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Plant–soil microbe feedbacks depend on distance and ploidy in a mixed cytotype population of *Larrea tridentata*

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Abstract

Premise: Theory predicts that mixed ploidy populations should be short-lived due to strong fitness disadvantages for the rare ploidy. However, mixed ploidy populations are common, suggesting that the fitness costs for rare ploidies are counterbalanced by ecological benefits that emerge when rare. We investigated whether differences in ecological interactions with soil microbes help to maintain a tetraploid–hexaploid population of *Larrea tridentata* (creosote bush) in the Sonoran Desert, California, United States, where prior work documented ploidy-specific root-associated microbes.

Methods: We used a plant–soil feedback (PSF) experiment to test whether host-specific soil microbes can alter the outcomes of intraploidy vs. interploidy competition. Host-specific soil microbes can build up over time; thus, distance from a host plant can affect the fitness of nearby plants.

Results: Seedlings grown in soils from near plants of a different ploidy produced greater biomass relative to seedlings grown in soils from near plants of the same ploidy. Moreover, seedlings grown in soils from near plants of a different ploidy produced more biomass than those grown in soils that were farther from plants of a different ploidy. These results suggest that the ecological consequences of PSF may facilitate the persistence of mixed ploidy populations.

Conclusions: This is the first evidence, to our knowledge, that is consistent with plant–soil microbe feedback as a viable mechanism to maintain the coexistence of multiple ploidy levels in a single population.

KEYWORDS

Janzen-Connell distance-dependent, microbe-mediated, minority cytotype exclusion, mixed-ploidy, plant-soil feedback, polyploidy

Polyploidy—whole-genome duplication—is the presence of more than two haploid-genome copies within an organism. Polyploidy is common in plants, although it is found in all domains (Brownfield and Kohler, 2011; Soppa, 2014; Campbell et al., 2016; Baduel et al., 2018). Theory predicts that mixed ploidy populations should be transient and sparse (Anneberg et al., 2023) because newly arisen ploidies are rare and have few reproductively compatible mates in a population since crosses between different ploidies typically produce inviable or sterile hybrids (i.e., minority cytotype exclusion; Levin, 1975). Despite this theoretical expectation, ploidy varies across the ranges of many species, but also can vary within populations (Sudová et al., 2014; Muñoz-Pajares et al., 2018; Plue et al., 2018; Kiedrzyński et al., 2021; Anneberg et al., 2023). For example, among 1209 populations of scentless chamomile, *Tripleurospermum inodorum* that were assayed across Central Europe, mixed-ploidy populations were found at an incidence of 22% at a landscape- and 33–43% at a regional spatial scale. A subset of the mixed populations ranged from balanced frequencies of two ploidies, to combinations of up to three ploidies at varying

frequencies (Čertner et al., 2017). Most research on the fitness disadvantage experienced by rare ploidies in mixed-ploidy populations has focused on plants; however, recent investigations have also documented ploidy variation within populations of different animal lineages, including snails (*Potamopyrgus antipodarum*; Neiman et al., 2011), fish and amphibians (reviewed by Mable et al., 2011), fungi (reviewed by Gerstein and Sharp, 2021), and bacteria (Pecoraro et al., 2011), although the last two adhere to different definitions of populations and do not face the same causes of potential rare ploidy disadvantage.

Minority cytotype exclusion is predicted to occur when a new ploidy (i.e., a new cytotype) arises in a population through genetic mechanisms or immigration but experiences a fitness disadvantage by having few reproductively compatible mates, resulting in localized extinction. For example, a tetraploid arising within a diploid population would be predicted to produce few viable and fertile offspring from matings with the more frequent diploids and to go extinct without other mechanisms facilitating an increase in tetraploid frequency. Several models exist to explain how the fitness disadvantage experienced by rare ploidies in mixed populations may be lessened or eliminated by niche and fitness differences between ploidies (Felber, 1991; Van Dijk and Bijlsma, 1994; Li et al., 2004; Oswald and Nuismer, 2011; Gaynor et al., 2023). Niche and fitness differences, in this context, could be realized by environmental partitioning that increases intraploidy competition compared to interploidy competition resulting in reproductive outputs that increase the frequency of the rare ploidy. For example, fitness advantages may arise for a recently derived, rare cytotype if it exhibits greater pathogen resistance (Oswald and Nuismer, 2007; Mehlferber et al., 2022), better adaptation to the local habitat (Li et al., 2004; Garmendia et al., 2018), or ploidy-specific pollinator differentiation, such that intraploidy competition is stronger than interploidy competition (Rodríguez, 1996; Fowler and Levin, 2016; López-Jurado et al., 2019). However, there are few empirical tests of such models (Husband, 2000; Chrtek et al., 2017), in part due to the challenges associated with conducting such experiments.

Interploidy vs. intraploidy competition in mixed ploidy populations is analogous to interspecific vs. intraspecific competition, allowing the application of modern coexistence theory (Chesson, 2000) to mixed-ploidy populations. One assertion of coexistence theory is that specialized natural enemies, including pathogenic soil microbes, can build up on common species (or genotypes) over time and that rare species (or genotypes) may possess a relative fitness advantage as a result (i.e., Janzen-Connell hypothesis; Connell, 1961; Janzen, 1970). Evidence from the black cherry tree, *Prunus serotina*, suggests that distance-dependent seedling mortality around adult trees may be due to a buildup of *Pythium* fungal pathogens around the adult soil-conditioning trees (Packer and Clay, 2000). Moreover, a study of hackberry trees, *Prunus padus*, indicate that fungal pathogens decrease in relative abundance with increasing distance (1–20 m) from adult trees (Liu et al., 2015).

On the basis of the predictions of coexistence theory, we tested whether the accumulation of soil microbes around common ploidies may facilitate the maintenance of mixed-ploidy populations with an adaptation of classic plant soil microbe feedback (PSF) experiments (Liu et al., 2015). Although originally conceived as a framework for examining species co-occurrences, PSF may similarly help explain the prevalence of mixed-ploidy populations. Typically, PSF experiments manipulate either plant environment (home vs. away; Klironomos, 2002) or plant density/frequency (e.g., Chung and Rudgers, 2016). Here, we substituted distance for frequency, adapting methods published by Packer and Clay (2004). Distance can stand in for frequency because ploidy-specific soil microbes accumulate near hosts as a function of the nature of movement through soil, and their density decreases with increasing distance from the plant (i.e., near plant mimics high density; far from plant mimics low density (Eppinga et al., 2022). When one ploidy is numerically or spatially rare in a population, similar to rare species in a community, it may escape harmful, specialized pathogens, and thereby gain a fitness advantage sufficient to overcome minority cytotype disadvantage. However, relatively few prior studies have demonstrated a ploidy-specific soil microbe interaction, and it remains unclear if findings of microbiome differences among co-occurring cytotypes contribute to fitness differences that would facilitate co-occurrence.

