



# Eco-evolutionary processes affecting plant–herbivore interactions during early community succession

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## Abstract

The quality and outcome of organismal interactions are not only a function of genotypic composition of the interacting species, but also the surrounding environment. Both the strength and direction of natural selection on interacting populations vary with the community context, which itself is changed by these interactions. Here, we test for the role of interacting evolutionary and ecological processes in plant–herbivore interactions during early community succession in the tall goldenrod, *Solidago altissima*. We use surveys in a large-scale field experiment with repeated plots representing 6 years of early oldfield succession and reciprocal transplant common garden experiments to test for the relative importance of rapid evolution (genetic) and environmental changes (soil quality) in affecting mean plant resistance and growth phenotypes during community succession. While plant growth varied strongly with soil quality over the first 5 years of agricultural abandonment, plant secondary metabolism, and herbivore resistance varied minimally with the soil environment. Instead, mean composition and abundance of plant secondary compound bouquets differed between *S. altissima* plants from populations collected in communities in the first (“early”) and sixth (“intermediate”) years of oldfield succession, which was reflected in the feeding preference of the specialist herbivore, *Trirhabda virgata*, for early succession lines. Moreover, this preference was most pronounced on poorer quality, early succession soils. Overall, our data demonstrate that plant quality varies for insect herbivores during the course of early succession and this change is a combination of altered genotypic composition of the population and phenotypic plasticity in different soil environments.

**Keywords** Plant defense · Herbivory · Natural selection · Belowground interactions · Soil · Phenotypic plasticity

## Introduction

Insect herbivores can modify plant community dynamics both by altering competitive interactions among members of the community (Carson and Root 2000) and through natural selection and the resulting population-specific changes in plant defense trait expression (Bode and Kessler 2012; Agrawal et al. 2012; Uesugi and Kessler 2016). Thus, herbivores, continuously or intermittently, alter the context in which their interactions with plant populations are played out. For example, the intensity of biotic stresses, such as herbivory and competition, vary along environmental gradients in space (geographically) and time (community succession), and consequently so do their roles as interacting agents of natural selection on associated plant traits, such as resistance to herbivores and inter- and intra-specific competitive ability (Walker and del Moral 2003; Hodkinson 2005; Schemske et al. 2009; Hakes and Cronin 2012). In addition, plants exhibit phenotypic plasticity to both biotic and abiotic

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This is one of the first studies addressing eco-evolutionary feedbacks in plant–herbivore interactions and, specifically, in the expression of plant chemical defenses.

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conditions in their environments (Callaway et al. 2003). Thus, the expression of a plant phenotype (e.g. chemical defenses, competitive ability) and its relative or associational status within the community is a function of the community composition, the population genetic composition, and the presence of biotic and abiotic factors inducing changes in plant metabolism (phenotypic plasticity). In a natural environment, all of those factors interact and consequently make plant chemistry-mediated interactions context dependent. The outcome of biotic interactions, such as those between plants and herbivores, can consequently be predicted to be subject to a reciprocal interaction between ecological and rapid evolutionary processes (Agrawal et al. 2013; Travis et al. 2014).

### Phenotypic plasticity and rapid evolution in *Solidago altissima*

The tall goldenrod, *Solidago altissima* L. (Asteraceae), is a particularly interesting species in which to study ecological and rapid evolutionary dynamics. This species dominates early succession habitats, such as oldfields and naturally open habitats in northeastern North America, as well as frequently across its invasive range in Europe and Asia (Meyer et al. 2005; Etterson et al. 2008). *Solidago altissima* has long been used as a community ecology study system to understand complex interactions between plants and insect herbivores and predators (Weis and Abrahamson 1986; Maddox and Root 1987; Crutsinger et al. 2006). In some such oldfield communities, insect herbivory has been shown to be the major driver of community dynamics because herbivory reduces the competitiveness of the dominant *S. altissima* relative to other plant species in the community and so maintains higher species diversity (Carson and Root 2000). At the same time, the members of the diverse arthropod community associated with *S. altissima* differentially prefer different plant genotypes as hosts (Maddox and Root 1987). This phenomenon is suggested to underlie the finding that high genotypic diversity within *S. altissima* populations begets arthropod community diversity (Crutsinger et al. 2006).

Recent studies have demonstrated not only that the relative dominance of *S. altissima* populations within oldfield communities results from complex interactions between herbivory and competition with other plant species (Carson and Root 2000), but that differential exposure to these two different types of antagonism (herbivory and competition) affects the genetic composition of the plant population and consequently the mean expression of defense and competition phenotypes. While herbivory selects for increased expression of defense metabolites and herbivore resistance (Bode and Kessler 2012; Uesugi and Kessler 2016), relief from herbivory selects for the increased expression of traits mediating increased competitive ability, such as increased growth

under competition and increased production of allelopathic polyacetylenes, such as dehydromatricaria ester (Johnson et al. 2010; Uesugi and Kessler 2013, 2016). These changes in mean defense and competitive phenotypes in herbivore inclusion and exclusion plots, respectively, were detected after a 12-year maintenance of an herbivore exclusion treatment (Bode and Kessler 2012; Uesugi and Kessler 2013) suggesting rapid evolutionary processes with both insect herbivores and competition with neighboring plants as the major agents of natural selection (Uesugi et al. 2017).

Community context and rapid evolutionary processes affecting the population genetic composition both likely interact with phenotypic plastic responses. *Solidago altissima* has strong induced and very specific secondary metabolic responses to insect herbivory (Bode et al. 2013; Uesugi et al. 2013; Morrell and Kessler 2016) and insect-derived chemical cues (Helms et al. 2013; Uesugi et al. 2016). This strong inducibility of resistance combined with a volatile organic compound-mediated chemical information transfer from plant to plant (Morrell and Kessler 2016) has been shown to result in a more even distribution of herbivory among plants of the same population (Rubin et al. 2015). Strong context dependency means then that the outcome of plant–animal interactions, such as those described above, and thus natural selection on the associated traits should vary with community composition and population genetic structure.

