Plant-Pollinator Interactions over 120 Years: Loss of Species, Co-Occurrence, and Function

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comparison, methods for maintaining diverse wild insects for crop pollination are less developed, and research on such pollination services is more recent (*3*, *16*, *17*, *20*, *26*, *27*) (table S1). Although honey bees are generally viewed as a substitute for wild pollinators (*3*, *6*–*8*), our results show that they neither maximize pollination nor fully replace the contributions of diverse wild insect assemblages to fruit set for a broad range of crops and agricultural practices on all continents with farmland. These conclusions hold even for crops stocked routinely with high densities of honey bees for pollination, such as almond, blueberry, and watermelon (Fig. 2 and table S2). Dependence on a single species for crop pollination also carries the risks associated with predator, parasite, and pathogen development (*4*, *20*, *28*).

Our results support integrated management policies (*29*) that include pollination by wild insects as ecosystem service providers, along with managed species—such as honey bees, bumble bees (*Bombus* spp.), leafcutter bees (*Megachile* spp.), mason bees (*Osmia* spp.), and stingless bees (Meliponini)—as agricultural inputs, where they are not invasive species. Such policies should include conservation or restoration of natural or seminatural areas within croplands, promotion of land-use heterogeneity (patchiness), addition of diverse floral and nesting resources, and consideration of pollinator safety as it relates to pesticide application (*3*, *16*, *17*, *20*, *27*). Some of these recommendations entail financial and opportunity costs, but the benefits of implementing them include mitigation against soil erosion as well as improvements in pest control, nutrient cycling, and water-use efficiency (*30*). Without such changes, the ongoing loss of wild insects (*4*, *5*) is destined to compromise agricultural yields worldwide.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1230200/DC1
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References (*31*–*79*)
Database S1

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Plant-Pollinator Interactions over 120 Years: Loss of Species, Co-Occurrence, and Function

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Using historic data sets, we quantified the degree to which global change over 120 years disrupted plant-pollinator interactions in a temperate forest understory community in Illinois, USA. We found degradation of interaction network structure and function and extirpation of 50% of bee species. Network changes can be attributed to shifts in forb and bee phenologies resulting in temporal mismatches, nonrandom species extinctions, and loss of spatial co-occurrences between extant species in modified landscapes. Quantity and quality of pollination services have declined through time. The historic network showed flexibility in response to disturbance; however, our data suggest that networks will be less resilient to future changes.

Almost 90% of flowering plant species, including many important crop species (*1*), rely on animal pollinators (*2*). Plant-pollinator interaction networks may be particularly susceptible to anthropogenic changes, owing to their sensitivity to the phenology, behavior, physiology, and relative abundances of multiple species (*3*). Alternatively, the overall structure of plant-pollinator networks might be robust to perturbations because of a high degree of nestedness and redundancy in interactions (*4*).

Several authors have speculated about how changes in biodiversity (*5*) and phenology (*6*–*8*) might translate into changes in the structure (*9*, *10*) and stability (*11*) of complex interaction networks. However, there has been a lack of historical data on plant-pollinator networks and phenologies for both plants and insects in the same community. By using an extensive and unique data set, we were able to examine changes in plant-pollinator network structure and phenologies of forbs and bees across more than a century of anthropogenic change.

In the late 1800s, Charles Robertson meticulously collected and categorized insect visitors to plants, as well as plant and insect phenologies, in natural habitats near Carlinville, Illinois, USA (*12*–*14*). Over the next century, this region experienced severe habitat alteration, including conversion of most forests and prairies to agriculture, and moderate climatic warming of 2°C in winter and spring. In 2009 and 2010, we revisited the area studied by Robertson and re-collected data on the phenologies and structure of a subset of this network—26 spring-blooming forest understory forbs and their 109 pollinating bees (*15*). Hence, we could quantify changes in network structure, local bee diversity, and phenologies of forbs and bees. Further analyses and a null model determined the degree to which changes in network structure and bee diversity were attributed to species' traits, phenological mismatches, and land-use factors that spatially separate interacting species. To examine shifts in the quantity of pollinator services, we used a second historical data set from Carlinville collected in the early 1970s (*16*), examining the diversity and visitation rate of bees to the most important floral resource in this network (*Claytonia virginica*).

