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Genetic Relatedness Can Alter the Strength of Plant-Soil Feedbacks

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GENETIC RELATEDNESS CAN ALTER THE STRENGTH OF PLANT-SOIL FEEDBACKS

by

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ABSTRACT

Intraspecific variation may play a key role in understanding the relationships between plants and their interactions with soil microbial communities. The effects that soil-microbes have on individuals can venerate variation across individuals in their responsiveness. I explored how relatedness alters plant-soil feedbacks in established *Solidago altissima* clones grown in a common garden. Seedlings of known parentage were inoculated with soils from the maternal, paternal, or unrelated clones and compared to autoclaved control inocula. I found that the soil inocula generated from *S. altissima* had an overall negative effect of seedling biomass. Furthermore, seedlings inoculated with maternal or paternal soils experienced a larger negative effect than seedlings inoculated with unrelated soils. Relatedness to the culturing plant strongly negatively affected seedling growth, whereas unrelated soils were slightly more variable but less negative. My data argue that genetic relatedness represents a largely unexplored source of heterogeneity in plant-soil feedbacks.

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INTRODUCTION

Soil microbes play diverse roles in plant communities, altering plant community composition and productivity (Horner-Devine et al., 2003). Many plant species alter their surrounding soil communities resulting in plant-soil feedback (Bever, 1994) that may then alter plant fitness, species abundances, and long-term population persistence (van der Putten et al., 2013). Individual plant genotypes may influence the community composition of soil microbes differently due to intraspecific variation in microbial associations and plant traits (Paul & Clark, 1996). For this reason, changes in plant traits, even within a species, can change soil microbial communities and alter plant-microbe interactions (Schweitzer et al., 2008).

Intraspecific trait variation has become a major research theme in ecology (Violle et al., 2012) because of its importance in altering trait-mediated interactions. Intraspecific variation affects several ecological processes such as plant-herbivore interactions, food web structures, and arthropod diversity, (Ruel & Ayres, 1999: Bolnick et al., 2011; Crutsinger et al., 2006), and may determine plant species interactions and community responses to those interactions (Moran et al., 2015). While trait variation has been explored extensively, the importance of intraspecific variation in plant-microbe interactions has been much less investigated.

Intraspecific variation in plant chemistry can alter plant defense against predators, pathogens, and parasites (Glassmire et al., 2016). It should then be expected that an individual plant's genotype or traits may also produce intraspecific variation in plant-microbe interactions (Schweitzer et al., 2008). Such variation may represent an important source of heterogeneity in ecological systems (Peacher & Meiners, 2020). As the volume of soil influenced by a single genotype is much larger in clonal plant species, intraspecific variation in plant microbe interactions should have a much larger spatial extent than non-clonal species (de Witte &

Stocklin, 2010). As a result, intraspecific variation in clonal plants may impact the microbial interactions of a larger suite of associated plant species.

Key to an organisms' success is the expression of traits and their contribution to fitness, a combination of genetic variation and phenotypic plasticity in response to local conditions. An additional source of variation may be transferred through non-genetic, parental effects. Seed characteristics, embryo development, and the early growth are greatly influenced by maternal effects (Mazer, 1987a, b; Roach & Wulff, 1987). When in competition, maternal effects can still be seen in mature plants (Schmid & Dolt, 1994) illustrating their potential, long-term impacts. Maternal effects responsible for seed development and fitness can be altered by soil resource levels, temperature, drought, plant hormones, and even paternal effects (Wilson & Price, 1980; Parrish & Bazzaz, 1985; Marshall et al., 1986; Stratton, 1989; Schmid & Dolt, 1994; Roach &Wulff, 1987).

Paternal effects may also play a role in determining offspring phenotype, but fewer studies have observed these effects (Antonovics & Schmitt, 1986; Roach & Wulff, 1987; Lyons et al., 1989). It is thought that maternal effects play a larger role in seed development and juvenile plants than paternal effects (Roach & Wulff, 1987; Mazer & Gorchov, 1996; Schmid & Dolt, 1994). Paternal effects can also play a role in seed development but are often overlooked because of the strength of maternal effects (Roach & Wulff, 1987). However, paternal effects play a large role in determining offspring pollen production (Young & Stanton, 1990; Delph et al., 1997; Aizen & Raffaele, 1998). Although rarely compared, differences between the strength of maternal and paternal may be quite small (Galloway, 2001).

An extensive study by Schmid & Dolt (1994) found the soil environment in which *S. altissima* (Syn. *S. canadensis*) grew influenced offspring phenotype. Seedling biomass and

height was greater when the maternal plants were grown in sand rather than in soil with no effect of maternal and paternal genotype. Plants grown in sand produced heavier seeds than individuals grown in soil. However, offspring produced from paternal plants grown in sand had a lower mass of stems and leaves. However, there was no indication that the paternal or maternal contributions differed (Schmid & Dolt, 1994).

Though much work has been done on maternal and paternal effects in plants, there is little information on how relatedness may alter plant-soil microbe interactions. This study aims to determine if the microbial community of the maternal and paternal parents influences plant-microbe interactions of their offspring. I will specifically use *Solidago altissima* to assess this as the species' potential for maternal and paternal effects and its responsivity to soil microbes have been confirmed.