### Plant–herbivore interactions changing along successional gradients

Within this framework, gradients of community succession are particularly interesting, because they represent this ever-changing community context and have been associated with a number of seminal hypotheses on the evolution of chemical anti-herbivore defenses (McKey 1974; Coley et al. 1985; Herms and Mattson 1992). For example, growth rates and defensive trait expression can vary dramatically with the succession of a habitat (Walker and del Moral 2003), with late successional species growing more slowly than early colonizers, but having stronger herbivore resistance (Herms and Mattson 1992; Bergelson 1994). However, while these patterns have been studied in species comparisons, they have rarely been investigated as genetically correlated traits within species. In those few within-species comparisons, a genotype's competitiveness was usually negatively correlated with its resistance to herbivores (Bode and Kessler 2012; Agrawal et al. 2012; Uesugi and Kessler 2013). Most importantly, evolutionary shifts in the mean phenotypes happened over surprisingly short periods of time (3–12 years), suggesting that rapid evolutionary and ecological processes underlying plant–herbivore interactions reciprocally affect each other in similar ways as has been found in artificial and

natural systems, such as rotifer–algae chemostats and guppy life history evolution interacting with nutrient cycling (Post and Palkovacs 2009).

Specific relationships between ecological and rapid evolutionary processes can be predicted to shift during community succession and thus affect the outcome of species interactions on the population level, not only because species composition and diversity of the interacting community may change, but also because the abiotic conditions may have shifted as an emergent property of community composition and diversity (Post and Palkovacs 2009). For example, soil conditions have been found to change over relatively short periods of time with the changing plant community, involving changes such as soil microbial community shifts, soil organic matter, and nutrient availability (Bazzaz 1979; Maharning et al. 2009). Similarly, changes in plant community composition may affect how a specific plant population is exposed to herbivory as a function of abundance (e.g. resource concentration, (Root 1973) and associational resistance mechanisms (Barbosa et al. 2009)). Thus, the community context in which plant–insect interactions are played out gradually changes during succession and differential natural selection on *S. altissima* populations can be predicted.

However, a recent reciprocal transplant experiment did not detect a genotype effect, but instead found a pure environment effect causing plants to express high resistance and low tolerance when transplanted in early successional communities and relatively lower resistance but higher tolerance in late succession communities (Hakes and Cronin 2012). Nevertheless, the same study found signs of directional natural selection on herbivore resistance, suggesting that population genetic composition and so the distribution of defense phenotypes should change over succession. Hakes and Cronin (2012) suggested that the focus of their study on mid (5 year) and late (15 year) succession may have contributed to the lack of detection of genotypic effects, since high population genotypic diversity and recruitment from seeds is highest (Hartnett and Bazzaz 1985; Maddox et al. 1989), and hence natural selection is potentially more easily detected in the first 5 years of succession.

Here, we address this apparent contradiction and assess the relative importance of environmental vs. population genetic factors as mechanisms determining the differences in mean resistance and growth phenotypes of *S. altissima* in the early years (years 1 and 6) of community succession using a common garden soil reciprocal transplant experiment. We hypothesized that soil conditions and plant population origin (early vs. intermediate succession habitats) will be major factors affecting mean growth and resistance phenotypes of *S. altissima* along successional gradients. Focusing on soil quality as a major environmental factor changing during community succession, we reciprocally transplanted genotypes from early and late succession habitats into common

gardens with soils from those habitats and measured growth and insect resistance phenotypes. To explain natural herbivore distribution and plant growth patterns that we observe in plant populations during early oldfield succession (year 1–6), we specifically examined genotypic and soil environment-induced differences in plant secondary metabolism, as well as insect herbivore choice.

## Materials and methods

### Study system

The tall goldenrod, *S. altissima*, is a common forb that often dominates early succession plant communities and is particularly common in oldfields. Even though it is an early colonizer, *S. altissima* can dominate oldfield habitats for a long time and persist for 50–75 years (Hartnett and Bazzaz 1985; Maddox et al. 1989). However, recruitment from seeds is thought to be limited to the first 5 years of the establishment of a typical forb community after which plants reproduce largely vegetatively (Hartnett and Bazzaz 1985; Maddox et al. 1989). *Solidago altissima* is associated with a very diverse arthropod herbivore community (Maddox and Root 1987) of which some affect plant species persistence and dominance to the point of altering plant community composition (Carson and Root 2000). Thereby, the persistence of *S. altissima* genotypes within a population is a function of the interaction between herbivore resistance and plant competitive ability (Uesugi and Kessler 2013, 2016). One major plant community-altering herbivore is the specialist chrysomelid beetle *Trirhabda virgata*, which has also been suggested as a major agent of natural selection on plant chemical resistance (Bode and Kessler 2012; Uesugi and Kessler 2016). Periodic and localized population outbreaks of *T. virgata* are common during early succession (Maddox and Root 1987), but the factors driving outbreak cycles are unknown. Another common herbivore in the study area is a specialist chrysomelid beetle *Microrhopala vittata*, whose larvae are leaf miners and adults leaf chewers.

### Field experiment

For this project, we utilized a long-term early oldfield succession chronosequence located in Dunlop meadow, Brooktondale, NY (42°23'13"N, 76°24'00"W), established in 2002. Each of 6 years in a succession sequence (1–6) after 3 years of agricultural use is represented with two 30 × 30 m plots (total of 12 plots) separated by 10 m mowed grassland buffer. After 6 years of succession, the experimental plots are planted with maize for 3 years following conventional agricultural practices (Fig. S1). In the first year after agricultural use, the plant community establishes through

natural recruitment from seed. The gene pool from where the recruitment occurs is likely to be very similar across years because of the close proximity of the plots to one another. Potential differences in recruitment due to differences in biotic and abiotic conditions between years are mitigated by continuing recruitment of *S. altissima* over the first 5 years of succession (Maddox et al. 1989).