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Finally, to estimate changes in potential quality of pollination services through time, we identified pollen grains from the bodies of preserved specimens of the most important pollinators of *C. virginica* (*Andrena* sp.) collected during each of these studies (1888 to 1891, 1971 to 1972, and 2009 to 2010).

We observed considerable shifts in overall network structure from the late 1800s (Robertson's historical data) to 2009 to 2010 (Fig. 1). Only 24% of the original interactions (125 of 532) are still intact. However, we observed 121 novel forb-bee interactions in the contemporary data, such that the absolute difference of interactions lost was 46% (246 of 532). Reasons for shifts in interactions could include extirpations of species participating in the interaction, lack of spatial co-occurrence of species in modern fragmented landscapes, and changes in phenology, abundance, behavior, or physiology that alter the propensity for particular interactions to occur.

Bee extirpations contributed substantially to the observed shifts in network structure. Of the 407 lost interactions, 45% (183) were lost because bee species were extirpated from the study region; all 26 forbs remained present. It is unlikely that the dramatic loss of bees observed in the contemporary data set resulted from differences in sampling effort between the historic and contemporary studies. Robertson observed the pollinators of each forb species for 1 to 2 years before moving on to other species. In our intensive resurvey over 2 years, we found less than half (54 of 109) of those bee species. Although Robertson's sampling effort in each season is unknown, we were able to extrapolate our data based on sampling effort (*17*) and found that our observations were close to the "true" richness (table S1). If Robertson's sampling was less intense on a per plant species basis than ours, then the bee extirpations are a conservative estimate. Furthermore, the loss of bees was nonrandom, such that bees that were specialists, parasites, cavity-nesters, and/or those that participated in weak historic interactions were more likely to be extirpated (table S2), congruent with other findings (*18*, *19*). Specialists were lost more than generalists (even after correcting for potential observation bias), despite the fact that their host plants were still present (table S2 and fig. S1). This pattern may result from lower specialist abundances in Robertson's time (fig. S1) and/or their higher sensitivity to fluctuations in floral resources (*20*) and habitat loss (*21*). Parasitic species (mostly in the genus *Nomada*; family Apidae) were lost more than solitary or social bees, possibly because of the greater sensitivity of higher trophic levels to habitat loss and other perturbations (*22*). Additionally, cavity-nesting species (many in the Megachilidae family) (fig. S2) were lost disproportionately (table S2), potentially related to landscape management that reduces the availability of woody debris for their nests. Persisting bee species participated in stronger interactions historically [i.e., greater mean phenological

