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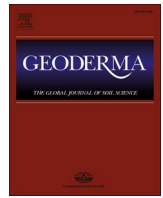
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Effects of soil biota on growth, resistance and tolerance to herbivory in *Triadica sebifera* plants

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ABSTRACT

Interactions with soil microbes can strongly affect plant growth and defense against aboveground herbivores. Plant species often accumulate specific soil pathogens in their rhizosphere, leading to reduced growth of plants in soils originating from stands of conspecific plants compared to soils from heterospecific plants. However, whereas effects of such conspecific vs. heterospecific soil biota on plant growth have been well documented, their effects on plant resistance and tolerance to aboveground insect herbivores have not. We compared growth and defense of *Triadica sebifera* plants from populations where the species is native (China), when grown in sterilized soils, or in soils harbouring belowground biota from conspecific (native *Triadica*) or heterospecific (native grass) soils. In each of these soils, plants were exposed to a 15-day period of foliar herbivory by a specialist weevil (*Heterapoderopsis bicallosicollis*), a specialist caterpillar (*Gadirtha inexacta*), or no herbivory (cage), followed by a 60-day recovery period. Soil biota from conspecific and heterospecific soils differed in their effects on plant growth and defense. First, in the absence of herbivory, soil biota from heterospecific soils slightly enhanced plant growth, whereas those from conspecific soils strongly reduced plant growth. Second, soil biota from conspecific soils strongly affected plant resistance and tolerance to foliar herbivory, whereas soil biota from heterospecific plants did not. The effects of soil biota on plant defense were herbivore-specific. In particular, conspecific soil biota reduced resistance to caterpillar but not to weevil feeding, whereas they enhanced tolerance to weevil but not to caterpillar feeding. Conspecific soil biota also mitigated induction of root flavonoids by herbivores and led to reduced root phenolics in response to herbivory. Conversely, caterpillar feeding increased AMF colonization, but under these conditions, AMF colonization was negatively associated with plant biomass. In addition to testing effects on native plants, we also tested effects of native soil biota on growth and resistance of plants from the introduced range (North America). Plants from the introduced range had higher shoot production, shoot-to-root ratio, and leaf phenolic and flavonoid production than plants from the native range, but their interactions with soil biota showed only minor differences compared to plants from the native range. Our results suggest that incorporating the effects of soil biota in interactions between plants and foliar herbivores is critical for understanding variations in growth, defense, and performance among plant populations at local and broader geographic scales.

1. Introduction

Over the past 30 years increasing attention has been paid to multi-trophic interactions occurring in both aboveground (i.e., shoots) and belowground (i.e., roots) domains of plants (Masters et al., 1993;

Bardgett et al., 1998; van der Putten et al., 2001; Bezemer and van Dam, 2005; Heil, 2011; Soler et al., 2012; Johnson et al., 2013). These interactions involve an array of micro- and macro-invertebrate fauna occurring in both domains. It is now well established that changes in growth and defense of plants in response to belowground microbes can

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have profound effects on their interactions with aboveground herbivores and vice versa (Biere and Bennett, 2013). For instance, many beneficial soil microbes such as plant growth promoting rhizobacteria and mycorrhizal fungi induce systemic resistance in plants, that can result in reduced performance of particular groups of aboveground insect herbivores (Vannette and Hunter, 2009; Jung et al., 2012; Pieterse et al., 2014). Hence, plants are important mediators of interactions between soil microbes and aboveground herbivores (Bezemer and van Dam, 2005; Rosa et al., 2018; Hannula et al., 2019) and the interactive effects among soil microbes, foliar herbivores and plants are an important factor shaping the structure of belowground and aboveground communities (Bardgett et al., 1998; van der Putten et al., 2001; Soler et al., 2012; Heinen et al., 2018). From the plant's perspective, the consequence of such intricate interactions is that in many cases the impact of aboveground and belowground biota on plant growth and defense will not simply be the sum of their independent effects but will be shaped by their interactive effects.

Plant growth may differ considerably when grown in soils that differ in the relative abundances of various groups of soil organisms, including mutualists (e.g., arbuscular mycorrhizal fungi, hereafter AMF), pathogens (e.g., fungal or bacterial pathogens), and decomposers (Bever et al., 1997; van der Putten et al., 2013). Differences in plant performance may be caused by soils having different 'histories' of plant association, due to the accumulation of, or escape from, taxon-specific soil pathogens and their associated toxic chemicals. The effects of taxon-specific soil pathogens on plant growth and fitness have been reported in many plant species (Reinhart and Callaway, 2006; Reinhart et al., 2010). Generally, plants perform more poorly when grown in soils that have an historical association with conspecific plants (hereafter referred to as "conspecific soil"), compared with plants grown in soils that have historical associations with heterospecific plants (hereafter referred to as "heterospecific soils") (van der Putten et al., 2013). This phenomenon is commonly referred to as a 'negative plant-soil feedback' (Ehrenfeld et al., 2005). Moreover, variation in the history of the plant-soil association, or the net effects of soil biota, may affect interactions between plants and aboveground antagonists, such as herbivores (Heinen et al., 2018, 2020).

The ability of plants to deploy defense responses to herbivores, such as resistance (the ability of plants to reduce the performance of antagonists and the damage they inflict) or tolerance (the ability of plants to maintain growth and reproduction at a given level of damage, Strauss and Agrawal, 1999), can be strongly affected by soil biota (Jung et al., 2012; Pieterse et al., 2014; Biere and Goverse, 2016). Plants have evolved an array of responses to reduce the impacts of herbivory and thus enhance plant fitness (Bennett and Wallsgrrove, 1994). For example, most plants produce secondary metabolites, either constitutively or in response to herbivory, that influence the behavior, growth, and/or survival of herbivores (Bennett and Wallsgrrove, 1994). Such chemicals can act as repellents or toxins to herbivores, and often reduce plant digestibility (Wang et al., 2012; Xiao et al., 2019), enhancing plant resistance. Soil biota such as growth-promoting rhizobacteria and arbuscular mycorrhizal fungi can have a large impact on resistance by activating Induced Systemic Resistance (ISR) that systemically primes plants for enhanced resistance (Jung et al., 2012; Pieterse et al., 2014). In addition to their ability to induce systemic resistance (Jung et al., 2012), many AMF can also enhance plant tolerance to herbivory by absorbing nutrients and water which increase compensatory growth in plants (Bennett et al., 2006). The effects of soil biota on plant resistance and tolerance to insect herbivory will depend on many factors. One is the composition of the soil community. For instance, plants grown in soils having higher levels of AMF may be more resistant or tolerant to herbivory than those grown in soils having less AMF (Balog et al., 2017; Heinen et al., 2018). On the other hand, soils that have higher pathogen loads may limit the ability of plants to recover from herbivore damage (Ballhorn, 2011; Lazebnik et al., 2014; Biere and Goverse, 2016). The outcome of these effects on plant tolerance to herbivory will therefore likely depend on the relative strength of these negative and positive

effects. Another factor that can strongly affect the effect of soil biota on plant resistance and tolerance to herbivory is the identity of the herbivore. For instance, whereas beneficial microbes often enhance the resistance to leaf chewing insects, they may actually enhance susceptibility to sap sucking insects such as aphids (Jung et al., 2012; Pineda et al., 2012).

Plants face shifts in the composition of their soil biota and herbivore communities every generation following seed dispersal to new localities. Major shifts may occur when plants are introduced into a novel range. Novel selection pressures imposed by the altered set of abiotic environments and biotic interactions in the introduced range can lead to evolutionary changes in traits of introduced plants in their novel range (Blossey and Nötzold, 1995) that can result in increased tolerance to abiotic factors (fundamental niche expansion; Yeh and Price, 2004) and herbivory (Huang et al., 2012). For example, introduced populations of *Triadica sebifera* (synonym *Sapium sebiferum*; hereafter "*Triadica*") have higher herbivory tolerance than native populations (Wang et al., 2016). Moreover, introduced *Triadica* populations have higher frequencies of mycorrhizal associations than native populations (Yang et al., 2015). Thus, the higher tolerance of introduced *Triadica* populations to herbivory may in fact be due to a higher rate of mycorrhizal association. Furthermore, plants from different populations may differ in their responses to herbivores and pathogens due to geographic differences in associations with AMF, pathogens, and herbivores (Bossdorf et al., 2005). Thus we predict that the outcome of interactions between plants, insect herbivores, soil mutualists and antagonist microbes may vary with herbivore identity, soil microbial composition, and plant species or genotypes (Bennett et al., 2006). To date, however, studies on these complex interactions are rare.

