Plant–soil feedbacks contribute to an intransitive competitive network that promotes both genetic and species diversity

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Summary

1. Plant communities are generally thought to follow strict competitive hierarchies, in which species can be linearly ordered according to their ability to compete for a few limiting resources. Such strict hierarchies should lead to reduced diversity, since the single best competitor will eventually exclude the other species. However, more complex dynamics may emerge if different plant species or genotypes compete in different ways, such as through the release of toxic allelochemicals or the alteration of soil microbial communities.

2. Brassica nigra genotypes and their competitor species show a ‘rock-paper-scissors’ like dynamic, in which no one type is competitively superior to all others. This intransitive dynamic can maintain both species diversity and genetic variation in an allelochemical trait of B. nigra.

3. Here we show that feedbacks with soil biota, probably involving arbuscular mycorrhizal fungi, facilitate these dynamics. Brassica nigra genotypes that invest heavily in the allelochemical sinigrin reduce mycorrhizal abundance of surrounding soils, which reduces the growth of heterospecific competitors. Since B. nigra is non-mycorrhizal, investment in sinigrin does not improve a genotype’s ability to compete with other conspecifics.

4. Synthesis. These results highlight the importance of complex soil communities for the maintenance of both genetic and species diversity in plant communities.

Key-words: allelopathy, arbuscular mycorrhizal fungi, below-ground interactions, Brassica nigra, genetic variation, glucosinolates, intransitivity, plant–soil feedbacks, soil microbes

Introduction

Intransitive competitive networks occur when no one species (or genotype) is superior to all others, such as in a game of rock-paper-scissors, whereas transitive hierarchies occur when all competing species or genotypes can be linearly ordered according to their competitive abilities. Transitive hierarchies are expected to reduce diversity, as the best competitor eventually dominates, but intransitive networks promote diversity because no single species is able to out-compete all others. Intransitive networks can maintain either allelic or species diversity in mathematical models (Laird & Schamp 2006; Vellend & Litrico 2008), and occur in systems ranging from microbial communities to coral reefs (Buss & Jackson 1979; Sinervo & Lively 1996; Kerr et al. 2002).

At the species level, plant communities generally form transitive hierarchies (Grace, Guntenspergen & Keough 1993; Keddy, Gaudet & Fraser 2000). Since most plants compete for a similar set of resources (light, water, soil nutrients), the species best able to acquire those resources should outcompete all others. For example, when species compete for a single limiting resource, the species that can maintain a positive population growth rate at the lowest level of that resource (known as their $R^*$) will exclude all others (Tilman 1982). However, intransitive hierarchies require more complex competitive dynamics than simple resource competition, such as a difference in competitive strategies among species (Callaway & Howard 2007). For instance, some species may compete by depleting shared resources (known as scramble competition), whereas others may compete by directly interfering with the growth or...
resource acquisition abilities of its neighbours through the release of toxic allelochemicals. In such a system, the R* of the species may not predict the competitive outcome, as an allelopathic species may outcompete other species that are sensitive to its allelochemicals even if it does not drive resources to low levels.

Development of different competitive strategies may be mediated by non-competitive interactions between plants and other organisms, such as the soil microbiota. Plant–soil microbiota feedbacks can influence plant species diversity, relative abundance patterns, community succession and species invasions (Klironomos 2002; Wolfe & Klironomos 2005; Kardol, Bezemer & van der Putten 2006; Kardol et al. 2007; Rudgers, Fischer & Clay 2010). If plant species differ in the soil communities they cultivate and in their response to those communities in non-transitive ways, then these relationships could result in intransitive competitive networks among plant species.

Interactions with soil biota could also result in apparent competition (Holt, Grover & Tilman 1994; Holt & Lawton 1994), for example if one plant species causes an increase in the microbial pathogen load experienced by a competitor. Additionally, plant alterations of soil biota could lead to interference competition similar to allelopathy if a decrease in mutualists caused by one plant species reduces the resource acquisition ability of a competitor (Stinson et al. 2006).

Recent research has documented an intransitive network among several plant species (referred to as heterospecifics hereafter) and genotypes of another species, Brassica nigra, that produce either high or low levels of a secondary compound, sinigrin (Lankau & Strauss 2007). High sinigrin B. nigra genotypes could invade patches of heterospecifics, but not patches of low sinigrin B. nigra genotypes (Fig. 1 transitions B and F). Heterospecifics could invade patches of low, but not high, sinigrin B. nigra (Fig. 1 transition C and A), and low sinigrin genotypes could invade patches of high sinigrin B. nigra (Fig. 1 transition E) but not heterospecifics (Fig. 1 transition D). Simulations suggest that this intransitivity could promote both species and allelic diversity over the long term (Lankau 2009). Further analysis of the field data in this system suggests that the different B. nigra genotypes and their heterospecific competitors may utilize different competitive strategies (i.e., scramble versus preference, see Appendix S1 in Supporting Information). However, the mechanisms underlying these competitive interactions are not clear.

