scintillation vial and labelled. A hand lens with razor blade or tweezers was used at the laboratory to remove florets from involucres. Anthers and pollen were placed into tin capsules (8 × 5 mm) and weighed on a Perkin-Elmer autobalance. Overall, four to six floral branches were collected from each of 350 individual plant specimens in the USA and Canada that spanned a time period from 1842 through to 1998. Florets from each branch were analysed separately then averaged for a given herbarium sample. Additional field samples were processed from Maryland (2008, 2012, 2014) and Texas (2012, 2014).

(c) Experimental: floral demography and insect visitation

To provide a more updated assessment of autumn floral demographics and to quantify *Solidago* populations, we measured the frequency of flowering stems and mapped their distribution using a 24 × 24 m area gridded into 1 m^2 quadrats in Williamstown, Berkshire County, Massachusetts. We identified all stems to the species level and for quadrats with less than 15 stems, we recorded the *x*- and *y*-coordinates; for quadrats with more than 15 stems, we assigned coordinates so that stems were evenly distributed in the quadrat. To determine the type and frequency of flower visitors to autumn-blooming Asteraceae, we marked out ten 1 m^2 quadrats and directly scored visitors to flowers in five 3 min observations periods (15 min) for 10 quadrats for a total of 750 min. We recorded the identity of each insect that visited a flower in the quadrat. Both sets of observations were from 2010; September and early October, up until the first frost.

(d) Experimental: field trials

Solidago canadensis was grown in assemblages of prairie plants along a pre-industrial (subambient) to projected C_a gradient (500 ppm) in the Lysimeter CO_2 Gradient (LYCOG) facility located in central Texas USA (31°05′ N, 97°20′ W). The LYCOG consists of two transparent and tunnel-shaped chambers, aligned

in parallel along a north–south axis. At present, this is the only field-based facility capable of exposing plant assemblages to a continuous gradient of C_a spanning pre-industrial to elevated concentrations [44,45].

Each chamber in LYCOG is divided into 10 consecutive compartments each 5 m long and 1.2 m (LYCOG) wide and tall. Chambered vegetation was enclosed in a transparent polyethylene film. Photosynthesis by enclosed vegetation progressively depleted the CO_2 concentration in air as it was moved by blowers towards the air outlet of each chamber to create daytime CO_2 gradients of 500–395 ppm (elevated chamber) and 395–250 ppm (subambient chamber). Night-time CO_2 concentrations were regulated at 130–150 ppm above daytime values along each chamber. Air temperature and vapour pressure deficit were regulated near ambient values by cooling and dehumidifying air at 5 m intervals along chambers. CO_2 treatments are maintained each growing season from April through to mid-November.

The LYCOG facility was constructed atop 1.2 m wide × 1.6 m deep steel containers that were buried to 1.2 m depth and into which were placed intact soil monoliths (each 1 × 1 × 1.5 m deep) of three soil types: silty clay, clay and sandy loam [44]. Four perennial C_4 grass species and three perennial C_3 forb species, all characteristic of tallgrass prairie in central Texas, were transplanted into each monolith in June 2003, 3 years prior to CO_2 treatment [44,45]. Eventual dominants included the C_4 grasses *Bouteloua curtipendula* (Michx.) Torr. and *Sorghastrum nutans* (L.) Nash and the forb *S. canadensis* (Canada goldenrod). CO_2 treatments were initiated in 2006. Each monolith in the LYCOG was irrigated twice weekly during each growing season. Irrigation was applied to simulate the seasonal distribution and average of growing season precipitation in central Texas (560 mm). *Solidago canadensis* was sufficiently abundant to assess relationships between pollen N and C_a only for monoliths from the clay soil.