In a previous study of a common garden 24 *Solidago canadensis* clones, significant intraspecific variation was found in microbial feedbacks on unrelated *S. altissima* seedlings (Foster, Unpublished thesis). In my study, I determined whether plant relatedness to the genotype that generated the soil microbial community mitigated their impact on plant performance. To do this, I used hand pollinations of the clones in that common garden to generate seedlings of known parentage. The seedlings produced by these crosses were inoculated with either microbes from the maternal clone, paternal clone, all unrelated clones, or sterile soil. I used this experiment to determine (1) if the relatedness of a seedling to a conditioning plant effects the plants soil feedback and (2) if seedling interactions differ when growing in soil inoculated with microbes representing the maternal or paternal environments.

METHODS

Study species. *Solidago altissima* (Syn *S. canadensis*) is a perennial forb native to Eastern North America commonly be found in old fields and open areas such as prairies (Werner et al., 1980; Uesugi et al., 2019). The herbaceous plant forms a pyramidal inflorescence which contains numerous small bright yellow flowers that open uniformly (Weber, 2000; Weber & Jacobs, 2005). The species first colonizes new habitats by seeds and, once established, spreads clonally via rhizomes (Schmid et al., 1988). Initial populations contain many genetic individuals, but this diversity is lost during clonal expansion and sorting (Hartnett & Bazzaz, 1985). *Solidago altissima* is self-incompatible and must be pollinated by a different genotype to produce viable seeds (Taylor & Hastings, 2005; Davis, et al., 2004). *Solidago altissima* is an aggressive invader in many parts of Europe (Schmid & Dolt, 1994) and Asia, but less successful in Australia where sufficient genotypes for reproduction are lacking (Uesugi et al., 2020).

Solidago altissima has been studied extensively for its chemical ecology (Uesugi et al., 2019; Zhang et al., 2007; Heath et al., 2014) plant-microbe interactions (Awaydul et al. 2018; Peacher & Meiners, 2020; Dong et al., 2021; Foster et al., Submitted), and parental effects (Schmid & Dolt 1994), making it a useful model organism to address parental effects on plant-microbe interactions.

Common garden. Rhizomes/stem segments from twenty-four genets of *Solidago altissima* were collected from Douglas-Hart Nature Center (Mattoon, IL 39° 29’ N; 88° 17’ W) in the spring of 2014. The area where the rhizomes were collected was used for row crop agriculture three years prior to collection. Following agricultural use, the area was restored to prairie but *S. altissima* was not part of the initial seeding. Thus, all the *S. altissima* genotypes collected represent natural

colonization's from the surrounding area. To ensure genetic uniformity of the genotypes collected, the rhizome/stem segments were collected within 0.5 meters making sure the patches were isolated (> 2.0 m) and distinct from others (Foster et al., 2020 unpublished).

The site selected for the common garden was a level piece of land in Clark County, IL (39 19' N; 87 55' W). In 2013 the land was used to grow corn. The following spring, each genotype was planted into a 1.5 m x 1.5 m plot enclosed with aluminum flashing buried 15 cm to prevent rhizome spread. Each plot was spaced 2 m from each other and the area between mown regularly. In each plot, 5 ramets from each genotype were planted with one in each corner and one in the center. Flowering heads were removed from all stems during the first two years to prevent seed set and allow clones to become established without colonization of new genotypes. However, other species were allowed to naturally colonize to assess clone's competitive ability (Foster, Unpublished Thesis 2020). Starting in the spring of 2020, plots were weeded to remove all other plants and to generate genotype-specific soil microbial communities.

Hand pollinations. During the end of September and early October, crosses between pairs of clones of *S. altissima* were made via hand pollinations to produce seeds of known parentage (Schmid & Dolt 1994; Table 1). To prevent external pollination, pollinator bags made of fine white polyester mesh were tied onto three different inflorescences on each clone, prior to flower maturation. One inflorescence was used as the pollen donor, one as the seed producer, and the third remained unopened to assess the effectiveness of pollinator exclusion. The unopened inflorescence was paired with a similarly sized inflorescence that remained open to pollinators. Clones to be crossed were selected based on shared bloom time.

To pollinate the seed-producing inflorescence, the pollinator exclusion bag was removed from the pollen donor, the inflorescence removed, rubbed across the seed-producing inflorescence, and re-bagged until seed set (Figure 1). Bags that blew off or inflorescence stems that broke with the pollination bags during high winds resulted in the loss of many treatments. Crosses were only assessed if the pollinator exclusion bags remained on the seed producer, pollen donor, and third inflorescence. If a bag was removed at any time during the experiment, other than for hand pollinations, seeds produced from that clone were not used in the study. For simplicity, I refer to the seedlings from each cross by their maternal clone identity.

Greenhouse assay. On November 5, 2020, all inflorescences were harvested to collect seeds. The total fruit mass was removed from stem tissue and the biomass measured. On November 20, 2020, seeds from the hand pollinated clones were placed in plastic bags and stratified in a cool-moist environment (5 ºC) for 60 days to break seed dormancy.

On January 21, 2021, approximately 1 L of soil was collected from the top 10 cm of each plot in an area where *S. altissima* was the densest. Collecting supplies were washed and sterilized with a 5% bleach solution after each use. The soil was sifted to remove plant fragments and debris, and to homogenize the inocula. All processing materials were washed and sterilized with a 5% bleach solution and allowed to dry between clones. Once the inoculum was sifted, half was stored at 5 ºC to be used as live inoculum and half was autoclaved (120 °C for 20 minutes at 0.07 MPa) to use as sterilized inoculum.

January 25, 2021, 24 trays were filled with a moistened, soilless media (Pro Mix BX, Premier Horticulture, Quakertown, PA, USA) and laid out in the greenhouse. The soilless media used was not sterilized; however, it did not contain any plant-specific microbes and would be

uniform across clones. Seeds that were produced via hand pollinations were grown in these trays for three weeks. After three weeks, reproduction was evaluated and successful crosses were used in an inoculation experiment.

