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Agricultural soil legacy influences multitrophic interactions between crops, their pathogens and pollinators

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Soil legacy influences plant interactions with antagonists and below-ground mutualists. Plant–antagonist interactions can jeopardize plant–pollinator interactions, while soil mutualists can enhance plant–pollinator interactions. This suggests that soil legacy, either directly or mediated through plant symbionts, affects pollinators. Despite the importance of pollinators to natural and managed ecosystems, information on how soil legacy affects plant–pollinator interactions is limited. We assessed effects of soil management legacy (organic versus conventional) on floral rewards and plant interactions with wild pollinators, herbivores, beneficial fungi and pathogens. We used an observational dataset and structural equation models to evaluate hypothesized relationships between soil and pollinators, then tested observed correlations in a manipulative experiment. Organic legacy increased mycorrhizal fungal colonization and improved resistance to powdery mildew, which promoted pollinator visitation. Further, soil legacy and powdery mildew independently and interactively impacted floral traits and floral reward nutrients, which are important to pollinators. Our results indicate that pollination could be an overlooked consequence of soil legacy and suggests opportunity to develop long-term soil management plans that benefit pollinators and pollination.

1. Introduction

Ecological history leaves legacy effects on soil properties and subsequent plants' traits such as resistance to antagonists, including herbivores and pathogens [1–4]. Plant antagonists can negatively influence plant–pollinator interactions, and therefore may mediate soil legacy effects on pollinators [5]. Despite the critical role that pollinators play in ecosystem function [6], the lingering impacts of long-term soil conditioning on pollinators are poorly characterized. Consequently, it is challenging to predict environmental contexts that shift the relationship between antagonists and pollinators, [7] to identify mechanisms by which soil legacies affect plant community composition [1], or to evaluate whether soil management has lasting repercussions on pollinators or pollination services.

Plant antagonists influence the ecological outcomes of plant–pollinator interactions. Herbivory and pathogen infection can affect pollen, nectar quality and floral traits, often jeopardizing plant–pollinator interactions [5,8,9]. For herbivory, factors such as feeding site vary in the strength and direction of their effects on pollinators [10]. The comparatively smaller body of work on plant–pathogen–pollinator interactions focuses disproportionately on floral pathogens rather than foliar pathogens, which may affect floral traits indirectly [11,12]. Moreover, we have a poor understanding of how the outcomes of plant–antagonist–pollinator interactions depend on broader ecological contexts, such

as soil environment. While poor soil conditions could amplify the negative effects of antagonists on floral traits, high quality soil could buffer or even reverse these negative effects through overcompensation in the form of increased flower production or pollinator attraction [13–15]. Therefore, the costs and benefits of different agricultural soil management practices may depend on how these practices affect plant biotic interactions.

Long-term soil conditioning affects soil properties, future plant growth and resistance to antagonists, often in ways that are distinct from the effects from short-term conditioning [16–20]. In agriculture, long-term organic management and cover cropping can increase nutrient availability and promote microbial mutualists such as mycorrhizal fungi [21–25]. Crucially, some of the same nutrients and soil microbes affected by soil legacy also shape crop–antagonist interactions. For instance, residual excesses or deficits in soil nutrients [26] can affect leaf composition, and consequently, resistance to herbivores and pathogens [27,28]. Additionally, microbial isolates from soils under long-term organic management enhance plant resistance to herbivores [3]. While not typically considered in this framework, soil legacy likely also affects floral traits and plant–pollinator interactions.

Soil legacy could indirectly or directly influence plant–pollinator interactions. We expect organic soil legacy to benefit floral traits and pollinator attraction by improving plant resistance to antagonists or by preserving microbial mutualisms (e.g. mycorrhizal fungi) that promote floral quality [29,30]. Short-term studies demonstrate that inadequate or excessive soil fertility can negatively impact floral reward nutritional quality [31–33]. However, little work has investigated the legacy effects of long-term management practices on flowers, which may attenuate or magnify with soil conditioning duration or time since application [16,34–39]. Therefore, current evidence is insufficient to develop a predictive framework for how long-term soil management influences floral traits, pollinator fitness, or pollination. Furthermore, soil legacy effects may differ based on pollinator diet breadth because bees that specialize on a narrow range of plant taxa may have decreased ability to forage flexibly in response to changes in diet quality.

Our objective was to understand whether soil legacy affects pollinators, and if so, to identify possible mechanisms. In Experiment 1, we assessed how organic versus conventional soil legacy affects plant interactions with mycorrhizal fungi, powdery mildew, herbivores and pollinators. As soil management practices within organic and conventional management systems vary widely, we also compared the effects of different nutrient regimes within each management type. Within organic systems, we compared a legacy of high- versus low-nutrient amendments. Within conventional systems, we compared a legacy of green manures (tilled-in cover crops) versus synthetic fertilizer inputs. To test direct, indirect and interactive effects of (a) soil legacy, (b) plant antagonists, (c) pollen quality and (d) floral abundance on pollinator abundance, we used structural equation modeling (SEM), a statistical approach that evaluates complex relationships among multiple potentially correlated variables (figure 1).

Because Experiment 1 suggested that soil legacy indirectly affects floral traits and pollinators by changing plant resistance to powdery mildew, we inoculated plants grown in the different legacy soils with powdery mildew in Experiment 2. This manipulation in Experiment 2 tests how soil legacy affects powdery mildew resistance and how soil, powdery mildew, and their interaction affects floral traits important to pollinators. To understand whether soil microbes might mediate soil effects on aboveground interactions, we quantified mycorrhizal colonization. Finally, in Experiment 3, we fed bumble bees with pollen collected from plants grown in Experiment 2 to test whether soil- or powdery mildew-driven changes to pollen quality affect bee colony development.

2. Material and methods

Squashes (genus *Cucurbita*; Cucurbitaceae) are globally cultivated, pollinator-dependent crops [40,41] with economically important herbivores and pathogens [42,43]. We documented the herbivorous beetles *Diabrotica virgifera*, *Diabrotica barberi*, *Acalymma vittatum* (Chrysomelidae) and the squash bug *Anasa tristis* (Coreidae). We also documented powdery mildew (*Podosphaera xanthii)* [44], a primarily wind-borne pathogen of Cucurbits [45]. For pollinators, we observed the specialist squash bee *Peponapis pruinosa* (Apidae), the generalist bees *Apis mellifera*, *Bombus spp.* (Apidae), *Lasioglossum spp.*, *Augochlorella spp.* and *Agapostemon spp.* (Halictidae).