In early October of 2012 and again in 2014, we collected 10–15 floral branches (3–6 cm in length) with pollen-bearing anthers from monoliths of the clay soil along the C_a gradient (i.e. from 280 to 500 ppm). (Samples were also taken in 2013, but were not analysed owing to the government shutdown.) Floral collections were accessed through zippered-openings in the polyethylene film enclosing vegetation. Samples were collected from each inflorescence present in each monolith (more than three flowering stems/monolith), combined for a given monolith, and stored in labelled vials. All vials were sent to Beltsville, MD, USA where they were processed as with the historical samples. The C_a to which plants in each monolith was exposed was calculated from the linear relationship between C_a and the physical position of each monolith along the gradient [44,45]. The effect of C_a on floral quality did not differ as a function of year, so both years were combined for analysis; however, *S. canadensis* biomass production was not correlated with C_a for either year ($p = 0.26$ and 0.87 in 2012 and 2014, respectively). The forb contributed an average of 12.5% of above-ground production of prairie assemblages along the C_a gradient.

(e) Carbon, hydrogen, nitrogen analysis

Elemental concentrations of carbon and nitrogen were determined using a Perkin-Elmer 2400 CHN/O analyser (Perkin-Elmer, Waltham, MA, USA). Samples from all floral branches for a given specimen/sample were collected and pooled, and three subsamples were run to establish an average value. Nitrogen and carbon content were determined as a percentage of the dry weight of the sample. Because it has been used previously for assessing nitrogen to protein conversion for pollen in bee diets, a conversion factor (N to protein) of 6.25 was used [46]. The protein concentration reported for *S. canadensis* in this study for ambient C_a is consistent with that of other studies [47].

(f) Statistical considerations

For analysis of both historical and experimental data, we used regression analysis to test for significance between C_a and the response variable using SigmaPlot (v. 12, 2014). We tested different functions for data fitness and selected the model with the highest adjusted r^2. If no differences were evident, we report results for the linear 'best-fit' model.

3. Results

Although there are a number of older quantitative estimates of demography and predominance of *S. canadensis* and related taxa (e.g. *S. altissima*) [38]; we wished to update these data by documenting the floral dominance and pollinator visits for *Solidago* during the autumn, including domesticated and wild bees. We observed that of 11 718 flowering stems, 10 993 (87%) are *Solidago* with *S. altissima* representing 5964 (50%) and *Apis*, or honeybees, being the dominant pollinator (figure 1*a*,*b*). The relative abundance of *Solidago* observed was consistent with previous observations (e.g. [38]).

Having confirmed the dominant role of *Solidago* in pollen availability during the autumn; we then determined whether C_a influenced the nutritional value of *S. canadensis* pollen. We employed two independent methodological approaches. Both approaches; one historical, one experimental, make use of a continuous C_a gradient. Results from continuous C_a gradients can be highly informative, as plant properties, including tissue chemistry, do not always respond in a linear manner with ambient C_a versus 2× ambient C_a comparisons [48].

Historical data were obtained through the Smithsonian Institution's National Museum of Natural History archives. These archives contain floral *S. canadensis* plant samples collected between 1842 and 1998 across a wide range of biogeographic locations throughout the USA and southern Canada. In addition, we supplemented these historical data with *S. canadensis* samples obtained *in situ* from Maryland (2008, 2012, 2014) and Texas (2012, 2014). The increase in C_a, from the onset of the industrial revolution to the beginning of the twenty-first century, was highly correlated with the observed decline in pollen protein ($r^2 = 0.81$, $p < 0.001$) with overall pollen protein declining by approximately one-third (from approx. 18 to 12%; figure 2*a*). Although the entire C_a record is over a 170 year period, the bulk of the C_a increase has, in fact, occurred since the latter half of the twentieth and early twenty-first century (i.e. C_a has risen from approx. 315 ppm in 1960 to 398 ppm in 2014); consequently, the largest decrease in pollen protein for *S. canadensis* has occurred during that time. The observed decrease in protein is concomitant to a parallel increase in the ratio of carbon to nitrogen (figure 2*b*). Such an increase is consistent with previous studies and is likely to indicate more substantial increases in carbohydrate to protein ratio as increasing C_a tends to increase the concentration of starch and sugars while reducing the concentration of protein (nitrogen) in plant tissues (e.g. [5,9]).