In this study, we use *Triadica* as a model system to investigate the interactive effects of different soil communities and herbivory by aboveground insect herbivores on plant performance and biomass allocation. We specifically explore how soil biota impact the ability of plants to reduce the amount of damage incurred by herbivores (resistance) and how they affect the ability of plants to cope with damage during a regrowth period (tolerance). We further examine plant leaf and root secondary compounds (flavonoids and phenolics) that may mediate these interactions. Flavonoids and phenolics play an important role in plant-herbivore interactions (Bennett and Wallsgrrove, 1994; Treutter, 2005) and soil biota such as AMF are known to affect root and shoot levels of flavonoids and phenolics (Eftekhari et al., 2012; Zhang et al., 2013; Kaur and Suseela, 2020). Conversely, AMF are themselves affected by the concentrations of such metabolites in plant roots (Piotrowski et al., 2008; Pei et al., 2020). There is thus ample scope for interactions between soil biota and herbivores mediated by these secondary compounds. However, it is unknown to what extent soil biota modulate the extent of induction of these metabolites by herbivores and whether this differs between soil biota from different origins. We performed a common garden experiment in which we grew *Triadica* from native and introduced populations in four different soils and subjected them to attack by two specialist foliar herbivores. Soils contained one of four types of live soil biota: soil biota from conspecific plants (native *Triadica*) with the full spectrum of soil biota, soil biota from conspecific plants excluding fungi (fungicide treatment), soil biota from heterospecific plants (native grasses), or no soil biota (sterilized soil). We address the following three questions. (1) Do the soil biota from con- and heterospecific plant species affect the growth of *Triadica*? (2) Do the soil biota affect plant resistance (leaf damage) and tolerance (regrowth) to native aboveground insect herbivores and do these effects depend on herbivore identity (caterpillars vs. weevils)? (3) Do the soil biota affect the metabolic profiles of *Triadica* leaves and roots? (4) Do the responses of *Triadica* plants differ between plants from the native and the introduced range? Based on the commonly observed negative plant soil feedback observed for soil biota originating from soils of conspecific plants, we hypothesize that conspecific soil biota have a negative effect on the growth of *Triadica*, but heterospecific soil biota do not.

Furthermore, based on the ability of beneficial soil microbes to enhance resistance and tolerance of plants, we hypothesize that soil biota promote resistance and tolerance to herbivory, that these effects are partly mediated by microbially-induced changes in plant secondary metabolites, and that the effects differ among herbivores with different life styles. Finally, we hypothesize that the effects of soil biota originating from native *Triadica* soils will be different for *Triadica* plants from the native range, with which they have a shared and continued coevolutionary history, than for *Triadica* plants from the invaded range. The latter, after invasion, have evolved under novel abiotic and biotic selection regimes, which is likely to affect their response to the original soil biota from their native area.

2. Materials and methods

2.1. Study system

Triadica sebifera is a rapidly growing, polycarpic tree. It is native to China, and was first introduced to Georgia in the United States in the late 18th century for agricultural and ornamental purposes, then to Texas, Florida and Louisiana in the early 20th century (Pile et al., 2017). *Triadica* is invasive in the southeastern US, aggressively displacing native plants and forming monospecific stands. We used first-year *Triadica* seedlings, as previous studies suggest that this early stage plays an important role in its invasion success (Pile et al., 2017). In addition, young plants may be especially susceptible to the effects of herbivory (Reader, 1992).

In November 2013, we hand-collected *Triadica* seeds from seven populations across China (hereafter referred to as native populations) and eight populations across the southeastern United States (hereafter referred to as introduced populations, Table S1). Molecular studies indicate that populations in the introduced range come from two distinct introduction events and our collections encompassed both introductions and their likely source populations (DeWalt et al., 2011). For each population, we collected seeds from four to ten randomly selected *Triadica* trees. We removed the waxy seed coats by soaking them in water with laundry detergent (10 g / L) and we surface sterilized seeds by treating them with 10% bleach [0.6% sodium hypochlorite]. To evaluate the potential impacts of seed provisioning on seedling performance, we weighed 20 seeds from each population but there was no difference in seed mass of introduced and native populations [nested analysis of variance (ANOVA), $F_{1,13} = 1.65$, $P = 0.25$]. We germinated seeds in sterilized (autoclaved) soil (122 °C, 1 h with the center of the soil at this temperature) in April 2014.

Heratopoderopsis bicallosicollis (Coleoptera: Attelabidae) (hereafter “Weevil”) is a leaf-rolling specialist weevil, having 4 to 5 generations per year in the Hubei Province in China. Adults feed on leaves by nibbling holes in them but they avoid veins and lay eggs inside rolled leaves (nidi). One nidus usually contains one or two eggs. Larvae and pupae live inside the nidus until they emerge as adults (Wang et al., 2009). *Gadirtha inexacta* (Lepidoptera: Noctuidae) (hereafter: “Caterpillar”) is a multivoltine moth. The eggs of the moth overwinter on branches or leaves and hatch in May. The larvae of this specialist may consume entire *Triadica* leaves, causing severe damage, especially in the last three instars (Wang et al., 2009).

We collected *G. inexacta* and *H. bicallosicollis* in fields in Luotian, Hubei, China in July 2014 and reared them in cages separately (24 °C in insectarium) at Wuhan Botanical Garden, Hubei, China (31°32' N, 114°24' E). We used the offspring of these field collected insects for the experiment.

We collected soils from four locations in Dawu, Hubei, China (31°32' N, 114°24' E) in April 2014. Soils immediately adjacent to each of five *Triadica* trees were mixed to create a single *Triadica* soil (conspecific soil). We removed surface litter before collecting topsoil to a depth of 10 to 15 cm and removed larger sticks, rocks and root fragments by passing soil through a one cm mesh screen. In addition, we collected soil from

native grassland areas at this site using the same methods in April 2014 and mixed it to create a single grassland soil (heterospecific soil). The grass soil was chosen as a representative of a heterospecific soil as it represents a very common type of vegetation into which *Triadica* spreads in its native range. We analyzed selected basic properties of the *Triadica* conspecific and heterospecific soils (0–15 cm; Table S2).

In May, we created four soil biota treatments. Since our aim was to study effects of soil biota while minimizing any confounding effects of other soil factors such as texture, pH or nutrients, all pots were filled with the same soil mixture, that consisted of a 1:1 (volume) mixture of *Triadica* and grassland soil. The soil biota treatments were created by sterilizing either the *Triadica* soil, or the grass soil (or both) before mixing the two soil parts, thus resulting in soils that only contained the live soil biota of the grass soil or the *Triadica* soil (or neither of them), respectively. Specifically, we created the following four soil biota treatments: (1) Sterilized soils (“Sterile”) were created by autoclaving both parts (122 °C, 1 h with the center of the soil at this temperature). We did not measure the potential effects of sterilization on soil nutrients, but it is possible that there was a flush of nutrients (that may have been higher in the sterilized soil than in the other soils in which only half the soil volume was autoclaved). Next, we created two types of soil with live soil biota from conspecific plants. (2) Full conspecific (*Triadica*) soil (“*Triadica*”) was created by mixing active *Triadica* soil and sterilized grassland soil. (3) Reduced conspecific soil (“*Triadica*-F”) was also created by mixing active *Triadica* soil and sterilized grassland soil, but in addition the soil was treated with fungicide. This treatment was chosen to enable us to specifically assess the role of soil fungi in conspecific soils. As fungicide we used iprodione (rovral, 0.32 g active ingredient / m²), applied as drench prior to seedling planting and biweekly thereafter. Iprodione is traditionally used as a broad-spectrum contact fungicide, preventing the germination of fungal spores, though it may also have non-target effects on soil enzymes and bacteria (Zhang et al., 2017). (4) Heterospecific soils (“*Grass*”) were created by mixing active grassland soil and sterilized *Triadica* soil.

2.2. Experimental design

The experiment was a complete factorial design with four soil biota treatments (Sterile, *Triadica*, *Triadica*-F and Grass), three herbivore treatments (Weevil, Caterpillar and Control, i.e. no herbivores added), and two plant origins (China, “native” and N. America, “introduced”). In addition, the experiment included 15 plant population origins (7 populations from China and 8 populations from N. America) nested within plant continent origin. Three replicates were used for each treatment combination (soil, herbivore, population), totaling 540 plants. In May, we filled 540 pots (diameter = 15 cm, height = 9 cm) with one of the four soil types, transplanted a single seedling into each pot, caged each seedling in a mesh cage, and arranged pots randomly within an open-sided glasshouse, and watered them twice daily.

In July 2014, each seedling was subjected to one of the three herbivory treatments (control, caterpillar, and weevil herbivory). No herbivores were placed in cages of seedlings in the control treatment. For seedlings in the herbivory treatment, we placed one first instar caterpillar or 15 adult weevils in cages. After 15 days, we removed the herbivores and visually assessed leaf area chewed (we used the percent leaf damage as a relative damage measure for each leaf). We allowed plants to regrow for 60 days and then excised them at ground level, separated leaves and stems, dried them at 50 °C for 6 days, and then weighed them. We washed root systems from the soil, and collected fine root fragments from each to measure levels of mycorrhizal colonization. We dried and weighed the remaining roots.