Larrea tridentata (DC.) Coville (Zygophyllaceae) is a long-lived, perennial evergreen shrub comprising a polyploid complex with diploids (2*x*), autotetraploids (4*x*), and autohexaploids (6*x*). Ploidy distributions roughly align with the Chihuahuan Desert (diploid), Sonoran Desert (tetraploid), or Mojave Desert (hexaploid) of North America (Laport et al., 2012). Both single ploidy and mixed ploidy *L. tridentata* populations have weakly uniform spacing between plants (Phillips and MacMahon, 1981). Contact zones between ploidies are relatively broad (i.e., spanning several kilometers; Laport and Ramsey, 2015) and comprise complex mixed-ploidy populations that exhibit different patterns of spatial dispersion by co-occurring cytotypes (Laport and Ramsey, 2015). Tetraploid and hexaploid *L. tridentata* also support distinctive communities of root-associated fungi (i.e., fungi from rhizosphere soils and fine roots) in areas where they co-occur. Indicator operational taxonomic units (OTUs) for these communities are divergent, with indicator OTUs associated with tetraploids belonging to *Knufia*, *Mortierella*, and *Cystobasidium* and indicator OTUs associated with hexaploids belonging to the Sordariomycetes (Gerstner et al., 2023). However, it remains unclear whether these ploidy-specific microbes might offset any fitness disadvantages experienced by the minority cytotype in these populations through the net fitness benefits the rare cytotype gains by escape from specialist microbial pathogens.

Here we use a modified plant–soil microbe experiment to test whether ploidy-specific soil microbe interactions may promote ploidy co-occurrence by influencing the relative fitness of autotetraploid and autohexaploid cytotypes from a mixed-cytotype population of *L. tridentata*. Unlike allopolyploid

species, which are formed by hybridization between two species, tetraploid and hexaploid *L. tridentata* are hypothesized to have arisen via autopolyploidization (Barbour, 1969; Yang et al., 2000). Thus, soil microbe differences should more closely reflect differences due to polyploidization per se, plus subsequent post-polyploidization adaptation rather than the effects of combining two partially divergent genomes. We focused on plant interactions with soil fungi because past studies have found fungi to have a stronger influence as drivers of PSF than bacteria, which have faster turnover, greater dispersal, and therefore are less likely to accumulate as specialists (Bever, 2002; Klironomos, 2002; Van Der Putten et al., 2013). We hypothesized that plant–soil microbe interactions in mixed-ploidy populations would result in net positive fitness outcomes for numerically or spatially rare cytotypes when growing near plants of the numerically or spatially dominant co-occurring cytotype. We predicted that (1) host ploidy specialist microbes impact plant growth, (2) that the degree of impact is specific to plant ploidy, and (3) that different-ploidy seedlings grow better than same-ploidy seedlings in soils collected from near a host plant, consistent with the idea that specialist microbes decrease in density with distance from the host plant.

MATERIALS AND METHODS

Study system

Larrea tridentata reproduces primarily via seed, but can also grow clonally (for hundreds or thousands of years) under specific environmental conditions (Mabry et al., 1977). Prior research using flow-cytometric analyses to infer DNA content has identified multiple mixed ploidy *L. tridentata* populations comprising permanently marked plants (Laport et al., 2012; Laport and Ramsey, 2015). However, the three ploidies have relatively well-defined geographic distributions, likely maintained by abiotic environmental variation, but also potentially determined by interactions with galling midges and pollinator-mediated assortative mating at cytotype contact zones (O'Connor et al., 2019; Laport et al., 2021). In mixed-ploidy populations, 4*x* plants of *L. tridentata* tend to be found in denser vegetation associations than 6*x* plants, which tend to be found at higher elevations and in more species-rich communities on coarser soils (Laport et al., 2016). Tetraploids tend to flower earlier and produce more flowers than 6*x* plants (Laport et al., 2016). The size of morphological structures tend to increase with ploidy (e.g., larger-diameter pollen grains, longer stamens and pistils, and longer, wider petals and leaves) though 4*x* plants tend to be taller than 6*x* plants (Laport et al., 2016).

Field soil collections for distance dependence

In April 2021, we collected soils at eight distances along a transect outward from adult soil-conditioning plants of known ploidy from two 4*x*–6*x* sites (Algodones N4; 33.00°, −115.07° and Algodones S3; 32.81°, −114.87°; Laport and Ramsey, 2015; Figure 1). Soil-conditioning plants represent the numerically or spatially dominant cytotype in our experiment, and "condition" the soil with their root zone-associated microbiome that may affect other plants recruiting into nearby soils. We combined the two nearby 4*x*–6*x* sites in our analyses and hereafter treat and refer to them as a single population to balance sample sizes for each ploidy from the asymmetrically mixed sites (Laport and Ramsey 2015). Sampling distances were standardized from the shrub dripline as 0 m, then sampled at −0.1 m (under the shrub canopy) and at 0, 0.25, 0.5, 0.75, 1.0, 1.5, and 2.0 m. We set soil-sampling transects in directions that minimized