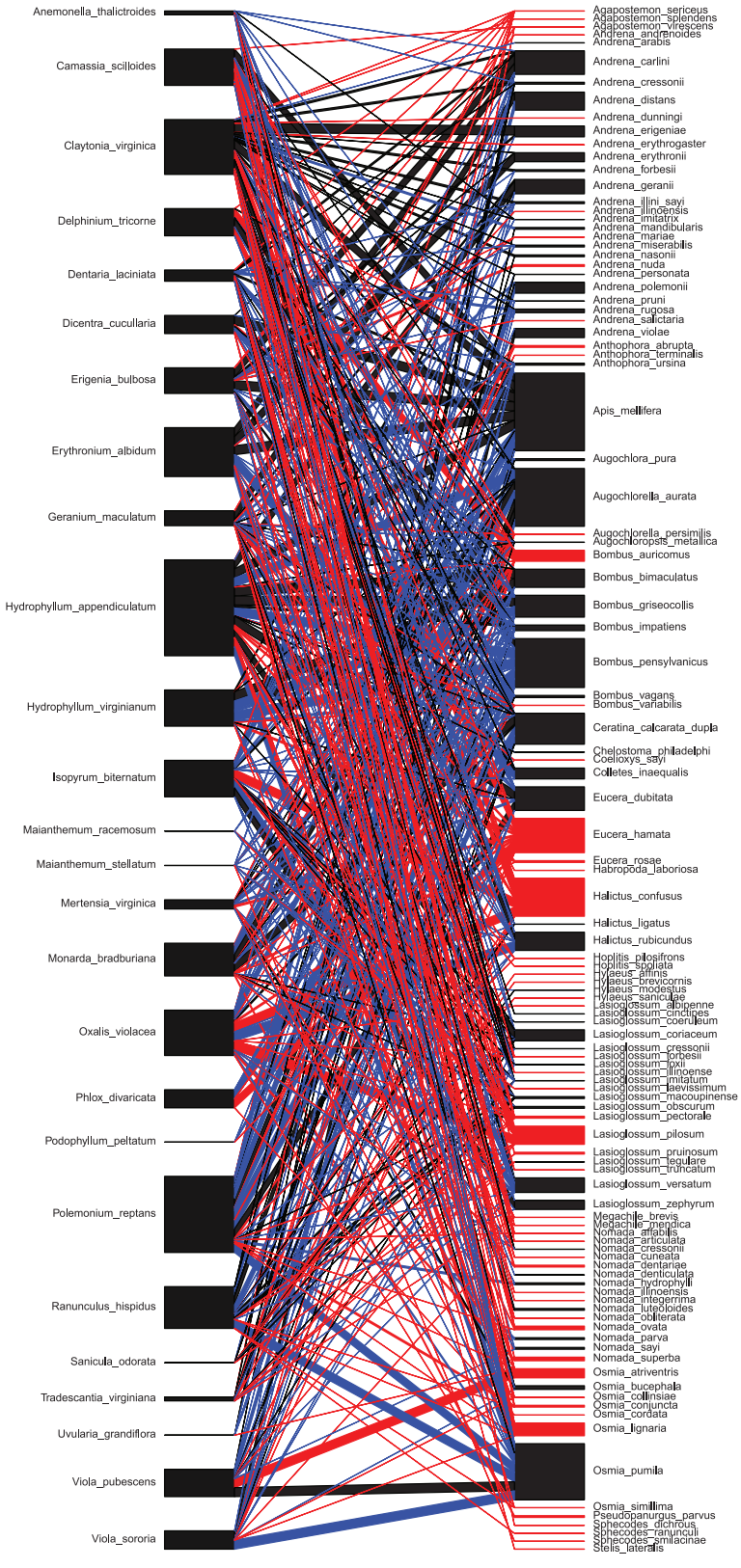

Fig. 1. Robertson's forb-bee interaction network included 532 unique interactions. Black lines (125 of 532 interactions; 24%) represent interactions that were observed in Robertson's time and persisted to the present; red lines (183 of 532; 34%) indicate interactions that were lost through the extirpation of bee species; and blue lines (224 of 532; 42%) represent cases where interactions were lost for other reasons, despite continued persistence of potentially interacting species in the Carlinville system. The thickness of the interaction lines represents the frequency categories of the interactions that were assigned by Robertson: present, frequent, or abundant. Bee species in red were extirpated.

overlap (*23*) by over 4 days (table S2)]. Many of these factors are not significantly associated with extinction when information on phylogenetic relationships are incorporated into statistical analyses (table S2), possibly because traits tend to be clustered in a few clades and/or few contrasts are available in the taxonomic phylogeny (fig. S2).

Historic sampling occurred in a relatively continuous forest landscape, whereas our modern observations were constrained to remaining forest fragments within a matrix of agricultural, commercial, and residential lands. Of the 224 lost interactions not explained by the extirpation of bee species, 41% (91 of 224) were explained by either lack of spatial co-occurrence (38 of 91), lack of temporal co-occurrence (48 of 91, phenological mismatches), or both (5 of 91) (Fig. 2). The contemporary networks are vulnerable to future perturbations because remaining interactions often occur at only a single study site and across a very short temporal period (e.g., 73% occurred during <1 week).

Few studies have examined phenological changes in both forb and bee communities in the same location across a long period (*8*). Recent literature syntheses suggest that forb and bee communities should shift synchronously, because the phenologies of both are strongly influenced by temperature (*9*). Alternatively, it is possible that bees rely more on temperature for their development and activity (*9*), whereas forbs use a more diverse suite of cues (*24*, *25*), resulting in phenological mismatches. We found evidence for the latter. Peak forb bloom was 9.5 days earlier (t_{25} = 3.91, P = 0.0007), and peak bee activity was 11 days earlier (t_{29} = 5.92, P < 0.0001) in 2009 to 2010; both results are on par with previous observations from other systems [plants (*6*, *7*); pollinators (*8*, *26*, *27*)]. However, phenologies of bee species active earliest in the spring shifted the most ($F_{1,29}$ = 5.89, P = 0.022, r = 0.42) (fig. S3), whereas there were no differences in phenological shifts among forb species ($F_{1,25}$ = 0.0001, P = 0.99, r = 0.0024) (fig. S3). Moreover, bloom periods were 8 days shorter (t_{25} = 3.18, P = 0.0042) and flight periods were 22.5 days shorter (t_{29} = 4.67, P < 0.0001), likely from physiological responses and/or reduced population sizes with truncated phenological variance (*28*). These results compounded to weaken interaction strengths [i.e., phenological overlap (*23*)] through time (t_{657} = 2.55, P = 0.011).