(a) Experiment 1: field mesocosm experiment

We evaluated plant interactions with herbivores, pathogens, and pollinators from 16 June to 19 August 2020, in a mesocosm experiment using potted plants. We collected soils at 15 cm depth from 25 organic and conventional plots at the Musgrave Research Farm (Aurora, NY: 42°44′02.8″N 76°39′04.3″W) and the Homer C. Thompson Research Farm (Freeville, NY: 42°31′07.2″N 76°20′06.2″W) (electronic supplementary material, figure S1). Soils from conventional plots with a synthetic fertilizer legacy had received primarily synthetic fertilizer annually for at least 3 years ($N = 5$ plots; 3 from Freeville and 2 from Musgrave). Soils from conventional plots with a cover cropping legacy had received primarily green manures (tilled-in cover crops) with 0–1 total applications of synthetic fertilizer for at least 3 years ($N = 4$ plots; 3 from Freeville and 1 from Musgrave). Soils from organic plots came from two long-term organic management trials that each had low- and high-nutrient regimes comprised of a poultry manure and cover crops (Musgrave), and poultry manure, cover crops and compost (Freeville) (high nutrient: $N = 8$ plots; 4 plots per site, and low nutrient: $N = 8$ plots; 4 plots per site). Experimental organic plots were established in 2005 (Musgrave) and 2014 (Freeville) and were certified organic by the Northeast Organic Farming Association of New York (NOFA-NY).

At Freeville, cropping history for the previous three years included *Brassica sp.*, *Cucurbita sp.* and *Lactuca sativa* in organic high and low nutrient plots; *Cucurbita sp.*: *Zea mays*, *Solanum tuberosum*, *Solanum lycopersicum*, *Trifolium pratense* and *Secale cerale* in conventional cover crop plots; and *Brassica sp.*, *Cucurbita sp.*, *S. tuberosum*, *Sorghum bicolor* (L.) Moench × *Sorghum sudanense* (Piper) Stapf, *S. cerale*, and *Trifolium repens* in conventional synthetic plots. At Musgrave, cropping history for the previous three years included *Z. mays*, *Glycine max*, *S. cereale*, *Triticale*, *Lolium perenne*, *T. pratense*, *Melilotus officinalis and S. bicolor* in organic high- and low-nutrient plots; *Z. mays* and *G. max* in conventional synthetic plots; and *T. pratense* and *Tritucum aestivum* in conventional cover crop plots. Therefore, while there were broad differences in management, there was also variation in cropping history (electronic supplementary material, table S1).

Using soils described above, we conducted a common garden mesocosm experiment at Bluegrass Research Center in Ithaca, NY, USA (42°27′34.4″N 76°27′38.7″W). We seeded summer squash (*Cucurbita pepo*) (‘Success PM’, High Mowing Seeds, Wolcott, VT, USA) in 11.4-litre pots (5–10 pots for each