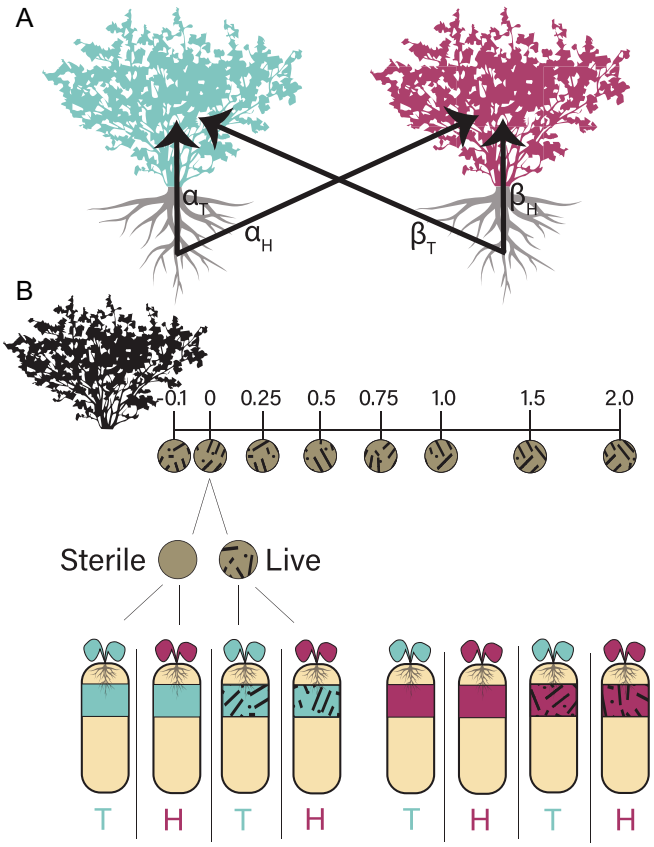

FIGURE 1 Simplified plant–soil microbe feedback experiment. (A) Depiction of plant–soil microbe feedback variables for tetraploid (teal, T) and hexaploid (maroon, H) plants of *Larrea tridentata*. α_T represents the direct relative effect of tetraploid soil microbes on tetraploid plants, α_H the indirect relative effect of tetraploid soil microbes on hexaploid plants, β_T the indirect relative effect of hexaploid soil microbes on tetraploid plants, and β_H the direct relative effect of hexaploid soil microbes on hexaploid plants. (B) Simplified sampling scheme and greenhouse experimental setup. Each brown circle represents a field-collected soil sample at one of eight locations along a 2.1-m transect; the plant silhouette is the conditioning plant. On the transect, zero was set at the shrub dripline to standardize the sampling across plants with varying crown sizes. "Sterile" and "Live" refer to the type of soil inoculum (either sterilized or not, respectively) used. Eight unique pots were used for each distance and each conditioning pair. The plant was either a tetraploid (teal) or hexaploid (maroon), the band under the plant is the soil inoculum (sterile, solid color; live, cross hatched), and the inoculum ploidy is assigned to match the ploidy of the conditioning plant on which the transect originated.

obstacles (i.e., avoided other plants). Soil collection was done by twisting two 50-mL sterile tubes into the soil (approximately 11.5 cm deep) at each sampling distance, inverting the tube and capping. When the soil surface layer was impenetrable, we brushed aside the top layer (e.g., top 1–2 cm of pebbles) using a soil knife. Although the roots of adult *L. tridentata* penetrate much deeper into the soil than 11.5 cm, potentially affecting later-stage or lifetime fitness outcomes for these long-lived shrubs, we aimed to capture and assess the initial soil microbe interactions with a seedling because this is the life history stage at which PSF might have the greatest effect on fitness (i.e., recruitment and growth; Drees et al., 2023). We sterilized all sampling equipment between samples with a 10% v/v bleach solution and allowed each to dry completely. In total, we collected 288 soil samples, 16 each from nine soil-conditioning tetraploids and nine soil-conditioning hexaploids. We focused on soils 2 m from existing plants because studies that quantify spatial variation of soil microbes in associations with plants often do so between 0 and 2 m and have found the greatest turnover in microbe community composition occur within 0.5 m (reviewed by Chung, 2023).

Soil samples were stored on ice in coolers for up to 72 h before being refrigerated (4°C) at the University of New Mexico. The two tubes of soil collected from each distance were combined, and the total volume was divided in half; one half remained refrigerated for later use as live inoculum, and the other half was sterilized for use as sterile inoculum. All sterilizations were performed at 121°C in an autoclave on a gravity cycle with a 180-min sterilization and 60-min dry period.

Seed material

To assess the effect of the microbe-conditioned soil on seedling recruitment, we collected mature fruits containing seeds directly from plants in May 2021 in zones of known single-ploidy plants. Tetraploid seeds were collected from CA-O (32.91°, −115.27°) and hexaploid seeds were collected from CA-S (33.11°, −114.90°; Laport and Ramsey, 2015). All fruits were stored in paper envelopes and transported to the lab at the University of New Mexico. To rule out an influence of seed-borne fungi, we assessed seed stock for culturable fungi. We randomly selected 30 seeds of each ploidy for assays of surface and endophytic fungi. We examined surface fungi on nonsterilized seeds by placing 10 seeds of each ploidy on malt extract agar plates with penicillin and streptomycin. We observed nominal culturable fungi (two across all seeds) after 2 weeks, which we hypothesize is due to the presence of condensed tannins in the seeds (Hyder, 2002; Appendix S1) and thus did not surface-sterilize seeds. We examined surface-sterilized seeds for fungal endophytes with seeds sliced in half and placed on malt extract agar plates with penicillin and streptomycin. After 2 weeks, there was no fungal growth on any of the plates, suggesting no fungal endophytes were present within the seed stock (Appendix S1).

Experimental design

We used a paired design to assess plant–soil microbe feedback (PSF), with each pair comprising soils from one tetraploid and one hexaploid soil-conditioning plant from the field (Figure 1). The resulting nine soil-conditioning pairs comprised eight cone-tainers representing either the tetraploid or hexaploid soil-conditioning plant, live or sterile inoculum, and seed ploidy for each of the eight soil sampling distances (Figure 1B). The full experimental design comprised 576 cone-tainers representing the nine tetraploid-hexaploid pairs. Cone-tainer position was randomized for inoculum, distance, conditioning-plant ploidy, and seed ploidy across 16 racks in the greenhouse.

Greenhouse setup

We sterilized cone-tainers (3.8 × 21 cm, SC10R Stuewe & Sons, Tangent, Oregon, USA) by soaking them for 12 h in 10% v/v bleach and allowed them to dry in bleach-sterilized tubs. In the greenhouse, we positioned between 24 and 48 sterilized cone-tainers per cone-tainer rack (RL98, Stuewe & Sons), leaving an empty space on all sides. We then added an autoclaved cotton ball to the cone-tainer bottom and added autoclaved 30/70 soil/sand mixture to ca 90% of total pot volume, then a layer of sterile or live inoculum (ca 10% of cone-tainer volume). Each cone-tainer was marked with a unique plant tag identifier. Preliminary trials with the cone-tainers had showed that the substrate compacted after the first 2 weeks with the top substrate surface at 1–2 cm below the top of the cone-tainer, and a similar amount of compaction occurred during our experiment.

We removed seeds from their fruit capsules and placed them on moistened paper towels in plastic clamshells and incubated them in the dark at room temperature for 12–24 h until the radicle began to emerge. We then used sterile forceps to place a germinated seed on the inoculum surface and capped the inoculum with an autoclave-sterilized sand layer (~1–2 mm). We immediately misted cone-tainers until saturation (i.e., water dripped from the bottom of the cone-tainer).