Ecological interactions which could mediate these differences in competitive strategies include herbivory, direct allelopathy and soil microbial feedbacks (Schenk 2006; Callaway & Howard 2007). Arbuscular mycorrhizal fungal (AMF) infection potential was significantly lower in soils from underneath randomly assigned, experimentally planted patches of high sinigrin B. nigra compared with low sinigrin heterospecific patches (Lankau & Strauss 2007). Glucosinolates, including sinigrin, break down into by-products toxic to a wide range of organisms (Kliebenstein 2004), including mycorrhizal fungi (Schreiner & Koide 1993; Roberts & Anderson 2001). Most flowering plants benefit from associations with AMF, in which, among other benefits, the fungus aids in nutrient acquisition in exchange for fixed carbon from the plant (Smith & Read 1997; Sikes, Cottenie & Klironomos 2009; Sikes, Powell & Rillig 2010). However, B. nigra, like most members of the Brassicaceae, does not form mycorrhizal connections, and so a reduction in AMF abundance could provide it with a competitive advantage against AMF dependent heterospecifics. These differences in AMF communities suggested that interactions with soil biota may partly underlie the observed intransitivity in this system.

In this study, we performed several experiments to determine whether different B. nigra genotypes and their heterospecific competitors cultivated different soil communities, and, in turn, if differences in these communities mediated some or all of the competitive interactions seen in previous field studies. We characterized field soils under high and low sinigrin genotypes, and under a mixed heterospecific community, with respect to their microbial communities. High and low sinigrin B. nigra genotypes were created by artificial selection on leaf sinigrin levels (Lankau & Strauss 2007; Lankau & Kliebenstein 2009). Since community treatments were randomly assigned to field locations in this experiment, we can be confident that the differences in AMF communities among plots were caused by genetic differences between the experimental plants. The exact mechanisms by which selection on leaf sinigrin concentrations lead to alteration of AMF communities are unclear, but could have resulted from root or leaf litter exudates of high sinigrin plants or from traits highly genetically correlated with leaf sinigrin expression.

We then grew each plant type (high or low sinigrin B. nigra genotypes and heterospecifics) in greenhouse pots inoculated with these soil types in a factorial design. In addition, to elucidate the role of AMF, relative to the broader soil microbiota, on observed growth effects, we grew the heterospecific plants in both intact soil inocula and in a microbial filtrate from which AMF had been removed.

The natural history of this system (summarized in Fig. 1) leads to several specific hypotheses about how soil mediated interactions may vary between different competitor types:

![Fig. 1. Schematic of the intransitive competitive network among high and low types of B. nigra and heterospecific species. Arrows mark invasion pathways, starting from the invader plant type and leading to the invaded community type. Solid arrows represent allowed transitions while dotted arrows with ‘forbidden’ symbol represent un-allowed transitions, according to previous experimental work in Lankau & Strauss (2007).](image-url)
1. If high sinigrin *B. nigra* genotypes possess allelopathic or anti-mycorrhizal traits, then one would expect heterospecifics to grow poorly in high sinigrin soils if heterospecific competitors are strongly mycorrhiza-dependent (Fig. 1 transition A). If alteration of mycorrhizal communities is the primary mechanism, then heterospecific fitness should vary in soils with intact mycorrhizal communities but not in filtered soils. We also expect low levels of root colonization by mycorrhizal fungi on plants grown in soils conditioned by high sinigrin *B. nigra* genotypes.

2. If, as suggested by the prior study, low sinigrin genotypes do not reduce mycorrhizal infectivity of soils, we predict high growth and higher levels of root colonization by AMF of heterospecifics grown in low sinigrin *B. nigra* soils (consistent with Fig. 1 transition C).

3. If altered soil microbial communities also determine whether *B. nigra* individuals can invade communities of heterospecific plants, then we predict that high sinigrin *B. nigra* genotypes should grow well, but low sinigrin genotypes should grow poorly, in heterospecific soils (Fig. 1B,D). Since *B. nigra* is non-mycorrhizal, these patterns should be evident in the microbial filtrate.

4. Since *B. nigra* is non-mycorrhizal, any mycorrhiza-inhibiting traits possessed by high sinigrin *B. nigra* plants should not provide any advantage against low sinigrin *B. nigra* genotypes. Thus, we predict that competition among *B. nigra* individuals will be driven primarily by simple resource competition rather than indirect interactions with mycorrhizal fungi (Fig. 1, transitions E,F).

