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Global Change Biology

RESEARCH ARTICLE

# Eco-Evolutionary Dynamics of Plant–Soil Feedbacks Explain the Spread Potential of a Plant Invader Under Climate Warming and Biocontrol Herbivory

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**Received:** 30 October 2024 | **Revised:** 26 January 2025 | **Accepted:** 12 February 2025

**Funding:** Y.S. was supported by National Natural Science Foundation of China (#32201438) and the Scientific Research Foundation for Returned Scholars, Huazhong Agricultural University (#11042110026). H.M.-S. received funding from Swiss National Science Foundation (SNSF project 31003A_166448). D.S. received funding from ETH Zurich and the Swedish Foundation for Strategic Environmental Research MISTRA within the framework of the research programme BIOPATH (F 2022/1448).

**Keywords:** *Ambrosia artemisiifolia* | biological control | biological invasions | causal models | climate warming | eco-evolutionary plant–soil feedback | experimental evolution | *Ophraella communa*

## ABSTRACT

Plant–soil feedbacks (PSFs) can contribute to the success of invasive plants. Despite strong evidence that plant genetic traits influence soil microbial communities and vice versa, empirical evidence exploring these feedbacks over evolutionary timescales, especially under climate change, remains limited. We conducted a 5-year field study of the annual invasive plant, *Ambrosia artemisiifolia* L., to examine how selection under climate warming and biocontrol insect herbivory shapes plant population genetics, soil properties, and microbial communities. After four generations under warming and herbivory, we collected seeds of the $F_4$ plant populations together with their conditioned soil for a common garden PSF experiment to explore how resulting PSFs patterns are influencing the performance and spread potential of *Ambrosia* under changing environmental conditions. This is especially relevant because our recent predictions point to a northward spread of *Ambrosia* in Europe and Asia under climate change, outpacing the spread of its insect biocontrol agent. We discovered that warming and herbivory significantly but differentially altered plant genetic composition and its soil microbial communities, with less pronounced effects on soil physicochemical properties. Our results indicate that both herbivory and warming generated negative PSFs. These negative PSFs favored plant growth of the seeds from the persistent soil seed bank growing in the conditioned soil under insect herbivory, and by this maintaining the *Ambrosia* population genetic diversity. They also enhanced the spread potential of warming-selected plant offspring, especially from warmer (southern) to colder (northern) climates. This can be explained by the observed decrease in soil pathogens occurrence under insect herbivory and by the especially strong genetic changes in plant populations under climate warming. Our findings provide insights into how climate warming and biocontrol management affect eco-evolutionary interactions between invasive plant populations and their soil environments, which are critical for predicting invasion dynamics in the context of global change.

## 1 | Introduction

The question of how populations change in size and genetic makeup has long intrigued ecologists (Birch 1960). Our understanding of plant population dynamics has primarily been shaped by examining the partitioning of abiotic resources, lately increasingly in the frame of global climate change (Parmesan and Yohe 2003; Sanczuk et al. 2023), and by focusing on the mediation of higher trophic level organisms, such as herbivores and pathogens (Allsup et al. 2023; Biere and Goverse 2016). More recently, progress has been made in understanding how plant population dynamics and genetic variation can alter the soil biotic and abiotic properties in which they grow, and how these modifications can feedback to influence the natal (here plants from the persistent seed bank) or different (here plants from the spreading populations) plants (Tedersoo et al. 2020; Thakur et al. 2021; Ware et al. 2019). Plant–soil feedbacks (PSFs) have now become an important concept for explaining vegetation dynamics, terrestrial ecosystem responses to global change, and the invasion success of introduced exotic species (Allen et al. 2021; van der Putten et al. 2016). In a case study, Callaway et al. (2004) showed that *Centaurea maculosa* L. (= *C. stoebe*) switches from negative PSFs in its native European range to neutral, or even positive PSFs in the introduced North America range by escaping pathogens and accumulating beneficial microbes. Thus, the eco-evolutionary dynamics in PSFs can play a crucial role in the success of colonization and spreading by invasive alien plant species (Levine et al. 2006; Ware et al. 2019; Zhang et al. 2020), especially under rapid environmental changes, such as climate warming or biocontrol management (Müller-Schärer et al. 2020).

Invasive alien plant species provide a unique opportunity to explore eco-evolutionary interactions between plant population genetics and associated soil microbiota. Plant evolutionary history particularly matters for temporally separated populations at the same site. For instance, natal seeds from persisting seed banks may recruit into established populations that, in the meantime, may have already undergone selection caused by rapid environmental changes, which in turn may have affected their soil microbiota. Additionally, invasive plant populations frequently experience multiple introductions or secondary invasion within the introduced range over time, which influences their population genetic composition. Together with repeated dispersal from source populations into the expanding invasion front (Capinha et al. 2023; Wilson et al. 2009), this will result in genetically distinct populations often encountering spatially separated new soil microbiota. Thus, as invasive plants spread, these populations will interact with their yet unconditioned soil. Exploring the outcomes of PSFs of such eco-evolutionary interactions can reveal and add a further mechanism to the multiple processes affecting the performance and spread of invasive plant populations. Climate change and plant herbivores can impose strong selection on plant populations, driving evolutionary changes even over relatively short ecological timescales. Importation biocontrol involving the deliberate release of specialist natural enemies, mostly arthropods and pathogens, from the weed's native range has been initiated against some 175 widespread plant invaders (Winston et al. 2020) and has proven that it can be highly effective. Yet, only a little more than one third of the targeted plant invaders in each country experienced 'heavy impact' based on the impact of a single agent (Sun et al. 2022). There are many well-documented cases of evolutionary changes in invasive alien plants post-introduction, but yet only a few experimental studies of evolutionary responses in biocontrol systems (Müller-Schärer et al. 2020). Invasive species pose a substantial threat to biodiversity and ecosystem health, highlighting the necessity to understand how weed biocontrol management (biotic interference) affects PSF processes in the face of climate change (abiotic interference). This knowledge can help unravel the complex interactions between plant population genetics and their soil microbiota, advancing both fundamental research and practical applications.

There is strong evidence that plant traits and population genetics affect the soil microbial community (Semchenko et al. 2021), and that soil microbes can impose selection on plant traits and genotypes (Allsup et al. 2023; Banerjee and van der Heijden 2023). For instance, in a model *Populus* system, Schweitzer et al. (2008) found that plant genotype influenced soil microbial community composition, explaining up to 70% of the variation in soil biota community composition. In turn, variation in soil biota can be an important selective agent, causing differential fitness and local adaptation in *P. angustifolia* genotypes (Smith et al. 2012). Moreover, the direction of PSFs can alter plant community structure, which can impose further selection on plant traits (Delory et al. 2024). It is well documented that pathogenic soil bacteria, fungi, and nematodes can greatly reduce plant viability (Biere and Goverse 2016; Savary et al. 2012), while rhizosphere bacteria and AMF are known to improve the survival, growth, and reproduction of plants (Newman and Reddell 1987; Tedersoo et al. 2020). A previous study also showed that PSFs can favor the expansion of *P. angustifolia* beyond its current range limits (Van Nuland et al. 2017). All of this suggests that there is ample potential for eco-evolutionary feedbacks between plants and soil microbes, but direct empirical evidence of such feedbacks has so far only rarely been addressed. Experiments in this area are not easy, but the rise of metagenomics and genomics has allowed unprecedented access to the dynamics of microbial community structure and function, as well as to the plant population genomic makeup, which are the basic ingredients to unravel the dynamics of eco-evolutionary plant–soil feedbacks (terHorst and Zee 2016; Van Nuland et al. 2016).

We combined a 5-year field experimental evolution study using the worldwide highly invasive annual plant, common ragweed *Ambrosia artemisiifolia* L. (Asteraceae; *Ambrosia* in the following) with soil inoculum collected from their experimental field soils to now measure plant–soil conditioning and feedbacks in a common garden (Figure 1). In our previous study, we observed distinct genetic clustering among treatments, with greater genetic variation among populations than within, confirming true evolutionary shifts driven by selection. Specifically, four genetically diverse replicated *Ambrosia* populations (Figure 1b) and their soils were subjected to four treatment combinations, i.e., ambient and climate warming, in combination with or without biocontrol herbivory with the ragweed leaf beetle *Ophraella communa* LeSage (Chrysomelidae; *Ophraella* in the following). Thus, the previous and now also the present experiment captures both the direct effects of the four treatment combinations as well as effects mediated by changes in the genetic composition of *Ambrosia* over four generations. With our common garden