## Field survey

We surveyed herbivory, growth, seed production, and abundance of *S. altissima* in the experimental succession plots in 2016. As all observations were made during the same growth season, comparisons between “years” refer to comparisons between communities of different successional stages, but not the age of individual plants. In mid May, when the *S. altissima* had started growing, we marked 20 ramets in each plot chosen at random. Ramets were evenly distributed across each plot with 5 m to the edge of the plot and 4–5 m between each ramet. We measured the height of the marked ramets and recorded the occurrence of the main herbivores *T. virgata* (number of larvae) and *M. vittata* (number of eggs) every 2–3 weeks from late May to July and once a month from August onward. In the fall once the flowers opened and growth ceased, we collected, dried (at 60 °C for 4 days), and weighed the inflorescences, stems, and the remaining leaves to get an estimate of seed production and leaf biomass. During the survey, 8–16% of the ramets died in the plots in successional years 4–6, whereas the mortality was much lower (0–3%) in the early succession plots. To estimate the abundance of *S. altissima* we counted the number of *S. altissima* ramets in four 50 by 50 cm quadrats in each plot in mid-July.

To assess changes in soil properties over succession, we collected six 10 cm soil cores from plots that had undergone 1 and 5 years of oldfield succession (and were beginning their 2nd and 6th years of succession, respectively) on 07 June 2017. We dried the samples at 50 °C for 4 days and sieved them to 2 mm, and then sent them to the Cornell Nutrient Analysis Lab (Ithaca, NY, USA) for analysis of soil organic matter, nutrients (K, P, Ca, Mg), and pH using standard methods (USDA 2014).

## Soil transplant experiment

To assess the effects of changes in the environment (soil successional origin effect) and genetic structure of the plant population over succession (plant successional origin effect) on plant growth and defense phenotypes, we performed a transplant experiment in which plant lines from years 1 and 6 of succession were reciprocally planted into soils from years 1 and 5 of succession in an outdoor (screenhouse) common garden at Cornell University

(Ithaca, NY, USA). We collected rhizomes of *S. altissima* plants from plots representing successional years 1 and 6 of oldfield succession from Dunlop meadow at the end of the *S. altissima* growing season in summer or fall 2015. To avoid collecting individuals from the same plot that were genetically identical, we avoided collecting ramets that appeared to be part of the same clonal stand and collected plants that were at least 3 m away from each other (*S. altissima* are not expected to clonally spread more than 0.6 m away from the original plant after 5 years (Cain 1990)). Because *S. altissima* are self-incompatible, it is highly unlikely that plants collected from farther distances, and especially different plots, would be genetically identical, even if they colonized from seeds of the same maternal plant. An earlier test using the analysis of 16 microsatellite loci verified that this collection method ensures that different genotypes are sampled (Uesugi et al. 2017). In an attempt to remove potential effects of previous acclimation and plant ontogeny, we vegetatively propagated plants from young rhizome cuttings through at least two reproductive cycles in a standard potting media (Lambert’s LM-111, Quebec, Canada) in a glasshouse at Cornell University following an established protocol (Uesugi and Kessler 2013). The transplant experiment utilized 10–13 genotypes per plant and soil successional origin combination with 1–3 biological replicates.

We collected topsoil (top 10 cm) on 23 May 2017 from multiple locations in one 1st and one 5th year plot (going into their 2nd and 6th years of succession, respectively), and passed it through a 5 mm sieve to remove rocks, roots, and other debris. Because the soil was collected at the beginning of the field season, we considered the soil to have a successional age of 1 or 5 years, rather than 2 or 6 years, whereas we considered that plant lines that we collected to be the age of their plot as designated in Fig. S1, as they were collected at the end of the growing season. We manually homogenized each soil on a tarp with a shovel for 25 min and then portioned it into standard 15 cm standard pots (1.7 L) and stored it outdoors until planting. We randomly assigned the plants to soil treatments and to pots within each soil treatment and planted them on 28 May 2017 as crown cuttings (~ 6 cm length) dipped in rooting powder (0.80% indole-3-butyric acid, Hormex rooting powder #8). We arranged the pots in a randomized block design, watered ad libitum, and weeded weekly. The use of crown cuttings further reduces potential ontogenetic or environmental legacy effects.

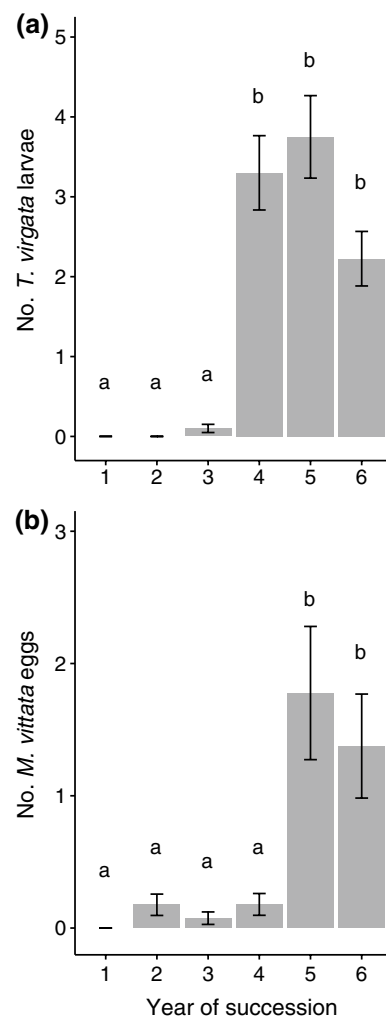
To assess the effects of successional stage of the plant population and soil on plant growth, we measured the heights of each stem of each plant every 3 days during the early growing season (29 June to 11 July in 2017) and calculated growth rates based on increases in height (cm) for each 3-day interval.

## Feeding choice assays

To assess the preference of herbivores for plants from populations of different successional stage or grown in soils from different years in succession, we performed a series of feeding choice tests with adult *T. virgata* beetles and *Trichoplusia ni* caterpillars on 14 July 2017. We collected *T. virgata* from near Beebe Lake (Ithaca, NY, USA) and starved them for 9 h prior to the experiment. We purchased *T. ni* from Benzon Research, Inc. (Carlisle, PA, USA) and fed them on cucumber plants until approximately the fourth instar, and starved them for 3 h prior to the experiment. To assess the effects of soil on feeding choice, we gave the insects a choice between two 13 mm diameter leaf discs cut from the second youngest fully expanded leaves of plants of the same successional origin (year 1 or 6) grown in year 1 or 5 soil. Similarly, to assess the effects of plant successional origin on choice, we presented them with a choice between leaves from plants of different successional origin grown in a common soil (from either successional year 1 or 5). We performed ten tests, each with different plant genotypes and individual insects for each plant origin–soil origin combination for each herbivore species, each in a 9 cm diameter Petri dish arena with a ~ 3 mm layer of agar. After 2 h of feeding, we measured the area of the leaf discs eaten using ImageJ (Schneider et al. 2012). We used choice bioassays, rather than non-choice assays to better relate the resulting data to naturally occurring herbivore distribution patterns that find fundamentally higher herbivore numbers on intermediate succession plots (4–6 years), although plots of all stages are in close proximity to each other (Fig. 1).