### Materials and methods

**STUDY SPECIES**

*Brassica nigra* is a Eurasian annual introduced around the world, including California’s Central Valley (Lankau & Strauss 2007). Members of the Brassicaceae, including *B. nigra*, produce glucosinolates, a class of secondary compounds that break down into by-products toxic to insects, bacteria, fungi and other plants (Kliebenstein 2004). In *B. nigra*, sinigrin (ally-glucosinolate) comprises 90–99% of the total glucosinolate content. We used three other annual species *(Amsinckia menziesii, Sonchus oleraceus, and Malva parviflora)* as heterospecific competitors; all three commonly co-occur with *B. nigra* and form connections with AMF (observed and documented below). *Brassica nigra*, like most members of the Brassicaceae, does not form AMF associations (Schreiner & Koide 1993).

**FIELD EXPERIMENT**

To create two different *B. nigra* communities, we used *B. nigra* lines artificially selected for high or low sinigrin levels for three generations (for details of artificial selection scheme see Lankau & Kliebenstein 2009); sinigrin concentrations after three generations of selection were still within levels observable in natural populations. We also used three species of heterospecifics to create three types of experimental communities: (i) all high sinigrin *B. nigra*, (ii) all low sinigrin *B. nigra*, or (iii) a mix of the three heterospecific species. The three community types were factorially crossed with three types of invaders (high sinigrin *B. nigra*, low sinigrin *B. nigra* or one of three heterospecifics) with 10 replicates per *B. nigra* invader type per community type and seven replicates per heterospecific species (total 21 heterospecific invader replicates) per community type. Each 1.5 m² plot consisted of 24 neighbours surrounding one target invader (see Lankau & Strauss 2007 for further details).

In May 2006, we collected soil samples from 10 randomly selected field plots of each community treatment (without regard to the invader type). Three soil cores were taken from the central portion of the plots to avoid edge effects. In the mixed heterospecific plots, we took samples from the base of one individual of each species. These soils were used for microbial community profiling, as inocula for greenhouse experiments, and for measures of mycorrhizal infection potential (MIP). MIP was measured as the percent root colonization of *Sorghum bicolor* seedlings after 5 weeks of growth (Giovannetti & Mosse 1980; Lankau & Strauss 2007). Since community treatments were randomly assigned to field locations in this experiment, any differences in soil communities under high sinigrin plants could have resulted from root or leaf litter exudates of high sinigrin plants or from traits highly genetically correlated with leaf sinigrin expression.

**DESCRIPTIVE ANALYSIS OF SOIL MICROBIAL COMMUNITIES AND NUTRIENTS FROM EXPERIMENTAL FIELD PLOTS**

Community compositions for three microbial taxonomic groups – bacteria, general fungi and AMF – were evaluated using terminal restriction fragment length polymorphism (t-RFLP). t-RFLP is a PCR-based community fingerprinting technique in which individual restriction fragment lengths are considered to be representative of unique operational taxonomic units (OTUs) present within the sampled community (Thies 2007).

We extracted microbial DNA from 0.5 g of soil per sample (10 samples per plant community type) using a commercial kit (Fast-DNA SPIN Kit for Soil, MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer’s directions, followed by a chloroform:isoamyl alcohol purification step. We standardized DNA concentrations to 10 ng µl⁻¹ before use as template for PCR. We amplified segments of the ribosomal RNA region by PCRs using a Biometra t-Gradient (Biometra, Goettingen, Germany) using taxon-specific primers with 6-FAM fluorescent labelling of forward primers. Bacterial PCR targeted the 16S ribosomal RNA region using primer pair 8F and 1492R (Liu et al. 1997; Kits 2001) and general fungal PCR targeted the internal transcribed spacer (ITS) region of the ribosomal RNA gene segment using primer pair ITSf1 and ITS4r (Dickie, Xu & Koide 2002; St. Laurent, Merwin & Thies 2008). AMF PCR used a nested approach, first targeting the fungal 28S (large subunit) ribosomal RNA region using primer pairs LR1 and FLR2, followed by FLR3 and FLR4 for AMF-specific internal amplification (Gollette, van Tuinen & Atkinson 2004; Mummey & Rillig 2007, 2008). Five samples (three high sinigrin and two low sinigrin *B. nigra*) did not produce PCR product for the AMF primers and were removed from analysis of AMF community structure.

We digested PCR products with three restriction enzymes, one reaction per enzyme, to create three distinct community profiles per sample. Complete digestion of PCR product was confirmed by agarose gel electrophoresis before performing capillary electrophoresis on an ABI Prism 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA) using a product size matched fluorescent lane standard (ROX1000, LIZ600, and ROX500 for bacteria, fungi and AMF, respectively). Size-calling was performed using GeneMarker®.
software (SoftGenetics, State College, PA, USA) with one base-pair allele bins.