Seedlings from each cross were grown in live and sterilized inoculant from the maternal parent, paternal parent, and unrelated clones. Seedlings in unrelated inocula were grown in each of the 22 clones not used in the cross. Replication in related soils was 20 individuals for each parent and sterilization combination, for a total of 992 plants (Figure 1).

Seedlings from each cross were grown in inoculated cone-tainers (164 mL, Stuewe & Sons, Inc., Tangent, OR, USA). Two-thirds of each cone-tainer was filled with the same soilless potting medium that the seeds were started in. Then, 10 mL (approx. 6% volume) of one inoculum was incorporated into the top 2 cm of this medium and capped with 2 cm of the potting mix. This volume of inoculum should be sufficient to represent the majority of the natural soil biota (Howard et al., 2017) and should minimize abiotic differences across inocula.

Seedlings were transplanted from germination trays into the top 2 cm layer of potting medium to allow roots to grown downward into the inoculum layer. Racks of cone-tainers were placed on a single greenhouse bench and watered as necessary. All seedlings from the same maternal clone were kept together to minimize variation due to environmental differences. Seedlings that died within the first 10 days were replaced; seedlings that died after this resulted in a smaller sample size. After 60 days, the above- and belowground biomasses were harvested, dried at 60 °C for 4 days, and weighed.

Statistical analysis. To test if pollinator bags effectively prevented external pollination, a Wilcox test was ran using data from the seed biomass of the hand pollinated individuals and the

pollinator excluded individuals. Initial analyses used total seedling biomass (Appendix 1), but this analysis was heavily influenced by seedling vigor. To center the data appropriately, I calculated ln-transformed response ratios with each seedling scaled to its appropriate autoclaved control. This approach is widely used in similar studies (Hedges et al., 1999; Kempel et al., 2018) and should account for any abiotic differences among clones. Initial analysis of a full factorial ANOVA (Clone × relatedness × sterilization) of response ratios was followed with a 2-way ANOVA for each clone separately with Tukey's Post Hoc tests. Meeting of analytical assumptions was verified by examining the residuals. All analyses used R version 3.6.1 (R Foundation for Statistical Computing).

Table 1. Reciprocal crosses of *Solidago altissima* made by hand pollinations by each date. Seedlings used within the inoculation experiment are indicated with *.

September 20, 2020	
Male	Female
2	10*
10	2
4	11
11	4*
20	24
24	20
September 26, 2020	
3	19
19	3*
6	12*
12	6
7	17
17	7*
8	18*
18	8
14	22*
22	14
13	15
15	13
October 3, 2020	
1	21
21	1*
5	23
23	5
9	16
16	9

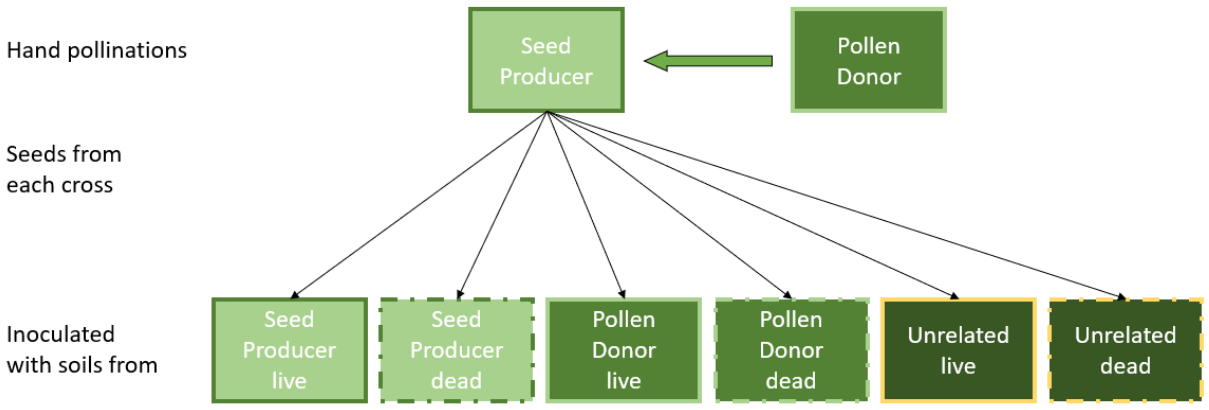

Figure 1. Experimental design depicting the crosses and the soil inocula seedlings were grown in.

RESULTS

Hand pollinated individuals produced much more reproductive mass (seeds and fruit) than individuals with pollinators excluded (~75% reduction; Wilcoxon value=124; df=15; P=0.0232). Large numbers of crosses were damaged by a period of heavy winds, precluding the use of reciprocal crosses in the experiment. The number of seedlings produced from the crosses varied dramatically across clones (Figure 2). Eight clones that produced more than 100 seedlings after the first two weeks were used for the greenhouse experiment; numbers were insufficient for other crosses.

All experimental factors, clone, sterilization, and relationship, as well as all interactions were significant in the full 3-way ANOVA of the response ratios (Table 2). Live soils consistently reduced the performance of *S. altissima* seedlings. When data were analyzed for each clone separately (Table 3), variation in their responses to experimental treatments became clear. For half of the crosses (Clones 1, 10, 12, and 18), live soil from unrelated clones had a less negative impact on plant growth than either live parental soil (Figure 3). Two clones (Clones 3 and 22) had the opposite result, with plant performance worse in unrelated microbial soils than at least one of the parental soils. Two clones (Clones 4 and 7) did not respond to relatedness alone or its interaction with sterilization (Figure 3).