## Leaf secondary metabolite analyses

To assess the effects of plant and soil successional origin on secondary metabolism, we analyzed leaves for phenolics and diterpene acids. We excised the second youngest fully expanded leaf at the petiole with a razor blade on 14 or 15 July 2017, removed the mid vein, flash-froze them in liquid N<sub>2</sub>, and stored at – 80 °C until extraction. We extracted approximately 125 mg of tissue from each sample in 1 mL of 90% methanol and homogenized the sample via a FastPrep® tissue homogenizer (MP Biomedicals®, Solon, OH, USA) twice at 6.5 m/s for 45 s with 0.9 g grinding beads (Biospec®, Zirconia/Silica 2.3 mm). We removed the leaf tissue from the extract by centrifugation at 14,000 rpm at 4 °C for 15 min and analyzed 15 µL of the recovered supernatant for secondary metabolites using an Agilent 1100 series high-performance liquid chromatography instrument with a Gemini C18 reverse-phase column (3 µm, 150 × 4.6 mm, Phenomenex, Torrance, CA, USA). The elution system consisted of aqueous 0.25% H<sub>3</sub>PO<sub>4</sub> and acetonitrile (ACN) which were pumped through the column



**Fig. 1** Number of **a** *Trirhabda virgata* larvae and **b** *Microrhopala vittata* eggs per *S. altissima* ramet in experimental succession plots in early June (least square means  $\pm$  SE). “Year of succession” refers to the number of years since agricultural abandonment. Differences between years ( $\alpha = 0.05$ ) were obtained from Tukey’s test and are denoted by letters above the bars

at a rate of 0.7 mL/min with increasing concentrations of ACN: 0–5 min, 0–20% ACN; 5–35 min, 20–95% ACN; and 35–45 min, 95% ACN. We identified peaks of 10 phenolics (caffeic and coumaric acid derivatives, and flavonoids) and 12 diterpene acids, listed in Table S1 to compound classes using UV spectra information and quantified their signal intensity at 320 nm (caffeic and coumaric acid derivatives) or 210 nm (flavonoids and diterpenes).

## Data analysis

We analyzed the number of *T. virgata* larvae per ramet, the number of *M. vittata* eggs per ramet, and dry mass of leaves and inflorescence to assess differences in herbivory and *S. altissima* growth in experimental succession populations.



We used a zero-inflated model with a negative binomial distribution (logit link function) for the herbivore data (*zeroinfl* in *pscl* package; Jackman 2017; Zeileis et al. 2008) because of the large number of zeros in the data. Flower and leaf biomass were analyzed with one-way ANOVAs (*lm* in *stats* package) with log-transformed data to meet the model assumptions of homogeneity of variances. We analyzed the number of *S. altissima* ramets per quadrat with a generalized linear model. We used negative binomial distribution (log link function) for the model because of moderate overdispersion of the data (*glm.nb* in *MASS* package; Venables and Ripley 2002). One-way ANOVAs were used to compare soils of different successional origin using the *lm* and *anova* in *stats* package. Year of succession (number of years passed since agricultural abandonment) was used as a factor in all analyses.

In the soil transplant experiment, we analyzed the height growth rate data using repeated measures ANOVAs with plant successional origin, soil successional origin, and date as main effects and individual plant ID, genotype, and block as random effects (*lmer* in *lme4* package and *Anova* in *car* package). We also inspected interactions between the main effects and included them in the model if  $P < 0.1$ .

In the insect choice tests, we determined preference within each trial based on which leaf disc had the greatest amount eaten (most area missing) and then statistically analyzed within each choice type (e.g., choice between plants of different successional origin grown in year 1 soil) using exact binomial tests with the null hypothesis proportion = 0.5 (*binom.test* in *stats* package). We omitted data from insects that did not make a choice within the 2 h period (two *T. virgata* in each soil effect tests and one in the plant successional origin choice in year 1 soil) from the statistical analysis.

For the leaf chemical analyses, we standardized the area of each peak to the weight of leaf tissue used for extraction. We removed one outlier plant, which had over a 100-fold higher concentration of compound 19 (a diterpene acid) than the mean for other plants from analyses of concentrations of compound 19 and total secondary metabolites. We analyzed the overall composition of compounds using nonmetric multidimensional scaling (Bray–Curtis distance matrix; *metaMDS* in *vegan* package; Oksanen et al. 2017) and tested for the effect of the plant and soil year on the composition with permutational multivariate analysis of variance (PERMANOVA; 999 permutations; *adonis2* in *vegan* package). We applied a square root transformation to the dissimilarity matrix because of negative eigenvalues. To assess the effects of plant and soil successional origin on the concentration of each individual compound, as well as total phenolics, total flavonoids, and total diterpenes, we used linear mixed models with main effects of plant origin, soil origin, and a plant origin–soil origin interaction and a random genotype effect

(*lmer* in *lme4* package and *Anova* in *car* package). If necessary, we applied a logarithmic transformation to the data so that residuals met the assumptions of normality and homogeneity of variance. To correct for multiple comparisons, we adjusted the  $P$  values for the 22 compounds using the false discovery rate (FDR) adjustment (*p.adjust* in package *stats*).