To characterize the abiotic components of soil samples, we had the samples analyzed for eight abiotic factors: percent organic matter, concentrations of nitrate, phosphorous, potassium, magnesium, and calcium, pH and cation exchange capacity (A & L Great Lakes Laboratories, Fort Wayne, IN, USA). Soil for some plots was unavailable following the microbial analysis, so we were only able to analyze soils from 10 high sinigrin, six low sinigrin, and four mixed heterospecific plots. We analyzed microbial community patterns separately for the three microbial taxonomic groups using constrained correspondence analysis (CCA) with the three plant community types as constraints (Ramette 2007). We used permutation tests to determine if the constrained axes explained more variation than would be expected by a random assignment of soils to community sources. We present presence/absence data as the results did not qualitatively differ between analyses based on fragment presence/absence, peak height, or peak area. We present the analysis from one restriction enzyme per group since all three enzymes showed similar patterns (using the enzyme which produced the most distinct OTUs). Ordinations and permutation tests were performed with the CCA programs in the vegan package of the R programming language (Oksanen et al. 2005). We tested for differences among the three plant community types for each abiotic soil factor using ANOVAs.

**EFFECTS OF PLANT COMMUNITY TYPE AND SOIL INOCULUM TREATMENT ON PLANT GROWTH AND FUNGAL COLONIZATION**

To test our first and second hypotheses, that heterospecific plants would grow poorly in soil from high sinigrin *B. nigra* plots due to reduced mycorrhizal abundance, but would grow well in soil from low sinigrin *B. nigra* plots with ample mycorrhizal propagules, we used soils collected from the same 30 field plots in a greenhouse experiment. We grew heterospecific plants in a factorial experiment with two soil treatments (whole live soil or a liquid microbial filtrate) crossed by three soil inoculum sources (high sinigrin *B. nigra* plots, low sinigrin *B. nigra* plots and heterospecific plots). The microbial filtrate treatment was intended to remove AMF spores and hyphae with minimal effects on other soil microbes, although it may have affected other fungal species and invertebrates (Ames, Mihara & Bethlenfalvay 1987). Additionally, even microbial species that pass through the filter may have been affected since some species may be better or worse at establishing in a new soil environment. Nevertheless, this technique has been used successfully in other studies to gain insights into the effects of AMF versus other soil microbes on plant growth (Klíronomos 2002; Collins & Foster 2009; van der Heijden 2010; Johnson et al. 2010).

This $2 \times 3$ factorial experiment (whole versus filtrate from three community types) was performed separately for two heterospecific species (*M. parviflora* or *S. oleracea*), with 10 replicates per treatment for each species (three soil sources $\times$ two treatments $\times$ 10 replicates $\times$ two species = 120 pots). We chose *M. parviflora* and *S. oleracea* because they represented the extremes in response to *B. nigra* sinigrin levels (showing no significant response or a strong decline in high sinigrin field plots, respectively; Lankau & Strauss 2007). We also included a sterilized soil treatment for each species (10 replicates each).

We performed an additional experiment comparing the growth of high and low sinigrin *B. nigra* genotypes to these soils to test our third hypothesis, that *B. nigra* individuals would be insensitive to the differences in microbial communities between the plant community types.

We grew 10 pots each with high and low sinigrin *B. nigra* individuals (each from an independent family) in soils with the microbial filtrate from each inoculum source as well as sterilized soils (four soil sources (including sterilized soils) $\times$ 2 sinigrin lines $\times$ 10 replicates $= 80$ pots). Unfortunately, our collections of whole soil were completely consumed performing the previously described heterospecifics experiments. Since brassicaceae plants do not form connections with AMF, our results should not be greatly affected. Nevertheless, results from this experiment should be interpreted with some caution as the microbial filtrate may not exactly represent the microbial communities faced by *B. nigra* individuals in the field.

To create the microbial filtrate for a given field plot, we first homogenized 150 mL of live field soil with 250 mL of Milipore ultra-purified water in a blender. The slurry was passed through a 45-μm filter and then through filter paper (Whatman, Inc., Sanford, ME, USA; 20-25 μm pore size) under vacuum. This process was performed separately for each of the soil samples from the 30 field plots, with equipment sterilized between samples.

Pots in the whole soil treatment were filled with 100 mL of sterilized background soil consisting of 50% clay loam collected from nearby the field site and 50% sand. These were homogenized and sterilized by autoclaving twice. We then added 300 mL of a live/sterilized mix (80 mL of live field soil mixed as inoculum with 220 mL of sterilized background soil), followed by another 100 mL of sterilized background soil to isolate inocula in pots. We used a low ratio of inoculum to total pot volume to minimize any abiotic differences among treatments. For pots in the microbial filtrate treatment we added 400 mL of the sterilized background soil, then 50 mL of the liquid filtrate, followed by another 100 mL of the sterilized background soil. Fifty millilitres of microbial filtrate represents c. 20 mL of original field soil. The sterilized treatment consisted of only the sterilized background soil.

Surface sterilized seeds were germinated in sterilized soil and then transplanted to the experimental pots within 1 week of growth. Plants were grown for 4 months, then above-ground biomass was removed, roots were washed free of soil and plant tissue was weighed after drying at 60°C for 48 h. Root material from each heterospecific plant was stained with trypan blue, and mounted on slides. Percent root length colonized by AMF was scored using the grid line intersect method (McGonigle et al. 1990). Fungal structures were counted as either AMF or non-AMF based on morphology.