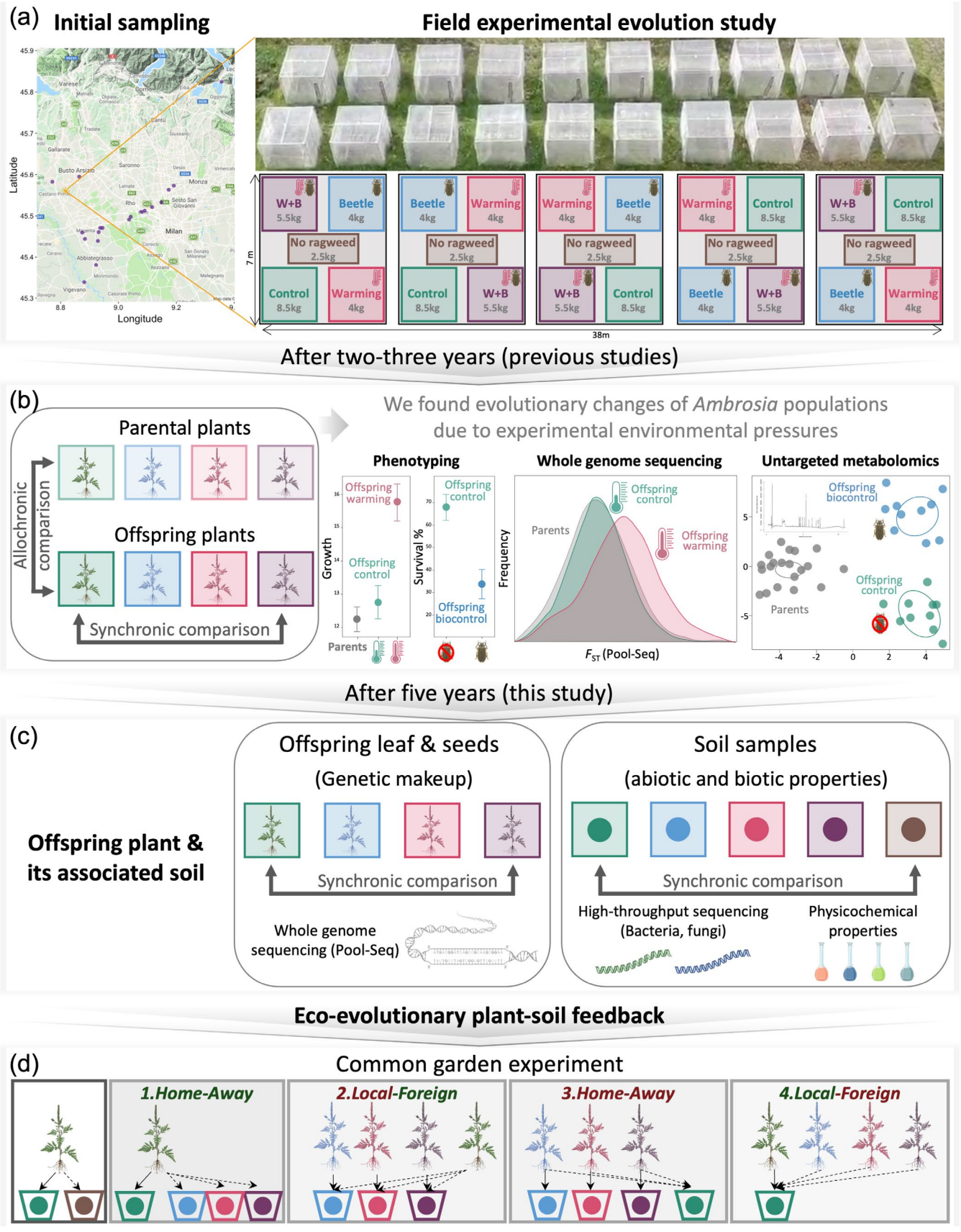


**FIGURE 1** | Legend on next page.

**FIGURE 1** | Flow diagram of the experimental design. A field experimental evolution study was conducted over 5 years. Sixty maternal families of *Ambrosia artemisiifolia* were collected from Northern Italy and planted together in experimental cages. These cages were exposed to climate warming and herbivory during 5 years and the resulting plant populations and soils were used as starting material (a). A previous study showed that field warming caused a significant change of offsrping growth and genetic composition (b; Sun et al. 2020, 2022). For this study, we used the offspring material after 5 years of experimental evolution. *Ambrosia* offspring were sequenced again to assess the genetic makeup, the soil microbial communities (bacteria and fungi) were sequenced, and soil physicochemical characteristics were measured to test the divergence of sol biotic and abiotic properties (c) and the eco-evolutionary plant–soil feedback was assessed in a greenhouse common garden experiment (d). Colours represents *Ambrosia* plant/conditioned soil under four treatments, i.e., control (green), with a history of herbivory (blue), with a history of warming (red), with a history of both warming and herbivory (pink), or outside cage without *Ambrosia* (brown).

PSF experiment, we now asked the following questions: (1) How do *Ambrosia* field selection through warming and herbivory affect the genetic composition and phenotypic performance of the offspring *Ambrosia* populations, soil abiotic properties, and soil microbial communities? (2) How do these changes in the plant genetics and the soil microbial communities mediate the outcome of PSFs? (3) What are the drivers that govern the interactions between plant genotypes, soil microbes, and abiotic soil parameters, particularly under varying environmental conditions? (4) How do historical selection pressures, such as those imposed by warming and herbivory, shape future interactions when previously separated populations—genetically distinct or temporally separated—come into contact again, and what are the implications for the invasion potential? To address these questions, we developed and tested four interaction scenarios on the hypothetical role of PSFs in invasion processes: (i) *Conditioned soil effects on original populations*: these arise when natal seeds from seed banks recruit into populations shaped by rapid environmental changes, altering their soil microbiota; (ii) *Adaptation of populations to their conditioned soil*: when coevolved populations adapt to the conditioned soil over time; (iii) *Evolved population responses to conditioned soil*: this reflects multiple introductions and when seeds disperse from source populations into the expanding invasion front; (iv) *Evolved population responses to unconditioned soil*: this happens when invasive plants encounter new soils, yet unconditioned by them, during their spread (cf. Figure 1). This approach will provide a better understanding of the PSFs' role in plant invasions under changing environmental conditions, using soil data to explain the observed PSF patterns. The study is particularly motivated by predictions of further northwards expansion of *Ambrosia* both in Europe and Asia under future climate change (Chapman et al. 2014; Hamaoui-Laguel et al. 2015; Sun, Brönnimann, et al. 2017; Sun, Zhou, et al. 2017), which is outpacing the spread of its insect biocontrol agent (Sun, Brönnimann, et al. 2017; Sun, Zhou, et al. 2017).

## 2 | Materials and Methods

*Ambrosia artemisiifolia*, a rapidly spreading outcrossing annual weed native to North America, has become a highly invasive species across Asia, Europe, and Oceania (Essl et al. 2015). This plant is notorious for its highly allergenic pollen and its resilience as a crop weed, making it difficult to manage (Müller-Schärer et al. 2018). An effective counter to *Ambrosia*'s spread is *Ophraella*, an oligophagous leaf beetle that prefers *Ambrosia* and is also native to North America (Futuyma and McCafferty 1990; Palmer and Goeden 1991). This beetle accidentally reached several countries in eastern Asia (Shiyake and Moriya 2005) and is now considered the most effective biocontrol agent against *Ambrosia* in China (Zhou et al. 2015) and has similarly impacted *Ambrosia* populations in Japan (Fukano and Doi 2013; Fukano and Yahara 2012). *Ophraella* was also accidentally introduced to Europe (northern Italy and southern Switzerland) in 2013 (Müller-Schärer et al. 2014), where it has shown the potential to significantly curb *Ambrosia* growth. Under optimal conditions, *Ophraella* can produce 4–7 generations annually (Augustinus et al. 2020; Zhou et al. 2014), enabling it to reach high densities and effectively control *Ambrosia* by causing complete defoliation and even death during the latter part of the growing season (Palmer and Goeden 1991; Zhou et al. 2014). In a recent study, Schaffner et al. (2020) predicted that *Ophraella* has the potential to reduce the number of patients in Europe suffering from *Ambrosia* pollen allergy by approximately 2.3 million and the health costs by Euro 1.1 billion per year.

### 2.1 | Field Experimental Evolution Study

In April of 2016, we set up 20 cages (2 × 2 × 2 m) organized into five blocks, with four cages per block, containing genetically similar experimental *Ambrosia* populations in a field in Magnago, Northern Italy (Figure 1; Table S1). Each caged population was founded by 120 individuals ($F_0$), with two individuals from each of 60 maternal families sampled from 19 invasive *Ambrosia* populations between 2013 and 2015. Herbivory by biocontrol candidate *Ophraella* was introduced by releasing 30 adults into half of the field cages in mid-June each year from 2016 to 2019. To maintain approximately 54% ± 2.9% visual leaf damage throughout the experiment (Table S2), beetle populations were adjusted by adding or removing individuals as needed. The populations were caged to enclose the released beetles, to protect the plants from unintentional herbivory, and to prevent pollen escape. Open-top Plexiglas chambers were installed in half of the cages to simulate warming, increasing daily mean temperature by 2.2°C while minimizing other ecological effects. To maintain *Ambrosia* monocultures, we hand-weeded the field cages monthly as necessary. Further details on the experimental setup and monitoring are given in (Supporting Information: Appendix A; Sun et al. 2020, 2022). At the end of the growing seasons each year from 2016 to 2019, plants were allowed to shed seeds ($F_1$–$F_4$) within each field cage. The same treatments were applied again to the naturally re-growing offspring plants in the following year. To collect offspring seeds for further common garden experiments, we harvested five mature seeds from each individual branch located in the centre of the field cage (100 × 120 cm). This collection typically represented

several hundred individuals at the end of each season, following differential mortality, growth, and reproduction of the originally sown genotypes.

### 2.2 | Offspring *Ambrosia* Genomic Analyses

To assess the genetic makeup of each offspring population, we collected 20 pooled leaf samples of $F_4$ populations. Each pool contained equal amounts of tissue from individual plants (~1 mg from a cut leaf from a total of 120 individuals per experimental field population in the central 100×120 cm, Supporting Information: Appendix B). Samples were processed for DNA sequencing on an Illumina NovaSeq 6000 Sequencing System S1 flow cell platform at the Interfaculty Bioinformatics Unit, University of Bern (see Supporting Information: Appendix B for details on DNA extraction, library preparation and raw data processing). Following the processing pipeline, sequencing data from 20 population pools yielded a total of 5,734,046,360 paired-end reads, with 3.77% of reads being unmapped and then removed. The final dataset corresponds to an average coverage of 36× with a GC content of 37.88% (Tables S1, S3 and S4). We followed 'PoolSNP' pipeline (https://github.com/capoony/PoolSNP, Kapun et al. 2020) for SNP calling (see Supporting Information: Appendix B for details) and excluded sites that did not pass the SNP calling criteria, resulting in 92,165,872 common SNPs across all populations.

To estimate genome-wide pairwise genetic differences $F_{ST}$, we used the method of Weir and Cockerham (1984) for all pairwise combinations of sample pools by following the protocol of Kapun et al. (2020). For each sample, we averaged pairwise $F_{ST}$ between that sample and the other 19 samples. We performed Principal Component Analysis (PCA) based on common SNP allele frequencies using the Euclidean distances as implemented in the *vegan* R package version 2.6.6.1 (Oksanen et al. 2018) to summarize the population genomic structure. The genetic variation among *Ambrosia* offspring populations was analyzed with permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) with the *adonis2* function in *vegan* (9999 permutations; Oksanen et al. 2018).

### 2.3 | Soil Collection and Analyses

Soil samples (0–10 cm in depth) were taken from >100 points using a stainless-steel soil borer (5 cm diameter, 10 cm length) in the central area (100×100 cm) of each of the 20 field cages and 5 similarly sized *Ambrosia*-free plots in between the cages in November 2019. All soil from each field cage was cleaned and mixed into one sample (25 samples in total, see Figure 1) and divided into subsamples for the analysis of abiotic properties, extractable DNA, and conducting the PSF experiment in the greenhouse (Table S1). All soil samples were stored at 4°C before DNA extraction and the plant–soil feedback experiment. One kilogram of soil from each sample was used for analyses of abiotic properties at Agroscope, Zürich, Switzerland. The soil physicochemical properties, i.e., texture, pH, organic C, and soil nutrients (P, K, Ca and Mg) were determined according to the Swiss reference methods of the Federal Agricultural Research Stations (FAL et al. 1996; Details are given Supporting Information: Appendix D). All soil physicochemical properties were analysed with linear mixed models, using the function *lmer* in the *lme4* R package, version 1.1.35.5, including field beetle treatment, warming treatment, their interaction, and *Ambrosia* history (based on soil sampling inside vs. outside cages, with outside-cage soils being *Ambrosia*-free) as fixed factors, and field cages nested within blocks as random effects. In cases where significant differences occurred ($p<0.05$), we carried out Tukey's post hoc tests using the *glht* function to compare among all treatments.