We washed the fine root subsamples on a 500 µm sieve and stored them in histocassettes in 50% ethanol until we processed them for AMF quantification. We cleared roots in 10% KOH at 80 °C for 45 min, rinsed them, soaked them in 10% bleach for 1 min, rinsed them again, acidified them in HCl (2.5%), stained them with trypan blue, and mounted 10 × 1

cm root fragments on slides. We estimated AMF colonization rates using a modification of the gridline intersect method (McGonigle et al., 1990). We examined roots for the presence of arbuscules, vesicles, coils, and hyphae of AMF under a compound microscope at 400 × magnification.

2.3. Chemical analyses

We ground leaf and root samples in a ball mill and then stored them at -20°C in sealed plastic bags before chemical analyses. We extracted samples (100 mg) with 1 ml of 60% (v/v) ethanol (1 h at room temperature (24°C)), sonicated them (1 h, 20 min three times), and then removed insoluble material by centrifugation (5 min, 10,000 r.p.m., 4°C). We estimated total phenolic content using the modified Prussian blue assay (Graham, 1992). We mixed sample extracts (100 μl) with 3 ml distilled water, 1 ml of 0.016 M $\text{K}_3\text{Fe}(\text{CN})_6$ and 1 ml of 0.02 M FeCl_3 . After 15 min, we added 5 ml of stabilizer (0.2% Gum Arabic in 17% H_3PO_4) and measured absorbance at 700 nm. We used commercial gallic acid monohydrate (Sigma-Aldrich) as a standard. We estimated total flavonoid content using the $\text{Al}(\text{NO}_3)_3$ colorimetric method (Jia et al., 1999). We mixed sample extracts (200 μl) with 2.4 ml ethanol, 0.2 ml of 5% NaNO_2 , 0.2 ml of 10% $\text{Al}(\text{NO}_3)_3$, and 2 ml 1.0 M NaOH . After 15 min, we measured absorbance at 510 nm. We used commercial rutin (Germany) as a standard. We expressed all chemical concentrations as mg g^{-1} dry weight.

2.4. Data analyses

We used linear mixed models (LMM; PROC MIXED, SAS 9.4, SAS Institute Inc., Cary, NC, USA) to examine the dependence of aboveground biomass, belowground biomass, total biomass, root:shoot ratio and plant chemistry (leaf and root concentrations of flavonoids and phenolics) on soil treatment (Sterile, *Triadica*, *Triadica*-F, Grass soil biota), herbivory treatment (control, weevil, caterpillar), plant origin (native, introduced), and their interactions as fixed effects. Plant population (nested within plant origin) was included as a random effect. Similarly, LMMs were used to assess the effect of these factors on damage incurred by herbivores (measured by the percent of leaf area eaten), excluding data from the no-herbivory treatment, and the effect of these factors on root colonization by AMF structures (hyphae, arbuscules, vesicles and coils), excluding data from sterile and *Triadica*-F soil treatments. Adjusted means partial difference tests were used to examine differences among means for significant factors with more than 2 levels. We checked that data met the assumptions for LMM. We square root transformed AM colonization data to fit these assumptions.

Additional analyses were performed to test effects of soil biota on plant tolerance to herbivory. Herbivory-exposed plants were subjected to a 15-day period of herbivory and then allowed to regrow for 60 days. The growth reduction (compared to non-herbivory plants from the corresponding soil/origin treatment) that was observed at final harvest is thus the result of their (in-)ability to reduce damage (resistance) and their (in-)ability to regrow following damage (tolerance). To disentangle the role of tolerance we classified plants into three damage classes (mild: 1–33%; moderate: 34–66%; severe: 67–100% loss of leaf area) and assessed the percent reduction in final yield compared to plants from the corresponding control (no-herbivore) treatment for each damage class. Tolerance was defined as 100 minus this percent reduction. An LMM was then performed to test effects of plant origin, soil biota treatment, and damage class as fixed effects on final plant biomass with plant population included as random factor nested within plant origin. Significant interactions between soil treatment and damage class will indicate differences in tolerance between plants grown with different soil biota. Likewise, interactions between plant origin and damage class will indicate differences in tolerance between plants from the native and introduced range.

All data analyses were performed with the statistical analysis software SAS, ver. 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Effects of plant origin, soil biota and herbivory on biomass production

Across all soil and herbivory treatments, *Triadica* plants from the introduced range on average produced 19% more shoot dry weight than plants from the native range (Table 1, “Plant origin” effect, $P < 0.01$), independent of soil or herbivory treatment (no interactions between plant origin and soil or herbivory, Table 1). By contrast, their root biomass production was similar (Table 1). As a result, plants from the introduced range on average produced a higher total biomass (mean \pm se: 3.75 ± 0.12 g) and a lower root-to-shoot ratio (0.57 ± 0.03) than plants from the native range (3.35 ± 0.12 g and 0.67 ± 0.03 , respectively).

Soil biota significantly affected total, aboveground and belowground plant biomass (Table 1, Fig. 1). Soil biota originating from conspecific soils from the native range (*Triadica*) on average reduced total *Triadica* biomass by 21% when compared to growth on sterile soils (Fig. 1). The extent of this reduction was on average higher for plants from the native range (28%) than for plants from the introduced range (13%), but this difference was not statistically significant due to large variation among populations from the introduced range (Fig. S1). The reduction in total biomass by biota from conspecific soils was entirely due to the fungal component of these soil communities, as soil biota from conspecific soils that were treated with fungicide (*Triadica*-F) actually enhanced biomass by 8% (Fig. 1). A similarly modest but significant growth promotion (9%) was observed for plants grown in the presence of heterospecific soil biota that originated from grass soils (Grass, Fig. 1). Soil biota affected shoot and root biomass to a similar extent, hence they did not affect root-to-shoot ratio (Table 1).

3.2. Interactive effects between herbivores and soil biota on plant damage and biomass production

The two herbivore species differed in the amount of damage that they inflicted on plants during the 15-day feeding period (Fig. 2). Weevils consumed on average 25% of the leaf area of plants, independent of plant origin or soil treatment. By contrast, caterpillars on average consumed a much larger proportion of leaf area (58%) and the extent of their damage depended on soil treatment (Table S3, “Soil biota origin \times herbivory” interaction, $P = 0.018$). Specifically, plants growing on soils with conspecific *Triadica* biota suffered more caterpillar damage (71%) than plants grown in sterilized soil (56%) (Fig. 2). Plants grown on conspecific soils that had been treated with fungicide did not suffer such increased caterpillar damage (49%), suggesting that the increased susceptibility of plants on soils with conspecific soil biota was mainly due to interactions with the fungal component of this soil community. Plants growing on soils with grass soil biota soil suffered similar damage (56%) as plants grown on sterilized soil (Fig. 2), indicating no specific effect of soil biota from these soils on caterpillar damage.

After the 15-day feeding trial, plants were left to regrow for 60 days. Effects of herbivory on final biomass therefore encompass the ability of plants to withstand damage (resistance), as well as their ability to regrow following damage (tolerance). Herbivory significantly reduced final plant total biomass (Table 1, “Herbivory” effect, $P < 0.001$, Fig. 1), i.e., plants were not able to fully compensate for the inflicted damage. Surprisingly, despite the fact that weevils had consumed less than half of the leaf area compared to the amount that was consumed by caterpillars, the reduction in final plant biomass after 60 days of regrowth was roughly similar for the weevil and caterpillar treatments (weevils: 23% reduction; caterpillars: 28% reduction, Fig. 1). As this suggests that plants were overall less tolerant to weevil damage than to caterpillar damage, we inspected whether there was a steeper decline of final biomass on percent damage for weevil damage than for caterpillar damage. Intriguingly, these relationships appeared to be non-linear, and

Table 1

Linear Mixed Models analyzing the effects of plant origin (China, USA), soil biota origin (*Triadica* soil, *Triadica* soil -fungi, grass soil, sterile), herbivory (weevil, caterpillar, control), and their interactions on *Triadica sebifera* performance (total dry weight, shoot dry weight, root dry weight, and root-to-shoot ratio (R:S)). Effects of plant population (nested within plant origin), were included as random factor in the analysis). Significant results are in bold.