The overall response was that the clones grown in unrelated live soil had a less negative effect on plant growth than the parental soil microbes. Overall, maternal and paternal soil microbial communities affected the seedling growth equally, not differing in impact experiment-wide (Figure 4).

Table 2. Influence of clone identity, sterilization (live vs. autoclaved) and relatedness (maternal, paternal, or unrelated) on the performance of *S. altissima*. Analysis based on ln-transformed response ratios of each treatment relative to the appropriate control. Significant p-values indicated in bold.

	df	MS	F	P	R^2
Clone	7	27.58	34.14	**<0.0001**	0.4266
Sterilization	1	273.70	338.85	**<0.0001**	
Relatedness	2	9.90	12.25	**<0.0001**	
Clone*Sterilization	7	10.94	13.54	**<0.0001**	
Clone × Relatedness	14	4.30	5.32	**<0.0001**	
Sterilization × Relatedness	2	8.44	10.44	**<0.0001**	
Clone × Sterilization × Relatedness	14	4.37	5.41	**<0.0001**	
Residuals	836	0.81			

Table 3. Response of each cross to the different factors tested with a two-way ANOVA using the ln-transformed response ratios. Significant p-values indicated in bold.

	df	MS	F	P	R^2
Clone 1					0.1538
Relatedness	2	2.01	3.11	**0.0485**	
Sterilization	1	9.67	14.95	**0.0002**	
Relatedness × Sterilization	2	1.59	2.46	0.0934	
Residuals	111	0.65			
Clone 3					0.1589
Relatedness	2	5.29	3.47	**0.0352**	
Sterilization	1	20.98	13.73	**0.0004**	
Relatedness ×Sterilization	2	2.47	1.62	0.2039	
Residuals	95	1.53			
Clone 4					0.0142
Relatedness	2	0.03	0.02	0.9815	
Sterilization	1	1.11	0.78	0.3807	
Relatedness ×Sterilization	2	0.12	0.08	0.9227	
Residuals	68	1.43			
Clone 7					0.3563
Relatedness	2	0.19	0.24	0.7898	
Sterilization	1	43.43	55.18	<**0.0001**	
Relatedness X Sterilization	2	1.19	1.51	0.2266	
Residuals	106	0.79			

Clone 10					0.3654
Relatedness	2	7.47	11.29	<**0.0001**	
Sterilization	1	10.46	15.82	**0.0001**	
Relatedness X Sterilization	2	9.20	13.91	<**0.0001**	
Residuals	115	0.66			
Clone 12					0.7570
Relatedness	2	16.57	33.89	<**0.0001**	
Sterilization	1	109.76	224.51	<**0.0001**	
Relatedness X Sterilization	2	16.88	34.53	<**0.0001**	
Residuals	116	0.49			
Clone 18					0.3610
Relatedness	2	2.45	3.28	**0.0413**	
Sterilization	1	34.35	46.02	<**0.0001**	
Relatedness X Sterilization	2	3.99	5.34	**0.0061**	
Residuals	112	0.75			
Clone 22					0.6944
Relatedness	2	4.85	8.92	**0.0003**	
Sterilization	1	122.73	225.62	<**0.0001**	
Relatedness X Sterilization	2	3.61	6.64	**0.0019**	
Residuals	113	0.54			