We devised a null model approach to disentangle the likely contributions of these phenological shifts versus other possible mechanisms in bee extirpation and interaction losses. The null model uses real data about historic interactions and phenology and observed phenological shifts in extant forbs and bees. Model scenarios examine a range of possible shifts in bee phenology (because the phenology of extirpated bees is not known) and circumstances under which bees and forbs forge novel interactions. Null-expected bee extirpations and loss of interactions due solely to phenological shifts ranged from 17 to 55% and 14 to 44% of those observed, respectively (figs. S4 and S5). Both the

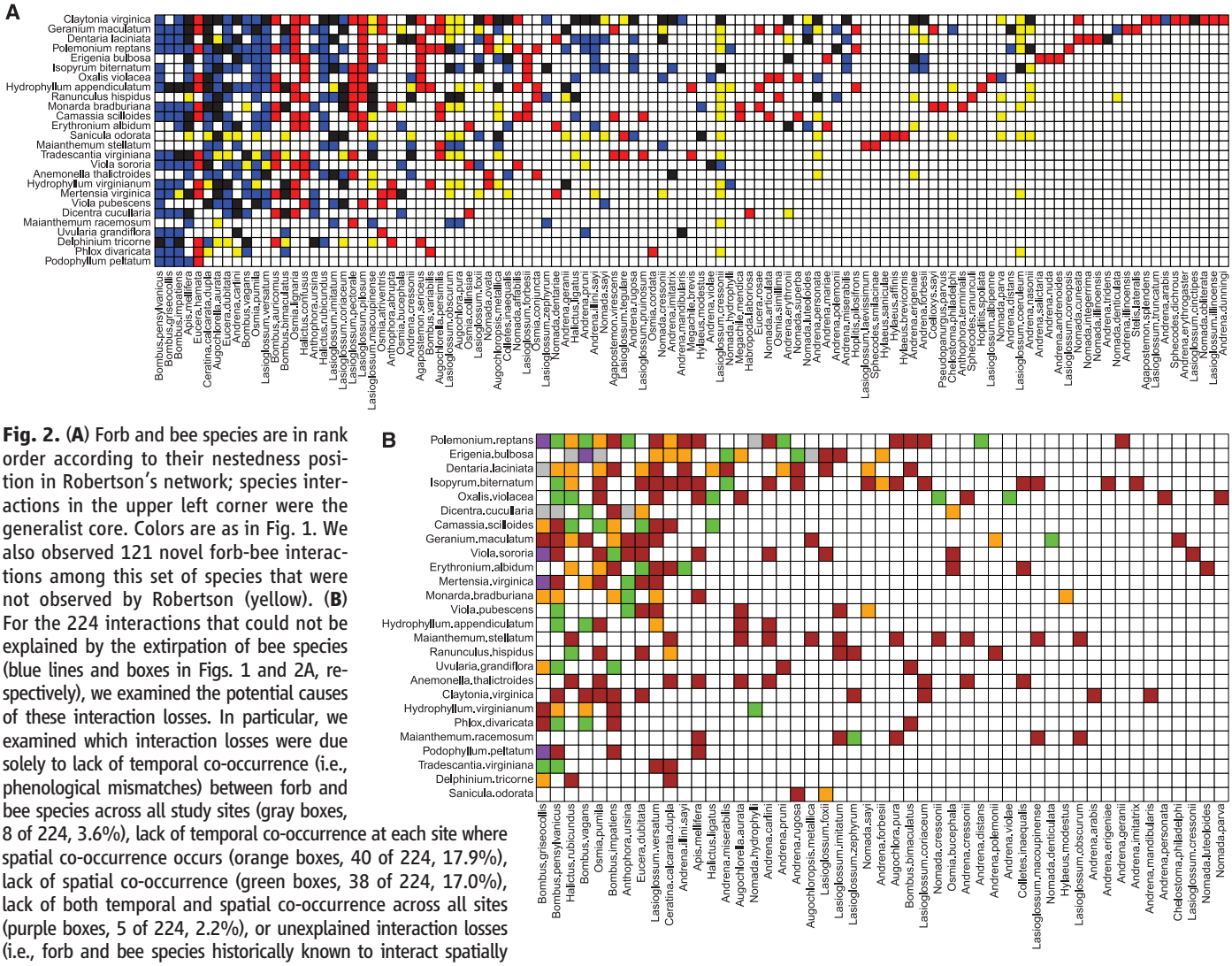

Fig. 2. (**A**) Forb and bee species are in rank order according to their nestedness position in Robertson's network; species interactions in the upper left corner were the generalist core. Colors are as in Fig. 1. We also observed 121 novel forb-bee interactions among this set of species that were not observed by Robertson (yellow). (**B**) For the 224 interactions that could not be explained by the extirpation of bee species (blue lines and boxes in Figs. 1 and 2A, respectively), we examined the potential causes of these interaction losses. In particular, we examined which interaction losses were due solely to lack of temporal co-occurrence (i.e., phenological mismatches) between forb and bee species across all study sites (gray boxes, 8 of 224, 3.6%), lack of temporal co-occurrence at each site where spatial co-occurrence occurs (orange boxes, 40 of 224, 17.9%), lack of spatial co-occurrence (green boxes, 38 of 224, 17.0%), lack of both temporal and spatial co-occurrence across all sites (purple boxes, 5 of 224, 2.2%), or unexplained interaction losses (i.e., forb and bee species historically known to interact spatially and temporally co-occur but do not interact; lack of interaction may be explained by physiological, behavioral, or relative abundance reasons; brown boxes, 133 of 224, 59.4%).