Two subsamples (0.25 g) of each mesocosm were taken for DNA extraction with the DNeasy PowerSoil Pro Kit (Qiagen) following the manufacturer's protocol (see Supporting Information: Appendix C for details). Operational taxonomic units (OTUs) were generated with *UPARSE* (usearch version 10.0.024, Edgar 2013) following the tutorial (drive5.com/uparse/) (see Supporting Information: Appendix C for details). Amplification of 16S rRNA gene fragments yielded an initial set of 22,346 OTUs. After removing OTUs with minimal 50 total read counts across all samples or with counts in fewer than three samples (8039 OTUs), 14,307 OTUs remained. Of these, 11,624, 36 and 2647 were classified as bacteria, archaea and unknown, respectively. We removed unknown for all the subsequent analyses. Within the bacterial domain, the 10 most abundant phyla accounted for 98.2% of all OTUs. Amplification of ITS rRNA gene fragments yield an initial set of 1761 OTUs. After removing OTUs with minimal 10 total read counts across all samples or with counts in fewer than three samples (511 OTUs), 1250 OTUs remained. Variation in OTU relative abundance was analysed with a generalized linear model in R with the package *DESeq2* version 1.24.0 according to factorial designs (Love et al. 2014). For each model, *p*-values were adjusted for multiple testing (Benjamini-Hochberg), and OTUs with an adjusted *p*-value (false discovery rate [FDR]) below 0.05 and a minimal $\log_2$ fold-change of 1 (i.e., the difference between the $\log_2$ transformed, normalized OTU counts) were considered to be differentially abundant. Normalized OTU counts for all other analyses were calculated accordingly with *DESeq2*. Normalized counts were $\log_2(x+1)$-transformed to obtain the normalized OTU abundances. Sequencing data were not rarefied (McMurdie and Holmes 2014) except for calculating the diversity indices and testing the community structures (see below). For the analyses with the diversity indices and the community structure, data were rarefied to the sample with the lowest number of counts in the data set. The phylogeny was generated using FastTreeMP version 2.1.11 (Price et al. 2010). OTU richness was quantified as the number of individual OTU per composite sample, Shannon diversity and inverse Simpson diversity was used to calculated bacterial and fungal diversity based on the abundance of individual OTU per composite sample, rarefied richness was calculated in the *vegan* package using the function *rarefy* (Oksanen et al. 2018), Faith's phylogenetic diversity was calculated as the sum of branch lengths in their phylogenetic tree (Faith 1992), and Pielou's evenness ($J'$) specifically as a measure for the ratio of observed over maximum diversity for each sample, which calculated as diversity division by $\log_e$-transformed richness. These indices were then analysed with linear mixed model, with function *lmer*, including field beetle treatment, warming treatment, their interaction and *Ambrosia* history as fixed factors, field cages nested within blocks as random effects. In cases

where significant differences occurred ($p < 0.05$), we carried out Tukey's post hoc tests using the *glht* function to compare among all treatments. Moreover, we applied Canonical Correspondence Analysis (CCA) to the normalized data using package *vegan* in R. The objective of these analyses was to identify the best ordination models to detect shifting microbiotic patterns among field treatments over four generations of selection. Permutational multivariate analysis of variance (PERMANOVA) was then applied to test significant differences between groups, using the function *Adonis* with 9999 permutations in the package *vegan* (Oksanen et al. 2018) in R. We also performed a PERMANOVA to analyze dissimilarities in the relative abundance of soil biotic communities (i.e., soil pathogens) based on OTUs, using Bray–Curtis distances with the *vegan* R package.

### 2.4 | Plant–Soil Feedback Experiment and Analyses

All $F_4$ seeds were transported to the University of Fribourg, Switzerland, and stored at 4°C for stratification over 8 weeks to break dormancy (Willemsen 1975). In 2020, stratified seeds from each of the 20 offspring populations were placed into Petri dishes containing two filter papers wetted with distilled water in a growth chamber for germination, supplemented by metal halide bulbs, following a 12/12 h day/night at 20/10°C cycle (Leiblein-Wild et al. 2013). Seeds were watered with distilled water if necessary. Germinated seeds were transplanted into seedling trays with 150 (10 × 15) cell plugs of 15 mL volume filled with autoclaved potting soil (121°C at 1.1 atm for 60 min; Trevors 1996) for initial growth for 2 weeks. We prepared 550 1 L pots, which were filled with a mixture of 0.9 L standardized medium, i.e., autoclaved sand, autoclaved vermiculite (Vermica AG, Bözen, Switzerland) and a low nutrient commercial potting soil (Fenaco Genossenschaft, Bern, Switzerland) sterilized by X-ray with 25–90 kGy doses (Synergy Health, Däniken, Switzerland), in the ratio 1:1:2 by volume and 0.1 L field soil (10% of total volume, to avoid significant nutrient differences). We moisturized all prepared pots and kept them in a greenhouse at the University of Fribourg for over a month before transplanting to let the soil microorganisms spread. 50 seedlings (2 individuals × 5 plant field blocks × 5 soil field blocks) were transplanted into one of the 11 plant–soil treatment combinations (two replicate seedlings per cage), resulting in total of 550 pots (Figure S1). Dead seedlings were replaced in the first week after transplanting. All plants were placed in the greenhouse and experienced natural light conditions supplemented by metal halide bulbs, following a 16/8 h day/night at 28/18°C cycle. Each plant received 150 mL tap water every 2 or 3 days, and to avoid position effects, the pots were randomized every fortnight.

We measured the plant initial height after transplanting and used as covariate in the analyses. Because of the highly allergenic pollen of *Ambrosia*, regional health regulations prohibited us from allowing plants to flower in the greenhouse. This restricted growing plants for additional generation in the greenhouse, which would have been preferable for reducing potential maternal effects on plants. Instead, we harvest each plant just before it began to produce pollen, i.e., as length of the first male inflorescence reached 1.5 cm, which was 58–152 days after transplanting. Previous studies indicate that *Ambrosia* plants typically achieve maximum height and biomass at flowering (Lommen et al. 2018; Sun and Frelich 2011; Sun et al. 2020), and their biomass was found to be highly correlated with per-capita seed and pollen production in a field study across 39 sites in Europe (Lommen et al. 2017). Therefore, we use biomass at harvest as a proxy of fitness. At harvesting, we recorded days to flowering and counted the numbers of developing male inflorescences of each plant at harvest as a proxy for potential male reproductive output. We measured the plant final height and assessed the relative height growth rate (RHGR) of each plant species by calculating $\mathrm{RHGR} = (\ln H_1 — \ln H_0)/\mathrm{days}$, where '$H_1$' is the height of the plant at harvest, '$H_0$' is the initial height at the beginning of the experiment and 'days' refer to the days to harvesting. Three leaves per plant were detached in low (1/5 of height), middle (1/2 of height) and high (4/5 of height) position to determine specific leaf area (SLA). Dry weight (DW) was then measured at 60°C after 72 h. Leaf area (LA) was obtained from the scans using ImageJ software. SLA were calculated as the mean value of three leaves per plant as follows: $\mathrm{SLA}\left(\mathrm{cm}^2 \cdot \mathrm{mg}^{-1}\right) = \overline{\mathrm{LA}/\mathrm{DW}}$. We harvest both above and below ground biomass, the total biomass was calculated as the sum of aboveground and root biomass together of the three leaves used above; and calculated the root:shoot ratio by dividing root biomass by shoot biomass. At harvest, we also took three replicated measurements of soil moisture content (SMC; using Delta-T Devices, Cambridge, UK) per pot in all pots to assess differences in water use of the plants.

Plant–soil feedback is the performance of plant grown in conspecific condition versus the performance of plant grown in heterospecific conditions. Our design allowed for both home-away (soil-centric) and local-foreign (plant-centric) approaches to assess the PSF (Figure 1). Specifically, '1.Home-Away' is calculated as $\mathrm{PSF}_{1.\ \mathrm{Home\text{-}Away}} = \ln C_c - \ln C_t$, '2.Local-Foreign' is $\mathrm{PSF}_{2.\mathrm{Local\text{-}Foreign}} = \ln T_t - \ln C_t$, '3.Home-Away' is $\mathrm{PSF}_{3.\ \mathrm{Home\text{-}Away}} = \ln T_t - \ln T_c$, and '4.Local-Foreign' is $\mathrm{PSF}_{4.\mathrm{Local\text{-}Foreign}} = \ln C_c - \ln T_c$, where $C_c$ is the total biomass of *Ambrosia* offspring from control treatment grown in its own soil, $C_t$ is the total biomass of *Ambrosia* offspring from control treatment in conditioned soil of *Ambrosia* experienced one of the three treatments (i.e., beetle, warming or warming + beetle), $T_t$ is the total biomass of *Ambrosia* offspring from one of the above three treatments grown in their own soil, $T_c$ is the total biomass of *Ambrosia* offspring from one of above three treatments grown in control soil. Moreover, the pairwise PSF is calculated as following: $I_s = \ln C_c - \ln C_t - \ln T_c + \ln T_t$ (Bever et al. 1997). We adapted the interspecific pairwise PSF framework to assess interactions among *Ambrosia* populations with distinct evolutionary histories. Thus, each value of $I_s$ represents the average pairwise PSF of a given *Ambrosia* pair in the respective treatment. Negative $I_s$ arises when plants alter soil communities to favour populations with different evolutionary history over their own (conspecifics), hence stabilising coexistence through conspecific negative density dependence. In contrast, positive $I_s$ occurs when populations influence soil communities in a way that favours conspecifics over others with different evolutionary histories, reducing diversity through conspecific positive density dependence (Bever et al. 1997).

We analysed the *Ambrosia* growth data, including days to flowering, number of male inflorescences, total biomass, root:shoot

ratio, internode length, SLA, and SMC, with (generalized) linear mixed models, using Poisson error distributions or normal distributions with the functions *glmer/lmer* in the R package *lme4*, which uses maximum likelihood to estimate model parameters (Bates et al. 2014). The models included the warming treatment and beetle treatment in the field experiment, and their interactions, as fixed factors, with initial plant height as a covariate, and field experiment cages as random effects. To validate model fit, we checked each Poisson model for potential over-dispersion of the residuals. If this was the case, then we corrected for over-dispersion by adding an observational-level random term to the model by serially numbering each observation (Harrison 2014). We also checked the normality of the residuals in all models using QQ-plots. For plotting figures, the fitted means and standard errors of fixed-effects parameters were extracted using the *fixef* and *devfun2* functions with stderr from the R package *lme4* (Bates et al. 2014).