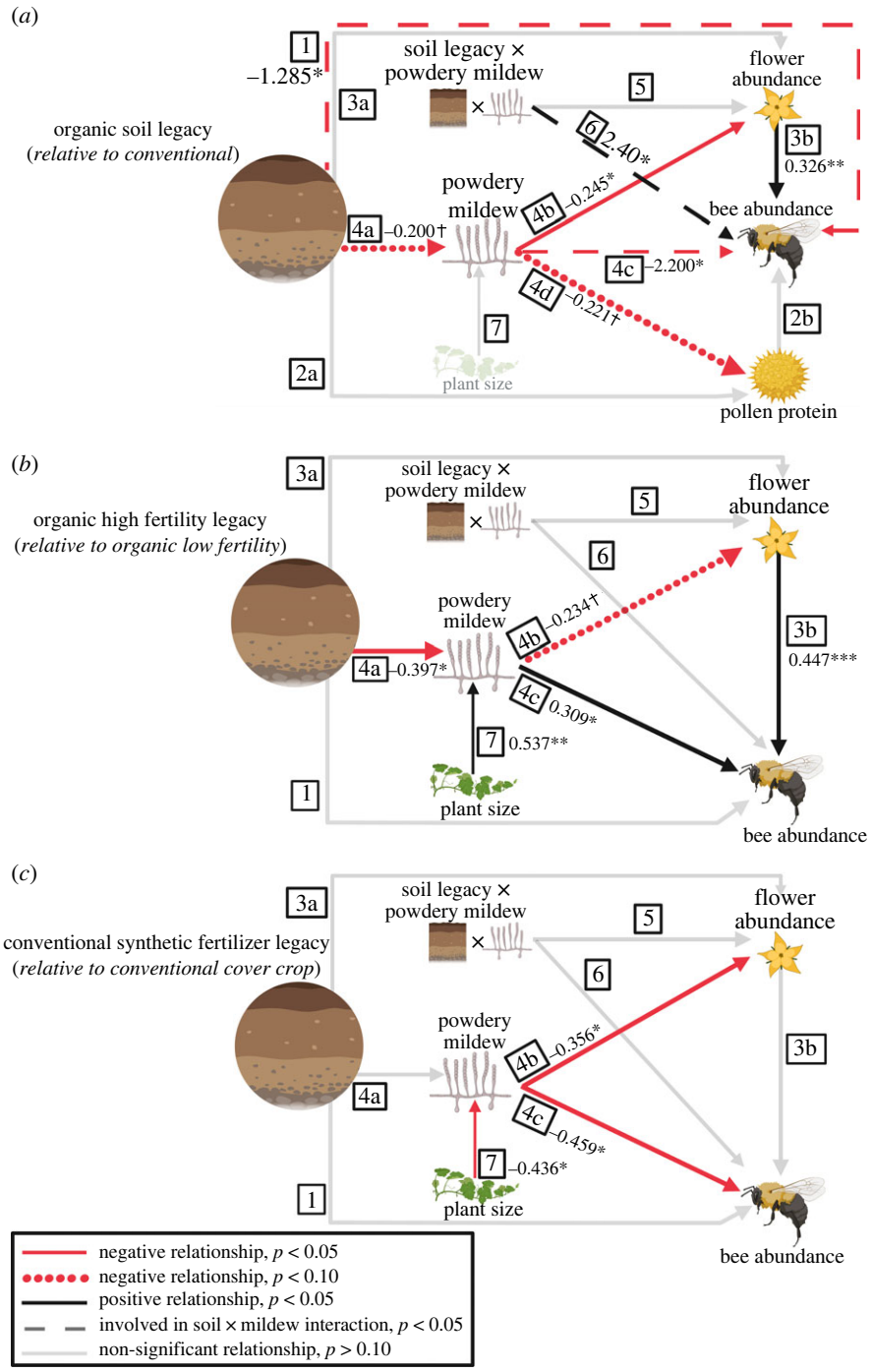

Figure 1. Hypothesized and observed relationships between soil legacy and pollinator visitation. We tested the hypothesis that an organic compared with conventional management legacy would compromise plant–pollinator interactions. We predicted this would happen directly (H1) and indirectly by increasing pollen quality (H2a, H2b) and/or increasing floral abundance (H3a, H3b). We hypothesized reduced powdery mildew due to soil (H4a) would increase floral abundance (H4b), bee visitation (H4c) and pollen quality (H4d). Additionally, we hypothesized that plants in organic legacy soils would compensate for negative effects of powdery mildew on floral abundance (H5) and bee abundance (H6). All comparisons between soil and response variables are made in reference to (*a*) conventional soil legacy, (*b*) organic low fertility and (*c*) conventional cover crop, and line colour denotes direction of the effect (e.g. a red line between soil and powdery mildew indicates a negative effect of organic compared with conventional soil legacy on powdery mildew infection). Solid lines indicate significant relationships ($p < 0.05$). Circular-dashed lines indicate relationships where $p < 0.1$, and faded grey lines indicate nonsignificant relationships ($p < 0.10$). Rectangular-dashed lines indicate that the variable is involved in a significant soil × powdery mildew interaction; in (*a*), the positive effect of the interaction term indicates that the negative relationship between powdery mildew and bee abundance in conventional soils was nullified for plants in organic soils. Values represent standardized coefficients, which provide an estimate of the relative effect size of variables measured at different scales. Asterisks represent significance level (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, † $p < 0.10$).

of the 25 plots for a total of 137 replicates, 130 of which survived the entire experiment) in a randomized design on 16 June 2020.

We measured vegetative growth, powdery mildew infection, herbivory, bee visitation, pollen protein and flower abundance. We calculated the proportion of herbivore- and powdery mildew-damaged leaves by dividing the number of damaged or infected leaves by the total number of leaves. To evaluate pollinator visitation, we conducted two-minute timed trials, visiting each plant three times during peak bloom in August between 6.30 AM and 12.30 PM, on sunny, low wind days (18°C to 29°C). We recorded the taxa of pollinators that landed on flowers to the highest possible resolution. We collected pollen in July and August by brushing pollen off anthers with a metal spatula, then stored it at −80°C. To measure pollen protein content,

we used a Pierce BCA protein quantification kit (electronic supplementary material, material S1).

(b) Experiment 2: greenhouse manipulative experiment

To pinpoint the role of powdery mildew, we conducted a greenhouse experiment. We manipulated soil type and powdery mildew, then measured powdery mildew infection intensity (proportion leaf area infected), flowering phenology, pollen and nectar quality, and floral morphology. We collected soil from the Freeville organic trials and from adjacent conventional plots (electronic supplementary material, table S2). There were no differences between synthetic fertilizer and cover crop legacy in the mesocosm experiment. Therefore, we did not make this comparison again in the greenhouse experiment. Cropping history for the previous three years in conventional plots included *Brassica sp.*, *Cucurbita sp.*, *Beta vulgaris*, *Spinacia oleracea*, *Solanum lycopersicum*, *S. cereale*, *T. pratense*, *S. bicolor*, *Triticum aestivum*, *Hordeum vulgare*, *Allium sativum* and *Z. mays*. Cropping history for the previous three years in organic high- and organic low-nutrient plots included *Brassica sp.*, *Cucurbita sp.*, *Lactuca sativa*, and *Beta vulgaris*.

On 16 May 2021, we seeded summer squash (Success PM) in 11.4-liter pots in four greenhouses at Cornell University, in a fully factorial design manipulating soil legacy and powdery mildew infection. To prevent contamination, powdery mildew-inoculated plants were kept separate from control plants in two greenhouses each. We collected conventional soils from 7 plots and organic soils from 8 plots (4 high-fertility plots and 4 low--fertility plots) with between 5 and 9 replicate plants for each of the 15 plots. Plants were arranged in a randomized array, and we accounted for spatial position within each greenhouse by assigning plants to spatial blocks.

When plants were three weeks old, we inoculated powdery mildew treatment plants with *Podosphaera xanthii* (McGrath Laboratory, Cornell Long Island Research and Extension Center, Riverhead, NY, USA). We used a flame-sterilized disposable pipette to transfer fungal spores from the edge of the powdery mildew culture by gently brushing first the sporulating culture and then the leaf with the pipette tip onto three leaves per plant [46]. We repeated the same procedure on control plants using a sterile pipette.

When plants were 6–7 weeks old (approx. 3 weeks post-inoculation), we measured powdery mildew area using calipers. We used LeafByte [47] to calculate average leaf area using a young, middle-aged, and old leaf. To estimate whole plant leaf area, we multiplied average leaf area by the number of leaves on the plant, then calculated the proportion of powdery mildew by dividing powdery mildew area by total leaf area. We measured pollen protein following methods described above, and corolla width using digital calipers (Bioquip Products, Inc., Compton, CA, USA). We measured nectar volume using calipers and microcapillary tubes (Drummond Scientific, Broomall, PA, USA) and sugar concentration with a refractometer (Neta Scientific, Hainesport, NJ, USA). Nectar samples were diluted with 100 uL of distilled water and Brix values back-calculated if volume or sugar concentration were too low or too high respectively for the refractometer to provide an accurate reading. At the end of the experiment on 9 September 2021, we harvested roots for mycorrhizal fungi quantification (electronic supplementary material, material S2).