The experimental study was conducted *in situ*, using parallel, elongated chambers to maintain a continuous C_a gradient spanning pre-industrial to projected mid-twenty-first century (500 ppm) concentrations [44,45]. Carbon and nitrogen were quantified and pollen protein estimated for *S. canadensis* flowers grown along this C_a continuum during 2012 and 2014. Although the absolute numbers differed, the

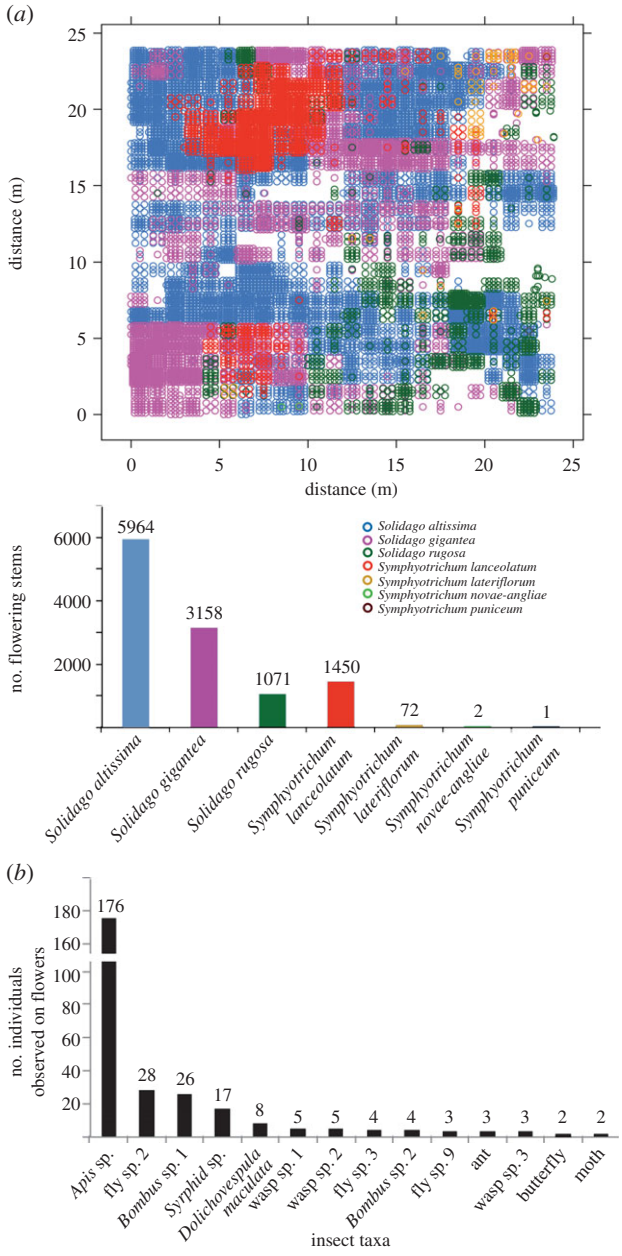

Figure 1. Widely distributed over North America, goldenrod (*Solidago* spp.) is a key floral pollen source for pollinator populations just prior to winter. Canada goldenrod (*S. canadensis*) is the largest goldenrod taxon and includes a number of taxa that have species status such as *S. altissima* [36] (http://plants.usda.gov/core/profile?symbol=soal6). (*a*) Map of all flowering stems (Sep–Oct) in a 24 × 24 m area plotted by species. (*b*) Frequency distribution of insect visitors to 1 m^2 quadrats within map based on 750 min of observations (Sep–Oct). Among pollinator populations, *Apis*, or honeybees are the most frequent visitors. See Material and methods for additional details.