For PSF analyses, we took a random bootstrap sample of the plant biomass values for the home soil treatment and a second random bootstrap sample for the corresponding foreign soil treatment. We calculated the feedback value and repeated this 10,000 times by sampling with replacement. We then constructed 95% bootstrap confidence intervals to determine whether the mean coefficient of the biomass ratio was significantly different from zero. Moreover, we explored how the strength and direction of feedbacks were related to variation in soils and plants, as feedbacks result from changes to the soil environment and plant genotypes. We compared soil variation and *Ambrosia* genotypes in the field experiment to *Ambrosia* performance across all treatments in the greenhouse experiment. We correlated the PSF with the distance among soil microbial communities and the genetic differentiation among *Ambrosia* populations, and *Ambrosia* total biomass with the genomic composition of $F_4$ plants and the abundance of soil microbial communities for conspecific (plant grown in its own soil) and heterospecific (same genotypes grown in different soils, or different plant genotypes grown in the same soil) types, respectively. Finally, we then constructed a Restricted Maximum Likelihood (REML) model with PSF or total biomass as the response, soil microbiome or plant genomic composition as fixed effects, and field cage nested within block (in the field experiment) as random effects.

## 2.5 | Causal Models Using Directed Acyclic Graph (DAG) With Bayesian Linear Mixed Models

We used a graph theory framework to estimate the confounder- and bias-corrected effect of our experimental variables on PSFs (Pearl 1995, 2009). Associational assumptions were depicted using a Directed Acyclic Graph (DAG), which allowed us to assess the plausibility of the articulated assumptions (Textor et al. 2011). A DAG is a graphical model that comprises a series of hypotheses about the causal processes generating the variables of interest. An arrow pointing from one variable to another represents the hypothesis that altering the first variable would directly influence the second, and that changing the first variable by external intervention changes the second as well (Shrier and Platt 2008). We developed this graphical representation of the processes that govern four groups of variables, i.e., the soil abiotic properties, soil biota communities, plant genomic attributes, and plant traits based on our field and common garden experiments (see Table S5 for all variables) using the *dagitty* R package version 0.3.4. This DAG served as a transparent representation of our assumptions, enabling scrutiny of our conclusions. We then compared this representation to empirical data to test its plausibility via the conditional dependencies implied by the diagram (Pearl 2009). Conditional dependencies refer to the statistical relationships between variables, where the value of one variable depends on the value of another, given the presence or absence of additional variables. If these conditional dependencies are consistent with empirical data—meaning the DAG suggests an association and the data confirms it—this can be seen as evidence supporting the DAG structure. Using the *localTests* function from the *dagitty* package, we found support for our DAG across all conditional dependencies. Finally, we used weighted Bayesian linear mixed models to account for confounders and quantify the unbiased effect of all variables on PSFs, whereby the formula of each model was derived from the DAG via the *adjustmentSets* function from the *dagitty* package. This approach enabled us to evaluate the direct effects of each variable within the ecological system while accounting for confounding influences based on the causal structure represented in the DAG (Arif and MacNeil 2023).

For this modeling approach, the best predictors of PSFs were selected on the basis of the Akaike Information Criterion (AIC) from the above four groups of variables. Each variable was tested in a separate linear mixed model as a predictor of PSF using the *lmer* function of the *lme4* package (Bates et al. 2014). $AIC_c$ scores and model weights for the full model set were calculated using the *dredge* function of the *MuMIn* package version 1.48.4 (Barton 2009; Shrier and Platt 2008). The predictors yielding the lowest $AIC_c$ scores within each variable set were retained for use in the causal models. Within the set of soil biotic and abiotic properties and plant traits, many variables were highly correlated and aligned closely with each other; therefore, we removed those with a variance inflation factor (VIF) greater than 10 using the *vif* function from the *car* R package version 3.1.2. We thus included four plant phenotypic traits, one plant genomic attribute, five soil biotic features, and three soil abiotic properties in the DAGs (Table S5).

We used the *dagitty* package in R to identify the path coefficients that were identifiable by regression and to determine the appropriate adjustment sets for each coefficient. Adjustment sets were derived to control for confounding effects along each path, ensuring that the direct effects could be accurately estimated. We generated all possible combinations of four group variables to create potential causal pathways and test different model specifications (Table S5). To estimate the effects of the causal relationships identified in the DAG, we used Bayesian linear mixed models implemented in the *brms* R package version 2.21.0 to assess the effect of all variables on PSFs (Bürkner 2017). We fitted individual models for each combination of variables and then

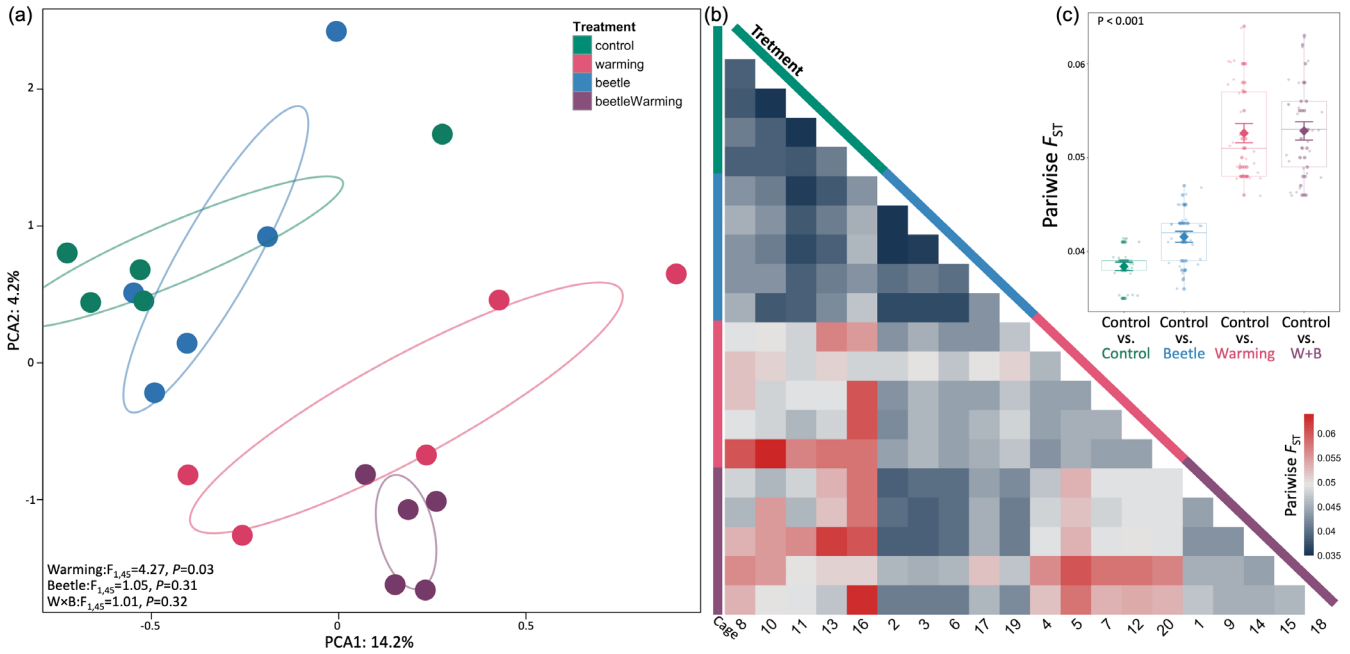


**FIGURE 2** | The effects of field treatments on the genetic makeup of *Ambrosia artemisiifolia* offspring populations. Genomic composition using allele frequencies of common polymorphic SNP markers (a) and pairwise $F_{ST}$ across all populations of *Ambrosia* $F_4$ offspring (b), and pairwise comparison between control and treatments (c) in the field experimental evolution study. Colours represents *Ambrosia* plant under control (green), *Ambrosia* with a history of herbivory (blue), with a history of warming (red), or with a history of both warming and herbivory (pink). Detail for pairwise $F_{ST}$ matrix can be found in Table S6. The dots represent each replicate. In panel (c), the diamond is the mean value, the thick bar is ±SE, and the thin box represents the interquartile range (IQR) with whiskers extend (1.5×IQR); each small dot represents one pairwise population comparison.

averaged posterior draws based on the fit to the empirical data via pareto smoothed importance sampling (Vehtari et al. 2017; Yao et al. 2018). We modelled the PSFs as below:

$$\text{PSF} \sim \text{Normal}(\mu, \sigma)$$

$$\mu \sim \alpha + \beta\text{Exposure} + \beta\text{Confounder}$$

$$(\beta\text{Exposure} + \beta\text{Confounder}) \sim \text{Normal}(0, 1)$$

whereby we presumed a normal distribution for PSFs and used non-informative prior distributions for the variables. For all models, we used four Monte Carlo Markov Chains with 4000 samples each. We inspected diagnostic model quantities after model fitting to ensure a successful model convergence. In total, we have seven models to quantify the coefficient for each of conspecific and heterospecific conditions (Figure S9).

All statistical analyses were performed using the R software (version 4.3.3, R Development Core Team). Data and R code are available at the Figshare data repository (Sun 2025).

## 3 | Results

### 3.1 | Offspring Plant Genomics

The PCA visualising dissimilarities among pooled population samples was clustered well within each treatment and showed a strong genetic composition effect of $F_4$ by warming treatments and no significant effects of beetle treatments (Figure 2a). Pairwise $F_{ST}$ was significantly higher for *Ambrosia* offspring populations for control versus warming and for control versus warming + beetle comparison than that for control versus beetle comparisons (Figure 2b,c).