Factor	df	Total mass		Shoot mass		Root mass		R:S	
		F	P	F	P	F	P	F	P
P: Plant origin	1,13	5.7	0.0324	12.5	0.0036	0.0	0.8579	7.7	0.0154
S: Soil biota origin	3,489	21.5	<0.0001	24.9	<0.0001	12.8	<0.0001	0.5	0.7026
H: Herbivory	2,489	49.7	<0.0001	46.6	<0.0001	40.7	<0.0001	7.6	0.0005
P × S	3,39	1.0	0.3864	0.8	0.5242	1.2	0.3222	0.6	0.6016
P × H	2,26	2.0	0.1626	2.3	0.1200	2.8	0.0767	6.1	0.0069
S × H	6,489	5.7	<0.0001	4.4	0.0003	6.6	<0.0001	5.5	<0.0001
P × S × H	6,78	0.4	0.8584	0.4	0.8803	0.6	0.7086	2.1	0.0585

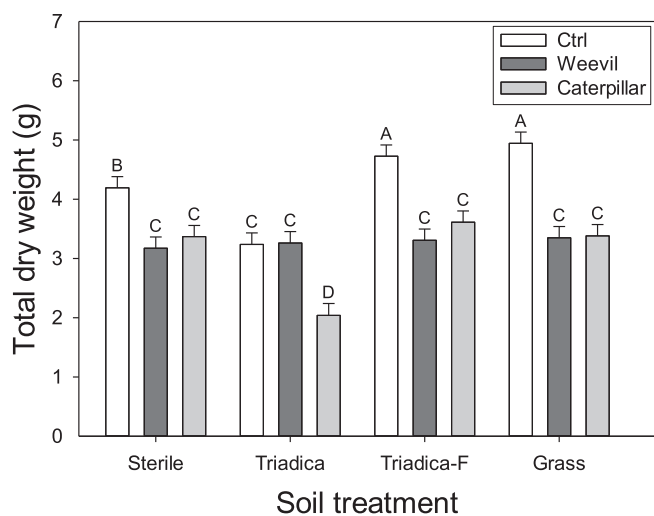


Fig. 1. Total dry mass produced by *Triadica sebifera* plants grown under different soil and herbivory treatments. Soils were either sterilized or harboured soil biota from conspecific *Triadica* soils that were either untreated (*Triadica*) or treated with fungicides (*Triadica-F*), or soil biota from heterospecific grass soils. Plants were either grown without herbivores (Ctrl, open bars) or subjected to 15 days of herbivory by specialist weevils (*H. bicallosicollis*, dark grey bars) or caterpillars (*G. inexacta*, light grey bars). Means \pm 1 SE. N = 45 for each treatment combination. Means sharing a common letter are not significantly different from each other in post-hoc tests.

plants showed distinct tolerance responses to weevil and caterpillar damage (Fig. 3; Fig. S2). Specifically, plant tolerance to moderate levels of damage (33–66% of leaf area consumed) was similar for damage inflicted by caterpillars (23% biomass reduction at final harvest) and weevils (29% reduction). However, plants exposed to mild damage (1–33%) showed high tolerance when the damage was inflicted by caterpillars (4% reduction) but not when it was inflicted by weevils (22% reduction). Conversely, plants exposed to severe damage (67–100%) showed high tolerance when the damage was incurred by weevils (31% reduction) but not when it was incurred by caterpillars (48% reduction). Thus, plants were not just overall less tolerant to weevil damage than to caterpillar damage, but rather were specifically less tolerant to the low levels of damage that were inflicted by these weevils.

The extent of the reduction in final biomass by caterpillars and weevils strongly depended on soil biota (Fig. 1; Table 1, “Soil biota origin \times Herbivory” interaction, $P < 0.001$). On soils with *Triadica* biota, the reduction in final plant biomass by caterpillars (compared to controls without herbivory) was almost twice as large (37%) as it was on sterile soils (20%). This stronger reduction was due to the fungal component of these soils, since the reduction on soils treated with fungicide was comparable to that on sterile soils (23%). The observed stronger reduction in plant final biomass by *Triadica* soil biota is in accordance

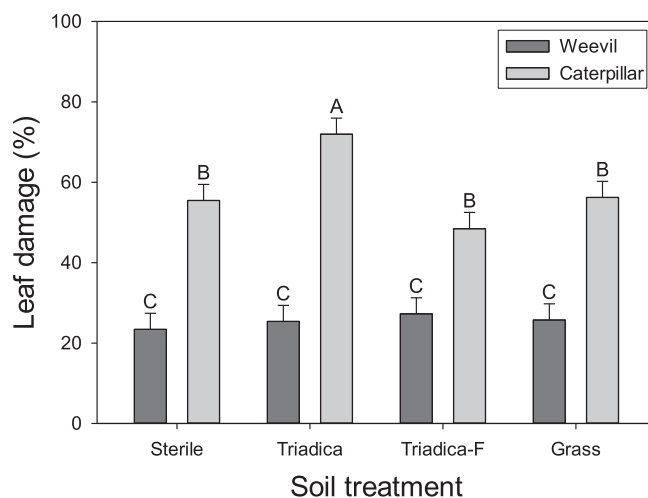


Fig. 2. Damage (% leaf area removed) incurred by *Triadica sebifera* plants during a 15-day period of herbivory by specialist weevils (*H. bicallosicollis*, dark grey bars) or specialist lepidopteran caterpillars (*G. inexacta*, light grey bars). Soils were either sterilized or harboured soil biota from conspecific *Triadica* soils that were either untreated (*Triadica*) or treated with fungicides (*Triadica-F*), or soil biota from heterospecific grass soils. Means \pm 1 SE. N = 45 for each treatment combination. Means sharing a common letter are not significantly different from each other in post-hoc tests.

with the larger damage incurred by caterpillars on these plants. This effect can thus be seen as a consequence of the reinforcing effects of fungi from *Triadica* soils on plant susceptibility (higher caterpillar damage), rather than an effect on plant tolerance to damage. The latter conclusion is corroborated by the lack of an effect of soil biota on tolerance to caterpillar feeding (Fig. S2B; Table S4, no significant interaction “Soil biota origin \times Damage class”). By contrast, on soils with *Triadica* biota the reduction in final plant biomass by weevils was close to zero, whereas reductions on the other soils, including the fungicide-treated soils, ranged from 24 to 32% (Fig. 1). As the damage inflicted by weevils was independent of soil biota (Fig. 2), this effect can thus be seen as a consequence of the positive effects of the fungal component of *Triadica* soils on regrowth after weevil damage (tolerance). This conclusion is corroborated by the significant impact of soil biota on tolerance to weevils (Fig. S2A; Table S4, significant interactions “Soil biota origin \times Damage class”, $P < 0.001$). Summarizing, soil biota thus affected both resistance and tolerance of plants.

The two shoot herbivores exerted stronger negative effects on root biomass than on shoot biomass when they fed on plants from the native range, resulting in a ca. 15% reduction in root-to-shoot ratio, independent of soil biota. This effect was not observed for plants from the introduced range, that did not change their root-to-shoot ratio in response to herbivory (Table 1, “Herbivory” and “Herbivory \times Plant origin” interaction for root-to-shoot ratio, Fig. S3)

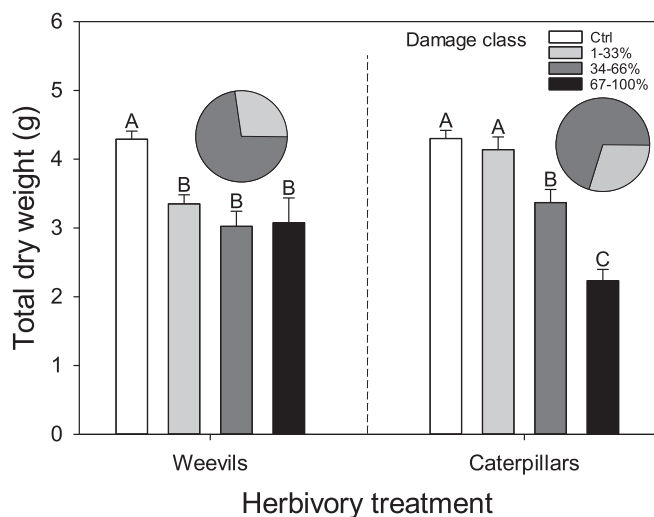


Fig. 3. Total dry mass of *Triadica sebifera* plants after a regrowth period of 60 days following 15 days of herbivory by weevils (*H. bicallosicollis*, left set of bars) or caterpillars (*G. inexacta*, right set of bars), averaged across different soils. The shading of bars indicate the amount of damage experienced during herbivory: open bars: 0% (control plants); light grey: 1–33%; dark grey: 34–66%; black: 67–100%. Pies above bars indicate the proportion of plants in each of the latter three categories. Means \pm 1 SE. Sample sizes for the four classes of increasing damage are 167, 120, 35 and 12 for weevils, and 167, 57, 47, and 69 for caterpillars, respectively. Means sharing a common letter are not significantly different from each other in post-hoc tests.

3.3. Effects of plant origin, soil biota and herbivory on defense compounds in leaves

Plants from the introduced range on average produced 14% higher levels of leaf flavonoids ($P < 0.01$) and 13% higher levels of leaf phenolics ($P < 0.05$) than plants from the native range (Fig. S4, Table S4). Herbivores significantly enhanced the levels of leaf flavonoids and phenolics (Fig. S4; Table S4, “Herbivory” effect $P < 0.001$). In response to caterpillar feeding, plants produced 34% more leaf flavonoids and 25% more leaf phenolics. By contrast, in response to weevil feeding, plants produced on average 22% more leaf flavonoids, but did not induce leaf phenolics. Soil biota did not affect leaf flavonoids or phenolics, nor did they affect the extent to which herbivores induced these compounds (Table S4).