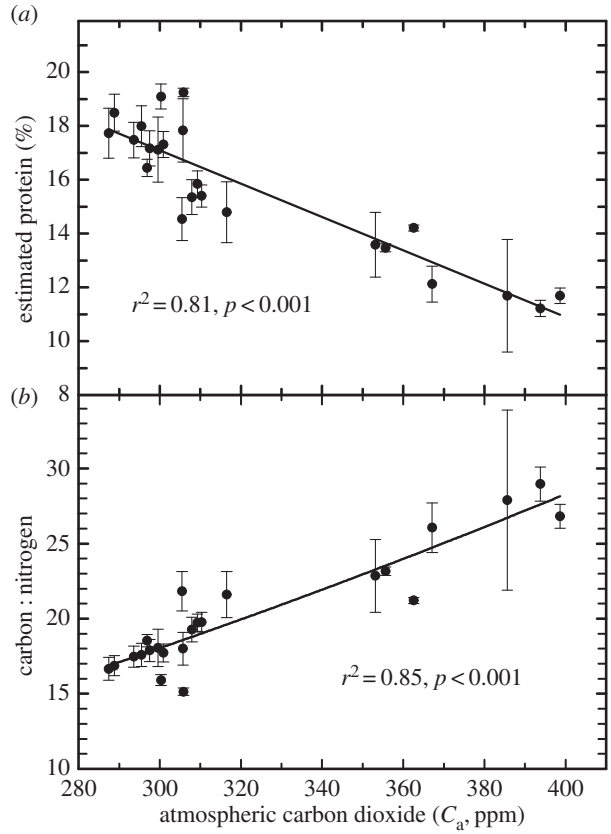

Figure 2. Average and variation (±s.e.) with time (1842–1998) in estimated protein concentration (*a*) and carbon to nitrogen ratio (*b*) for historical samples from floral (anthers and pollen) tissue for *S. canadensis* from the Smithsonian Natural History Museum. Atmospheric CO_2 (C_a) for a given set of sample dates were obtained prior to 1960 from [49]; after 1960 using http://www.esrl.noaa.gov/gmd/ccgg/trends/. Each point is the average of approximately 6–40 samples by year from different biogeographic regions within North America. See Material and methods for additional details.

pollen-C_a pattern observed was consistent with that derived from historical data; i.e. at higher C_a levels along the experimental tunnels, pollen protein concentration declined and carbon : nitrogen ratios increased (figure 3*a*,*b*). In addition, both approaches showed a similar decline in protein content and concurrent increase in carbon : nitrogen ratio for *S. canadensis* pollen in response to increased C_a.

4. Discussion

The decline in pollen protein concentration with C_a for *S. canadensis* observed in both the historical and experimental analyses is consistent with many studies and meta-analyses which have shown that increased C_a systemically reduces nitrogen and protein concentration in non-leguminous plant tissues [1–6,8,9]. Decreases in nitrogen concentration have also been reported for herbaria plant specimens in response to the increases in C_a during the twentieth century [50]. Overall, the data from the current study provide strong evidence that rising C_a since the start of the industrial age has, and will continue in the near term, to reduce the pollen protein concentration of *Solidago*, an important autumn pollen source for bees and other pollinators.

Because pollen provides all of the essential amino acids needed for bee development and can, in turn, affect hypopharyngeal gland and ovary development, pathogen susceptibility, immunocompetence and overall bee longevity [21,25,51–53], reductions in pollen protein concentration of *Solidago* have the potential to negatively affect bee health and survival.

These results for *Solidago* may be particularly relevant to potential health impacts for bees as it is the source of some of the last seasonal pollen acquired prior to winter, and thus constitute the nutrient load available for overwintering. Bees that overwinter require substantial pollen stores because late winter brood rearing occurs prior to the availability of spring pollen in temperate zones. For example, Farrar [54] found that the spring bee population as a percentage of the