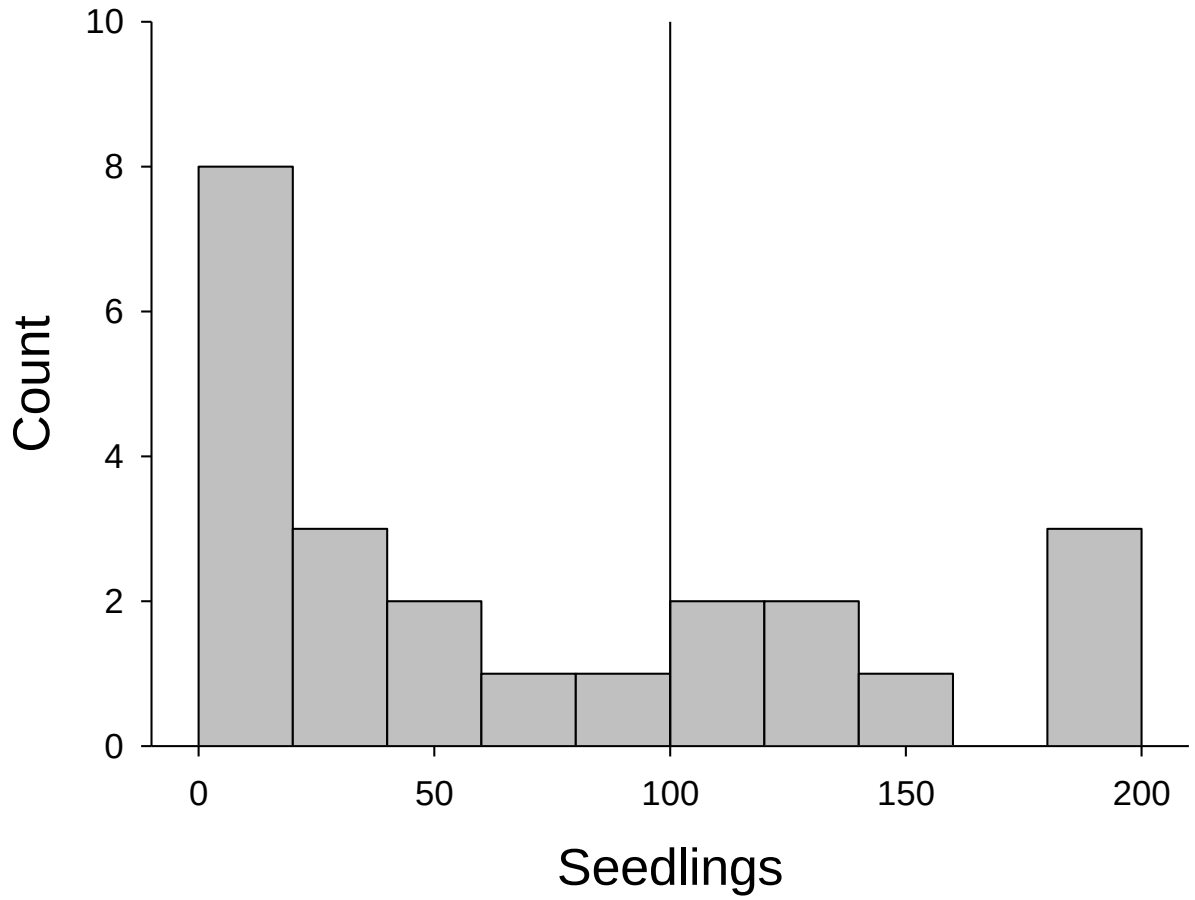

Figure 2. Frequency of seedling produced via hand pollinations. The crosses to the right of the reference line were those used in this study.

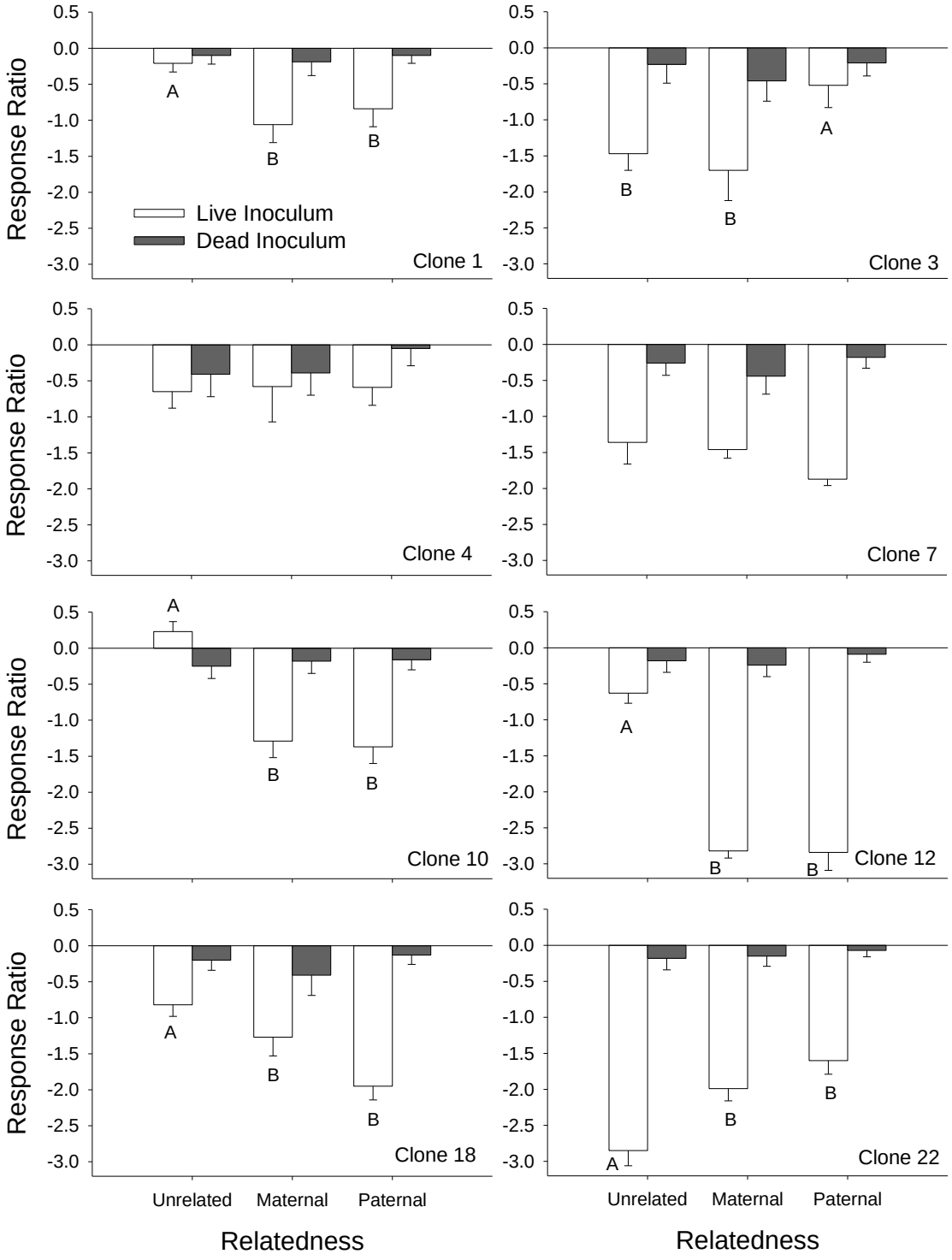

Figure 3. Response ratios of seedling growth for each individual clone in response to relatedness and soil sterilization. Data plotted are means ± 1 SE. Letters indicate significance in a Tukey's HSD test response ratio for each clone/soil are calculated relative to the matching sterilized control soil.