(c) Experiment 3: Bee bioassays

We tested whether pollen quality changes due to powdery mildew treatment or soil legacy affect adult bee survival and colony development. We fed bumble bee (*Bombus impatiens*) micro-colonies pollen from plants grown in Experiment 2. To prepare micro-colonies, we placed five workers of comparable size from three source colonies into 16 oz plastic deli cups with mesh bottoms. We reared micro-colonies for one week on a wildflower pollen diet and 30% sucrose before starting experimental pollen diet treatments. Each micro-colony was fed *ad libitum* 30% sucrose, plus one of six experimental pollen diets from plants grown in Experiment 2 under the different soil (organic high-fertility, organic low-fertility, or conventional legacy) and powdery mildew (inoculated or control) conditions ($N = 3$ to 5 micro-colonies per treatment combination). Experimental diets were mixed in a 1 : 1 ratio with wildflower pollen. For one week after initiating the experimental diets, we recorded adult survival, pollen consumption, and new brood cells daily. See electronic supplementary material, material S3 for detailed rearing conditions.

(i) Statistical analyses

We used RStudio v. 4.2.3 [48] for analyses. We used *piecewiseSEM* [49] and BioRender (see https://biorender.com/; accessed 29 September 2023) for SEM analyses and diagrams; *lme4* [50] and *DHARMa* [51] for mixed effects models, and *ggplot2* [52] to create graphs. In all analyses, we used AIC (Akaike Information Criterion) and parsimony to select the top model. To determine the effect of each predictor variable in mixed effects models, we used a likelihood ratio test to compare the full model to an identical model that excluded the variable of interest. Non-significant variables were removed from the final model if the simpler model yielded a lower AIC value. We evaluated pairwise differences using *emmeans* [53], correcting for multiple comparisons using false discovery rate. Where necessary, we transformed variables to meet model assumptions (electronic supplementary material, table S3).

(d) Field mesocosm experiment

All models contained field nested within soil collection site as random effects.

We built three types of SEMs [54] (figure 1). First, we compared organic with conventional management legacy, regardless of nutrient legacy. Then, we examined the effects of nutrient legacy within each management system. For each comparison, we ran two SEMs: one included powdery mildew and the other included herbivory as the 'plant antagonist'.

The SEMs comparing organic with conventional legacy were composed of the following models: (1) proportion leaf damage from plant antagonist dependent on soil, (2) floral abundance dependent on soil × plant antagonist damage, (3) pollen protein content dependent on soil and plant antagonist damage, and (4) bee visitation dependent on floral abundance, pollen protein and soil × plant antagonist damage. The SEMs comparing fertility legacies contained the same set of paths except for pollen protein due to sample size limitations introduced by the truncated datasets.

Because many plants never developed powdery mildew, we asked whether soil legacy improved resistance to any infection development or improved resistance once infected. We used a binomial model to test whether soil legacy, site or their interaction affected the presence of infection. Next, we used a beta regression to determine predictors of infection intensity only for plants that developed infection.

We tested the significance of soil legacy on abiotic soil traits with a permutation-based multivariate ANOVA using *vegan* [55] and the random forest classification algorithm [56] to identify variables of importance (electronic supplementary material, material S4).

(e) Greenhouse manipulative experiments

All models contained greenhouse/field as nested random effects, and where applicable, plant ID and sampling date as random

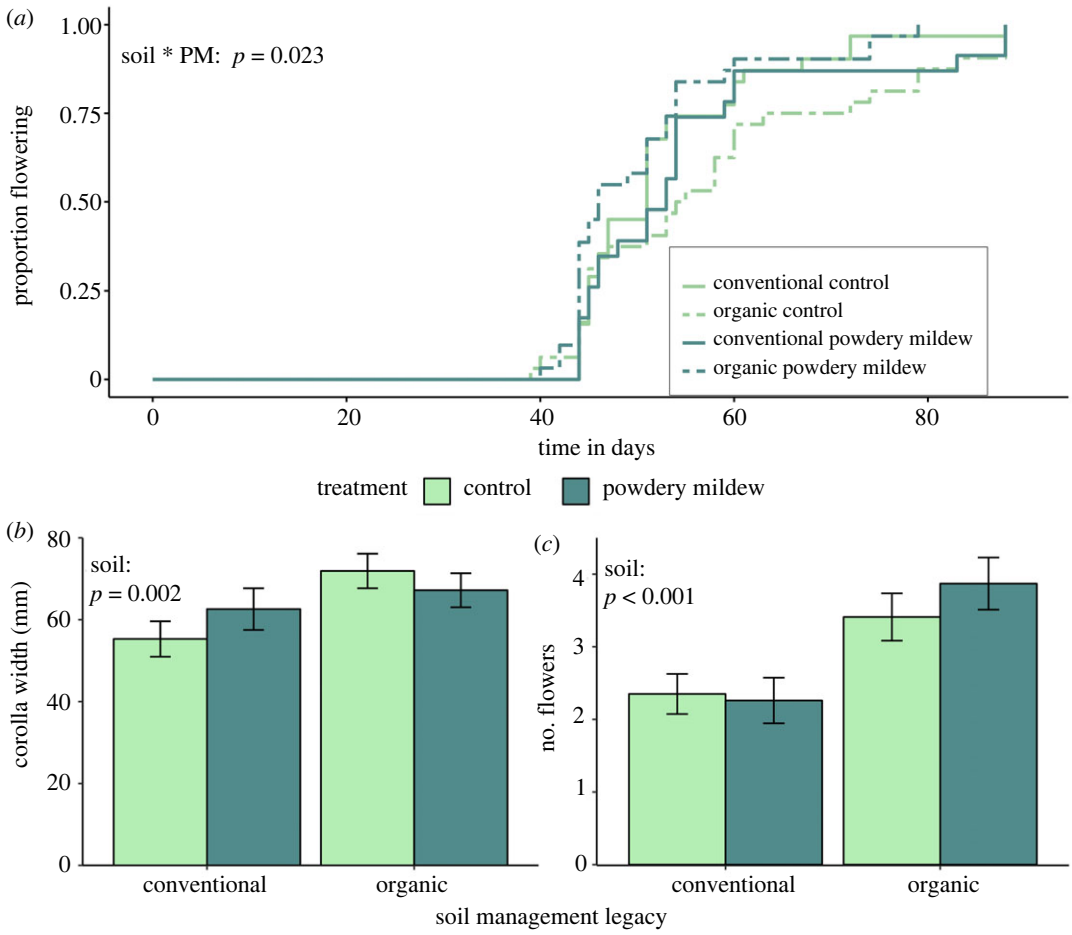

Figure 2. Effects of soil management legacy and powdery mildew on (*a*) flowering phenology, (*b*) flower abundance, and (*c*) flower size. (*a*) visualizes results from Cox proportional hazards model predicting soil and powdery mildew effects on plant flowering time. For (*b–c*), error bars represent model estimated marginal means ± 1 S.E.

effects (electronic supplementary material, table S3). Percent differences amongst groups were calculated using raw data.