3.4. Effects of plant origin, soil biota and herbivory on defense compounds in roots

Plants from the native and introduced ranges did not differ in their levels of root flavonoids or root phenolics (Table S4). In contrast to what was observed in leaves, effects of herbivores on secondary metabolites in the roots not only depended on herbivore identity but also on soil biota (Table S4, Fig. 4). On average, both weevils and caterpillars enhanced root flavonoid levels by 44%. However, the extent of this induction was modulated by soil biota (Table S4, “Soil biota origin \times Herbivory” interaction, $P < 0.05$). Caterpillar feeding enhanced root flavonoids by 83% when plants were grown on soils with biota from heterospecific (grass) soils, roughly similar to the extent of increase observed on sterile soils (65%), but did not significantly (14%) enhance root flavonoids on conspecific *Triadica* soils (Fig. 4A). Weevils also caused the strongest increase in root flavonoids on soils with biota from grass soils, but enhanced root flavonoids on soils with conspecific *Triadica* biota as well.

In contrast to root flavonoids, levels of root phenolics were not enhanced by herbivory, but instead were reduced, depending on soil treatment (Fig. 4B). On sterile soils, no effect of herbivory on levels of root phenolics was observed. However, caterpillars strongly reduced

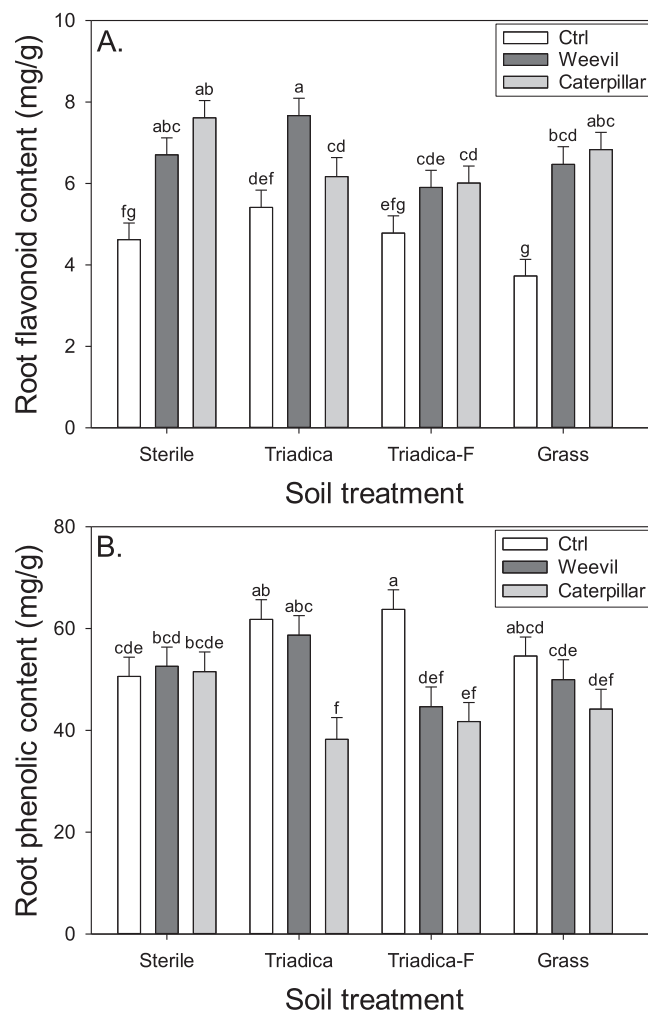


Fig. 4. Root concentrations of flavonoids (A) and phenolics (B) in *Triadica sebifera* plants grown under different soil and herbivory treatments. Soils were either sterilized or harboured soil biota from conspecific *Triadica* soils that were either untreated (*Triadica*) or treated with fungicides (*Triadica-F*), or soil biota from heterospecific grass soils. Plants were either grown without herbivores (Ctrl, open bars) or subjected to 15 days of herbivory by specialist weevils (*H. bicallosicollis*, dark grey bars) or lepidopteran caterpillars (*G. inexacta*, light grey bars). Means \pm 1 SE. $N = 45$ for each treatment combination. Means sharing a common letter are not significantly different from each other in post-hoc tests.

levels of root phenolic on *Triadica* soils (*Triadica*: 38%). This reduction was not due to the fungal soil component as it was observed in fungicide-treated *Triadica* soils as well (*Triadica-F*: 35%). A slight reduction in root phenolics was also seen in plants grown on soils with biota from heterospecific soils (Grass: 19%). By contrast, weevils only reduced root phenolic levels on *Triadica* soils treated with fungicide (Fig. 4B).

3.5. Effects of plant origin, soil biota, herbivory and root chemistry on AMF colonization

No root colonization by arbuscular mycorrhizal fungi (AMF) was observed in plants grown on sterilized or fungicide-treated soils, indicating that these treatments had been effective. Root colonization did not differ between plants originating from the introduced (54%) and native (52%) range (Fig. S5; Table S6). Plants were more strongly colonized by AMF when grown in soils with conspecific *Triadica* biota (60%) than in soils with live biota from heterospecific grass soils (46%) (Table S6, “Soil biota origin” effect for total colonization, $P < 0.001$). Interestingly, while plants grown in soils with *Triadica* biota were more

strongly colonized by hyphae compared to plants grown in soils with grass biota, they did not have a higher production of AMF functional modules (arbuscules, Table S6). Herbivory consistently increased total levels of AMF colonization, due to higher abundances of both hyphae and arbuscules (Fig. S5, Table S6). In plants grown with *Triadica* soil biota, caterpillar and weevil feeding enhanced total colonization from 54% to 63% and 64%, respectively. In plants grown with grass soil biota, colonization increased from 40% to 48% and 50%, respectively.

AMF root colonization was associated with root chemistry, but the direction of the association depended on plant origin (Fig. S6). In plants originating from populations from the native range (China), AMF colonization on grass soils was negatively associated with root concentrations of both flavonoids ($P < 0.002$) and phenolics ($P < 0.05$) (Fig. S6A, C). By contrast, in plants originating from populations from the invaded range (N. America), AMF colonization on grass soils was positively associated with root concentrations of flavonoids ($P < 0.05$), and unaffected by root concentrations of phenolics ($P = 0.13$) (Fig. S6B, D). On *Triadica* soils, no significant associations between AMF colonization and root chemistry were observed (Fig. S7).

Associations between AMF colonization rate and plant biomass depended on soil and herbivore treatment (Fig. S8). In the control (no-herbivory) and weevil treatment, AMF colonization rate was not significantly associated with biomass production (Fig. S8A-D). However, in plants that had been exposed to caterpillar feeding, root colonization was negatively associated with plant biomass. For plants grown on soil with *Triadica* biota (Fig. S8E), plant biomass decreased by 28 mg with every % increase in AMF colonization ($P = 0.012$); for plants grown on soil with grass biota, plant biomass also tended to decrease, by 26 mg with every % increase in AMF colonization (Fig. S8F, $P = 0.053$).

4. Discussion

4.1. Soil biota vary in their effect on plant growth

Soil biota from both conspecific and heterospecific soils were important drivers of variation in growth among *Triadica* plants. In the absence of herbivory, soil biota from grass soils slightly but significantly promoted growth, whereas soil biota from conspecific soils strongly reduced plant growth. Our results therefore support the general pattern observed in plant soil feedback studies, showing that the effects of soil biota on plant biomass production can vary from highly positive to highly negative, but that soil biota from conspecific soils commonly reduce plant performance (Van der Putten et al., 2013; Yang et al., 2013). Interestingly, when conspecific soils were treated with fungicide, plants experienced a similar growth promotion as plant grown on soils with soil biota originating from conspecific grass soils. This strongly suggests that conspecific soils harboured pathogenic fungi that shifted the balance from growth promotion to growth reduction (Yang et al., 2013, 2019, 2020). Due to their long lifespan, tree species like *Triadica* are likely to be prone to accumulation of specific soil pathogens that can have major ecological consequences such as reduced establishment of offspring around parental trees, contributing to local biodiversity (Janzen-Cornell hypothesis, see e.g. Petermann et al., 2008). Our results further indicate that the reduced growth of *Triadica* on conspecific soil compared to heterospecific grass soils is not due to a reduced availability of or propensity to form associations with AMF, as colonization rates were actually higher in conspecific than in heterospecific soil. This indicates that even if AMF from conspecific soils incurred a growth benefit to plants, this did not outweigh the negative effect of pathogenic fungi. Alternatively, AMF themselves may have been non-beneficial under the experimental conditions, but this is not supported by the absence of a relationship between plant biomass and AMF colonization rate in the absence of herbivory (Fig. S8A) and a positive association between AMF colonization and growth recently observed in *Triadica* in another study (Pei et al., 2020).

4.2. Effects of soil biota on plant resistance and tolerance to herbivory

Soil biota significantly affected the extent to which *Triadica* plants suffered from herbivory by the two tested specialist herbivores. They affected both the amount of damage that was inflicted by herbivores (resistance) and the ability of plants to regrow following damage (tolerance).

First, soil biota affected the resistance of plants. Specifically, fungi from *Triadica* soils increased the damage inflicted by caterpillar feeding. Since soil biota did not affect leaf flavonoids or phenolics, it is likely that this increased susceptibility was mediated by other, unmeasured, plant traits that were systemically affected by the soil fungi. In contrast to what we observed for caterpillar feeding, damage by adult weevils was independent of soil biota, illustrating that soil biota effects on resistance are herbivore-specific.