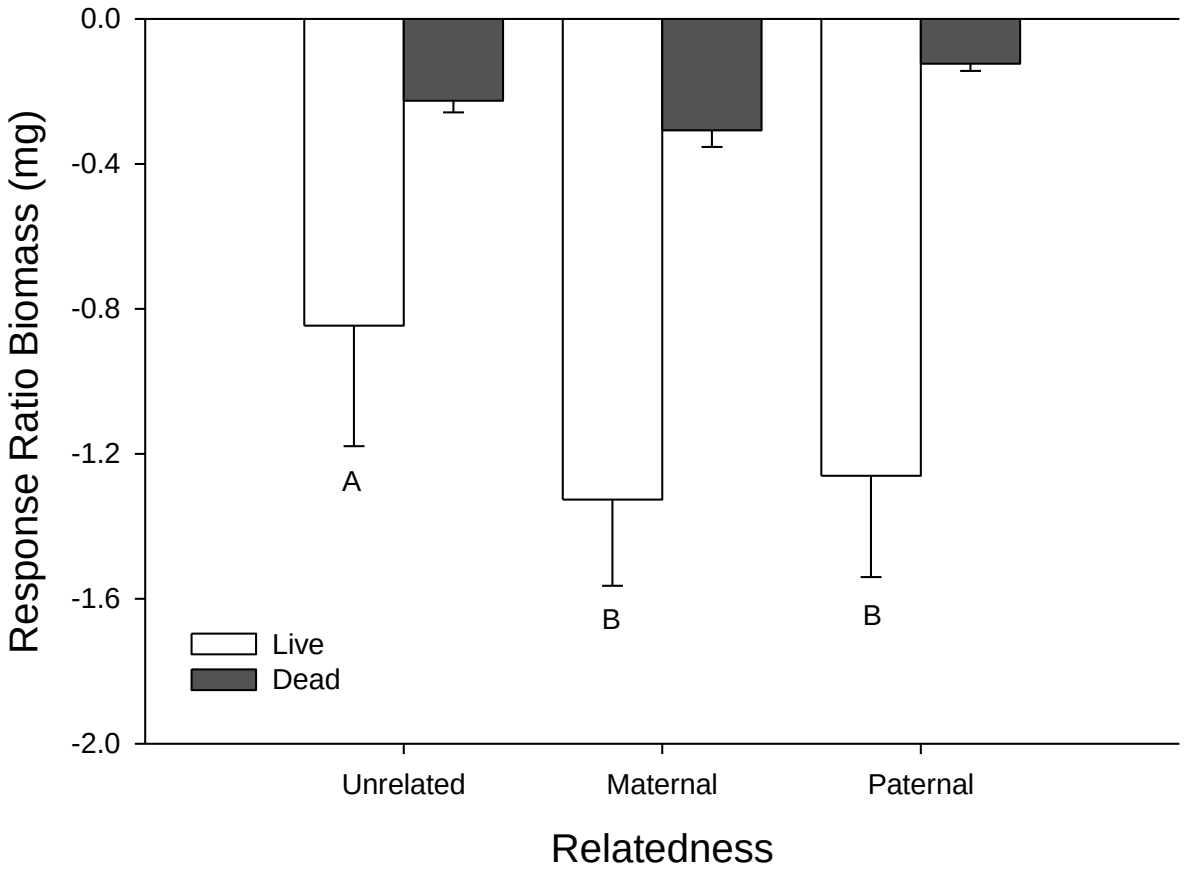

Figure 4. Overall response ratios of the crosses used in the greenhouse experiment. Data plotted are means ± 1 SE. Letters indicate significance in a Tukey's HSD test. Response ratios for each clone/soil are calculated relative to the matching sterilized control soil.

DISCUSSION

Solidago altissima is a self-incompatible species that requires crossing of two genetically different individuals via insect pollination (Gross & Werner, 1983). This self-incompatibility is responsible for strong Allee effects in many *S. altissima* populations in Australia (Uesugi et al., 2020), resulting in low seed set. In many of the crosses in this experiment, I observed either complete reproductive failure, or dramatically reduced reproduction. It is likely that several crosses made during this experiment did not produce many viable seeds because they were too closely related or were otherwise incompatible. Successful crosses produced many seedlings that overall grew well during the experiment, although variation occurred in biomass production with some (seedlings of Clone 7) having consistently poor growth, regardless of experimental treatment.

The plant-soil microbe interactions in this experiment were overall negative across all live soil inocula. These findings are similar to results of other studies finding that seedlings grown in local soils experienced inhibitory effects of plant-microbe interactions (Rasmussen et al., 2019; Kulmatiski et al., 2008). Such inhibitory plant-microbe interactions tend to promote turnover and facilitate diversity by preventing single species from dominating the community (Kulmatiski et al., 2008; Kardol et al., 2006). However, as *S. altissima* expands clonally once established, these effects do not seem sufficient to prevent long-term dominance of the species, particularly in successional communities (Howard et al., 2020).

Relatedness of the seedling to the plants that cultured the soil microbial community greatly influenced the direction and magnitude of plant-microbe interactions. Goldenrod soils produced by clones not directly related to the seedlings generally have a less negative effect on plant growth than parental soils. Soil microbes have been linked to certain genotypes. Therefore,

the antagonistic microbes that are present in the parental soils may not be present in unrelated soils that is why we are seeing lesser negative effects in unrelated soils. Overall, plants grown in live unrelated soils were less negatively affected than plants grown in parental soils. The antagonistic tendencies of the soil microbe communities from related plants represents an extension of Connell-Janzen dynamics (Conell, 1971; Janzen, 1970; Liu et al., 2015) to the individual genotype level. Similarly, this effect would favor the establishment of non-kin over kin if the clone were to die.