To evaluate nectar volume, sugar concentration, nectar sugar content (volume × sugar concentration) [57], floral width, floral abundance, pollen protein and mycorrhizae, we built mixed effect models with soil legacy, powdery mildew treatment and their interaction, and where applicable, nectar sampling time, as fixed effects. For nectar sugar models we also included whether the sample had been diluted as a random effect. We used a beta distribution for sugar concentration, a Poisson distribution for flower abundance, a binomial distribution for mycorrhizal colonization, and a Gaussian distribution for all other traits. For flowering phenology, we employed a mixed effects survival analysis [58]. In addition to testing whether the binary powdery mildew treatment affected floral traits, we also used these same modeling approaches to test whether infection intensity (proportion leaf area infected) correlated with floral traits for plants inoculated with powdery mildew.

(f) Bee bioassays

To test whether experimental diet affected adult bee survival, we used mixed effects survival analyses which can be used to model the likelihood that an event has occurred over time. We used soil legacy, powdery mildew treatment, and their interaction as fixed effects and source colony as a random effect.

3. Results

To display the degree to which soil legacy effects were consistent across experiments, we present the results of each comparison (i.e. organic versus conventional, organic high-fertility versus organic low-fertility, etc.) from all three experiments alongside one another.

(a) Organic versus conventional legacy

(i) Field mesocosm experiment

The SEM tested indirect and direct relationships between soil legacy and pollinator visitation, and suggested relationships between soil legacy, powdery mildew, flowers, and bees (figure 1*a*; Fisher's C = 2.936 $p = 0.817$, 6 d.f.). Plants in organic soils had marginally less powdery mildew than plants in conventional soils ($p = 0.093$), and plants with higher powdery mildew infection produced fewer flowers ($p = 0.030$). The effect of soil legacy on bee abundance per plant depended on powdery mildew infection intensity, such that bee abundance negatively correlated with infection intensity only for plants in conventional soils (figure 1*a*, electronic supplementary material, figure S3; interaction term $p = 0.014$). Finally, plants with more powdery mildew tended to produce pollen with less protein ($p = 0.057$).

Plants in organic soils were less likely to develop any powdery mildew infection compared with those in conventional soils (electronic supplementary material, figure S4*a*; $\chi^2 = 6.676$, 1 d.f., $p = 0.010$). However, organic plants that developed infection had higher proportion leaf area infected when grown in Musgrave soils (electronic supplementary material, figure S5*a*; $\chi^2 = 9.945$, 1 d.f., $p = 0.002$).

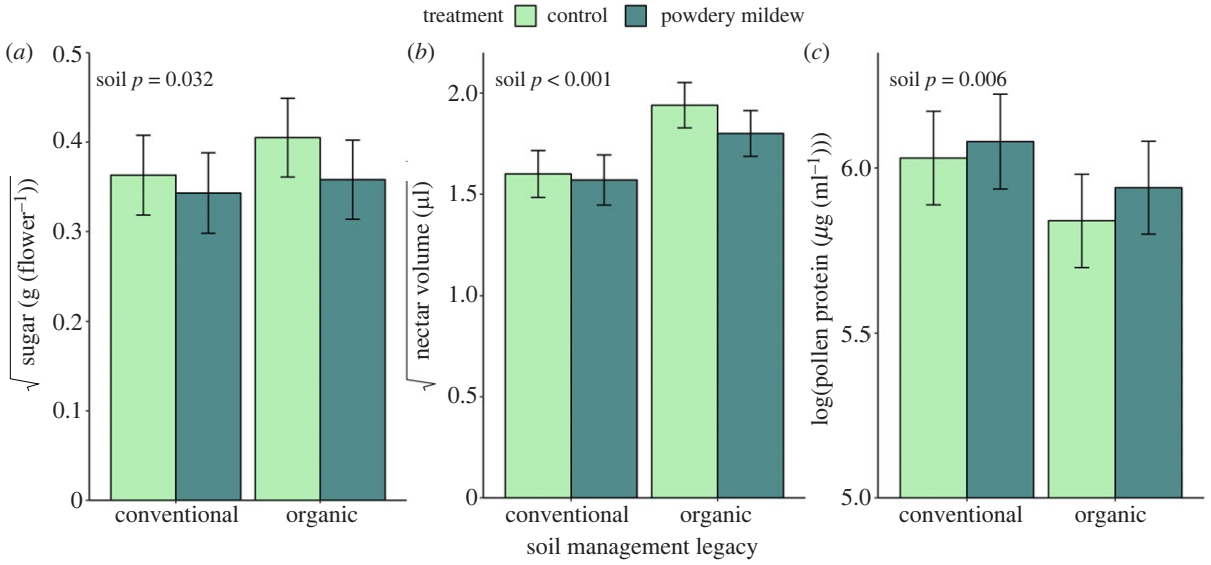

Figure 3. Effects of soil management legacy and powdery mildew on (*a*) total nectar sugar per flower, (*b*) nectar volume and (*c*) pollen protein content. Error bars represent model estimated marginal means ± 1 S.E.

Random forest identified aluminum, organic matter, and potassium content as important variables driving differences in soil composition (electronic supplementary material, material S4). Aluminum was lower in organic soils, while organic matter and potassium were higher in organic soils. Additionally, organic soils had higher phosphorus content ($\chi 2 = 5.642$, 1 d.f., $p = 0.018$).