null-expected (fig. S6) ($F_{1,98}$ = 27.35, P < 0.0001) and observed (above) results showed higher extinction for more specialized bees. Other nonrandom bee extirpations are not explained by phenological changes and may result from the major shifts in the landscape that occurred over the past 120 years.

Interaction gains, losses, and rewiring contributed substantially to the observed shifts in network structure. We observed large changes in the diet breadth of species that persisted. Changes in the species' relative abundances, behavioral shifts, and evolutionary responses [mutualism abandonment (*29*)] may all have contributed to these shifts. Studies examining plant-pollinator interactions across several years also report substantial rewiring resulting from fluctuations in species' relative abundances across years, showing that such changes in networks can occur even in shorter periods (*30*). However, we constructed networks by summing across years for the historic and contemporary sampling periods (albeit with few years within a sampling period), and some of the species in our network experienced population declines across decades (*31*). Historically, Apinae (primarily bumblebees) had significantly wider diet breadths than other bee groups ($F_{8,100}$ = 4.34, P = 0.0002) but have experienced the greatest loss of interactions ($F_{7,46}$ = 5.45, P < 0.0001). This was due in part to recent population declines of some species (*31*), such as *Bombus pensylvanicus*, the most connected bee in Robertson's data set; we only observed one individual in 447 hours of sampling, highlighting its severely reduced role in network structure. Interestingly, remaining and novel interactions were redistributed across bee species, not just historic generalists (figs. S7 and S8). As a result of the combined influence of bee extirpations, interaction losses, and diet breadth shifts (interaction rewiring), the overall structure of the forb-bee interaction network became less nested than it was historically (fig. S9), indicative of increased vulnerability of pollination services to future perturbations (*4*).