### 3.2 | Soil Physiochemical Properties

After growing *Ambrosia* over four generations, several fundamental changes in soil physiochemical properties were observed (Figure S2; Tables S7 and S8). Compared to soil without *Ambrosia*, the presence of *Ambrosia* significantly increased the pH of soil ($\chi^2 = 1.89$, $p < 0.001$), the percentage of silt ($\chi^2 = 4.74$, $p = 0.03$), basal soil respiration ($\chi^2 = 3.94$, $p = 0.05$), and significantly reduced total P and available P ($\chi^2 \geq 632$, $p \leq 0.01$). The warming treatment increased the pH ($\chi^2 = 7.48$, $p = 0.006$), significantly reduced the percentage of clay ($\chi^2 = 15.75$, $p < 0.001$), but increased the percentage of sand ($\chi^2 = 4.47$, $p = 0.03$), increased microbial biomass for both $C_{mic}$ and $N_{mic}$ ($\chi^2 \geq 4.31$, $p \leq 0.04$), and also increased basal soil respiration ($\chi^2 = 3.94$, $p = 0.05$). The beetle treatment had relatively less effect on soil properties; it reduced the percentage of clay ($\chi^2 = 5.70$, $p = 0.02$) and strongly reduced both total C and total N ($\chi^2 \geq 6.70$, $p \leq 0.01$). The interactions between warming and beetle treatments strongly affect soil pH, the percentage of clay, dry matter content, water content, and available P ($\chi^2 \geq 4.76$, $p \leq 0.03$).

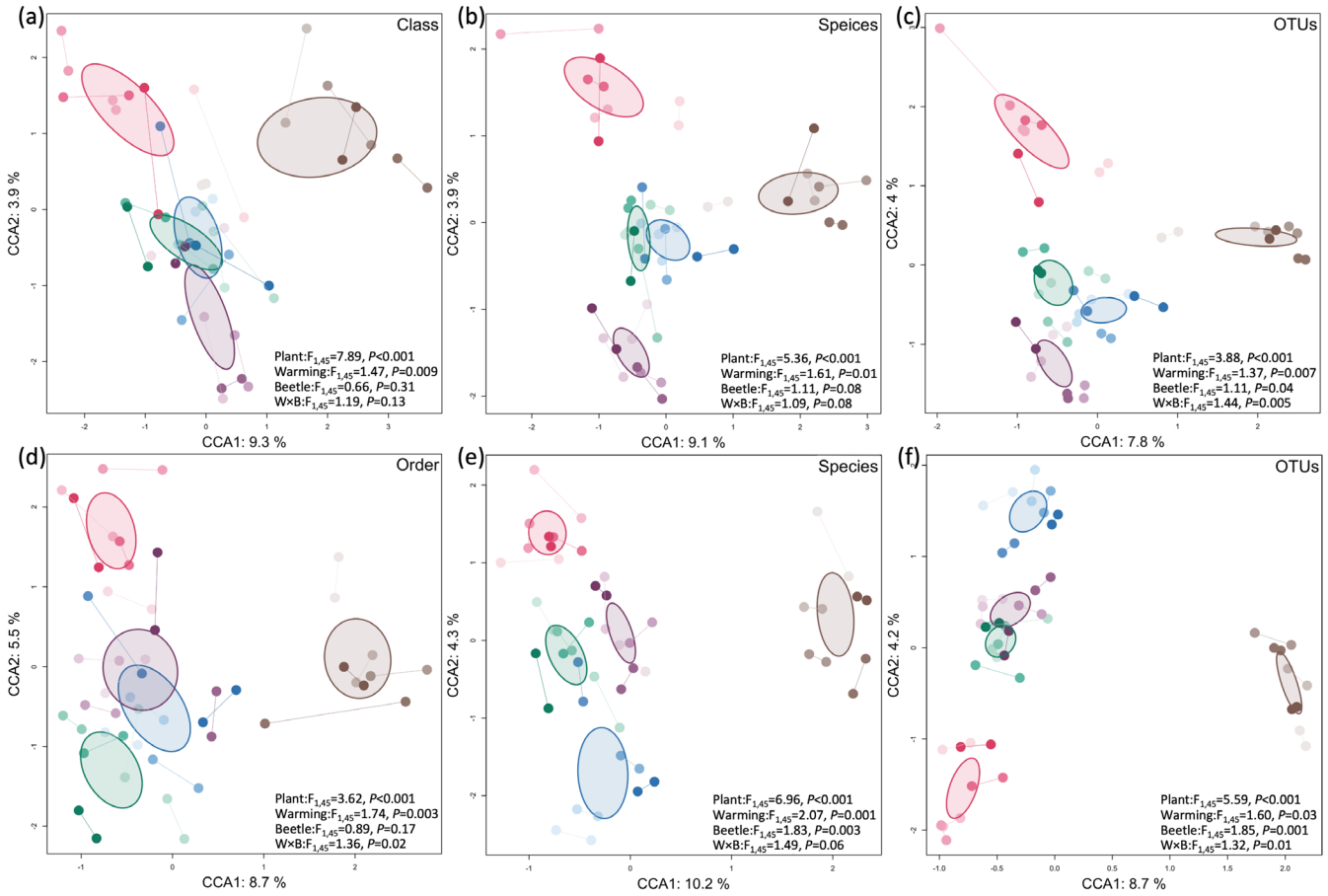


**FIGURE 3** | Bacterial (a–c) and fungal (d–f) community composition exposed to warming, herbivory (beetle), a combination of both warming + beetle, controls without warming and beetle or soil without *Ambrosia artemisiifolia*. The two-dimensional canonical correspondence analysis (CCA) ordination diagram with axes list taxonomic differences in microbial community composition in terms class (for bacteria), order (for fungus), species and OTUs. Significance of separation by treatments were determined using analysis of variance on distance matrices (adonis) and visualized with ellipses based on 95% confidence intervals of the centroid. Colour represents the microbial community in the rhizosphere of *Ambrosia* under control (greenish), *Ambrosia* with a history of beetles (bluish), with a history of warming (reddish), with a history of both warming and beetle (pinkish) and soil without *Ambrosia* growth (brownish). The figure for the complete taxonomy level can be seen in the Figure S7.

### 3.3 | Characterization of OTUs and Biodiversity of the Soil Microbial Community

Within the bacterial domain, the 10 most abundant phyla accounted for 98.25% of all OTUs (Table S9). There was a similar microbial composition at the phylum taxonomy level of bacteria and fungi, as well as for fungal trophic modes (Figure S3). The main bacterial phyla were Acidobacteria (28.18%), Proteobacteria (25.44%), Actinobacteria (19.32%), Planctomycetes (11.78%), and Verrucomicrobia (6.43%) (Figure S3a; Table S9). The main fungal phyla were Ascomycota (65.44%), Basidiomycota (16.09%), Mortierellomycota (9.67%), and Glomeromycota (5.62) (Figure S3b; Table S10). For the fungal community, the major trophic modes are undefined saprotroph (47.12%), animal pathogens (13.25%), plant pathogens (12.67%), arbuscular mycorrhizal fungi (11.73%) and ectomycorrhizal fungi (5.42%) (Figure S3c; Table S11).

For bacterial communities, *Ambrosia* presence significantly increased the species richness, Shannon diversity, Inverse Simpson diversity, rarefied species richness, and Faith's Phylogenetic Diversity ($\chi^2 \geq 7.9$, $p \leq 0.005$; Tables S12 and S13; Figure S4). Specifically, warming treatment increased the Shannon diversity, Inverse Simpson diversity and Faith's Phylogenetic Diversity ($\chi^2 \geq 3.94$, $p \leq 0.05$; Tables S12 and S13; Figure S4); and the beetle treatment increased the species richness, Shannon diversity, and Faith's Phylogenetic Diversity ($\chi^2 \geq 4.26$, $p \leq 0.04$; Tables S12 and S13; Figure S4); while there are no interactions between warming and beetle treatments across all diversity indices ($\chi^2 \leq 1.46$, $p \geq 0.22$; Tables S12 and S13; Figure S4). For fungal communities, we found no differences for any treatments on all diversity indices ($\chi^2 \leq 3.38$, $p \geq 0.07$; Tables S12 and S14; Figure S4). We found significant effects of the warming treatment on the species richness, Shannon diversity, and inverse Simpson diversity of pathotrophs, and on the Shannon diversity and the inverse Simpson diversity of plant pathogens ($\chi^2 \geq 4.16$, $p \leq 0.04$; Figure S5; Tables S15 and S16). The bacterial rarefied richness and fungal rarefied richness, bacterial diversity, and soil plant pathogen diversity and soil saprotroph diversity (both inverse Simpson index and Shannon index) were highly correlated across all treatments ($R^2 \geq 0.23$, $p \leq 0.015$, Figure S6).