In total, we evenly spaced 16 racks across a greenhouse bench under grow lights (Spydr 600, BML Horticulture, Austin, TX, USA) that were set to a 14 h/10 h day/night cycle for the experiment duration. Every other day for the first 4 weeks, we hand-watered the cone-tainers with a fine-mist sprayer from above until saturation. We then switched to an automatic mister system that watered for 5 min every 3 days for the experiment duration. If a seedling had not sprouted by 4 days after planting, we removed and replaced the seed, retopping with autoclave-sterilized sand. We repeated this step up to three times for each cone-tainer, after which no additional planting took place. We uprooted and discarded any additional seedlings that emerged (e.g., *L. tridentata*) from the soil seed bank in live-inoculum pots.

After 6 months of plant growth, leaf discoloration and leaf drop spiked, which we attributed to soil nutrient depletion. We trialed nutrient application to non-experimental plants, and observed leaves remained on the plant and returned to green. Thus, we applied once-monthly fertilization treatments of 5 mL general-purpose fertilizer at 250 ppm (20-20-20 Peters Professional, Everris Na, Dublin, OH, USA) directly to each cone-tainer for the experiment duration.

We harvested plants after 52 weeks of growth by cutting the plant stem at the soil surface. We dried aboveground material in paper envelopes at 60°C for 72 h before weighing the biomass. We also sieved roots through 2.36-mm mesh to remove soil. We recorded total root wet mass, then haphazardly removed roots from across the root system (~20 sections, each 2.5 cm long) and preserved them in tissue cassettes stored in 70% v/v ethanol. We recorded wet mass of the remaining roots and then dried and weighed remaining root biomass as done for shoot biomass to estimate total root dry mass. We removed reserved root material from ethanol, then stained for hyphae with black ink (Vierheilig et al., 1998) and made permanent slides of the roots using polyvinyl-lacto-glycerol. All slides were examined under 200 × magnification, recording observed hyphae for at least 80 (maximum 100) fields of view on each slide using standard methods (McGonigle et al., 1990).

Due to the 52-week growth period in the greenhouse, there was ample opportunity for environmental contamination (i.e., root fungal colonization). We estimated the prevalence of environmental contamination using plants grown in sterile inoculum that had roots colonized by fungi.

Analyses

Data sets

We used information on root colonization by fungi to create four data sets for analysis (Appendix S2). The first data set included plants grown in live inoculum with observed root colonization by fungi. The second data set included all plants grown in live inoculum. The third and fourth data sets included plants grown in sterile inoculum without and with root colonization, respectively. The first data set captured the effect of known root-associated fungi and bacteria, whereas the second data set captured a more general effect of the entire soil microbiome (i.e., soil fungi and bacteria). The third data set captured the effect of sterilization (i.e., fungal and bacteria absent soils), which allowed us to determine whether soil microbes cause any fitness advantage experienced by a seedling differing in cytotype from the soil-conditioning plant. The fourth data set captured the effect of suspected environmental contamination by allowing the detection of root-associated fungi. All analyses were performed in R 4.1 (R Core Team, 2022) using the R packages ggplot2 (Wickham 2016), tidyr (Wickham et al., 2023b), dplyr (Wickham et al., 2023a), stringr (Wickham, 2023), hablar (Sjoberg, 2023), splancs (Bivand et al., 2023) and MASS (Venables and Ripley, 2002).

Plant performance analyses

We used generalized linear modeling to examine the relationships between total plant biomass and seedling ploidy (total biomass ~ seedling ploidy), between aboveground and root biomass and conditioning-plant ploidy and seedling ploidy (aboveground biomass or root biomass ~ conditioning-plant ploidy + seedling ploidy) between biomass and conditioning-plant ploidy and soil microbe recruitment (aboveground or root biomass ~ conditioning-plant ploidy + microbes), and between belowground biomass and distance from conditioning plant (root biomass ~ conditioning-plant ploidy + seed ploidy + distance from conditioning plant).

Fitness advantage from soil microbes

We calculated the Bever's interaction coefficient (I_s) to examine plant–soil microbe feedback (PSF) relationships at each transect distance for each data set described above. The coefficient I_s summarizes the net effect of plant–soil microbe feedback and is useful to predict whether the ploidies will coexist through cyclical stability (Bever et al., 1997). A negative I_s indicates a fitness advantage is gained for the rare ploidy. A positive I_s indicates no fitness advantage is gained for the rare ploidy. I_s is the sum of the direct and indirect effect of the microbes associated with the hexaploid and the tetraploid plants calculated as:

Direct effect of tetraploid microbes on the tetraploid (T):

$$\alpha_T = G(T)_\alpha - G(T)_0 \quad (1)$$

Indirect effect of hexaploid microbes on the tetraploid (T):

$$\beta_T = G(T)_\beta - G(T)_0 \quad (2)$$

Indirect effect of tetraploid microbes on the hexaploid (*H*):

$$\alpha_H = G(H)_\alpha - G(H)_0 \quad (3)$$

Direct effect of hexaploid microbes on the hexaploid (*H*):

$$\beta_H = G(H)_\beta - G(H)_0, \quad (4)$$

where G(T) and G(H) are the total dry biomass (in grams) of a tetraploid and hexaploid, respectively, α and β are the soils conditioned by tetraploid and hexaploid plants, respectively, 0 are sterilized soils, α_T = tetraploid plants growing in soils conditioned by tetraploid plants, α_H = tetraploid plants growing in soils conditioned by hexaploid plants, β_T = hexaploid plants growing in soils

conditioned by tetraploid plants, and β_H = hexaploid plants growing in soils conditioned by hexaploid plants (Figure 1A).

The sum of these four values determines I_s as $\alpha_T - \beta_T - \alpha_H + \beta_H$:

$$\alpha_T - \beta_T - \alpha_H + \beta_H \quad (5)$$

Substituting in Equations 1–4 gives

$$I_s = [G(T)_\alpha - G(T)_0] - [G(T)_\beta - G(T)_0] - [G(H)_\alpha - G(H)_0] + [G(H)_\beta - G(H)_0],$$

which can be simplified as

$$I_s = G(T)_\alpha - G(T)_\beta - G(H)_\alpha + G(H)_\beta.$$

To test for distance-dependent effects, we examined the relationship of I_s across soils from the 2.1-m transect using a linear regression ($I_s \sim$ distance).

There are two scales to consider when examining I_s in our experiment, first as a single point estimate at each transect distance from the soil-conditioning plant, and second, as the slope of the average I_s line across the distance transect. The sign of I_s at any single sampling distance predicts whether the rare ploidy experiences a fitness disadvantage ($I_s > 0$) or advantage ($I_s < 0$) due to PSF. A significant non-zero slope of the average line indicates whether rare ploidy advantage/disadvantage is dependent on distance from the target soil-conditioning plant.