Changes in network structure and species abundance might be expected to alter both the diversity of visitors to forbs and the service pollinators are providing (quantity and quality of pollen delivered). In particular, bee extirpations may result in lowered interspecific competition among remaining species, decreasing fidelity (*32*). Alternatively, if community-wide declines in floral resources resulted in heightened competition among bees, fidelity may increase. To examine these patterns more explicitly, we focused on bee visitors to *Claytonia virginica*, one of the most important floral resources during early spring, both in terms of abundance and diversity of pollinators. We used a second historical data set on the pollinators of this species in 1971 (*16*) from the same field sites as those visited in 2009 to 2010. First, we found that the richness of bee species visiting *C. virginica* did not change between Robertson's studies and 1971 but declined by over half in the past 40 years (Fig. 3 and table S3), which appeared to be largely driven by changes in forested habitat area (change in forested habitat during the past 40 years was significantly related to change in bee species richness visiting *C. virginica*; $F_{1,11}$ = 6.62, P = 0.028, r = 0.63, Δbee richness = 0.073 + 0.000093 × Δforest area). Second, we found that rates of bee visitation to *C. virginica* were more than four times as high in the early 1970s as in the contemporary data (0.59 and 0.14 bees per minute, respectively; t_{11} = 3.76, P = 0.0031). Third, *C. virginica* bee community composition was nested across sampling sites in 1971 (i.e., poor sites housed subsets of species that were found at better sites; P = 0.03), but they were not significantly nested in 2010 (P = 0.67) (fig. S10), suggesting a loss of redundancy in bee species that is characteristic of more intact communities. Finally, we quantified the proportion of *C. virginica* pollen grains on the bodies of representative specimens of six *Andrena* species that were captured during visits to *C. virginica* during the same three time periods and found that bee pollinators have almost three times lower fidelity now than 120 years ago (Fig. 4) ($F_{2,483}$ = 166.65, P < 0.0001). Thus, each of these metrics showed that pollination service on *C. virginica* consistently declined.

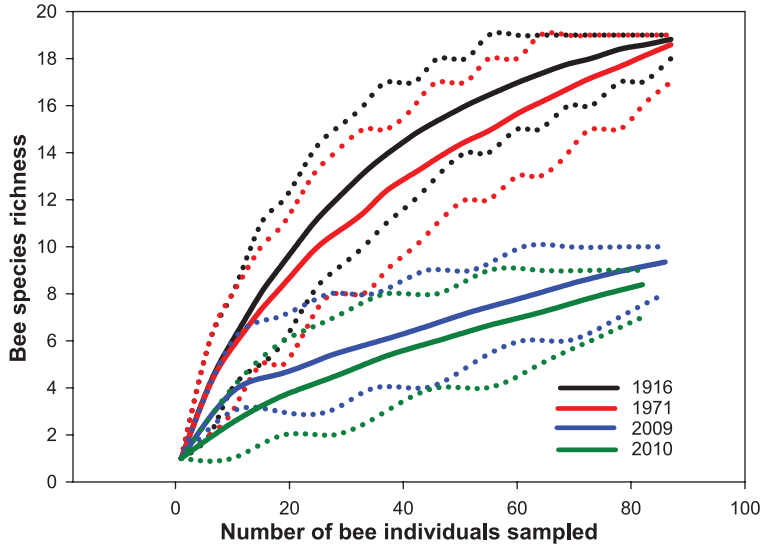

Fig. 3. The rarefied richness (solid lines) and 95% confidence intervals (dotted lines) of bee species visiting *Claytonia virginica* was more than twice as high in both 1916 and 1971 compared with 2009 and 2010.

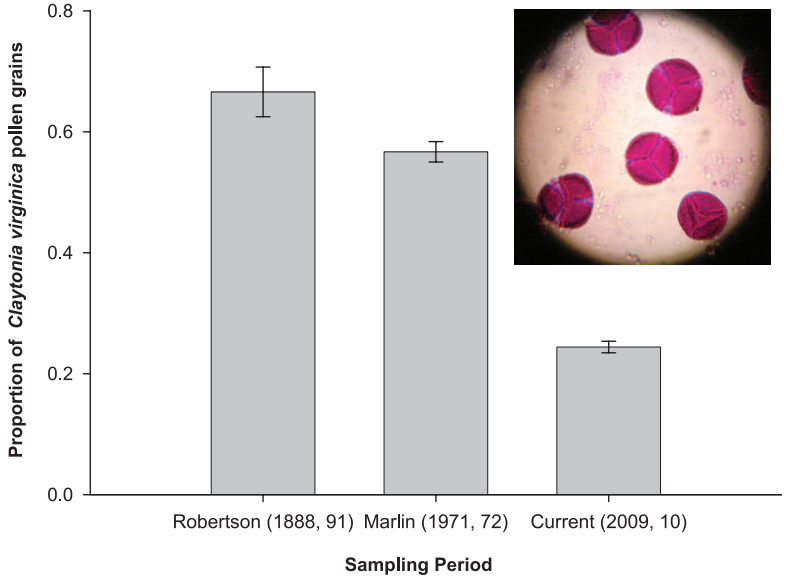

Fig. 4. Across six bee species, the proportion of *Claytonia virginica* pollen grains on the bodies of bee individuals captured visiting open flowers of that forb species declined over time, suggesting decreased fidelity and probability of successful pollination. Least-squared means are reported ± SE. Inset is a photo of *C. virginica* pollen grains.