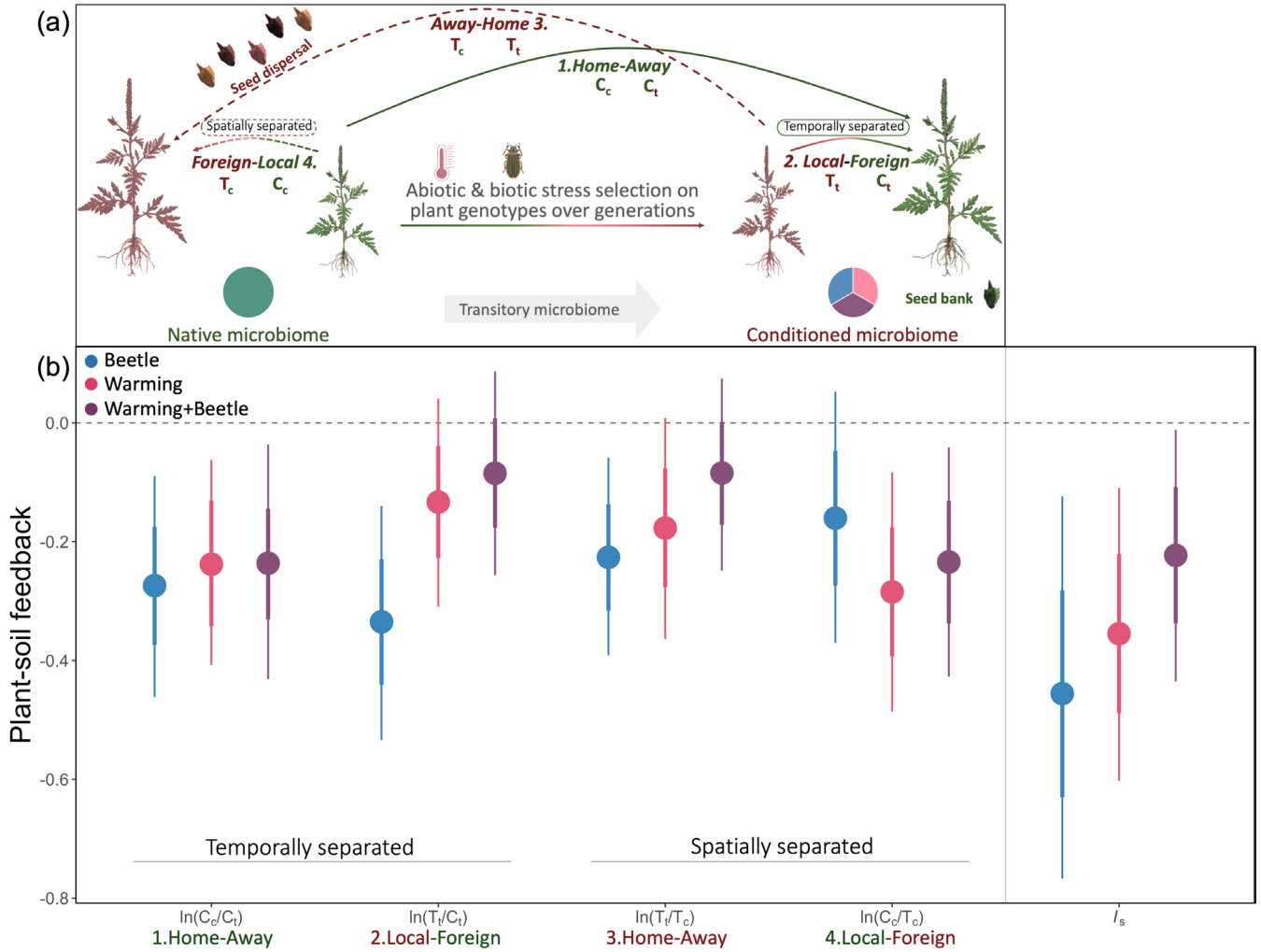


**FIGURE 4** | Specific and pairwise plant–soil feedback on plant biomass by type of microbiota inoculum and *Ambrosia artemisiifolia* origin (a, b). $C_c$ is the performance of *Ambrosia* offspring from control treatment in its own soil, $C_t$ is the performance of *Ambrosia* offspring from control treatment in soil of *Ambrosia* experienced beetle (blue), warming (red) and warming + beetle (pink) treatments, $T_t$ is the performance of *Ambrosia* offspring from three treatments in their own soil, $T_c$, is the performance of *Ambrosia* offspring from three treatments in soil of *Ambrosia* from control treatment, and $I_s$ is the pairwise plant soil feedback (see details in Section 2). Colours represents field treatment with herbivory (blue), with warming (red), or with both warming and herbivory (pink). The dot is the mean value, the thick line is ±SE, and the thin line is 95% bootstrap confidence intervals. Confidence intervals not overlapping the zero line (grey dash line) indicate PSF effects significantly different from zero at $p < 0.05$.

### 3.4 | Overall Treatment Differences in the Microbial Community Composition

Both bacterial and fungal communities differed between soils with and without *Ambrosia* growth across all taxonomic levels (Figure 3; Figure S7). Specifically, the bacterial community in the rhizosphere of *Ambrosia* under a history of warming (marginally) differed from all other treatments with *Ambrosia* at the class, family, genus, and species levels. While under the beetle history, differences were (marginally) observed only at the species level. When subjected to both warming and beetle history, (marginally) differences were found at the family, genus, and species levels (Figure 3a–c; Figure S7a). The fungal community in the rhizosphere of *Ambrosia* under warming differed at the order, family, and species levels. With beetle herbivory, differences occurred at the family, genus, and species levels. When both warming and beetle herbivory were present, (marginally) differences were detected at the order, family, genus, and species levels (Figure 3d–f; Figure S7b). Both bacterial and fungal communities clustered according to all treatments in terms of the normalized abundances of OTUs (Figure 3). The fungal community differed between soils with and without *Ambrosia* growth, and marginally in the rhizosphere of *Ambrosia* with a history of warming (Figure S7b).

### 3.5 | Plant–Soil Feedback Across Soil and Plant History

To investigate whether *Ambrosia* from the control treatment is favoured when grown in the conditioned soil from *Ambrosia* with warming or beetle history, we compared the performance of *Ambrosia* offspring from the control treatment in its own versus conditioned soil communities (Figures 1d and 4a:

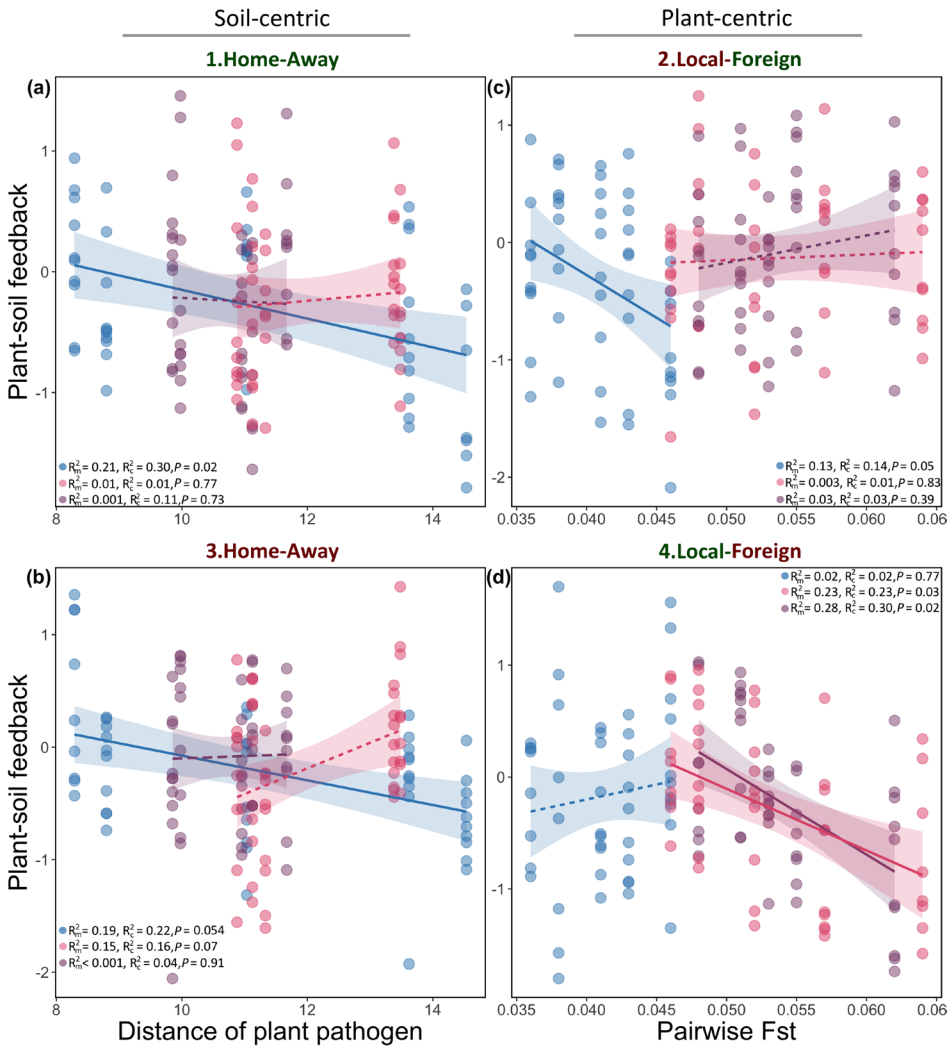


**FIGURE 5** | Relationship between plant–soil feedback for home-away comparisons with paired plant pathogen distance and pairwise $F_{ST}$. Panels (a, b, c, and d) are '1.Home-Away', '2.Local-Foreign', '3.Home-Away' and '4.Local-Foreign' PSF, respectively (see Figure 4 and details in Section 2). Colours represent beetle (blue), warming (red) and warming + beetle (pink) treatments. Solid and dashed regression lines represent significant and non-significant regressions, respectively.

1.Home-Away). We found significant negative PSFs in beetle, warming, and warming + beetle treated soil, indicating here negative conspecific effects, but escape of control *Ambrosia* offspring from their own soil and performing better in soils conditioned by other treatments (Figure 4b; $C_c/C_t$; Tables S17 and S18). Besides using the soil-centric 'home-away' approach to compare PSF, we also applied the plant-centric 'local-foreign' approach. We compared *Ambrosia* offspring from warming, beetle, or warming + beetle treatments grown in its own soil versus *Ambrosia* offspring from the control treatment grown with the same soil communities (Figures 1 and 4a: 2.Local-Foreign), and found a negative PSF only for the beetle treatment, and no effects for warming or warming + beetle treatment (Figures 1 and 4b; $T_t/C_t$; Tables S17 and S18). This suggests that the *Ambrosia* offspring from the control treatment had a better performance compared to offspring from beetle treatment when they grew in the soil communities conditioned with biocontrol history. To further explore whether dispersed seeds of *Ambrosia* offspring from abiotic and biotic stressed history would benefit when they grow in the unconditioned soil away from warming and beetle herbivory, we compared the performance of *Ambrosia* offspring from beetle, warming, or warming + beetle treatments in its own versus control soil communities (Figures 1 and 4a: 3.Home-Away), and we found a negative PSF for the beetle treatment only, with no effects for warming or warming + beetle treatment (Figure 4b; $T_t/T_c$; Tables S17 and S18), indicating that only dispersed seeds of *Ambrosia* offspring from the beetle treatment are doing better when grown in unconditioned soil away. Furthermore, we compared the performance of *Ambrosia* offspring from the control treatment in its own soil versus *Ambrosia* offspring from beetle, warming, or warming + beetle treatments grown with the same soil communities (Figures 1 and 4a: 4.Local-Foreign), and found no significant effect for the beetle treatment, and negative feedback for warming and warming + beetle treatments (Figure 4b; $C_c/T_c$; Tables S17 and S18). Thus, the selected warming and warming + beetle *Ambrosia* offspring will perform better when dispersed into soil away, represented here by soil conditioned by control plants. To quantify the contribution of PSF to plant coexistence, we calculated the derivative metric ($I_s$) of net pairwise PSF. We found a coexistence-stabilizing negative

pairwise PSF between *Ambrosia* offspring from control and beetle, warming, and warming + beetle treatments (Figure 4b; Tables S17 and S18).

### 3.6 | Feedback Drivers

We found that the soil-centric PSF were negatively correlated with the dissimilarity between soil plant-pathogens calculated as Bray–Curtis distances, where increasing differences of plant-pathogens led to more negative PSF for the beetle treatment, including two '*home-away*' comparisons (blue lines, $R^2_m \geq 0.19$, $R^2_c \geq 0.22$, $p \leq 0.054$; Figure 5a,b; Table S19). While, the plant-centric PSF were negatively correlated with the pairwise $F_{ST}$ for the beetle treatments of the type 2.Local-Foreign and for the warming and warming + beetle treatments of type 4.Local-Foreign (blue line, $R^2_m = 0.13$, $R^2_c = 0.14$, $p = 0.05$; Figure 5c; red line, $R^2_m = 0.23$, $R^2_c = 0.23$, $p = 0.03$; Figure 5d; purple line $R^2_m = 0.28$, $R^2_c = 0.30$, $p = 0.02$; Figure 5d; Table S19).