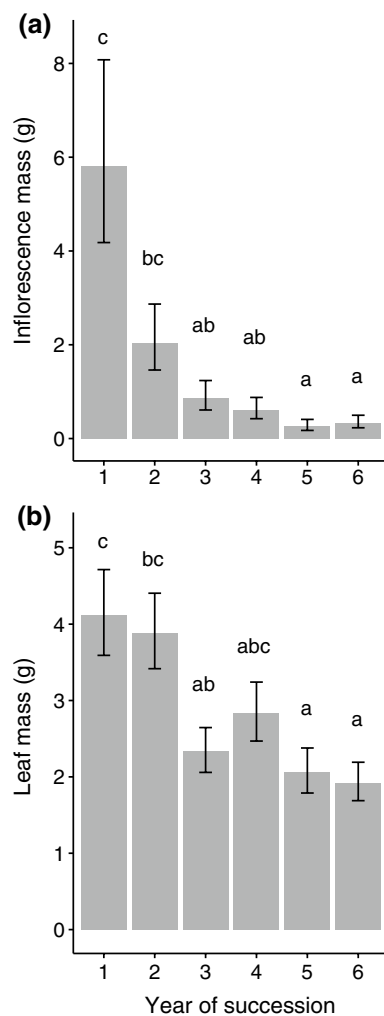
We assessed the relationship between growth and secondary metabolite production via the Pearson correlation between mean total height-based growth rate (average over the early growth period) and total leaf secondary metabolite production (combined total phenolics and diterpenes) for individual plants (*cor* in *stats* package). We modeled the effects of growth rate and plant and soil successional origin on total secondary metabolite production with a linear mixed model with a random genotype effect (*lmer* in *lme4* package) and used a likelihood ratio test to assess the effect of growth rate on total secondary metabolites (*anova* in *stats* package). All statistical analyses were performed using R (R Core Team 2017).

## Results

Both herbivores *T. virgata* and *M. vittata* were virtually absent in the plots after 1–3 years of succession, but occurred and were abundant in plots of 4–6 years of succession (*T. virgata*:  $df = 5$ ,  $\chi^2 = 50.43$ ,  $P < 0.001$ ; *M. vittata*:  $df = 5$ ,  $\chi^2 = 46.33$ ,  $P < 0.001$ ; Fig. 1). The inflorescence and leaf biomass were highest in early succession when herbivory was low (inflorescence biomass:  $F_{5,103} = 10.46$ ,  $P < 0.001$ ; leaf biomass:  $F_{5,209} = 5.89$ ,  $P < 0.001$ ; Fig. 2). *Solidago altissima* became and remained abundant in the plots that had undergone three or more years of succession ( $df = 5$ ,  $\chi^2 = 149.75$ ,  $P < 0.001$ ; Fig. 3).

With the progression of community succession, the soil became enriched with organic matter and potassium (Table 1), and promoted a higher growth rate in *S. altissima* in a soil transplant experiment (Fig. 4). The successional stage from which soil, but not from which plants originated, affected early season plant growth rate in a common garden, as measured in increases in stem height with plants growing consistently better in the intermediate succession soils (soil origin effect:  $df = 1$ ,  $F = 8.60$ ,  $P = 0.0067$ , plant origin effect:  $df = 1$ ,  $F = 0.0033$ ,  $P = 0.9546$ , date effect:  $df = 3$ ,  $F = 16.2836$ ,  $P < 0.0001$ ; soil origin–date interaction:  $df = 3$ ,  $F = 2.2399$ ,  $P = 0.08487$ ; Fig. 4).

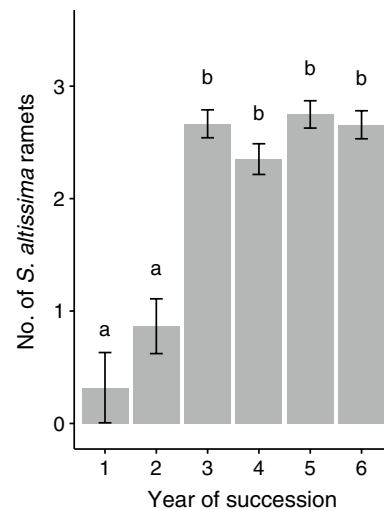
Insect herbivores generally consumed a greater proportion of leaf tissue from plant genotypes originating from early succession plots and from plants grown in early succession soil in choice experiments, although the only statistically significant preference observed was that of *T. virgata* for plant genotypes from the first year of succession



**Fig. 2** Dry mass of **a** inflorescences and **b** leaves of *Solidago altissima* ramets at the end of the growing season in experimental succession plots (least square means  $\pm$  SE). “Year of succession” refers to the number of years since agricultural abandonment. Differences between years ( $\alpha = 0.05$ ) were obtained from Tukey’s test and are denoted by letters above the bars

populations, over plants originating from sixth year succession, but grown in year 1 succession soil ( $P = 0.03906$ , Fig. 5).

*Solidago altissima* leaves contained many different phenolic compounds and diterpene acids (Table S1). Overall, the leaf secondary metabolite composition was affected by the successional origin of the plant, but not by the origin of the soil (Fig. 6; Table 2). The concentrations of several individual compounds were influenced by plant and soil successional origin, particularly one phenolic (#12) and two diterpene compounds (#16 and 22), although after correcting for multiple comparisons (22 compounds were assessed) there were no significant effects of plant or soil origin at the  $\alpha = 0.05$  significance level (Fig. 6; Table S2). There were no overall effects of plant or soil successional origin



**Fig. 3** Abundance of *Solidago altissima* in experimental succession plots as the number of ramets in 50  $\times$  50 cm quadrats (least square means  $\pm$  SE). “Year of succession” refers to the number of years since agricultural abandonment. Differences between years ( $\alpha = 0.05$ ) were obtained from Tukey’s test and are denoted by letters above the bars