**ANALYSIS**

We used ANOVA to compare plant biomass across the different inoculum treatments. For *S. oleracea* and *M. parviflora*, the model included the soil treatment (whole or microbial filtrate), the soil inoculum source (high sinigrin *B. nigra*, low sinigrin *B. nigra*, or hetero-specific), and their interaction as factors. For *B. nigra*, the model included the selection line of the target plants (high or low sinigrin) and the microbial filtrate source (high sinigrin *B. nigra*, low sinigrin *B. nigra*, heterospecific, and sterilized), and their interaction.

To explicitly test the four hypotheses posed above related to the pattern of observed and never observed transitions in the field experiment (Fig. 1), we performed a series of *a priori* contrasts corresponding to each arrow. For each transition, we compared the growth of a plant type in its own soil (the base of the arrow) versus its growth in the soil of the community it invades (the point of the arrow). We hypothesized that heterospecifics would grow more poorly in high sinigrin versus their own soil (Fig. 1 path A, hypothesis 1), and better in low sinigrin versus their own soil (path C, hypothesis 2). Additionally, we hypothesized that high sinigrin
B. nigra genotypes would grow better in heterospecific versus their own soil (path B), but low sinigrin genotypes would show the opposite pattern (path D, hypothesis 3). Finally, we hypothesized that soil microbes would have little effect on competition between B. nigra genotypes; therefore, we predicted that both high and low sinigrin genotypes would grow equally well in low or high sinigrin soil (paths E, F, hypothesis 4). For S. oleraceus and M. parviflora we used the whole soil treatment for these tests since that treatment includes all soil components and is most representative of the field. For B. nigra genotypes we used growth in the microbial filtrate since these plants were not grown in whole soil. Linear models were performed with the lm function in the base R package (R Development Core Team 2005).

To further evaluate a role for AMF in plant growth, we compared the growth of S. oleraceus and M. parviflora plants with (i) AMF community composition in the original soil inocula, (ii) percent root colonization by AMF at the end of the experiment, and (iii) the MIP of the original inocula [which was previously shown to be lower in high sinigrin versus low sinigrin or heterospecific soils (Lankau & Strauss 2007)]. We used the adonis function in the R package vegan to compare plant growth with the multivariate distance between AMF communities through permutation tests (Oksanen et al. 2005). Next, we regressed the final biomass for each plant in the greenhouse experiment against percent root length colonized by AMF for that individual after 4 months of growth. Finally, we regressed the biomass of S. oleraceus and M. parviflora in the greenhouse experiment against the MIP of Sorghum grown in soil collected from the same field plots.

Results

DESCRIPTIVE ANALYSIS OF SOIL MICROBIAL COMMUNITIES AND NUTRIENTS FROM EXPERIMENTAL FIELD PLOTS

Microbial communities separated into distinct groups based on the plant community treatments. The constrained axes explained more variation than would be expected by a random assignment of treatments to soil communities for bacteria, general fungal and AMF taxa (Fig. 2a–c, P < 0.001, <0.005, and <0.005, respectively). Although significant, none of the constrained axes explained a large amount of variation in community composition (all <7%). However, the significant effects suggest that all three taxonomic groups contained some taxa that responded to the experimental plant communities.

In contrast to soil microbial communities, soils from the three plant communities did not differ significantly in any of the eight abiotic factors measured, although power was lower for this comparison (see Appendix S2 in Supporting Information). Thus, differences among soil treatments that we observed are most parsimoniously interpreted to be a result of changing soil microbial communities in these treatments.

EFFECTS OF PLANT COMMUNITY TYPE AND SOIL INOCULUM TREATMENT ON FUNGAL COLONIZATION OF ROOTS

For both M. parviflora and S. oleraceus, AMF colonization was nearly absent from the microbial filtrate and sterilized soil treatments (percent root length infected of <1% for all inoculum sources) and was significantly higher in the whole soil treatment (16.6% ± 4.82 SE for M. parviflora, 33.6% ± 4.77 SE for S. oleraceus, Table 1); thus our filtering and sterilization treatments effectively removed AMF from soil inocula. AMF colonization of roots did not differ significantly among community types for either species in the whole soils (Table 1).

Non-mycorrhizal fungal structures on plant roots were significantly more abundant in the whole soil versus microbial
filtrate or sterilized soil treatments for *M. parviflora* (Table 1). For *S. oleraceus*, these fungi did not differ between the whole and microbial filtrate treatments, but were significantly more abundant in the microbial filtrate treatment versus sterilized soil (14.5% ± 2.53 SE versus 5.77% ± 2.98 SE, \( F_{1,50} = 4.49, P = 0.039 \)). The abundance of these fungi did not differ significantly among plant community types for either plant species (Table 1).