By examining how the genomic composition of the *Ambrosia* populations and microbial taxa relate to *Ambrosia* performance, we found that plant genomic composition positively correlated with the biomass of *Ambrosia* for both conspecific and heterospecific types ($p < 0.001$; Figure 6a; Tables S20 and S21), indicating the pronounced effect of the genetic plant population composition. Abundances of three higher plant pathogen families, i.e., Microascaceae, Diaporthaceae, and Sclerotiniaceae were negatively correlated with the biomass of *Ambrosia* for heterospecific conditions ($p \leq 0.05$; solid regression lines in Figure 6b–d; Tables S20 and S21), but not for conspecific conditions ($p \geq 0.29$; dotted regression lines in Figure 6b–d; Tables S20 and S21).

### 3.7 | Drivers of PSFs Using a Causal Inference Framework

To explore the effects of soil abiotic properties, soil biotic communities, *Ambrosia* phenotypic traits, and *Ambrosia* genomic composition on PSFs, we implemented a recently emerging causal inference framework. We used this approach that relies on a DAG to formalize the hypothesized causal structure of our study system (Arif and MacNeil 2023). Based on our DAGs (Figure 7a), we applied the backdoor criterion to determine adjustment sets required to address specific causal queries regarding whether, and to what extent, these four groups of variables influenced *Ambrosia* PSFs. Our analysis revealed that plant genome, plant traits, and soil biota all had direct effects on PSFs, which corroborated our hypotheses. Specifically, plant traits and genomic variables exhibited both a strong influence on PSFs, while the effects of soil biota were present but relatively weaker. These effects were larger under conspecific than heterospecific conditions (Figure 7b).

## 4 | Discussion

There is strong evidence that genetic variation in plant populations can influence the soil microbial community (Fitzpatrick et al. 2015), but little is yet known when considering the evolutionary history, i.e., when accounting for evolutionary changes under abiotic and/or biotic stress affecting the soil microbial community and the subsequent PSFs (Ware et al. 2019). By using soil data to explain the observed PSF, our study provides a better understanding of the role of PSFs in the *Ambrosia* invasion under climate warming and/or biocontrol herbivory management.

### 4.1 | Climate Warming and Beetle Herbivory Effects on Plant Genomic Composition, Soil Abiotic Properties, and Soil Microbial Communities

The results for abiotic and biotic selection are consistent with previous studies demonstrating a strong change in the genetic composition of *Ambrosia* populations in response to climate warming (Sun et al. 2020; van Boheemen et al. 2019), as well as a weak genetic plant population response to beetle herbivory selection (Sun et al. 2022). The latter was attributed to a transgenerational induced resistance with higher amounts of Sesquiterpene lactones, biologically active compounds often involved in plant resistance, in the *Ambrosia* offspring following biocontrol management (Sun et al. 2022). In addition, we observed distinct shifts in microbial communities in *Ambrosia*-conditioned soils exposed to warming or biocontrol herbivory, with notable changes in both bacterial and fungal taxa. Specifically, field warming led to higher-level taxonomic changes in both bacterial and fungal communities compared to insect herbivory, implying that warming may have a more profound impact on soil microbial diversity and composition. In general, only the bacterial communities showed increased diversity in responding to the warming or beetle treatments, suggesting that ecological stress had a more substantial impact on bacterial communities than fungal communities. This observed differential response pattern between bacterial and fungal communities is consistent with a large body of research showing that soil bacterial communities respond more distinctly to various ecological drivers than fungal communities (López-Angulo et al. 2020; Wang et al. 2021). The increased diversity of bacterial communities under warming treatments may be explained by the increased above-ground plant biomass observed under climate warming (Sun et al. 2020, 2022). The observed pattern is also in line with a large body of work demonstrating that moderate herbivory greatly increased soil bacterial richness and Shannon diversity (Jing et al. 2015; Wang and Tang 2019; Zhan et al. 2020). This could be linked to effects on soil C and N cycling under insect herbivory (Kristensen et al. 2020), but underlying biological drivers remain poorly understood.

### 4.2 | Changes in the Soil Microbial Communities Affecting the Outcome of Plant–Soil Feedbacks

Plant interactions with soil communities are critical factors that can influence the success and pace of alien plant invasion and spread. This requires investigating the processes driving PSF changes across various ecological contexts. From a temporal perspective, our results show that the history of soil conditioning by warming and/or beetles substantially impacts *Ambrosia* offspring performance. Interestingly, *Ambrosia* from control offspring, i.e., germinating from long-term seed storage in the soil, escape their negative PSF and perform

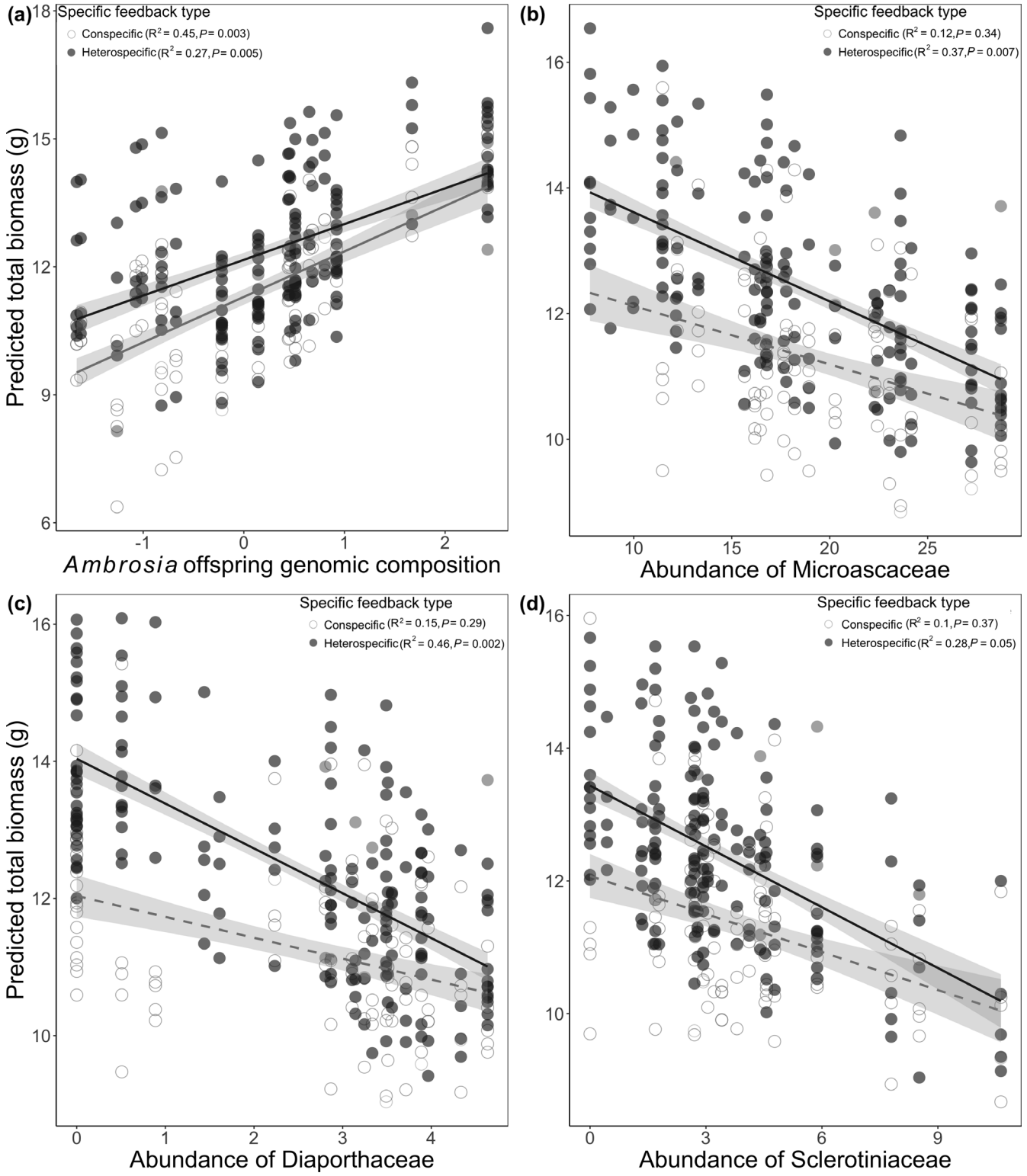


**FIGURE 6** | Relationship of plant total biomass with the genomic composition (a) and abundance of three pathogen families [Microascaceae; Diaporthaceae; Sclerotiniaceae; (b–d)]. Open dots represent single pots under conspecific conditions with grey regression lines, solid dots represent single pots under heterospecific conditions with black regression lines. Solid and dashed regression lines represent significant and non-significant regressions, respectively. The total biomass is extracted from linear mixed models.

better in newly trained soil by *Ambrosia* subjected to warming and/or beetle treatments compared to their own unconditioned soil. This implies that warming and/or biocontrol stress may have conditioned the soil microbiota, resulting in a more favorable environment for the natal seed bank, i.e., control offspring. The plant-centric '*local-foreign*' comparisons further demonstrate that *Ambrosia* development from the seed bank is consistently greater in beetle-conditioned soils compared to *Ambrosia* offspring populations that have undergone beetle herbivory. This implies a potential mechanism for