**Table 1** Comparison of soil properties between plots having undergone 1 or 5 years of oldfield succession

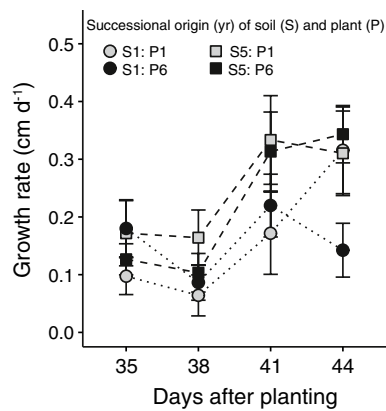
Property	Year 1	Year 5	$F_{1,10}$	$P$ value
Organic matter	2.93 ( $\pm$ 0.18)	3.40 ( $\pm$ 0.11)	<b>5.0574</b>	<b>0.04827</b>
pH	5.67 ( $\pm$ 0.06)	5.67 ( $\pm$ 0.10)	0.0002	0.9891
K	95.2 ( $\pm$ 9.3)	136.6 ( $\pm$ 11.9)	<b>7.4793</b>	<b>0.02102</b>
P	2.60 ( $\pm$ 0.36)	2.82 ( $\pm$ 0.28)	0.2205	0.6487
Mg	112.4 ( $\pm$ 9.6)	114.9 ( $\pm$ 4.6)	0.0538	0.8213
Ca	660 ( $\pm$ 48)	694 ( $\pm$ 33)	0.3439	0.5706

Means ( $\pm$  SE) values of soil properties are shown with the results of one-way ANOVAs comparing each property between successional years. Organic matter is expressed as a percentage and nutrients are expressed in mg/kg. Bolded values indicate significant differences ( $P < 0.05$ ) between successional years ( $n = 6$  samples per year)

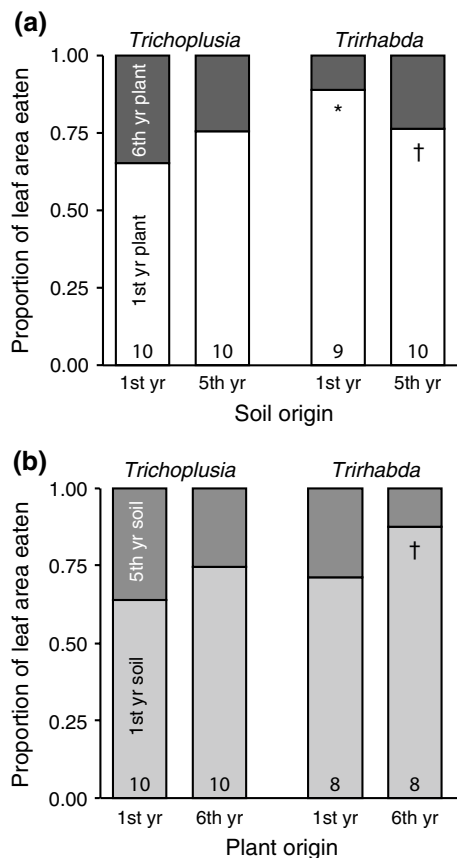
on secondary metabolite production at the compound class level (Table S3). However, there was a positive correlation between plant growth and overall secondary metabolite production (Pearson’s  $\rho = 0.4625$ ) and faster growing plants produced leaves with higher concentrations of phenolics and diterpenes ( $\chi^2 = 26.57$ ,  $P < 0.0001$ ).

## Discussion

We observed high densities of insect herbivores on *S. altissima* plants in communities in the 4–6th years of oldfield succession, in stark contrast to their earlier succession counterparts, which were nearly free from herbivores (Fig. 1).



**Fig. 4** Comparison of mean ( $\pm$  SE) growth rates of the 1st and 6th year successional plant lines grown in soil from plots in year 1 or 5 of succession. Growth rates were measured every 3 days as increases in shoot height (cm/day) during the early growing season (35–44 days after planting)



**Fig. 5** Preference of insect herbivores, fourth instar *Trichoplusia ni*, and adult *Trirhabda virgata* for leaves from the first or sixth successional year lines grown in the first or fifth successional year soil. Bars indicate the mean proportion of leaf discs eaten in two-way choices **a** between plants from year 1 or 6 of succession, both grown in year 1 or 5 soil; and **b** between plants grown in year 1 or 5 soil, both from either a year 1 or 6 successional plant origin. Asterisks indicate significant preference (\* $P < 0.05$ ; † $P < 0.08$ ) by binomial exact tests. Number of choice tests are denoted inside bars

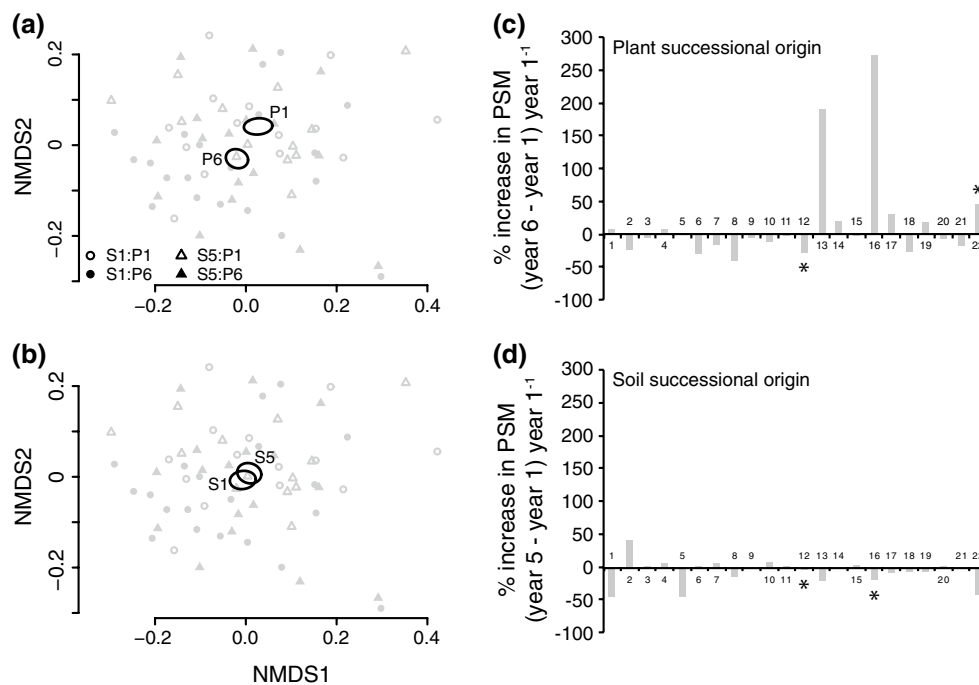
This distribution of herbivores among our experimental field plots, which are separated by as little as 10 m (Fig. S1), suggests that *S. altissima* defense phenotypes are shifting over succession. There are two non-mutually exclusive mechanisms through which phenotypic changes occur: genetic differentiation and phenotypically plastic shifts in trait expression. In a soil reciprocal transplant experiment investigating the mechanisms of phenotypic change, we found that plant successional origin affected plant secondary metabolite production (Fig. 6) and herbivore resistance (Fig. 5). Successional soil environment affected plant growth, but not resistance, suggesting that defense trait shifts are primarily genetic, rather than plastic responses to soil quality.