RESULTS

Plant performance

In total, 90% of cone-tainers had seeds that germinated (519 of 576), 84% of the seedlings survived to harvest (438 of 519) and 52% of the survivors had the expected response to the live inoculum (root colonization in 150 soils with live inoculum, no colonization in 79 soils with sterile inoculum). However, roots were colonized in approximately the same number of seedlings grown with live (150) and sterile inoculum (151) and was similar for both ploidies. Environmental contamination (i.e., root fungal colonization) occurred in 66% (151) of surviving plants grown in sterile inoculum (Appendix S2). Total dry biomass was used for calculating I_s and ranged from 0.011 g to 1.387 g. Biomass of tetraploid seedlings (0.49 g) did not differ significantly from that of 6*x* seedlings (0.52 g) ($F_{1,436} = 1.71$, $P = 0.28$). All seedlings allocated more biomass to roots (60%) than shoots (40%), independently of any soil microbe recruitment or the seed ploidy (Appendix S2). Overall, seedlings grown in live inoculum tended to have more root biomass (0.31 g) than seedlings grown in sterile inoculum (0.29 g), but their biomass did not differ significantly among each other ($F_{1,436} = 1.25$, $P = 0.27$). Mean root biomass was lower for seedlings grown in sterile inoculum collected near the soil-conditioning plant compared to seedlings grown in live inoculum collected near the same soil-conditioning plants, based on observations from Figure S2 in Appendix S2.

Ploidy-specific differences and plant–soil feedback

Soil microbes had strong effects on the biomass of seedlings grown in soils surrounding adult soil-conditioning plants of different co-occurring ploidies. Seedlings grown in soils (and with observed fungal root colonization) collected near (within ~0.8 m) different-ploidy soil-conditioning plants had 41% greater total biomass compared to seedlings grown in soils collected near same-ploidy soil-conditioning plants ($I_s = 0.307x - 0.253$, $R^2_{adj} = 0.713$, $P = 0.005$, Figure 3A). The positive fitness effects on seedlings of different ploidy from the adult soil-conditioning plant (i.e., 4*x* seedlings grown in soil from near 6*x* plants; 6*x* seedlings grown in soil from near 4*x* plants) decreased with distance, with different-ploidy seedlings no longer having a growth advantage by ~0.8 m from the adult soil-conditioning plant. More generally, seedlings of different ploidy from the adult soil-conditioning plant grown in microbe-conditioned soils (i.e., 4*x* seedlings in soils from near 6*x* plants; 6*x* seedlings grown in soil from near 4*x* plants), regardless of known root-associated fungi, had a weak fitness advantage (12% greater total biomass) when grown in soils from near an adult soil-conditioning plant of a different ploidy. This fitness advantage decreased with distance but was not statistically significant (live inoculum with and without colonization, $I_s = 0.159x - 0.176$, $R^2_{adj} = 0.237$, $P = 0.125$).

For soils with the sterilized inoculum, we observed no fitness advantage for seedlings grown in soils originating near adult soil-conditioning plants of a different ploidy (slope and intercept were not significantly different from 0, $I_s = -0.024x - 0.149$, $R^2_{adj} = -0.162$, $P = 0.881$; Figure 3C). Suspected environmental contamination, likewise, was not likely to have driven the distance-dependent fitness advantage/disadvantage (slope and intercept were not significantly different from 0, $I_s = -0.049x + 0.087$, $R^2_{adj} = -0.132$, $P = 0.683$, Figure 3D) experienced by seedlings of a different ploidy from the adult soil-conditioning plants.

DISCUSSION

This is the first time, to our knowledge, that evidence for a distance-dependent soil microbe-mediated fitness disadvantage has been described for two interacting ploidies in a mixed-ploidy population. Mixed-ploidy populations are predicted to be short-lived due to the reproductive disadvantage experienced by minority cytotypes (e.g., Levin, 1975; Anneberg et al., 2023). Yet, high-throughput flow cytometry and genomic approaches has enabled more documentation of mixed ploidy populations in natural populations (e.g., 47% of 32 *Andropogon gerardii*

populations exhibit mixed ploidy, McAllister et al., 2015; 33–43% of 1209 populations of *Tripleurospermum inodorum* exhibit mixed ploidy, Čertner et al., 2017; and 40% of 449 *Cystopteris fragilis* populations exhibit mixed ploidy, Hanušová et al., 2019). Research over the last several decades has shown that co-existence by plants differing in ploidy may be facilitated by disturbance (Čertner et al., 2022), phenological differences (Diallo et al., 2023), differences in size and spatial arrangement (Mráz et al., 2022), and differential herbivore attack (Münzbergová et al., 2015; O'Connor et al., 2019), among other phenotypic differences that may differentially alter niche exploitation. Recent work has also shown that high selfing rates and pronounced reproductive isolation can counter the effects of minority cytotype exclusion, resulting in long-term cytotype coexistence (Gaynor et al., 2023). Our findings demonstrate that plant–soil microbe feedbacks (PSF) operating over relatively small spatial scales (~2 m) may similarly counter the fitness disadvantages experienced by rare ploidies in mixed ploidy populations due to a lack of reproductively compatible mates and potentially facilitate the long-term maintenance of mixed-cytotype populations.

Seedlings grown in soils collected from near adult soil-conditioning plants of different ploidy (–0.1 to 0.75 m; Figure 3A, B) produced greater biomass than plants grown in soils collected farther from adult soil-conditioning plants of different ploidy. This difference in biomass growth resulted in I_s values that predict ploidy coexistence at close spatial scales (–0.1 to 0.75 m), whereas at larger spatial scales from the adult soil-conditioning plants (from ~1–2 m), exclusion of different-ploidy seedlings is predicted (Figure 2). The relationship between distance from adult soil-conditioning plants and I_s suggests there is a relatively narrow zone around adult plants (~0.8 m) in which seedlings of different ploidy experience a fitness advantage, consistent with the effects of root-zone-associated microbes. In a study of greasewood (*Adenostoma fasciculatum*), a shrub with broadly similar life-history traits to *L. tridentata* in the California chaparral ecosystem, soil bacterial biomass peaked at 0.1 m, root biomass peaked at 0.3 m, and soil fungal biomass peaked at 0.5 m from a focal plant (Klironomos et al., 1999). These, and prior findings of unique root-associated microbe assemblages of *L. tridentata* (Gerstner et al., 2023), are consistent with other studies on plant–soil-microbe feedbacks that play an important role in structuring plant communities (reviewed by Inderjit et al., 2021) and argue for further studies that characterize root-zone microbial associates at high spatial resolutions near target plants.