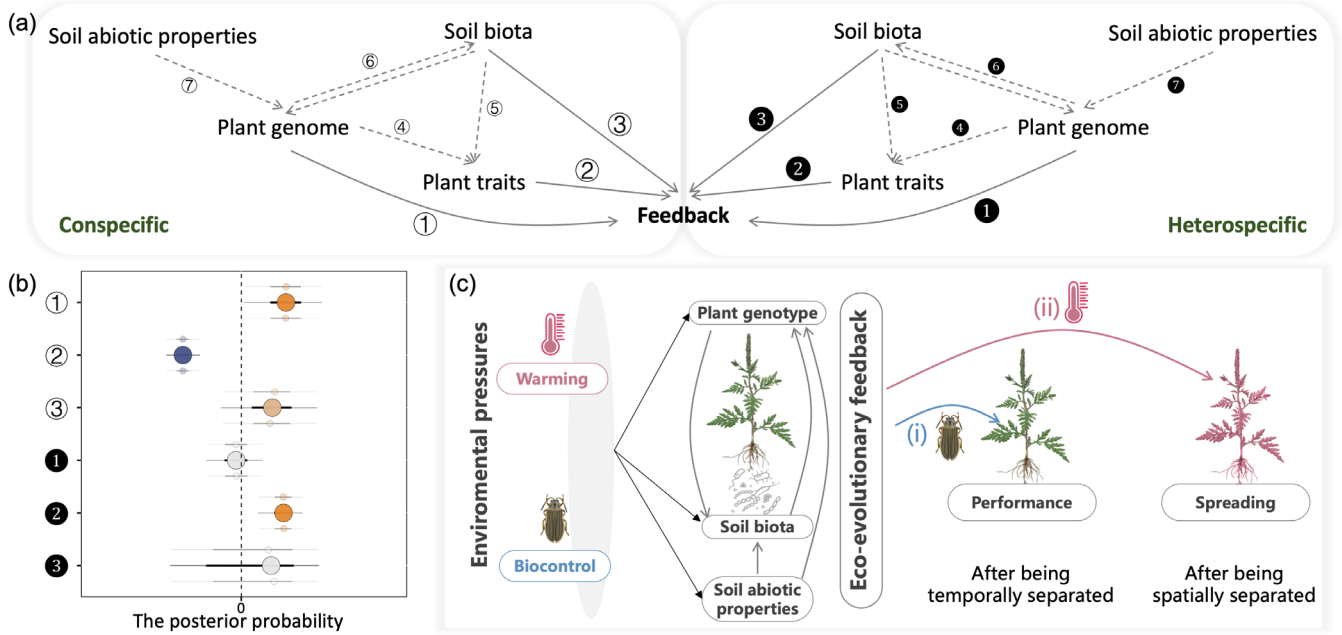


**FIGURE 7** | Causal effects of plant characteristics and soil properties on PSFs, with outcomes for the *Ambrosia* invasion. (a) Directed acyclic graphs (DAGs) illustrating the causal relationships. We hypothesize two DAGs, the only difference is path six, where we believe that both directions are possible (DAG1: Soil biota → Plant genome; DAG2: Plant genome → Soil biota; Figure S8). (b) Standardized effect sizes of drivers influencing feedbacks estimated from separate models, with the average posterior probabilities for relative importance of the direct three drivers. Parameter estimates are Bayesian posterior median values, 90% highest posterior density credible intervals (thin lines), and 50% credible intervals (thick lines). Dark circles indicate 90% credible intervals that do not overlap 0 and that the estimate was either positive (orange) or negative (navy-blue). Light circles indicate 50% of credible intervals not overlapping zero and that the estimate was either positive (light orange) or negative (light navy-blue). Grey circles indicate 50% credible intervals overlapping zero. The big points represent the average of two DAGs, while the two small points below and above the big one are DAG1 and DGA2, respectively. Details and indirect effect on feedback of the two DAGs are given in Tables S22 and S23 and Figures S9 and S10. (c) Eco-evolutionary feedbacks in *Ambrosia artemisiifolia* favours (i) invasion success by promoting the growth of natal plants germinating from the persistent seed bank under biocontrol herbivory management, and (ii) the spread of warming selected plants, especially from warmer to colder regions, e.g., from southern to northern regions.

maintaining *Ambrosia* population genetic diversity. Together, these results indicate that negative feedbacks generated by warming and biocontrol can enhance the success of invasive populations over temporal scales at a given site, ultimately promoting *Ambrosia*'s local invasion potential.

From a spatial perspective, our findings highlight that PSF dynamics are highly context-dependent. Specifically, dispersed seeds of *Ambrosia* populations that have experienced biocontrol management performed better in away conditioned control soils than in their own conditioned soils, suggesting that beetle herbivory may lead to soil microbial communities that are particularly inhibitory for future *Ambrosia* generations. This provides some evidence for the effectiveness of biocontrol management in limiting the establishment and growth of invasive *Ambrosia* offspring populations. While spreading into biocontrol-free soils allows them to escape from these negative PSFs, they will still perform poorly compared to control plants there. On the other hand, warming-related treatments (warming and warming + beetle) would outperform when dispersed seeds are grown in conditioned control soils compared to the seeds there, implying that warming-driven changes to soil biota might not be as persistent or detrimental as beetle herbivore-driven changes. The plant-centric '*local-foreign*' approach further underscores the unique impact of climate warming in shaping soil microbial communities and their role in invasion dynamics. While warming-related treatments induced negative feedbacks that may improve *Ambrosia*'s performance relative to control populations that have not yet experienced warming stress, potentially facilitating their spread, particularly from warmer (southern) to colder (northern) regions, beetle-induced feedbacks had no discernible negative impact. This implies that changes in soil conditions brought on by global warming may facilitate the invasion process by creating circumstances that are more conducive to *Ambrosia* expansion.

The coexistence-stabilizing negative pairwise PSF observed between *Ambrosia* offspring from control and warming and/or beetle treatments indicates that PSFs play a crucial role in regulating the dynamics of invasive populations. Negative pairwise PSF effects suggest that these interventions may contribute to maintaining *Ambrosia* population diversity by limiting the dominance of particular genotypes, thereby promoting coexistence. This discovery implies that biotic and abiotic stressors can influence soil microbial communities in ways that lessen the competitive advantage of invasive populations, which have important management implications for invasive species.

### 4.3 | Potential Drivers of PSFs by Plant Genetics and Soil Microbiota

Our findings highlight the role of plant genetics in the spread potential of *Ambrosia* invasions via eco-evolutionary dynamics of PSFs. Specifically, negative plant-centric PSFs correlated with pairwise $F_{ST}$ of *Ambrosia* offspring for warming-related treatments, suggesting that the strong selection under climate warming on *Ambrosia* populations, resulting in pronounced negative PSFs, allows them to thrive in unconditioned soil under an ambient temperature environment. Given our previous predictions of a northward spread of invasive *Ambrosia* outpacing the spread of its biocontrol agent in Europe and Asia under future climate scenarios, our results highlight that the invasion risk under climate change will be further promoted by the eco-evolutionary dynamics of PSFs, especially enhancing *Ambrosia* spread from warmer (southern) to colder (northern) regions.

Our analyses further highlight the effects of changes in soil plant pathogens and genetic differentiation among *Ambrosia* offspring populations in determining PSF outcomes. The soil-centric PSF analysis revealed a negative correlation between PSFs and the dissimilarity of soil plant-pathogens, particularly for the beetle treatments, indicating that increased differences in plant-pathogen communities led to stronger negative PSFs due to biocontrol management. Additionally, the decreased diversity of plant pathogens in soils with beetle herbivory history, along with the lower abundance of three pathogen families that reduced plant biomass, suggests that escaping specific pathogens may promote *Ambrosia* invasion, potentially enhancing its capacity to establish and spread in new environments. This reduction in pathogens can be explained by the higher levels of Sesquiterpene lactones observed in our previous studies (Sun et al. 2022). This is consistent with a previous study demonstrating that *Ambrosia's* remarkable invasion success in Europe was most likely benefited by escaping from specific pathogen enemies (Bieker et al. 2022). Above-ground herbivory has long been demonstrated to have an indirect effect on soil biotic communities by influencing plant root exudation and carbon allocation, or altering the quality of input of plant litter (Bardgett et al. 1998; van der Putten et al. 2001). The observed negative feedbacks across both '*home-away*' and '*local-foreign*' comparisons emphasize the importance of pathogen-mediated feedbacks in biocontrol contexts, where biocontrol management appears to reduce the soil pathogens that may facilitate future *Ambrosia* generations, particularly those from the soil seedbank.

In addition, our causal models exhibited strong effects by conspecifics compared to heterospecifics, which is in line with a study by Semchenko et al. (2018), suggesting that the dissimilarities in plant and soil characteristics might be more important predictors of plant growth responses in its own soil environment. Importantly, our causal model clearly revealed that, compared to the effect of the soil microbial communities, a higher direct contribution to PSF by plant population genomics and phenotypic traits was brought about by the strong selection imposed by climate warming on *Ambrosia* populations (Sun et al. 2020, 2022).

## 5 | Conclusion

We recognize that our study highlights just one, but so far neglected aspect of the impact of climate warming and biocontrol herbivory management on the invasion success of *Ambrosia*. However, this aspect could be crucial for understanding broader global change impacts on other plant invaders as well. We discovered that under warming and beetle herbivory, evolutionary changes in *Ambrosia* populations significantly and differentially altered the bacterial and fungal communities in the soil, which in turn contrastingly promoted *Ambrosia* invasion and spreading (Figure 7c). These results provide insights into how invasive species respond to abiotic and biotic stress through altered eco-evolutionary plant–soil interactions that are critical for predicting invasion dynamics in the context of biocontrol and global change scenarios.

#### Author Contributions

**Yan Sun:** conceptualization, data curation, formal analysis, funding acquisition, methodology, visualization, writing – original draft, writing – review and editing. **Daniele Silvestro:** formal analysis, funding acquisition, methodology, writing – review and editing. **Gregor H. Mathes:** formal analysis, methodology, writing – review and editing. **Marcel G. A. van der Heijden:** conceptualization, methodology, writing – review and editing. **Heinz Müller-Schärer:** conceptualization, data curation, funding acquisition, methodology, writing – original draft, writing – review and editing.

#### Acknowledgements

Y.S. acknowledges National Natural Science Foundation of China (#32201438) and the Scientific Research Foundation for Returned Scholars, Huazhong Agricultural University (#11042110026) for funding. H.M.-S. thanks funding through the Swiss National Science Foundation (SNSF project 31003A_166448). D.S. received funding from ETH Zurich and the Swedish Foundation for Strategic Environmental Research MISTRA within the framework of the research programme BIOPATH (F 2022/1448). We are grateful to the Comune di Magnago, Italy, for providing the experimental field site and to the many people who helped with the field and common garden studies, especially Elia Rossini and Antoine Roulin, and the local gardener Giuseppe Giambrone. We thank Florian Walder, Soil Quality/Soil Use group, Agroscope, Zürich, Switzerland, for the measurements of the soil abiotic properties, and Marc W. Schmid and Jun Liu for soil biotic community analysis. We also thank the support from Martin Kapun and Daniel Wegmann for the pool-seq analyses. We also thank Ragan M. Callaway for commenting on an earlier version of the manuscript.

#### Conflicts of Interest

The authors declare no conflicts of interest.

#### Data Availability Statement

The data and code that support the findings of this study are openly available in Figshare at https://doi.org/10.6084/m9.figshare.26347138. The raw sequence data are available in the NCBI Sequence Read Archive repository under the BioProject ID PRJNA1224024.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section.