There was great variation among crosses in their responsiveness to soil microbial communities. Seedlings of six of the eight clones responded to relatedness of the soil microbial community, and the other two were non-responsive. The two crosses that were non-responsive to the soil microbial treatments were seedlings of Clones 4 and 7. Clone 4 seedlings grew reasonably well in all three inocula types with little no impact of inocula sterilization. However, all Clone 7 seedlings grew poorly, gaining little biomass over the experiment and only responded to inocula sterilization suggesting a poor-quality cross. The non- responsiveness of seedlings of Clone 4 may be due to a lack of genotypically specific microbes in the soil sample whether they are beneficial (Gehring et al., 2017; Wang et al., 2019) or antagonistic (Liu et al., 2015; Eck et al., 2019).

Maternal and paternal soil microbial communities typically affected seedling growth to the same degree, resulting in an equivalent reduction in plant growth with two exceptions. Clone 3 seedlings grew significantly worse in maternal soils than paternal soils. Seedlings of Clone 22 grown in unrelated soils did worse than those that were grown in either parental soil. Soil microbes associated with these *S. altissima* clones vary greatly in their impacts on plant performance (Foster et al, Submitted). The soil microbial community of Clone 3 was

particularly antagonistic to *S. altissima* growth in that study, so poor performance in that inoculum is not surprising.

The four remaining clones that responded significantly (50% overall) showed that plants growing in unrelated soils were not as negatively affected by the soil microbes as much as the plants that were growing in either parental soil. In these clones, the maternal and paternal effects were equivalent. Because maternal and paternal effects were overall equivalent in strength, it suggests that there was genetically determined variation in seedling interactions with soil microbes. Maternal effects influence many aspects of the plant's offspring including seed production and germination, leaf production, early growth of seedlings (Roach & Wulff, 1987; Helenurm & Schaal, 1996) and even later development (Schmid & Dolt, 1994). The majority of these influences in offspring are generated by the maternal environment (Schuler & Orrock, 2012), here the soil microbial community. It is thought that maternal effects evolved to be beneficial to the seedlings preparing seedlings for environments similar to the maternal plant (Wolf & Wade, 2009; Schuler & Orrock, 2012), but this was not the case here. Not much research has been done on how soil-microbial communities influence paternal effects (Mazer & Gorchov, 1996). Overall, my results argue that maternal and paternal effects mediate by soil biota are equivalent (Galloway, 2001).

My experimental design should have minimized the contribution of abiotic differences to experimental results. The use of small amounts of inocula would have contributed minor amounts of soil nutrients or allelochemicals, even in autoclaved inocula. I also documented changes relative to the appropriate autoclaved inocula to statistically control for any abiotic changes that did occur. As the genetic relationships among the *S. altissima* genotypes in the common garden have not been verified, unrelated inocula likely contain a range of genetic

distances, causing less prominent effects on the seedlings (Liu et al., 2015). Lastly, the composition of the soil microbial communities have not been verified, preventing direct interpretation of the microbial effects.

Plant interactions with soil microbial communities have become appreciated as a primary driver of plant community structure and dynamics (Kulmatiski & Kardol, 2008). Variation across species relates to the abundances of species in communities (Kuiters, 2013), though this effect is often weak (Reinhart, Bauer, McCarthy-Neumann, et al., 2021). Phylogenetic relatedness of target plant species to the culturing species may control these effects, with more closely related species typically having similar responses to the same soil microbial community (Mehrabi & Tuck, 2014; Wandrag et al., 2020). My results expand this effect to the within-population scale (Liu et al., 2015), suggesting an additional source of biotically-generated heterogeneity that operates within plant communities. As intraspecific variation has become critical in functional ecology (Violle et al., 2012; Ruel & Ayres, 1999; Bolnick et al., 2011; Schindler et al., 2010; Crutsinger et al., 2006; Bischoff et al., 2010), it may also be important in understanding the role of soil microbes in determining plant population dynamics.

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APPENDIX A. BIOMASS ANALYSIS OF EXPERIMENTAL DATA

Table A1. Influence of clone identity, sterilization (live vs. autoclaved) and relatedness (maternal, paternal, or unrelated) on the performance of *S. altissima*. Analysis based on log-transformed total biomass of each treatment relative to the appropriate control. Significant p-values indicated in bold.

	df	MS	F	P	R^2
Clone	7	13.12	86.10	**<0.0001**	0.5708
Sterilization	1	50.87	333.90	**<0.0001**	
Relatedness	2	1.97	12.90	**<0.0001**	
Clone*Sterilization	7	2.05	13.48	**<0.0001**	
Clone × Relatedness	14	0.74	4.85	**<0.0001**	
Sterilization × Relatedness	2	1.59	10.44	**<0.0001**	
Clone × Sterilization × Relatedness	14	0.83	5.41	**<0.0001**	
Residuals	836	0.15			

Table A2. Response of each cross to the different factors tested with a two-way ANOVA using the log-transformed total biomasses. Significant p-values indicated in bold.

	df	MS	F	P	R^2
Clone 1					0.1964
Relatedness	2	0.44	3.64	**0.0296**	
Sterilization	1	1.82	14.95	**0.0002**	
Relatedness × Sterilization	2	0.30	2.46	0.0903	
Residuals	111	0.12			
Clone 3					0.2517
Relatedness	2	2.16	7.49	**0.0010**	
Sterilization	1	3.96	13.73	**0.0004**	
Relatedness ×Sterilization	2	0.47	1.62	0.2039	
Residuals	95	0.29			
Clone 4					0.0193
Relatedness	2	0.05	0.20	0.8203	
Sterilization	1	0.21	0.78	0.3807	
Relatedness ×Sterilization	2	0.02	0.08	0.9227	
Residuals	68	0.27			
Clone 7					0.3581
Relatedness	2	0.07	0.47	0.6239	
Sterilization	1	8.19	55.18	<**0.0001**	
Relatedness X Sterilization	2	0.22	1.51	0.2266	
Residuals	106	0.15			