(ii) Greenhouse manipulative experiment

Powdery mildew and soil management legacy interacted to affect flowering phenology (figure 2*a*; $\chi^2 = 5.202$, 1 d.f., $p = 0.023$); powdery mildew tended to advance bloom only for plants in organic legacy soils ($p = 0.08$). Soil legacy impacted flower abundance and size (figure 2*b*,*c*; $\chi^2 = 14.548$, 1 d.f., $p < 0.001$ and $\chi^2 = 9.837$, 1 d.f., $p = 0.002$); flowers were larger and more abundant in organic soils. There was no effect of powdery mildew or the soil*powdery mildew interaction on flower abundance or width (electronic supplementary material, table S4).

Soil legacy affected nectar sugar content, nectar volume and pollen protein content (figure 3*a*–*c*; $\chi^2 = 4.619$, 1 d.f., $p = 0.032$; $\chi^2 = 17.109$, 1 d.f., $p < 0.001$; $\chi^2 = 7.486$, 1 d.f., $p = 0.006$, respectively), but not nectar sugar concentration ($\chi^2 = 1.725$, 1 d.f., $p = 0.189$). Plants in organic legacy soils produced 17% more nectar sugar per flower than those in conventional soils and had 26% higher nectar volume than plants in conventional soils. Plants in organic soils produced pollen with 9% less protein than those in conventional soils. Neither powdery mildew treatment (inoculated versus control) nor its interaction with soil affected pollen or nectar traits (electronic supplementary material, table S4). However, powdery mildew infection intensity (proportion of leaf area infected) was negatively correlated with nectar sugar concentration and sugar content, regardless of soil type ($\chi^2 = 14.382$, 1 d.f., $p < 0.001$ and $\chi^2 = 15.048$, 1 d.f., $p < 0.001$, respectively). Additionally, infection intensity was negatively correlated with nectar volume for plants in conventional, but not in organic soils (interaction term: $\chi^2 = 6.381$, 1 d.f., $p = 0.011$). Neither powdery mildew infection intensity nor its interaction with soil legacy affected pollen protein content ($\chi^2 = 0.3607$, 1 d.f., $p = 0.548$ and $\chi^2 = 1.914$, 1 d.f., $p < 0.167$, respectively).

Soil legacy affected powdery mildew infection (figure 4*a*; $\chi^2 = 4.9831$ d.f., $p = 0.025$). Inoculated plants grown in organic legacy soils had 50% lower infection than those grown in conventional legacy soils. Additionally, plants grown in organic legacy soils had 37% higher mycorrhizal colonization than those grown in conventional legacy soils (figure 4*b*; $\chi^2 = 17.365$, 1 d.f., $p < 0.001$), likely driven by higher abundance of mycorrhizal vesicles and microsclerotia ($\chi^2 = 10.101$, 1 d.f., $p = 0.001$ and $\chi^2 = 18.994$, 1 d.f., $p < 0.001$, respectively). There was no effect of powdery mildew or its interaction with soil legacy on mycorrhizal colonization ($\chi^2 = 0.014$, 1 d.f., $p = 0.905$ and $\chi^2 = 0.110$, 1 d.f., $p = 0.740$, respectively).

(iii) Bee bioassays

Soil legacy and powdery mildew interacted to shape bee survival (figure 5; $\chi^2 = 4.625$ 1 d.f., $p = 0.032$). Bees in micro-colonies fed pollen from powdery mildew-infected plants grown in organic soils had higher survival compared with those fed pollen from powdery mildew-infected plants grown in conventional soils ($p = 0.049$). Additionally, colonies with higher pollen consumption had higher adult bee survival ($\chi^2 = 3.902$, 1 d.f., $p = 0.048$). There was no effect of soil, powdery mildew, or their interaction on micro-colony pollen consumption ($\chi^2 = 0.141$, 1 d.f., $p = 0.707$, $\chi^2 = 1.3691$, 1 d.f., $p = 0.242$ and $\chi^2 = 1.005$, 1 d.f., $p = 0.316$, respectively) or the time it took for micro-colonies to produce new brood cells ($\chi^2 = 0.460$, 1 d.f., $p = 0.498$, $\chi^2 = 0.022$, 1 d.f., $p = 0.882$ and $\chi^2 = 0.628$, 1 d.f., $p = 0.428$, respectively) (electronic supplementary material, table S5).

(b) Organic: high- versus low-fertility legacy

(i) Field mesocosm experiment

The SEM indicated indirect effects of organic fertility legacy on plant–pollinator interactions (figure 1*b*; Fisher's C = 6.854, $p = 0.335$, 6 d.f.). Plants in high-fertility soils had lower powdery mildew infection than plants in low-fertility soils ($p = 0.012$). Bees made more visits to plants with higher powdery