We had hypothesized that the buildup of herbivore populations would create strong selection for plant resistance, as previous studies on *S. altissima* have shown. In those studies herbivore pressure drove rapid evolution in *S. altissima* over the course of 12 years. The new data suggest that natural selection by herbivore insects may cause divergent patterns of resistance evolution in even shorter time periods, consequently causing interactions between ecological and evolutionary processes when species interact during community succession. This also means that the dramatic reorganization in community composition during early succession is paralleled in a population genetic shift on the species level.

### Natural selection on herbivore resistance specificity

In northeastern North American forb communities, insect herbivory mediates community composition and increases plant species diversity by reducing the dominance of the most common species *S. altissima* (Carson and Root 2000). Although the dominant herbivore species may vary by region, herbivore populations seem to commonly build up during the very early years of succession and some of them may enter outbreaks 4–7 years after the new community was formed (Root and Cappuccino 1992; Carson and Root 2000; Hakes and Cronin 2012). In the *S. altissima* system, groups of insect herbivores were identified that had preferences for specific plant genotypes so that the dominant species at a given time and place were hypothesized to drive most of the selection on plant defenses and competitive ability (Maddox and Root 1987). Indeed, population genetic differences seem to be specific to certain herbivores in this system (Bode and Kessler 2012) and the data presented here further support this hypothesis. The preference for early succession *S. altissima* plants was only observed for the specialist *T. virgata*, and when plants were grown in the poorer quality early succession soil (Fig. 5), despite the fact that soil environment overall had relatively small effects on defensive secondary metabolite production (Fig. 6). The chrysomelid beetle *T. virgata* is one of the most important herbivores in the system (Maddox and Root 1987; Carson and Root 2000) and





**Fig. 6** Overall plant secondary metabolite production in *Solidago altissima* leaves by soil and plant successional origin. NMDS plots depicting the effect of **a** plant origin and **b** soil origin on the overall secondary metabolite composition (stress 0.176). The points are individual plants, identified based on the successional year of the plant (P) and the soil in which it was grown (S), and ellipses are SEs.

**Table 2** Results of PERMANOVA comparing the effects of soil and plant successional origin on the overall leaf secondary metabolite composition (phenolic compounds and diterpene acids)

	<i>df</i>	Sum of sqs	<i>F</i>	<i>P</i>
Plant successional origin	1	0.289	<b>1.56</b>	<b>0.041</b>
Soil successional origin	1	0.196	1.06	0.350
Interaction	1	0.088	0.48	0.997
Residual	60	11.08		

Bolded values indicate significant differences ( $P < 0.05$ )

we found the mean resistance to this beetle, but not to the generalist *T. ni*, to be increased over the course of early succession (Fig. 5), suggesting selection for resistance specifically to the dominant herbivore(s). Indeed, the abundances of this beetle species and another dominant Chrysomelidae, *M. vittata*, reach significant numbers only in populations that have undergone 4–6 years of succession (Fig. 1) and are often found at outbreak levels in those years. At that same short initial 4- to 6-year phase of the community succession, the genetic diversity within the *S. altissima* population is expected to be at its peak because the population still recruits from an abundant and diverse seed bank, allowing for ample genetic variation on which natural selection can

Increases (%) in mean metabolite production in **c** year 6 compared to year 1 origin plants and **d** plants grown in year 5 versus year 1 successional soil. Asterisks indicate significant differences ( $P < 0.05$  based on raw *P* values in Table S2). A list of compounds with the corresponding numbers is given in Table S1

act (Ellstrand and Elam 1993). Recruitment from the seed-bank largely ceases after this initial phase, presumably due to overwhelming inter-specific (Hartnett and Bazzaz 1985; Maddox et al. 1989) and allelopathic compound-mediated intra-specific competition (Johnson et al. 2010; Uesugi and Kessler 2013). The emerging picture is that of dominant herbivores, such as *T. virgata* and *M. vittata*, selecting on *S. altissima* resistance traits while altering the plant community and thus the context in which the plant population is exposed to the entire herbivore community. Within these genetically different populations, the mean plant defense phenotype is further determined by the feeding activity of the herbivores (Uesugi et al. 2013), altered resource availability, and, most likely the community associational context in which resistance is expressed (Barbosa et al. 2009).

### Phenotypic plasticity along successional gradients

It is possible that, as a perennial plant that regrows from rhizomes each season, *S. altissima* phenotypes may not only be a result of genetics and environmental conditions, but also the age and previous experiences of the individual plants. In both the field and common garden experiments, we measured ramets produced in the current season, but it is unknown if there are interseasonal memory (Wiegand et al. 2004; Latzel

et al. 2016) or simple clone age effects in *S. altissima* that could affect plant phenotype expression in the field. In our common garden experiment, we are likely underestimating such long-term induction or ontogenetic effects. First, we used only young rhizome cuttings for propagation, which standardizes the starting age of the tissues for all propagates. Second, all clonal lines were propagated at least two times, which involves cutting rhizomes into small pieces and so a likely overwriting and standardization of a plant's previous experiences by this new severe damage. While these procedures make it unlikely that plant immunological memory and ontogenetic effects influence our measurements, they are likely to cause an underestimation of those processes as factors influencing the mean phenotype expression in the field. Future studies need to address the relative contribution of these longer-term phenotypic effects relative to the genetic and short-term induction effects we observed here.