### Table 1. Effects of soil inocula source and treatments on heterospecifics

<table>
<thead>
<tr>
<th>Source</th>
<th>Biomass</th>
<th>% Colonization – AMF</th>
<th>% Colonization – other fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>SS</td>
<td>F</td>
</tr>
<tr>
<td><em>Sonchus oleracea</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil inocula</td>
<td>2</td>
<td>5.046</td>
<td>1.14</td>
</tr>
<tr>
<td>Soil treatment</td>
<td>1</td>
<td>20.24</td>
<td>9.142</td>
</tr>
<tr>
<td>Soil inocula × soil treatment</td>
<td>2</td>
<td>16.17</td>
<td>3.652</td>
</tr>
<tr>
<td><em>Malva parviflora</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil inocula</td>
<td>2</td>
<td>0.186</td>
<td>0.05</td>
</tr>
<tr>
<td>Soil treatment</td>
<td>1</td>
<td>9.274</td>
<td>4.997</td>
</tr>
<tr>
<td>Soil inocula × soil treatment</td>
<td>2</td>
<td>1.945</td>
<td>0.524</td>
</tr>
</tbody>
</table>

**anova** results for biomass and % root length colonized by AMF or non-AM fungi for *S. oleraceus* and *M. parviflora*. Soil treatment refers to whole soil versus microbial filtrate, while inocula refers to the plant community (high or low sinigrin *B. nigra* or mixed heterospecific) from which the soil inoculum was taken. Bold values are significant at \( P < 0.05 \).

**EFFECTS OF PLANT COMMUNITY TYPE AND SOIL INOCULUM TREATMENT ON PLANT GROWTH**

When averaging across soils from all community types, both *M. parviflora* and *S. oleraceus* had higher total biomass in the whole soil versus the microbial filtrate treatments (Fig. 3a and b, Table 1). Biomass of *M. parviflora* did not respond differentially to soil inocula from the three plant communities, either in the whole soil or microbial filtrate treatments (Fig. 3a, Table 1). *Sonchus oleraceus* showed significant interactions between soils from different community treatments and soil fraction (Fig. 3b, Table 1).

Consistent with hypotheses 1 and 2, *S. oleraceus* grew marginally significantly worse in high sinigrin as compared with heterospecific whole soils and significantly better in low sinigrin whole soils as compared with heterospecific soils (Table 2). These differences were not apparent in the microbial filtrate (\( P > 0.05 \) for all comparisons, Fig. 3b). Growth of *M. parviflora*, which did not respond to sinigrin levels in the field experiment, did not differ significantly between any of the soil types (Table 2).

High and low sinigrin *B. nigra* lines did not differ in their overall response to soil community types, although high sinigrin genotypes were marginally larger than low sinigrin genotypes across all soil sources, (soil inocula, \( F_{3,30} = 1.23, P = 0.31 \); sinigrin line, \( F_{1,30} = 3.44, P = 0.07 \); soil inocula × sinigrin line, \( F_{3,30} = 0.21, P = 0.89 \), Fig. 4). As predicted, from the field pattern (Fig. 1), low sinigrin genotypes performed significantly worse in heterospecific microbial filtrates as compared with their own inocula (Table 2). However, the performance of high sinigrin genotypes did not differ between the heterospecific and their own soils and high and low sinigrin genotypes grew equally well in their own versus each other’s soil inocula (Table 2).
Can differences in AMF explain differences in plant growth among soil sources?

Multivariate permutation tests found no significant relationships between *S. oleraceus* or *M. parviflora* biomass in whole soils and the composition of the AMF community in the original inoculum (\(R^2 = 0.036, P = 0.639\); \(R^2 = 0.037, P = 0.606\), respectively). There were also no significant correlations between plant biomass and final percent root length colonized by AMF for either species (*S. oleraceus*, \(r = 0.19, P = 0.35\); *M. parviflora*, \(r = -0.31, P = 0.10\)).

On the contrary, MIP measured in soils from the same field plots used for the soil inoculum treatments was a strong predictor of *S. oleraceus* biomass in whole soils in the greenhouse (Fig. 5). In the greenhouse experiment, *S. oleraceus* biomass differed significantly among soil inocula from whole soils (\(F_{2,18} = 4.51, P = 0.03\)). When the MIP for each field plot was entered into that model as a covariate, the significant soil source effect was eliminated (\(F_{2,18} = 0.87, P = 0.44\)). MIP was not a significant predictor for *M. parviflora* performance in whole soils (Fig. 5). MIP also did not explain plant biomass in the microbial filtrate treatment for either species (\(R^2 < 0.02, P > 0.58\) for both).

**Discussion**

Intransitive competitive networks in plant communities can be powerful promoters of coexistence, but may entail more complex interactions among plants than simple resource competition (Callaway & Howard 2007). These complexities could arise through interactions with soil biota. A major aim of this research was to elucidate the possible mechanisms behind the competitive intransitivity observed in a previous study and outlined in Fig. 1 (Lankau & Strauss 2007). The results of this study suggest that plant–soil microbe interactions may underlie some, but not all of the observed interactions, as evidenced by the similarity in patterns of plant growth between the field and greenhouse experiment, despite the many differences in growing conditions (e.g. limited soil volume, less variable abiotic conditions, and lack of inter- or intraspecific competition.