Second, soil biota also significantly altered tolerance to herbivory. Notably, whereas weevils incurred the same amount of damage on plants regardless of the soil in which they grew, this resulted in a more than 30% loss of final biomass in plants grown on sterile soils, but did not incur any loss of final biomass in plants grown on *Triadica* soils. This indicates that native *Triadica* soil biota enhanced plant tolerance to herbivory by their specialist weevils. Since this effect was not observed when the *Triadica* soil had been treated with fungicides, we speculate that this effect was due to the fungal component of this soil, although iprodione is known to also affect other, non-target soil organisms. Although it is tempting to speculate that this increased tolerance could have been mediated by the enhanced levels of AMF colonization that were observed in plants exposed to weevil feeding, this hypothesis is not supported by the observation that there was no significant relationship between levels of AMF root colonization and final plant biomass in these plants (Fig. S8C). For plants exposed to feeding by caterpillars there was even a strongly negative association between AMF colonization and final plant biomass on *Triadica* soils (Fig. S8E), indicating that under conditions of caterpillar herbivory AMF compete for resources rather than benefit the regrowing plants.

Our results further revealed the interesting phenomenon that the extent to which plants could maintain biomass production after exposure to herbivory did not linearly decrease with the amount of damage that was incurred, as is generally assumed in studies of plant tolerance to herbivory, but in fact decreased non-linearly. Tolerance is operationally defined as the slope of the linear regression of a fitness proxy on damage (Stinchcombe and Rausher, 2002). This assumes that plants suffer a fixed proportion of fitness loss for every % of leaf area lost by herbivory. However, across all soils, *Triadica* plants could cope relatively well with low amounts of caterpillar damage, but suffered accelerating biomass loss when damage increased. By contrast, they suffered relatively large biomass loss even when they were only slightly damaged by weevils, but suffered decelerating biomass loss at higher levels of weevil damage. This means that the ranking of estimates of tolerance to weevil and caterpillar damage actually depends on the level of damage incurred by the herbivores. This cautions against the standard use of linear regressions of fitness on damage to estimate plant tolerance, and suggests tolerance might be a more complex phenomenon. The mechanisms underlying such non-linear responses need further study, but might be related to the different feeding modes of the herbivores. The weevil *H. bicallosicollis* usually nibbles on plant shoots, leaving feeding scars or feeding holes in *Triadica* leaves, but leaving the veins undamaged. By contrast, caterpillars of *G. inexacta* consume entire leaves or large parts of leaves including the veins. Thus, *Triadica* shoots and roots may exhibit different responses to different intensities of feeding by weevils and caterpillars that affect their ability to tolerate these damage levels.

4.3. Changes in plant secondary metabolite profiles in response to herbivores and the impact of soil microbes on these responses

Herbivory by both herbivore species resulted in higher levels of

flavonoids in the roots of their host plants, but in slightly lower levels of root phenolics. Reductions in root phenolics, notably hydrolyzable phenolic acids, upon aboveground herbivory are commonly observed, and have been shown to underlie the reduced preference of root-feeding herbivores, that use these compounds as feeding cue, for plants infested by aboveground herbivores in maize (Erb et al., 2015). Herbivore-induced increases in root metabolites may also have important consequences for interactions with soil microbes (e.g. Hu et al., 2018). For instance, herbivore-induced increases in root flavonoids could facilitate AMF colonization and inhibit specific soil pathogens (Hassan and Mathesius, 2012). Indeed, previous studies have shown that the level of AMF root colonization is positively correlated with root flavonoid content in *Triadica* (Pei et al., 2020). This is only partly corroborated by our results, as the association between root flavonoids and AMF colonization depended on soil and plant origin (discussed in the next section).

The observed herbivore-induced changes in root secondary metabolites in our study are in agreement with some of the earlier findings in *Triadica* (Li et al., 2016; Xiao et al., 2019) showing that specialist caterpillars of *G. inexacta* increase root flavonoids but not root phenolics, but oppose other findings in *Triadica* (Li et al., 2020) showing that these herbivores can actually reduce root flavonoids. Our results strongly suggest that one of the mechanisms underlying such variation in root biochemical responses to herbivory across studies could be variation in the soil microbial community. Notably, under some soil biota treatments (no soil biota, grass soil biota), plants strongly induced root flavonoids in response to caterpillars, but this response was repressed by soil biota from *Triadica* soils. Moreover, in sterile soils, plants did not alter root phenolics in response to caterpillars, but in soils with *Triadica* biota they decreased levels of root phenolics in response to caterpillar feeding. The important corollary of these observations is that root biochemical responses to aboveground herbivory are contingent upon soil biota. Therefore, in order to understand trade-offs versus facilitative effects of aboveground herbivores towards belowground herbivores (Kaplan et al., 2008; Johnson et al., 2012; Biere and Goverse, 2016) we need to take variation in the soil microbial community into account.

Interestingly, in contrast to their modulating effects on herbivore-induced changes in root biochemistry, soil biota did not interfere with herbivore-induced changes in phenolics or flavonoids in the leaves. In agreement with earlier studies (Xiao et al., 2019; Li et al., 2020), these compounds were strongly induced by caterpillars and to a lesser extent by weevil feeding, but the extent of this induction was independent of soil biota. This contrasts with many reports on the induction or priming of leaf defenses by beneficial soil microbes such as AMF and plant growth promoting rhizobacteria and fungi, including increases in leaf proteinase inhibitors and phenolic compounds active against leaf feeding herbivores (e.g. Avio et al., 2017; Selvaraj et al., 2020). However, it should be kept in mind that most studies on microbial priming of herbivore defenses are performed shortly after the initiation of herbivory. Our study focused on changes observed following a regrowth period of 60 days. The observed plant biochemical changes in response to herbivory are therefore likely to reflect a long-term response including changes due to altered leaf age distribution following regrowth, rather than short-term induction or priming effects in response to immediate herbivory. Furthermore, the absence of soil biota effects on herbivore-induced changes in leaf phenolics and flavonoids does not exclude the possibility that other leaf metabolites involved in plant-herbivore interactions were affected by soil biota, as soil biota did affect resistance to leaf damage by caterpillars.

4.4. Plants from the native vs. Introduced range differ in growth and defense but show only minor differences in their response to soil biota

Plants from the introduced range produced phenotypes that were quite distinct from those of plants from the native range. They produced more shoot biomass, had a lower root-to-shoot ratio and produced higher levels of leaf flavonoids and phenolics. These results corroborate

earlier studies (Huang et al., 2012; Siemann et al., 2017) showing that under controlled environmental conditions *Triadica* plants from the invaded range have higher biomass production than those from the native range. This suggests that introductions of *Triadica* have been non-random with regard to life-history traits, or that evolutionary shifts have occurred following invasion. In contrast to earlier studies (Wang et al., 2016), we did not observe that plants from the introduced range were more tolerant to herbivory. This can be inferred from the lack of significant interactions between plant origin and damage class effects in Table S4. Plants from the introduced range just produced proportionally more biomass for each level of damage that was assessed. However, whereas plants from the introduced range maintained their root-to-shoot ratio following herbivory, plants from the native range reduced their root-to-shoot ratio. This may indicate that plants from the native range prioritized shoot regrowth over root growth following herbivory. Under prolonged herbivory, this may deplete root resources and eventually result in lower tolerance.

The observation of higher levels of leaf phenolics and flavonoids in plants from the introduced range is in agreement with the shifting defense hypothesis (SDH, Doorduyn and Vrieling, 2011). *Triadica* plants in the invaded range appear to be released from many of their specialist enemies (Xiao et al., 2020). The SDH predicts that while such plants may invest less in costly defenses such as digestibility reducers that are active against specialists (EICA, Blossey and Nötzold, 1995), they may actually enhance investment in more specialized defenses such as phenolics that have been overcome by specialists, but that are still active against the generalists that they will encounter in their novel range.

In contrast to the higher flavonoid and phenolic concentrations observed in the shoots of plants from the invaded range, root concentrations were not different between plants from the native and introduced range. This contrast with the results from a recent *Triadica* study that used a different set of populations from the native and introduced range (Pei et al., 2020). For that set of populations, it was shown that plants from the introduced range had lower levels of root phenolics and higher levels of root flavonoids, associated with higher levels of AMF colonization, than plants from the native range. For our set of populations, we could not show that plants from the introduced range had higher root flavonoid levels, nor that they had higher levels of AMF colonization. Such variable results across studies are not unexpected given the large variation in the levels of these secondary metabolites that we observed among populations, especially among those from the native range (Fig S4), creating sampling effects. Interestingly, the associations between the concentration of root flavonoids and AMF colonization in our study differed between plants from the native and invaded range. Like in the study by Pei et al. (2020), higher root flavonoid levels were associated with higher AMF colonization in plants from the introduced range, but they were associated with lower levels of AMF colonization in plants from the native range. Further studies are necessary to reveal whether this result is due to differences in the composition of root flavonoids between plants from the native and introduced range. Root flavonoids comprise a diverse set of metabolites, whose composition may differ among species and whose biological properties strongly depend on e.g. sugar moieties of the molecules involved. Specific flavonoids have been shown to be associated with AMF colonization (Davies et al., 2005; Castillo et al., 2014) hence it is likely that variation in flavonoid composition will have a large impact on the association between flavonoid content and AMF colonization.