Interaction patterns mediated by plant induced responses to herbivory can be very complex (Karban and Baldwin 1997; Karban 2008) and their effects on plant fitness (Agrawal 1998; Baldwin 1998) may be as large as those mediated by purely genotypic differences. This is mainly because induced responses to herbivory act on multiple trophic levels. In *S. altissima*, resistance is strongly inducible, very specific to the attacking agent and mediates non-reciprocal interactions between different herbivore species (Helms et al. 2013; Uesugi et al. 2013, 2016) and neighboring plants (Morrell and Kessler 2016). Thus, the dominant herbivore species not only determine the genetic composition of the plant population through rapid evolutionary processes, but also the predominant defense phenotype in a density-dependent manner, and so overall community dynamics. Here, we did not specifically test for population differences in inducibility in response to differential natural selection along successional gradients, but in the light of the above-mentioned interactions, this will be an important area of future research.

In addition to responses to herbivory, differences in the immediate physical and chemical environments of plants caused by and mediating interactions with other plants and microbes can be hypothesized to be important for the distribution of herbivore defense phenotypes along successional gradients (Burghardt 2016; terHorst and Zee 2016; Hakes and Cronin 2011, 2012). Here, we focused on successional changes in the soil environment and its effects on plant resistance to herbivores because it is one of the factors predicted to undergo the most dramatic changes during succession and because *S. altissima* is known to produce allelopathic compounds with a broad activity spectrum (Inogouchi et al. 2003) and the potential to accumulate in the soil over time (Ito et al. 1998). Indeed, soil conditions changed significantly over this short 5-year period of succession with most remarkable increases in soil organic matter and potassium

concentration (Table 1). Increases in soil organic matter are commonly found in association with land use changes from agricultural land to native plant communities and continually accumulate over several years of succession (Post and Kwon 2000). Accumulated soil organic matter affects nutrient availability (Bezemer et al. 2006) and microbial activity and diversity (Kuzyakov and Blagodatskaya 2015). Similarly, changes of soil elemental composition are frequently observed during succession (Bezemer et al. 2006) and the availability of essential nutrients, such as potassium, is correlated with plant responses to biotic and abiotic stresses (Römheld and Kirkby 2010).

Soil microbial communities are expected to become increasingly dominated by fungi, rather than bacteria, over time, as changes in soil conditions and the plant community occur (Maharning et al. 2009; Hannula et al. 2017). Such changes in the soil microbiota over succession have the potential to influence plant growth and metabolism (Canellas et al. 2015), and consequently plant community interactions. A few studies have suggested that microbiota can affect plants' chemically mediated interactions with herbivores (Hol et al. 2010; Badri et al. 2013) as well as with other plants (Meiners et al. 2017), and thus understanding the extent to which soil microbial communities shift over early oldfield succession, and the consequences of such shifts on plant phenotypes, would be interesting to investigate.

In accordance with these fundamental changes in soil composition, we found some of the plant secondary metabolites differentially expressed in the different soils (Fig. 6; Table S2), although we did not observe successional changes in soil to affect the plants' overall production and composition of phenolic and diterpene bouquets (Fig. 6b, d). Associated with these relatively minor differences in plant secondary metabolism, herbivores showed only a minor preference for plants grown in earlier succession soils (Fig. 5). While soil did not substantially affect herbivore resistance, it did affect plant growth, with plants growing faster in the more nutritious later succession soil (Fig. 4). This trend is in opposition to our and other's observations (Carson and Root 2000) of smaller plants in later succession field populations. This latter pattern is likely driven by the increased density of competing conspecifics and mounting herbivore pressure during later succession (Figs. 1, 3), which was controlled in our common garden experiment. In support of this hypothesis of a competition- and herbivory-driven differential growth rate in early vs. late succession communities, we found no effect of plant origin on growth rate. Moreover, this finding also supports findings from herbivore exclusion experiments (Bode and Kessler 2012; Uesugi and Kessler 2013) that suggest that herbivory, rather than competition, may be the major selective force during early succession in the *S. altissima* system. However, as we did not measure

the plants in our soil transplant experiment under competition, it is difficult to draw conclusions about selection based on competitive ability from the current data. We also observed a positive correlation between plant growth and production of secondary metabolites, rather than the growth–defense trade-off that has been widely hypothesized (Herms and Mattson 1992). It is possible that in the early stages of growth, those plants that were best able to grow quickly had greater resources to use for metabolite production, which would be in support of the plant vigor hypothesis (Price 1991). Interestingly, another recent study on *S. altissima* also found a positive genetic correlation of plant growth indicators with specific secondary metabolite production (terpenoids), which was interpreted as a resource storage function of secondary metabolites to support later regrowth (Heath et al. 2014).

## Conclusions

During succession in native North American forb communities, both population genetic composition and the environmental circumstances in which species interactions are played out determine the dominant phenotype in plant populations, in our example, of tall goldenrod, *S. altissima*. Specifically, we demonstrated that differential natural selection during early and later succession results in different mean secondary metabolite and resistance phenotypes. Plant growth, secondary metabolism, and resistance are further altered by phenotypic plastic responses to the different soil environments in early and late succession, so that the mean phenotypes during different successional stages are a result of interacting evolutionary and ecological processes. Such “evolutionary connectionism” is predicted to underlie complex system-level behaviors (Watson et al. 2016) and, in the evo–eco framework, can potentially explain emergent ecosystem properties (Wu and Loucks 1995). Within this framework, it is, on one hand, particularly interesting to study the effects of dominant and keystone herbivore species on the rest of the community through their natural selection on plant growth and defense phenotypes (Poelman and Kessler 2016). On the other hand, research into how secondary metabolite responses to environmental biotic and abiotic factors, and thus the alteration of chemical information transfer among community member, affect species interaction and ecosystem services will lead to a better understanding of those emergent properties of biological communities (Kessler 2015). As this study suggests, the study of genetic and environmentally induced changes of plant populations along community succession gradients can function as a valuable model to tackle these large questions in ecology and evolutionary biology.

**Author contribution statement** All authors conceived and designed the experiments. MMH and AKalske conducted fieldwork and performed experiments. MMH and AKalske analyzed the data. All authors wrote the manuscript.

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