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**Table 2.** Soil inocula effects on specific competitive transitions

<table>
<thead>
<tr>
<th>Transition</th>
<th>Invader</th>
<th>Community</th>
<th>Tests of soil biota effects</th>
<th>F</th>
<th>P</th>
<th>Direction</th>
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<tr>
<td>A.</td>
<td>Heterospecific</td>
<td>High Sinigrin</td>
<td></td>
<td>3.22</td>
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<td></td>
<td><em>S. oleraceus</em></td>
<td></td>
<td></td>
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<td>0.37</td>
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<td>B.</td>
<td>Heterospecific</td>
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<td>+</td>
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<td></td>
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<td></td>
<td></td>
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<td>0.31</td>
<td>0</td>
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<tr>
<td>C.</td>
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<td>Low Sinigrin</td>
<td></td>
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<td>0.04</td>
<td>–</td>
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<tr>
<td>D.</td>
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<td>E.</td>
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<td>Low sinigrin</td>
<td></td>
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<td>0.75</td>
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</table>

Contrasts comparing the growth of a given plant type (Invader) in its own soil biota and soil cultured by a different type (Community). Transition refers to the lettered arrows in Fig. 1. Soil sources refer to microbial filtrates for *B. nigra* invaders (transitions B. and D–F) and whole soils for heterospecific invaders (transitions A and C). Direction indicates whether a type performed better (+), worse (–), or equally (0) in the new community versus its own soil. Bold values are significant at \(P < 0.05\).

**Fig. 4.** Mean and standard errors of total biomass of high (closed squares) or low (open squares) sinigrin *B. nigra* genotypes grown in soil inoculated with microbial filtrates from different soil sources (three plant community types and a sterilized control).

**Fig. 5.** Final biomass of (a) *S. oleraceus* and (b) *M. parviflora* in the whole soil treatments against the mycorrhizal infection potential of the original soil inoculum. Points are shaded according to the plant community from which the soil inoculum was derived.
in the greenhouse). In the previous field experiment, high sinigrin B. nigra genotypes competed strongly with heterospecific plants, especially S. oleraceus and A. menziesii and likely employed some form of interference competition against heterospecifics (Appendix S1), which could result from alterations of soil microbial communities. Variation in S. oleraceus performance among soil sources was evident in soils with an intact soil community, but not in filtered soils lacking AMF and other large soil components. Low levels of infective AMF propagules in the high sinigrin soil may play a role in preventing S. oleraceus from invading high sinigrin patches in field communities, as evidenced by a marginal inhibition of S. oleraceus growth in whole high sinigrin B. nigra soil. This hypothesis is also supported by power of the MIP of field soils (which were previously found to be lower in high sinigrin compared with low sinigrin or heterospecific soils Lankau & Strauss 2007) to predict S. oleraceus performance in our experimental inocula. In contrast to S. oleraceus and A. menziesii, M. parviflora was the one heterospecific species studied that did not show a significant response to high sinigrin B. nigra in the previous field experiment (Lankau & Strauss 2007), nor did it have a significant response to soil communities in the greenhouse. Malva parviflora may be generally less sensitive to AMF abundance or identity, since unlike S. oleraceus, growth of M. parviflora did not correlate significantly with the initial MIP of the field soils.

The composition of bacterial, fungal, and AMF communities all differed underneath the three plant community types. As a first step toward determining which of these groups had the strongest impact on plant growth, we used a filtering treatment to compare the effect of soil communities with and without AMF propagules with heterospecific growth. While the filters may have affected many taxa in addition to AMF (including protozoa, nematodes, and other fungi), several lines of evidence suggest that their dominant functional effect was due to the alteration of the AMF community. First, colonization of plant roots by AMF was common in the whole soil treatment, but very rare (and not significantly different from zero) in the filtered soil, whereas infection by non-mycorrhizal fungi did not differ between the treatments for S. oleraceus. Thus, propagules of some fungal species, but not AMF, were able to pass through the filters. Plant performance was equal or greater in the whole soil versus the filtered soil for both heterospecific species, suggesting that the primary functional effect of the treatment for plant growth was to remove a mutualist, even if various plant enemies were also affected. AMF are the most likely candidate in these soils for a mutualist that could not pass through the filtration steps. Finally, the mycorrhizal infection potential of soils from these same field plots was a strong predictor of S. oleraceus growth and explained most of the variation previously attributed to soil source when included as a covariate in the ANOVA model. Malva parviflora showed a reduced benefit from AMF compared with S. oleraceus (A. Bennett, unpublished data), which may explain why this species did not respond as strongly to the initial MIP of the soil inoculum or to B. nigra genotypes (in the field setting in a previous study, or to their cultured soils) as S. oleraceus.