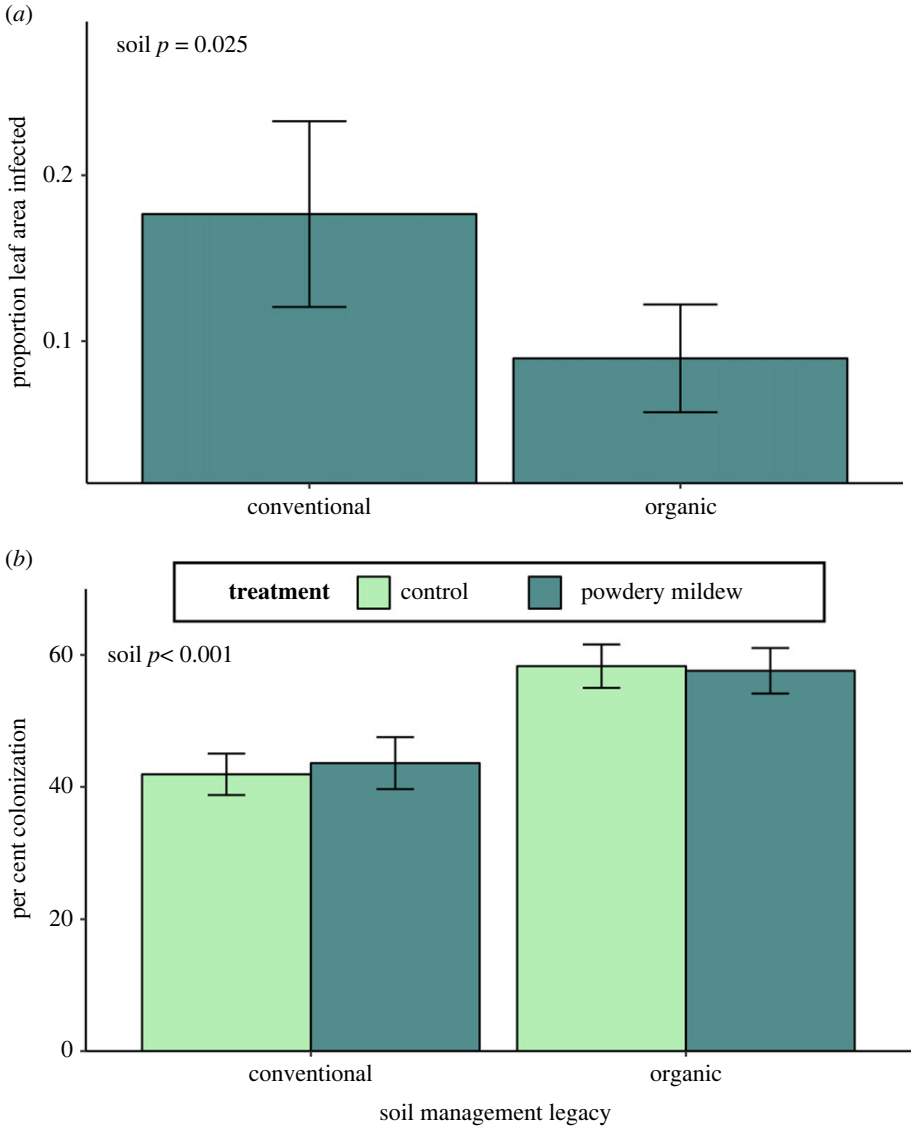

Figure 4. Effects of soil management legacy on (*a*) powdery mildew infection of plants inoculated with powdery mildew, and (*b*) mycorrhizal colonization. Error bars represent model estimated marginal means ± 1 S.E.

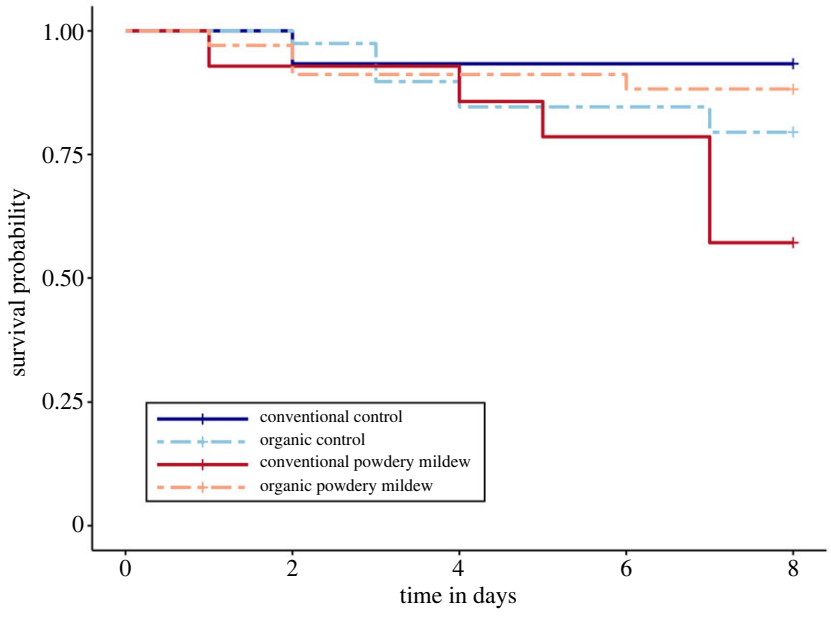

Figure 5. Effects on adult bee survival of pollen from plants grown in organic and conventional soils either infected or not infected with powdery mildew. Plot shows predicted survival of bees based on a Cox proportional hazards test.

mildew infection ($p = 0.025$). Plants with higher powdery mildew infection tended to produce fewer flowers, though this effect was non-significant ($p = 0.061$). Floral abundance was positively associated with pollinator visitation ($p = 0.002$).

Organic fertility legacy did not affect the likelihood of powdery mildew infection (electronic supplementary material, figure S4*b*; $\chi^2 = 0.0015$, 1 d.f., $p = 0.943$). In contrast, fertility legacy and field site affected infection intensity of infected plants (electronic supplementary material, figure S5*b*; $\chi^2 = 6.0974$, 1 d.f., $p = 0.014$ and $\chi^2 = 5.7353$, 1 d.f., $p = 0.017$, respectively); plants in low-fertility soils and plants grown in Musgrave soils had higher infection ($p = 0.017$ and $p < 0.001$, respectively).

(ii) Greenhouse manipulative experiment

Organic fertility legacy did not affect powdery mildew resistance ($\chi^2 = 2.089$, 1 d.f., $p = 0.148$). There was no effect of fertility legacy, powdery mildew treatment, or their interaction on total mycorrhizal colonization ($\chi^2 = 2.0514$, 1 d.f., $p = 0.152$, $\chi^2 = 0.177$, 1 d.f., $p = 0.674$, and $\chi^2 = 0.068$, 1 d.f., $p = 0.749$, respectively). However fertility legacy affected microsclerotia and vesicles, which were both more abundant in plants grown in high-fertility legacy soils ($\chi^2 = 57.003$, 1 d.f., $p < 0.001$ and $\chi^2 = 4.5324$, 1 d.f., $p = 0.033$, respectively).

Within organic soils, powdery mildew, but not fertility or their interaction, affected nectar sugar content ($\chi^2 = 3.870$, 1 d.f., $p = 0.049$, $\chi^2 = 0.557$, 1 d.f., $p = 0.456$, and $\chi^2 = 0.350$, 1 d.f., $p = 0.554$, respectively), with infected plants producing less sugar per flower ($p = 0.050$). Neither fertility legacy, powdery mildew, nor their interaction affected pollen protein, nectar volume, nectar sugar concentration or floral abundance (electronic supplementary material, table S4).

(iii) Bee bioassays

There was no effect of organic fertility legacy, powdery mildew or their interaction on adult bee survival, pollen consumption, or the time it took for micro-colonies to produce new brood cells (electronic supplementary material, table S5).