Interestingly, the strength of the relationship between I_s and distance from adult soil-conditioning plants not only increased with distance, but changed in sign from negative to positive indicating a shift from a fitness advantage to no fitness advantage. This pattern suggests PSF may facilitate tetraploid–hexaploid coexistence in the studied mixed-ploidy *L. tridentata* population through Janzen-Connell dynamics (e.g., negative distance dependence). The negative distance

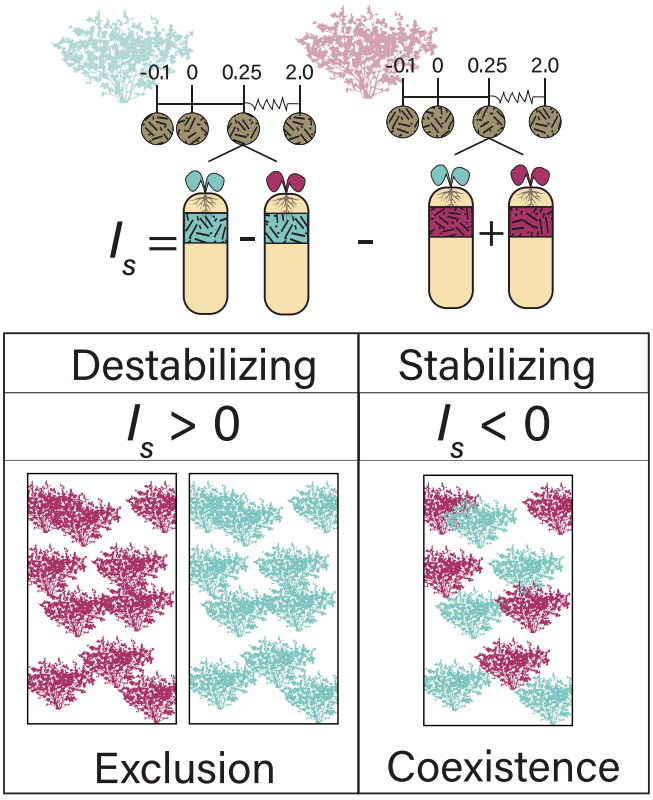

FIGURE 2 Conceptual framework linking plant–soil-microbe feedback with possible outcomes for rare ploidy disadvantage. The upper portion identifies the treatments necessary to calculate Bever's interaction coefficient (I_s); namely, a pair of plants must be used to calculate each value. Tetraploid: teal, hexaploidy, maroon. The lower portion identifies the primary interpretation for I_s: When I_s is positive, plant–soil feedback will not counter-balance rare ploidy disadvantage. When I_s is negative, plant–soil-microbe feedback will result in net stabilizing effects that may work against rare ploidy disadvantage and promote ploidy coexistence.

dependence suggests a hexaploid seed may have greater recruitment and growth success under and near a tetraploid plant than it would under or near a hexaploid plant. Similarly, a tetraploid seed may have greater recruitment and growth success under and near a hexaploid plant than it would under or near a tetraploid plant. In either case, the advantage experienced by seedlings of a different ploidy may be enough (in some cases) to offset the fitness disadvantage experienced by rare cytotypes due to a lack of reproductively compatible mates. Moreover, such PSF/Janzen-Connell dynamics may allow rare ploidies that disperse as seeds (via wind or animal) to become successfully established in previously single ploidy populations. Thus, at a regional scale, the effects of plant–soil-microbe feedbacks could result in a greater chance of hexaploid *L. tridentata* establishment in tetraploid populations or in tetraploid *L. tridentata* establishment in hexaploid populations, resulting in the broadening of mixed-ploidy contact zones. Indeed, the tetraploid–hexaploid contact zones in *L. tridentata* extend over several kilometers (Laport and Ramsey, 2015), consistent with our observations and the effects of PSF. However, it is not clear whether similar plant–soil-

microbe feedbacks operate in the diploid–tetraploid populations where the contact zones are apparently narrower (Laport and Ramsey, 2015). Further, it is unknown whether PSF contributes to the mixed-ploidy population dynamics in other species representing a range of life histories that also exhibit a range of spatial arrangements and ploidy composition (e.g., *Galax urceolata*, Burton and Husband, 2000; *Chamerion angustifolium*, Husband and Sabara, 2003; *Campanula rotundifolia*, Sutherland et al., 2018).

Spacing between individuals (i.e., shrubs) within *L. tridentata* populations (i.e., overdispersion of individuals) has long been posited to result from allelopathy or water and nutrient availability (Knipe and Herbel, 1966; Boyd and Brum, 1983; Brisson and Reynolds, 1994; Miller and Huenneke, 2000). While evidence suggests that such ecological interactions likely contribute to population structuring, our findings suggest another possible contributing factor: ploidy-specific soil microbe interactions with plants. One mechanism by which this may occur is the differential expression of defense genes in co-occurring ploidies in response to root-zone-associated microbes, as recently observed in diploid and tetraploid *Arabidopsis thaliana* in response to a model pathogen (*Pseudomonas syringae*, Mehlferber et al., 2022). The negative distance-dependent fitness outcomes (e.g., Janzen-Connell dynamics) we observed for seedlings grown in soil collected near a plant of the same ploidy would result in similar spatial patterning in both single- and mixed-ploidy *L. tridentata* populations. In single-ploidy populations, the decreased fitness of recruiting individuals in the soils near a plant of the same ploidy could result in successful recruitment only beyond ~1 m from the adult soil-conditioning plant. Similar effects on plant spacing would also be expected in mixed-ploidy populations, while facilitating cytotype co-occurrence due to the spatial heterogeneity of plant–soil-microbe feedbacks associated with where the seedlings of each ploidy have a fitness advantage or disadvantage.

Generally, ploidy-specific soil microbe interactions may structure mixed-ploidy populations by mechanisms other than ploidy-specific microbial pathogens and may be neither distance- nor density-dependent. Soil microbial communities may differ due to many factors, such as root morphologies, rooting depth, levels of root exudates (reviewed by Herms et al., 2022), canopy/gap structure (Kushwaha et al., 2021; Teachey et al., 2022) and leaf litter nutrient profile (Heděnec et al., 2023). Root exudates in *Solidago* (Asteraceae) have been shown to differ by ploidy (Wu et al., 2019) and root morphology and rooting depth in *Spartina* (Poaceae) have been shown to differ by ploidy and environment (Granse et al., 2022). These differences between ploidies may result in ploidy-specific soil microbe interactions with plants that may function to change the relative fitness relationship between ploidies and contribute to mixed-ploidy population persistence through space, time, and changing climate.