Clone 10					0.2925
Relatedness	2	0.24	1.95	0.1467	
Sterilization	1	1.97	15.82	**0.0001**	
Relatedness X Sterilization	2	1.74	13.91	<**0.0001**	
Residuals	115	0.12			
Clone 12					0.7338
Relatedness	2	1.20	13.07	<**0.0001**	
Sterilization	1	20.70	224.51	<**0.0001**	
Relatedness X Sterilization	2	3.18	34.53	<**0.0001**	
Residuals	116	0.09			
Clone 18					0.4359
Relatedness	2	2.10	14.92	<**0.0001**	
Sterilization	1	6.48	46.02	<**0.0001**	
Relatedness X Sterilization	2	0.75	5.34	**0.0061**	
Residuals	112	0.14			
Clone 22					0.6831
Relatedness	2	0.2430	2.37	0.0983	
Sterilization	1	23.15	225.62	<**0.0001**	
Relatedness X Sterilization	2	0.68	6.64	**0.0019**	
Residuals	113	0.10			

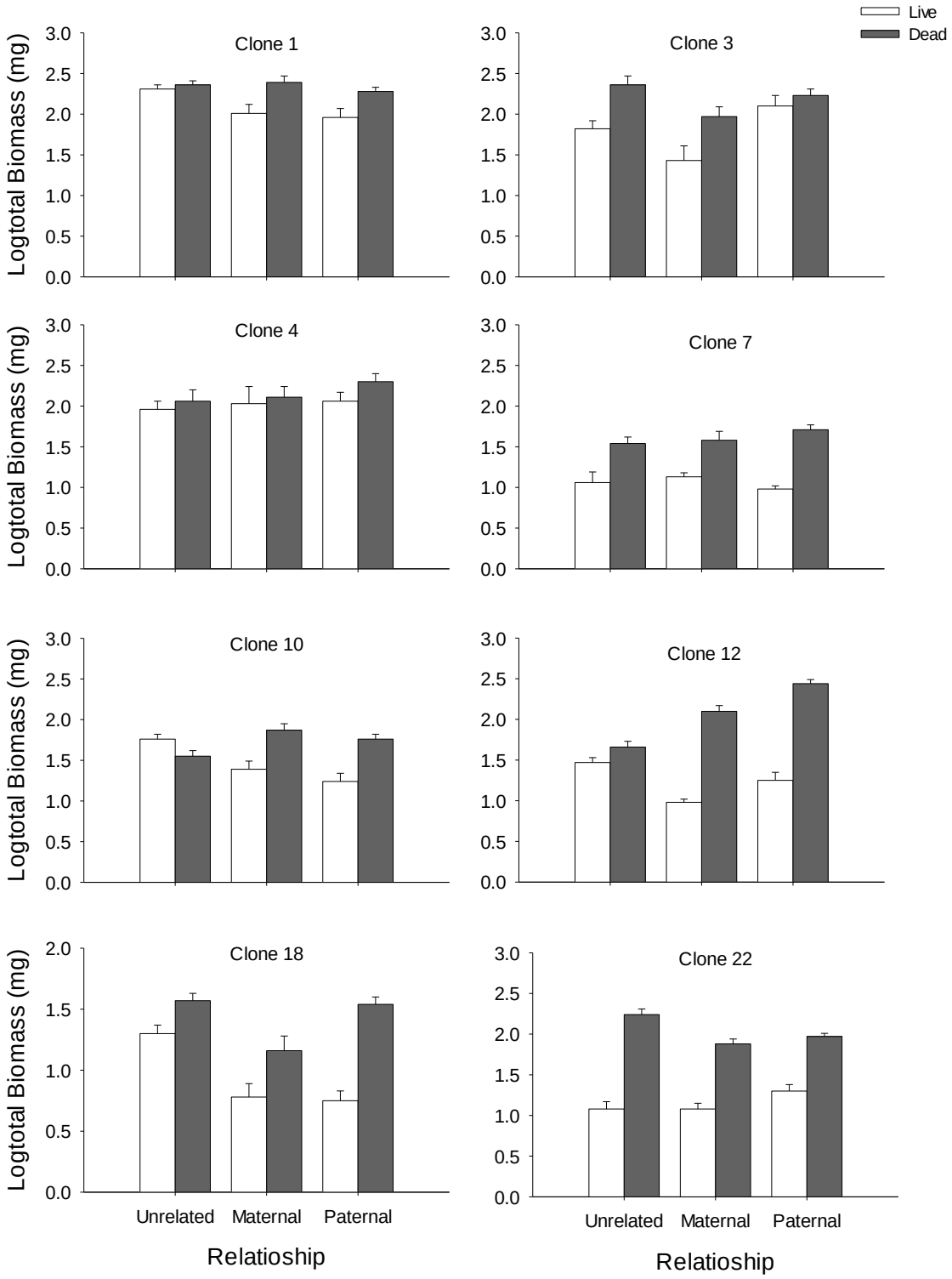

Figure A1. Log-transformed total biomasses of seedling growth for each individual clone in response to relatedness and soil sterilization. Data plotted are means ± 1 SE.