(c) Conventional: synthetic versus. green manure fertility legacy

As shown in figure 1*c*), conventional fertility legacy had no effect on powdery mildew, flowers, or bee abundance (see also electronic supplementary material, S5). Plants with more powdery mildew produced fewer flowers, and plants with more powdery mildew received fewer bee visits (figure 1*c*; $p = 0.024$ and $p = 0.007$, respectively). Smaller plants had lower powdery mildew infection ($p = 0.015$).

Soil legacy did not affect herbivore damage, so we present results of those SEM analyses in electronic supplementary material, figure S6. There was no effect of soil legacy on herbivore abundance (electronic supplementary material, S6).

4. Discussion

Our data support the hypothesis that soil legacy has direct and indirect effects on pollinators and traits important to pollinators, including floral display, nectar production and pollen protein content. This was sometimes mediated by powdery mildew, which influenced flowering phenology, nectar production, bee visitation and bee survival, often in negative ways for plants grown in conventional soils. Generally, organic soil legacy benefitted powdery mildew resistance, flower production and nectar sugar rewards. Our work provides new information on how long-term soil management affects mutualistic interactions in an agroecological context and suggests that enduring management practices could have strong effects on crop disease resistance, pollinator diet and crop–pollinator interactions.

Organic soil legacy modified the consequences of powdery mildew for floral traits and pollinators. This is evidenced by the fact that organically grown plants maintained attractiveness to pollinators despite high powdery mildew infection (figure 1*a–c*). Furthermore, organic soil legacy improved powdery mildew resistance, which may indirectly promote flower production and pollinator attraction. While the relationship between soil and powdery mildew infection intensity was only marginally significant in the SEM (figure 1*a*), there were general, repeated patterns of reduced powdery mildew in organic soils; powdery mildew incidence was lower in organic plants in the field mesocosm, and greenhouse plants inoculated with powdery mildew better resisted infection (electronic supplementary material, figure S4*a*; figure 4*a*). These results imply that continuous organic management may insulate future crops from yield loss both by reducing overall disease intensity and by buffering the consequences of disease for pollinator recruitment. However, more data are needed to assess the pollination consequences of our results.

Soil- and powdery mildew-driven changes to bee foraging behaviour in the field may reflect host plant suitability. Bee visitation negatively correlated with powdery mildew infection intensity for plants grown in conventional soils, even when floral abundance was accounted for. Reflecting this foraging preference, bees had lower survival when fed pollen from powdery mildew-inoculated plants grown in conventional compared with organic soil (figures 1 and 5). These results suggest that bees may detect powdery mildew-induced changes to pollen quality in plants grown in conventional soils [5,59–61]. In conventional soils, powdery mildew likely affected unmeasured components of floral reward chemistry, display, or scent that ultimately influenced bee attraction and survival [33,62].

The effects of soil legacy on floral reward quality and quantity could impact bee nutrition and population dynamics. Plants in organic legacy soils offered more nectar sugar but less pollen protein (figure 3*a–c*). Carbohydrates and protein are important for fueling bee flight and foraging [63,64], and pollen quality has strong effects on larval development [65,66]. Specialist species may be disproportionately affected by changes to pollen nutritional quality due to their higher reliance on specific host plant resources. However, given inadequate data on bee species- and life stage-specific nutritional requirements, it is hard to predict whether the changes to floral reward nutrients we observed will impact specialist bee larval development in positive or negative ways. Thus, it is important to determine whether soil management effects on floral reward quality impact bee reproduction, with particular focus on specialist species.

Organic and conventional soils varied in their compositions of nutrients and microbes that could have affected plant metabolism, disease resistance and floral traits. Organic

soils had higher mycorrhizal colonization, which could have led to primed or induced defense against powdery mildew [67] or promoted floral size and nectar sugar production [30]. Additionally, organic soils had higher phosphorus and potassium (electronic supplementary material S3), which can promote flower production and plant resistance to fungal pathogens, respectively [68,69]. However, soil legacy likely shapes other abiotic and biotic factors that could explain differences in floral traits and disease resistance between organic and conventional soils.

Within organic and conventional soils, effects of fertility legacy on powdery mildew, flowers and pollinators were less consistent. In the mesocosm, plants in organic high-fertility soils had lower powdery mildew infection than plants in organic low-fertility soils (figure 1*b*). Yet these effects were not repeated in the greenhouse. Within conventional soils, we saw no effect of management on powdery mildew, floral traits or bee visitation, suggesting that benefits of soil fertility practices may not manifest in the context of other conventional practices [70,71]. However, we may have been unable to observe more subtle effects of differences within management systems. Future work should test a broader range of management practices within both organic and conventional systems, ideally with greater replication.

We demonstrate that organic soil legacy promotes floral resources and plant–pollinator interactions directly and, in some cases, by changing interactions with a common plant pathogen. While soil conditions have previously been linked to pollinators [31–33], long- and short-term conditioning effects can differ dramatically. We provide rare documentation showing the lingering effects of long-term soil conditioning on floral traits and pollinators. We also provide one of only a few examples of a foliar plant pathogen affecting pollinator reward quality and behaviour. Our work implies that pollination could be an overlooked consequence of soil legacy with downstream effects on plant community composition and crop yield [1,72] and it suggests an opportunity to develop soil management plans that benefit pollinators and pollination. More broadly, this suggests that agricultural soil legacy could contribute to geographic selection mosaics in natural systems or abandoned agricultural fields by imposing differential selection pressures on plants or their antagonists [73–75]. Thus, the consequences of soil management legacy on crop disease resistance, floral traits and bee behaviour could affect both plant and pollinator population dynamics in natural and managed systems.

Ethics. No permits were required to conduct work with the invertebrates in this study.

Data accessibility. Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.f7m0cfz31 [76].

Supplementary material is available online [77].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. J.K.D.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, visualization, writing—original draft, writing—review and editing; A.D.C.: investigation, writing—review and editing; Z.L.G.-P.: conceptualization, methodology, writing—review and editing; H.L.G.: conceptualization, formal analysis, writing—review and editing; B.H.: investigation, writing—review and editing; R.M.M.: conceptualization, project administration, resources, writing—review and editing; C.J.P.: conceptualization, project administration, resources, writing—review and editing; A.R.: conceptualization, resources, writing—review and editing; M.R.R.: conceptualization, resources, writing—review and editing; T.A.U.: conceptualization, formal analysis, methodology, writing—review and editing; J.S.T.: conceptualization, funding acquisition, methodology, resources, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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