Prior tests of comparable distance-dependent plant–soil-microbe feedbacks (PSF) have focused on the interspecific population dynamics of several tree species making it difficult to draw comparisons to our study or make specific inferences about the consequences for ploidy co-occurrence. Generally, prior studies have identified similar patterns of PSF near the soil-conditioning plant that weaken with increasing distance (e.g., *Prunus serotina*, Packer and Clay, 2000; Reinhart and Clay, 2009; *Ormosia* spp., Liu et al., 2012, 2015). The magnitude of I_s values calculated for tetraploid and hexaploid *L. tridentata* are similar to those in prior studies on long-lived species ($I_s = 0$ to -0.5, McCarthy-Neumann and Ibáñez, 2013), as well as those focused on two interacting species in arid environments ($I_s = 0.25$ to -0.5; Reinhart, 2012; Chung et al., 2019a). One apparent pattern that does emerge is the scale varies at which a shift from a negative to positive I_s occurs around soil-conditioning plants. In tree species, shifts from negative to positive I_s occur between 2 and 30 m (Chung, 2023). For shrubs, or at least for *L. tridentata*, the shift from negative to positive I_s seems to occur at shorter distances (0.8–1 m; Figure 3A). This change in biomass with distance further supports the assertion that PSF arises from interactions with the root-associated microbiome at scales that are seemingly proportional to the size of plant root zones and has potentially significant consequences for the spatial dynamics of plant communities in a range of environments.

Caveats

Although our use of field-collected soils to assess plant–soil-microbe feedbacks is consistent with common experimental practices (Yan et al., 2022), the "gold standard" in PSF experiments is to condition soils in the greenhouse and then plant into the conditioned soils to ensure that the microbes present are a consequence of the soil-conditioning plant. *Larrea tridentata* are long-lived, deep-rooted shrubs that grow in very arid conditions, making it difficult to adequately mimic field conditions in the greenhouse. While our experimental setup appears to have captured important dynamics related to the early stages of seedling recruitment and growth (representing measures of fitness), follow-up studies are needed to better characterize the soil microbiomes of these plants. Doing so would help shed light on the identity of microbial constituents that may be responsible for plant–soil-microbe feedbacks, while helping to rule out other variables (e.g., other co-occurring soil microbes, persistent allelopathic chemicals) present in field-collected soils.

The limited spatial scale and single-time point of soil sampling in our approach may not adequately capture the dynamics of soil microbes. Soil microbial communities are known to vary with depth (reviewed by Naylor et al., 2022); thus, our sampling only near the soil surface may be appropriate for initial seedling recruitment but not for long-term fitness outcomes. It is also possible that the microbes that *L. tridentata* would encounter deeper underground could interact differently with the two co-occurring ploidies, potentially negating the initial seedling recruitment effects we observed. Further, sampling at only one time limits the

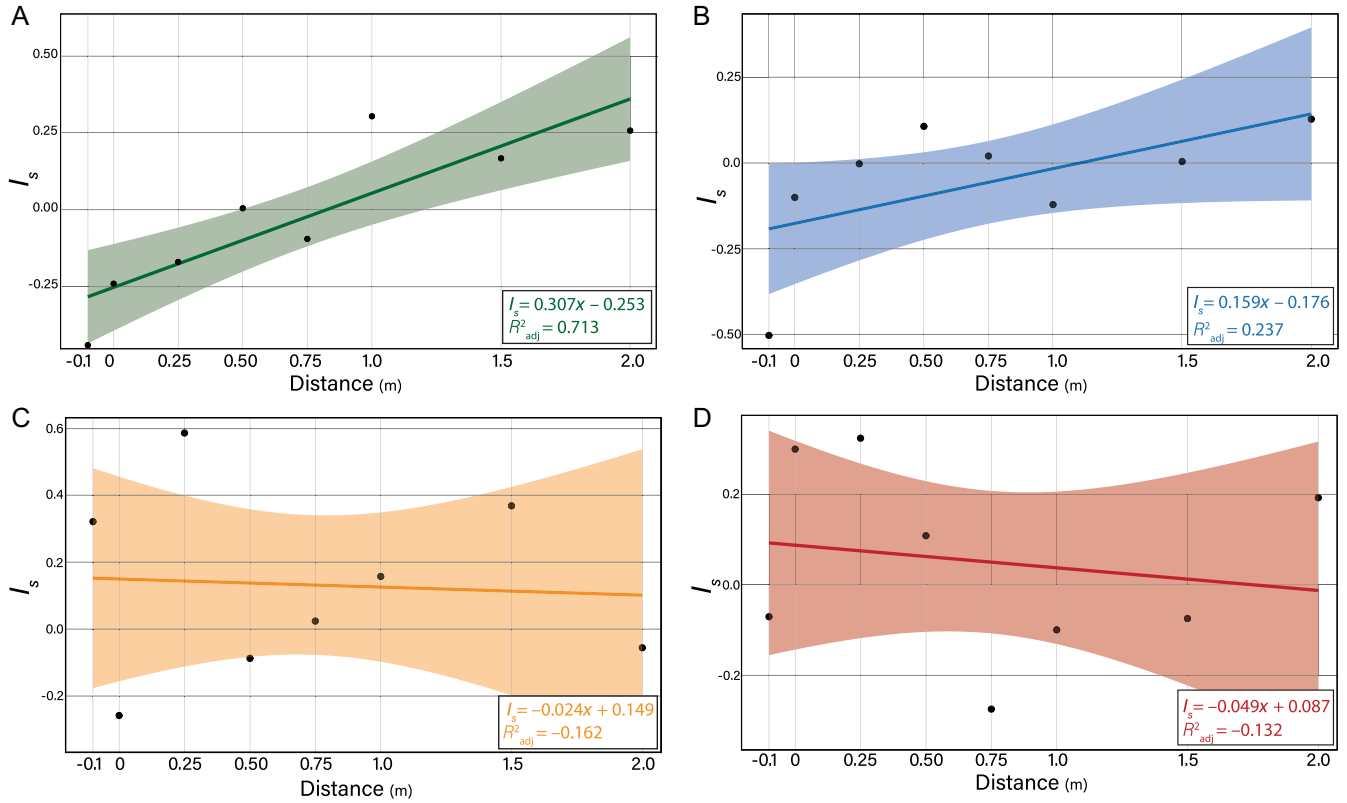

FIGURE 3 Average Bever's interaction coefficient (I_s) along distance transects from soil-conditioning plants showing the net effect of plant-soil microbes on the likelihood of cytotype co-occurrence. (A) Live inoculum with colonization: A significant positive relationship was observed across the transect, (I_s ~ distance, green line; 95% CI, shaded region; intercept = –0.253 [SE ± 0.072, P = 0.013], slope = 0.307 [SE ± 0.071, P = 0.005], R^2_{adj} = 0.713), showing a change in sign from negative to positive I_s values with increasing distance from the soil-conditioning plant. (B) All live inoculum: A nonsignificant positive relationship was fit by the linear model (I_s ~ distance, blue line; 95% CI, shaded region; intercept = –0.176 [SE ± 0.090, P = 0.099], slope = 0.159 [SE ± 0.089, P = 0.125], R^2_{adj} = 0.237). (C) Sterile inoculum without colonization: A nonsignificant weak negative relationship was observed across the transect (I_s ~ distance, yellow line; 95% CI, shaded region; intercept = 0.149 [SE ± 0.156, P = 0.374], slope = –0.024, [SE ± 0.155, P = 0.881], R^2_{adj} = –0.162). (D) Sterile inoculum with colonization: A nonsignificant weak negative relationship observed across the transect (I_s ~ distance, red line; 95% CI, shaded region; intercept = 0.087, [SE ± 0.118, P = 0.488], slope = –0.049, [SE ± 0.117, P = 0.683], R^2_{adj} = –0.132).