We have found major changes in a plant-pollinator network over the past 120 years. This is partly explained by the nonrandom extirpation of bee species that are expected to be the most vulnerable to land-use and climate change, such as rare and specialized species, species occupying higher trophic levels, and cavity-nesting species. We found large changes in phenology of both forbs and pollinators and the potential for interaction mismatches, and these phenological changes can explain some of the species and interaction losses observed in this system. Our more optimistic finding was that plant-pollinator interaction networks were quite flexible in the face of strong phenological change and bee species extirpations, with many extant species gaining interactions through time. However, the redundancy in network structure has been reduced, interaction strengths have weakened, and the quantity and quality of pollinator service has declined through time. Further interaction mismatches and reductions in population sizes are likely to have substantial negative consequences for this crucial ecosystem service.

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Supplementary Materials

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Materials and Methods
Supplementary Text
Figs. S1 to S10
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References (*33*–*37*)

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Roots and Associated Fungi Drive Long-Term Carbon Sequestration in Boreal Forest

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Boreal forest soils function as a terrestrial net sink in the global carbon cycle. The prevailing dogma has focused on aboveground plant litter as a principal source of soil organic matter. Using ^{14}C bomb-carbon modeling, we show that 50 to 70% of stored carbon in a chronosequence of boreal forested islands derives from roots and root-associated microorganisms. Fungal biomarkers indicate impaired degradation and preservation of fungal residues in late successional forests. Furthermore, 454 pyrosequencing of molecular barcodes, in conjunction with stable isotope analyses, highlights root-associated fungi as important regulators of ecosystem carbon dynamics. Our results suggest an alternative mechanism for the accumulation of organic matter in boreal forests during succession in the long-term absence of disturbance.

Globally, the boreal forest biome covers 11% of the land surface (*1*) and contains 16% of the carbon (C) stock sequestered in soils (*2*). Aboveground plant litter quality and decomposition rates have been proposed as the fundamental determinants of long-term soil organic matter accumulation (*3*–*6*). However, a large proportion of photosynthetically fixed C is directed belowground to roots and associated microorganisms (*7*, *8*), potentially affecting C sequestration either positively or negatively (*9*–*12*). A better mechanistic understanding of how the belowground allocation of C affects long-term sequestration rates is crucial for predictions of how the currently large C stock in boreal forest soils may respond to altered forest management practices, climate change, elevated CO_2 levels, and other environmental shifts.

Here we present evidence from a fire-driven boreal forest chronosequence that enables the study of soil C sequestration over time scales of centuries to millennia. The system consists of forested islands in two adjacent lakes, Lake Hornavan and Lake Uddjaure (65°55′ to 66°09′N; 17°43′ to 17°55′E), in northern Sweden. The islands in these lakes were formed after the most recent glaciation and have since been subjected to similar extrinsic factors. Larger islands, however, burn more frequently because they have a larger area to intercept lightning strikes (*6*, *13*); several large islands have burned in the past century, whereas some small islands have not burned in the past 5000 years. It has previously been shown that as the time since fire increases, soil and total ecosystem C accumulates unabated and linearly (*6*, *14*), leading to humus layers that can exceed 1 m in depth on the smallest islands. This has been attributed to a decline in the quality of aboveground litter inputs and impaired litter decomposition as the chronosequence proceeds (*6*, *14*, *15*). We studied organic soil profiles on 30 islands representing three size classes with increasing belowground C stocks (*14*): 10 large islands (>1.0 ha; on average, 6.2 kg of C m^{-2} accumulated belowground; mean time since fire 585 years), 10 medium islands (0.1 to 1.0 ha, 11.2 kg of C m^{-2}, 2180 years), and 10 small islands (<0.1 ha, 22.5 kg of C m^{-2}, 3250 years).

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