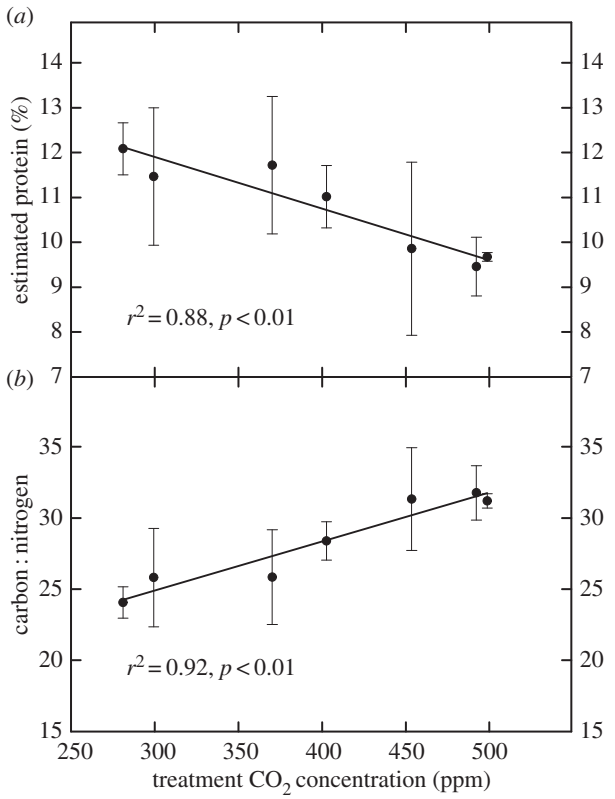

Figure 3. Average and variation (±s.e.) in estimated protein concentration (*a*) and carbon to nitrogen ratio (*b*) for experimental samples from floral (anthers and pollen) tissue for *S. canadensis* growing along a continuous gradient of C_a in clay soil for a mixed prairie community. C_a treatments were initiated in 2006. Data were averaged for 2012 and 2014 for each C_a treatment sampled within the LYCOG facility, $n = 6–14$ per C_a.

autumn population was positively correlated with the amount of stored pollen. In general, for temperate climates, early spring is recognized as a time of pollen protein shortages and colony starvation [22,27,35].

But can bees distinguish protein concentration among diverse pollen sources; and by doing so, compensate for any C_a-induced decrease? It has long been known that honeybees can differentiate sugar content among nectar sources and convey such information to the colony (e.g. the 'waggle dance'). However, while amino acid composition has been suggested as a learned aspect of honeybee protein foraging [55]; the overall consensus is that bees do not collect high-quality pollen preferentially [56,57].

There is widespread agreement then that: (i) pollen protein is an essential aspect of bee diet, and (ii) bees do not appear able to compensate by choosing other floral sources with higher protein concentration. Given the temporal importance of autumn pollen in pollinator life cycles in North America, the increase in C_a associated with climatic change and the resultant decline in pollen protein concentration of *S. canadensis*, could adversely affect bee health and overwintering capacity on a continental scale.

However, there are a number of caveats that need to be considered. First, it cannot be assumed that projected increases in C_a above those considered here will be proportionate to protein loss; i.e. there is the potential for saturating effects on protein concentration with rising C_a. Second, the subsequent influence of reduced protein concentration from *S. canadensis* on bee feeding, health or demographics has not been explicitly determined. Finally, whether C_a is also resulting in similar reductions in pollen protein in other floral species needs to be quantified (e.g. [58]). Overall the specific consequences of declining pollen concentration are likely to be dependent on these factors, as well as other environmental characteristics (e.g. Varroa, neonicotinoids), percentage of *Solidago* among flowering species in the autumn, etc. These environmental parameters and their potential interactions will require further elucidation in the context of C_a-induced nutritional changes in order to fully quantify impacts to bee health and population stability.

Although additional information is clearly needed, the current data do indicate a clear and unequivocal link, both historically and experimentally, between rising C_a and a qualitative decline in pollen protein for *S. canadensis;* among the most widely recognized and widely available food source for bees in North America (e.g. [43]). Given the economic and environmental importance of bees, and because the rise in C_a is global in nature, these data provide an urgent and compelling case for establishing the C_a sensitivity of pollen protein for other floral species and, in turn, quantifying the potential consequences for pollinator physiology around the globe.

Data accessibility. Data are available at http://dx.doi.org/10.5061/dryad.8bb66.

Authors' contributions. L.H.Z. conceived the experiments. A.C. provided the herbarium samples; J.E and J.E.H. provided the data on plant demographics of autumn *Solidago* and insect visitations; H.W.P. provided the LYCOG samples; M.B.T. did the CHN analysis; L.H.Z. wrote the paper; J.S.P, M.B.T., J.S.D., I.L. and H.W.P. analysed the data and/or edited the manuscript.

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