generality of our findings because seasonal changes in soil microbes could have a range of impacts on growth. Such temporal dynamics have been reported in *Salicornia*, where seasonal changes in rhizosphere fungal communities were observed through time (Gonçalves et al., 2022).

Our study supposes that current plant–soil microbe interactions are reflective of historical processes influencing cytotype co-existence, including post-polyploidization evolution and adaptation that may have shaped cytotype-specific associations. However, these associations, and any resulting niche divergence, may not mirror historical intraspecific interactions important during the development of tetraploid and hexaploid cytotypes. The life history of *L. tridentata* further complicates whether cytotype coexistence is facilitated by soil-microbe Janzen-Connell dynamics. Asexual reproduction (i.e., clonal reproduction) can ease the negative fitness consequences of minority cytotype exclusion and can promote cytotype coexistence. Similarly, phenotypic and physiological divergence among cytotypes in a polyploid complex may also contribute to cytotype-biased fitness advantages that could contribute to cytotype coexistence. Previous work in *L. tridentata* has documented such differences in reproductive (e.g., 6*x* have larger diameter pollen grains and longer stamens, pistils, and petals) and growth-related structures (e.g., 6*x* have longer, wider leaves; Laport et al., 2016) and abiotic niche differences (Laport et al., 2013). It is likely these are all cofactors that contribute to cytotype coexistence and are a rich system for further experimentation to tease apart which, if any, are the major contemporary drivers of cytotype coexistence. Plant biomass as a response metric estimating a component of fitness is reasonable for a 1-year growth experiment with a woody shrub (Younginger et al., 2017) but is complicated by ploidy. The gigas effect (Müntzing, 1936) could contribute to ploidy-specific biomass differences that arise from ploidy per se rather than the effects of soil microbes (Segraves, 2017). Further, it is unclear how ploidy-specific biomass differences would play out over long periods of time (like those experienced by long-lived species) where aboveground biomass may increase or decrease in response to environmental fluctuations (e.g., drought, wind damage). Our data, however, do not support a significant association between

total biomass and ploidy in *L. tridentata* seedlings, though this relationship may differ over the long lifespan of a typical *L. tridentata* plant, and other polyploid species may exhibit significant ploidy–biomass associations.

We observed a considerable degree of environmental soil-microbe contamination in our PSF experiment based on the fungal colonization of roots in the pots with sterile inoculum. Our pilot tests to culture any fungi present within the seeds indicated little signs of growth (Appendix S1); thus, the most probable source of contamination was airborne spores. The University of New Mexico greenhouse takes in outside air and conditions the temperature but does not filter out fine particulates. It is possible that the hyphae had greater success colonizing the initially sterile soil than the field-collected soil because of the lack of competitors. Additionally, contamination could have occurred during growth media or pot preparation in the greenhouse despite efforts to ensure sterile procedures. Without sequencing DNA from the soil and root-associated microorganisms, we cannot determine the identity of fungi in roots of sterile treatments; regardless, it is difficult to prevent contamination in a long-term growth experiment like ours, raising questions about the potential role of PSF from unintentionally introduced soil microorganisms in other long-term growth experiments. However, environmental contamination does not appear to have strongly affected the results of our experiment. The I_s values for cone-tainers containing sterilized growth media that exhibited colonization indicated no relationship between I_s and distance from the soil-conditioning plant (Figure 3D). This finding is encouraging, as it suggests that environmental contamination, which could also have affected live-inoculum pots, is not likely the underlying driver of the observed positive relationship between I_s and distance of soil origin from the soil-conditioning plant (Figure 3A, B) and that the patterns we observed are likely robust to environmental contamination.

Future directions

Given the ubiquity of plant–soil-microbe feedbacks among plant populations (Yan et al., 2022), it would be surprising if similar interactions were not occurring in other polyploid complexes. In follow-up work to test this idea, it would be prudent to include the effects of plant–plant competition (e.g., Thompson et al., 2015) allowing for the use of standardized frameworks of measuring niche and fitness differences due to ploidy-specific microbe associations (Kandlikar et al., 2019; Ke and Wan, 2020; Yan et al., 2022). Such standardization would permit comparisons of microbe-mediated niche and fitness differences between polyploid complexes. Future work should also endeavor to identify the microbes from field-collected soils, greenhouse soils and plant roots (see Chung et al., 2019b). Comparisons of microbe communities between these three environments could help expand our limited knowledge of the key players driving plant–soil-microbe feedbacks and how they vary with abiotic and biotic environments.

CONCLUSIONS

Without abiotic or biotic niche differences between ploidies, mixed-ploidy populations face a near certain fate: rare cytotype disadvantage and the ultimate exclusion of one cytotype. Here, we report the first evidence for plant–soil-microbe feedbacks as a biotic factor that may ease the fitness disadvantage of a rare cytotype by promoting niche differentiation over small spatial scales resulting in cytotype co-occurrence.

AUTHOR CONTRIBUTIONS

Leading (L), supporting (S), and equal (E) roles. Conceptualization: B.G. (L), R.L. (S), J.R. (E), and K.W. (E); data curation: B.G.; formal analysis: B.G. (L), J.R. (S), and K.W. (S); funding acquisition: B.G.; investigation: B.G. (L) and K.W. (S); methodology: B.G. (L), J.R. (S), and K.W. (S); project administration: B.G.; visualization: B.G.; writing original draft: B.G. (L), R.L. (S), J.R. (S), and K.W. (E); review and editing: B.G. (L), R.L. (E), J.R. (E), and K.W. (E).

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DATA AVAILABILITY STATEMENT

Data supporting this article can be found in Dryad: https://doi.org/10.5061/dryad.bk3j9kdk7 (Gerstner et al., 2024).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Examining seed stock for presence of fungal growth.

Appendix S2. Plant performance analysis, treatment effectiveness, and ratio of root to shoot biomass.

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