4.5. Conclusions

In conclusion, our study shows that soil biota are important drivers of variation in plant performance in *Triadica*. Whereas soil biota from heterospecific soils slightly promoted growth, fungi from conspecific soils strongly reduced plant growth. Moreover, soil biota had profound effects on both resistance and tolerance of plants to specialist insect herbivores. We show that the strength and direction of these effects are

contingent upon the identity of the herbivore and soil biota. We therefore conclude that inclusion of soil biota effects is indispensable to understand variation in plant resistance and tolerance to herbivory. Finally, despite enhanced colonization of plants by AMF following herbivory, we show that high levels of AMF colonization in this system are not associated with enhanced, but on the contrary with reduced performance under caterpillar herbivory. Whereas AMF may thus be beneficial to their host plants under some biotic conditions, they should be considered as competitors under others. Overall, our results nicely illustrate the intricate links that exist between aboveground- and belowground interactions (Biere and Goverse, 2016), showing that plant associations with soil biota such as AMF can affect damage caused by aboveground herbivores (reviewed in Koricheva et al., 2009), e.g. through soil biota-induced changes in the plant's biochemical responses to herbivore feeding, and that, conversely, aboveground herbivory can affect associations with beneficial soil microbes such as AMF (reviewed in Barto and Rillig, 2010).

Author contributions

QY, JD and ES conceived the experiments. QY carried out the experiments and secondary compound analysis. QY, ES and AB carried out data analysis. QY, JH, JD, ES and AB wrote the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2021.115191>.

References

- Avio, L., Sbrana, C., Giovannetti, M., Frassinetti, S., 2017. Arbuscular mycorrhizal fungi affect total phenolics content and antioxidant activity in leaves of oak leaf lettuce varieties. *Sci. Hortic.* 224, 265–271.
- Ballhorn, D.J., 2011. Constraints of simultaneous resistance to a fungal pathogen and an insect herbivore in lima bean (*Phaseolus lunatus* L.). *J. Chem. Ecol.* 37, 141–144.
- Balog, A., Loxdale, H.D., Balint, J., Benedek, K., Szabo, K.A., Janosi-Rancz, K.T., Domokos, E., 2017. The arbuscular mycorrhizal fungus *Rhizophagus irregularis* affects arthropod colonization on sweet pepper in both the field and greenhouse. *J. Pest. Sci.* 90, 935–946.
- Bardgett, R.D., Wardle, D.A., Yeates, G.W., 1998. Linking above-ground and below-ground interactions: How plant responses to foliar herbivory influence soil organisms. *Soil Biol. Biochem.* 30 (14), 1867–1878.
- Barto, E.K., Rillig, M.C., 2010. Does herbivory really suppress mycorrhiza? A meta-analysis. *J. Ecol.* 98 (4), 745–753.
- Bennett, A.E., Alers-Garcia, J., Bever, J.D., 2006. Three-way interactions among mutualistic mycorrhizal fungi, plants, and plant enemies: Hypotheses and synthesis. *Am. Nat.* 167 (2), 141–152.
- Bennett, R.N., Wallsgrove, R.M., 1994. Secondary metabolites in plant defense-mechanisms. *New Phytol.* 127 (4), 617–633.
- Bever, J.D., Westover, K.M., Antonovics, J., 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *J. Ecol.* 85 (5), 561–573.
- Bezemer, T.M., van Dam, N.M., 2005. Linking aboveground and belowground interactions via induced plant defenses. *Trends Ecol. Evol.* 20 (11), 617–624.
- Biere, A., Bennett, A.E., 2013. Three-way interactions between plants, microbes and insects. *Funct. Ecol.* 27 (3), 567–573.
- Biere, A., Goverse, A., 2016. Plant-mediated systemic interactions between pathogens, parasitic nematodes, and herbivores above- and belowground. *Annu. Rev. Phytopathol.* 54, 499–527.
- Blossey, B., Nötzold, R., 1995. Evolution of increased competitive ability in invasive nonindigenous plants - a hypothesis. *J. Ecol.* 83 (5), 887–889.
- Bossdorf, O., Auge, H., Lafuma, L., Rogers, W.E., Siemann, E., Prati, D., 2005. Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia* 144 (1), 1–11.
- Castillo, C.G., Fredericksen, C., Koch, R., Sieverding, E., 2014. Effect of seed treatment with natural products on early arbuscular mycorrhizal colonization of wheat by *Claroideoglossum claroideum*. *J. Appl. Bot. Food Qual.* 87, 117–123.
- Davies, F.T., Calderon, C.M., Human, Z., Gomez, R., 2005. Influence of a flavonoid (formononetin) on mycorrhizal activity and potato crop productivity in the highlands of Peru. *Sci. Hortic.* 106, 318–329.
- DeWalt, S.J., Siemann, E., Rogers, W.E., 2011. Geographic distribution of genetic variation among native and introduced populations of Chinese tallow tree, *Triadica sebifera* (Euphorbiaceae). *Am. J. Bot.* 98 (7), 1128–1138.
- Doorduyn, L.J., Vrieling, K., 2011. A review of the phytochemical support for the shifting defence hypothesis. *Phytochem. Rev.* 10 (1), 99–106.
- Eftekhari, M., Alizadeh, M., Ebrahimi, P., 2012. Evaluation of the total phenolics and quercetin content of foliage in mycorrhizal grape (*Vitis vinifera* L.) varieties and effect of postharvest drying on quercetin yield. *Industr. Crops Prod.* 38, 160–165.
- Ehrenfeld, J.G., Ravit, B., Elgersma, K., 2005. Feedback in the plant-soil system. *Ann. Rev. Env. Resour.* 30, 75–115.
- Erb, M., Robert, C.A.M., Marti, G., Lu, J., Doyen, G.R., Villard, N., Barrière, Y., French, B. W., Wolfender, J., Turlings, T.C.J., Gershenzon, J., 2015. A physiological and behavioral mechanism for leaf herbivore-induced systemic root resistance. *Plant Physiol.* 169 (4), 2884–2894.
- Graham, H.D., 1992. Stabilization of the Prussian blue color in the determination of polyphenols. *J. Agr. Food Chem.* 40 (5), 801–805.
- Hannula, S.E., Zhu, F., Heinen, R., Bezemer, T.M., 2019. Foliar-feeding insects acquire microbiomes from the soil rather than the host plant. *Nat. Commun.* 10.
- Hassan, S., Mathesius, U., 2012. The role of flavonoids in root-rhizosphere signalling: opportunities and challenges for improving plant-microbe interactions. *J. Exp. Bot.* 63 (9), 3429–3444.
- Heil, M., 2011. Plant-mediated interactions between above- and below-ground communities at multiple trophic levels. *J. Ecol.* 99 (1), 3–6.
- Heinen, R., Biere, A., Harvey, J.A., Bezemer, T.M., 2018. Effects of soil organisms on aboveground plant-insect interactions in the field: Patterns, mechanisms and the role of methodology. *Front. Ecol. Evol.* 6.
- Heinen, R., Biere, A., Bezemer, T.M., 2020. Plant traits shape soil legacy effects on individual plant-insect interactions. *Oikos* 129 (2), 261–273.
- Hu, L., Robert, C.A.M., Cadot, S., Zhang, X., Ye, M., Li, B., Manzo, D., Chervet, N., Steinger, T., van der Heijden, M.G.A., Schlaeppli, K., Erb, M., 2018. Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nat. Commun.* 9, 2738.
- Huang, W., Carrillo, J., Ding, J.Q., Siemann, E., 2012. Invader partitions ecological and evolutionary responses to above- and belowground herbivory. *Ecology* 93 (11), 2343–2352.
- Jia, Z., Tang, M.C., Wu, J.M., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 64 (4), 555–559.
- Johnson, S.N., Clark, K.E., Hartley, S.E., Jones, T.H., McKenzie, S.W., Koricheva, J., 2012. Aboveground-belowground herbivore interactions: a meta-analysis. *Ecology* 93 (10), 2208–2215.
- Johnson, S.N., Mitchell, C., McNicol, J.W., Thompson, J., Karley, A.J., 2013. Downstairs drivers - root herbivores shape communities of above-ground herbivores and natural enemies via changes in plant nutrients. *J. Anim. Ecol.* 82 (5), 1021–1030.
- Jung, S.C., Martinez-Medina, A., Lopez-Raez, J.A., Pozo, M.J., 2012. Mycorrhiza-induced resistance and priming of plant defenses. *J. Chem. Ecol.* 38, 651–664.
- Kaplan, I., Halitschke, R., Kessler, A., Rehill, B.J., Sardaneli, S., Denno, R.F., 2008. Physiological integration of roots and shoots in plant defense strategies links above- and belowground herbivory. *Ecol. Lett.* 11, 841–851.
- Kaur, S., Suseela, V., 2020. Unraveling arbuscular mycorrhiza-induced changes in plant primary and secondary metabolome. *Metabolites* 10 (8), 335.
- Koricheva, J., Gange, A.C., Jones, T., 2009. Effects of mycorrhizal fungi on insect herbivores: a meta-analysis. *Ecology* 90 (8), 2088–2097.
- Lazebnik, J., Frago, E., Dicke, M., van Loon, J.J.A., 2014. Phytohormone mediation of interactions between herbivores and plant pathogens. *J. Chem. Ecol.* 40, 730–741.
- Li, X.Q., Guo, W.F., Siemann, E., Wen, Y.G., Huang, W., Ding, J.Q., 2016. Plant genotypes affect aboveground and belowground herbivore interactions by changing chemical defense. *Oecologia* 182 (4), 1107–1115.
- Li, X.Q., Gao, X., Siemann, E., Shen, S.i., Guo, W.F., 2020. Effects of above- and belowground herbivory of specialists and generalists on the growth and defensive chemicals of introduced and native Chinese tallow seedlings. *Plant Soil* 455, 65–78.
- Masters, G.J., Brown, V.K., Gange, A.C., 1993. Plant mediated interactions between aboveground and belowground insect herbivores. *Oikos* 66 (1), 148–151.
- McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A., 1990. A new method which gives an objective-measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. *New Phytol.* 115 (3), 495–501.
- Pei, Y.C., Siemann, E., Tian, B.L., Ding, J.Q., 2020. Root flavonoids are related to enhanced AMF colonization of an invasive tree. *AoB Plants* 12 (1), plaa002.