This study utilized soils from a field experiment in which B. nigra genotypes artificially selected to express high or low concentrations of sinigrin in leaves were randomly planted into replicated plots in an old field. Therefore, the differences in soil biota among these plots, including the altered composition of bacterial, fungal, and AMF communities determined by T-RFLP analysis and the reduced MIP underneath high sinigrin plots, are most parsimoniously interpreted as having derived from the experimental treatments, which in this case was the genetic difference between the high and low sinigrin B. nigra genotypes. Measures of sinigrin content in the leaves of the field grown plants confirmed that the two selection lines maintained their differences in sinigrin expression in the field conditions (Lankau & Strauss 2007). While the toxic properties of glucosinolate breakdown products are well documented (Schreiner & Koide 1993; Kliebenstein 2004), the proximate mechanism by which the genetic differences in sinigrin expression in leaves caused the observed changes in soil microbial communities is not clear from this research. Sinigrin could have leached from leaves or litter, or leaf sinigrin levels could be genetically correlated with root sinigrin levels or some other unmeasured trait. Because the lines were created through multiple generations of divergent artificial selection on the same initial germplasm, any genetic correlations must result from pleiotropic effects or strong linkage. Thus, the ecological and evolutionary interpretation of the studies main findings, that evolutionary changes in sinigrin levels reflect competition between plant species indirectly by altering soil communities, are not dependent on any particular proximate link between sinigrin levels and soil communities.

In general, our data provide less information on how high sinigrin B. nigra genotypes are able to invade heterospecific communities (Fig. 1 transition B), since we found no signal of microbial inhibition or promotion. One possibility is that these genotypes tend to create high sinigrin levels in the areas near their own roots (via exudation, sloughing of root tissue, or localized throughfall or litterfall), which could potentially play a defensive function in reducing fine scale competition between B. nigra roots and mycorrhizal hyphae growing into the rooting zone. Such an effect would not have been apparent in the greenhouse experiment where plants were grown in isolation.

In the previous field experiment, the three heterospecific plants were able to invade communities of low sinigrin B. nigra, whereas the low sinigrin genotypes grew relatively poorly when invading heterospecific patches. Different mechanisms may underlie these two transitions. The high growth rate of S. oleraceus in low sinigrin whole soils (but not in the microbial filtrate) and the high MIP of these soils (Lankau & Strauss 2007) suggest that abundant AMF may have allowed the heterospecifics to acquire resources more effectively in those patches. On the contrary, the low sinigrin genotypes grew relatively poorly in soils inoculated with a microbial filtrate of heterospecific soils. This result suggests that non-AMF soil taxa may prevent the establishment of low sinigrin genotypes in heterospecific communities. One hypothesis is that low sinigrin levels may result in greater susceptibility to soil pathogens.

This hypothesis is also supported by the patterns of size symmetry in the field data (Appendix S1), which suggest that low sinigrin *B. nigra* genotypes face some form of interference when invading heterospecific communities.

Low sinigrin *B. nigra* genotypes were the best invaders of the *B. nigra* monocultures, and high sinigrin genotypes the worst in the previous field study. Soil biota seemed to have little effect on this interaction; if anything, high sinigrin genotypes grew better in those soils, in contrast to the field pattern. Accordingly, the field data suggests that competition among *B. nigra* genotypes is dominated by simple scramble competition for resources (Appendix S1). One possible explanation for this pattern is that producing sinigrin provides little advantage in intraspecific competition but entails some direct or indirect cost. We found no evidence for a direct allocation cost in this study, as high sinigrin genotypes tended to outperform low sinigrin plants in all soil sources, including sterilized soils. However, costs of secondary compounds are often only detectable in competitive situations (i.e., an opportunity cost, Van Dam & Baldwin 1998; Baldwin & Hamilton 2000). Sinigrin may also impose indirect ecological costs in the field that would not be present in a greenhouse; for instance, in previous studies high sinigrin genotypes faced higher levels of aboveground specialist herbivory under intraspecific competition (Lankau & Strauss 2008).

Although competitive intraspecificities can be powerful promoters of diversity, plant research has tended to assume transitive relationships partly because plants species are often thought to compete in similar ways for similar resources. However, recent research has shed light on the complexities of plant competitive strategies (Schenk 2006; Callaway & Howard 2007), suggesting that intraspecific networks may be more common than has been appreciated. Additionally, in the system we studied, the intransitive network occurred not between three species, but between one group of species and different genotypes of a single species. Thus while plant competition may be transitive at the species level, hidden intraspecificities may occur due to genetic variation in competitive ability within species (Aarssen 1989; Whitlock et al. 2007).

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References


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Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Size symmetry of competition among *B. nigra* genotypes and heterospecifics.

**Appendix S2.** Analysis of abiotic soil factors among plant communities.

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