- Petermann, J.S., Fergus, A.J.F., Turnbull, L.A., Schmid, B., 2008. Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology* 89, 2399–2406.
- Pieterse, C.M.J., Zamioudis, C., Berendsen, R.L., Weller, D.M., Van Wees, S.C.M., Bakker, P.A.H.M., 2014. Induced systemic resistance by beneficial microbes. *Annu. Rev. Phytopathol.* 52, 347–375.
- Pile, L.S., Wang, G.G., Stovall, J.P., Siemann, E., Wheeler, G.S., Gabler, C.A., 2017. Mechanisms of Chinese tallow (*Triadica sebifera*) invasion and their management implications - A review. *Forest Ecol Manag* 404, 1–13.
- Pineda, A., Zheng, S.J., van Loon, J.J.A., Dicke, M., 2012. Rhizobacteria modify plant-aphid interactions: a case of induced systemic susceptibility. *Plant Biol* 14, 83–90.
- Piotrowski, J.S., Morford, S.L., Rillig, M.C., 2008. Inhibition of colonization by a native arbuscular mycorrhizal fungal community via *Populus trichocarpa* litter, litter extract, and soluble phenolic compounds. *Soil Biol. Biochem.* 40 (3), 709–717.
- Reader, R.J., 1992. Herbivory, competition, plant mortality and reproduction on a topographic gradient in an abandoned pasture. *Oikos* 65 (3), 414–418.
- Reinhart, K.O., Callaway, R.M., 2006. Soil biota and invasive plants. *New Phytol.* 170 (3), 445–457.
- Reinhart, K.O., Tytgat, T., Van der Putten, W.H., Clay, K., 2010. Virulence of soil-borne pathogens and invasion by *Prunus serotina*. *New Phytol.* 186 (2), 484–495.
- Rosa, E., Woestmann, L., Biere, A., Saastamoinen, M., 2018. A plant pathogen modulates the effects of secondary metabolites on the performance and immune function of an insect herbivore. *Oikos* 127 (10), 1539–1549.
- Selvaraj, A., Thangavel, K., Uthandi, S., 2020. Arbuscular mycorrhizal fungi (*Glomus intraradices*) and diazotrophic bacterium (*Rhizobium* BMBS) primed defense in blackgram against herbivorous insect (*Spodoptera litura*) infestation. *Microbiol. Res.* 231, 126355.
- Siemann, E., DeWalt, S.J., Zou, J.W., Rogers, W.E., 2017. An experimental test of the EICA hypothesis in multiple ranges: invasive populations outperform those from the native range independent of insect herbivore suppression. *AoB Plants* 9, plw087.
- Soler, R., Van der Putten, W.H., Harvey, J.A., Vet, L.E.M., Dicke, M., Bezemer, T.M., 2012. Root herbivore effects on aboveground multitrophic interactions: Patterns, processes and mechanisms. *J. Chem. Ecol.* 38 (6), 755–767.
- Stinchcombe, J.R., Rausher, M.D., 2002. The evolution of tolerance to deer herbivory: modifications caused by the abundance of insect herbivores. *Proc. Roy. Soc. B-Biol. Sci.* 269 (1497), 1241–1246.
- Strauss, S.Y., Agrawal, A.A., 1999. The ecology and evolution of plant tolerance to herbivory. *Trends Ecol. Evol.* 14 (5), 179–185.
- Treutter, D., 2005. Significance of flavonoids in plant resistance and enhancement of their biosynthesis. *Plant Biol.* 7 (6), 581–591.
- Van der Putten, W.H., Bardgett, R.D., Bever, J.D., Bezemer, T.M., Casper, B.B., Fukami, T., Kardol, P., Klironomos, J.N., Kulmatiski, A., Schweitzer, J.A., Suding, K. N., Van de Voorde, T.F.J., Wardle, D.A., 2013. Plant-soil feedbacks: the past, the present and future challenges. *J. Ecol.* 101 (2), 265–276.
- Van der Putten, W.H., Vet, L.E.M., Harvey, J.A., Wackers, F.L., 2001. Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends Ecol. Evol.* 16 (10), 547–554.
- Vannette, R.L., Hunter, M.D., 2009. Mycorrhizal fungi as mediators of defence against insect pests in agricultural systems. *Agr. Forest Entomol.* 11 (4), 351–358.
- Wang, Y., Ding, J.Q., Wheeler, G.S., Purcell, M.F., Zhang, G.A., 2009. *Heterapoderopsis bicalloscollis* (Coleoptera: Attelabidae): A potential biological control agent for *Triadica sebifera*. *Environ. Entomol.* 38 (4), 1135–1144.
- Wang, Y., Siemann, E., Wheeler, G.S., Zhu, L., Gu, X., Ding, J.Q., 2012. Genetic variation in anti-herbivore chemical defences in an invasive plant. *J. Ecol.* 100 (4), 894–904.
- Wang, Y., Zhu, L., Siemann, E., Ding, J.Q., 2016. Repeated damage by specialist insects suppresses the growth of a high tolerance invasive tree. *Biocontrol* 61 (6), 793–801.
- Xiao, L., Carrillo, J., Siemann, E., Ding, J.Q., 2019. Herbivore-specific induction of indirect and direct defensive responses in leaves and roots. *AoB Plants* 11 (1).
- Xiao, L., Ding, J.Q., Zhang, J.L., Huang, W., Siemann, E., 2020. Chemical responses of an invasive plant to herbivory and abiotic environments reveal a novel invasion mechanism. *Sci. Tot. Env.* 741, 140452.
- Yang, Q., Carrillo, J., Jin, H.Y., Shang, L., Hovick, S.M., Nijjer, S., Gabler, C.A., Li, B., Siemann, E., 2013. Plant-soil biota interactions of an invasive species in its native and introduced ranges: Implications for invasion success. *Soil Biol. Biochem.* 65, 78–85.
- Yang, Q., Ding, J.Q., Siemann, E., 2019. Biogeographic variation of distance-dependent effects in an invasive tree species. *Funct. Ecol.* 33 (6), 1135–1143.
- Yang, Q., Wei, S.J., Shang, L., Carrillo, J., Gabler, C.A., Nijjer, S., Li, B., Siemann, E., 2015. Mycorrhizal associations of an invasive tree are enhanced by both genetic and environmental mechanisms. *Ecography* 38 (11), 1112–1118.
- Yang, Q., Biere, A., Harvey, J.A., Ding, J.Q., Siemann, E., 2020. Antagonistic interactions between above- and belowground biota reduce their negative effects on a tree species. *Plant Soil* 454, 379–393.
- Yeh, P.J., Price, T.D., 2004. Adaptive phenotypic plasticity and the successful colonization of a novel environment. *Am. Nat.* 164 (4), 531–542.
- Zhang, R.Q., Zhu, H.H., Zhao, H.Q., Yao, Q., 2013. Arbuscular mycorrhizal fungal inoculation increases phenolic synthesis in clover roots via hydrogen peroxide, salicylic acid and nitric oxide signaling pathways. *J. Plant Physiol.* 170 (1), 74–79.
- Zhang, M.Y., Wang, W.J., Zhang, Y.L., Teng, Y., Xu, Z.H., 2017. Effects of fungicide iprodione and nitrification inhibitor 3, 4-dimethylpyrazole phosphate on soil enzyme and bacterial properties. *Sci. Total Environ.* 599, 254–263.