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Nutrient supply alters goldenrod's induced response to herbivory

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Summary

1. Recent interest in using trait-based approaches to understand and predict ecosystem processes and evolutionary responses to environmental change highlights the need to understand the relative importance of genetic and environmental sources of intraspecific trait variation within local populations of dominant species.

2. Here, I combine plant defence theory with functional approaches to quantify genetic trait variation and phenotypic trait plasticity of nine goldenrod (*Solidago altissima*) genotypes derived from a local field population in Connecticut, USA, to herbivory along a nutrient supply gradient.

3. I found that increasing nutrient supply changed the dominant plant defence strategy from tolerance to induced resistance. Induced resistance was detected through decreased herbivore growth rates and a behavioural feeding shift of grasshoppers to older leaf tissue. This could not be fully accounted for through stoichiometric changes in leaf tissue quality.

4. A multidimensional phenotype approach revealed that abiotic and biotic environments (nutrients and herbivory) accounted for almost as much whole-plant trait variation (31%) as did plant genotype (36%). Increasing nutrient supply and herbivory resulted in independent and differential effects on whole-plant trait expression. Increasing both treatments concurrently produced a unique plant phenotype with increased leaf carbon content and allocation to asexual reproduction ($E \times E$).

5. Notably, individual genotypes exhibited different magnitudes of multivariate trait plasticity to nutrient and herbivory gradients. However, the population of genotypes as a whole within a given environment expressed an approximately equal magnitude of trait variation across both permissive (high nutrient, no herbivory) and stressful (low nutrient, high herbivory) environments.

6. Quantifying plasticity in defensive strategy in concert with correlated whole-plant trait expression changes across multiple abiotic and biotic factors may be key to providing a mechanistic understanding of how heterogeneous landscapes impact community interactions and ecosystem processes.

Key-words: genetic variation, goldenrod, herbivory, induced plant defence, multidimensional phenotype, nutrient fertilization, phenotypic plasticity, tolerance

Introduction

Recent concerted effort in terrestrial ecology focuses on characterizing plant species based on their functional traits and then determining how such traits influence community and ecosystem functioning (Lavorel & Garnier 2002; Violle *et al.* 2007). Within this approach, species are routinely characterized in terms of their mean trait values (Bolnick *et al.* 2011; Kazakou *et al.* 2014). Yet, there is abundant evidence that variation around the mean species value and/or changes in the mean across environments (i.e. intraspecific variation) may be key to producing accurate predictions of the nature and level of community and ecosystem processes (Miner *et al.* 2005; Wright & Sutton-Grier 2012). Understanding intraspecific variation within dominant species may be especially important, as these species often have larger proportional effects on such processes (Smith & Knapp 2003; Whitham *et al.* 2006).

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An important suite of plant functional traits are those associated with antiherbivore defence expression because they may alter both fitness, by influencing the degree to which plants can fend off or tolerate herbivores, and ecosystem processes, by altering the quality or quantity of plant organic matter entering the soil for decomposition (Choudhury 1988; Chapman, Schweitzer & Whitham 2006; Frost & Hunter 2008). Plant defence theory is steeped in a rich history of quantifying variation in trait expression by assessing plant phenotypic plasticity both to herbivore presence and across soil nutrient gradients (Cronin & Hay 1996; Hawkes & Sullivan 2001; Stamp 2003; Hay, Poore & Lovelock 2011). As a result, it may serve as a useful framework for mechanistically quantifying and contextualizing intraspecific variation.

Plants may engage in two different defensive responses to herbivores. Plant responses that decrease herbivore damage or lower herbivore performance are collectively known as *resistance* traits [e.g. spines, tough tissue, toxic compounds (Feeny 1976)]. These traits are expressed at a baseline level in the absence of herbivory (constitutive resistance) and may increase after a plant is attacked (induced resistance) (Agrawal & Karban 1998). Alternatively, plants may minimize the negative impact of herbivores on plant fitness through traits that increase the recovery of photosynthetic capacity, known as *tolerance* (Rosenthal & Kotanen 1994; Strauss & Agrawal 1999). These responses are not mutually exclusive; recent work suggests that plants may engage in, and herbivores select for, mixed defensive strategies (Carmona & Fornoni 2013).

The capacity of plants to express resistance and tolerance may depend on soil nutrient availability (Coley, Bryant & Chapin 1985; Darrow & Bowers 1999). Many experiments focus on between-species variation in defence expression across nutrient availability (Stamp 2003). However, species are also not typological. Across nutrient environments, the cost-benefit trade-off of defending tissue with N-rich and C-rich defensive compounds changes, creating situations where the best performing allocation strategy in one environment may be maladaptive in another (Herms & Mattson 1992; Burghardt & Schmitz 2015). Within-species variation in tolerance and resistance traits may arise from genetic differences (G), environmentally based differences or plasticity (E), as well as genetic variation for plasticity ($G \times E$) (Whitman & Agrawal 2009). Typically, (E) is used to refer to variation attributable to all environments. However, $E \times E$ interactions may occur whereby the developmental environment (such as nutrients, light or water) alters the direction or magnitude of the plastic response of a genotype in response to a later environment (such as herbivory). Through this mechanism, herbivore-induced differences may only be expressed within certain developmental environments, altering the population-level trait variance between environments (Cipollini & Bergelson 2001; O'Donnell, Fey & Cottingham 2013). Lastly, there may be genetic variation in response to the interactive effects of the two environments ($G \times E \times E$).

Often functional trait studies aggregate these sources of intraspecific variation together, but understanding the relative magnitude of each may be important for understanding local processes (Hakes & Cronin 2011) and evolutionary implications (Cortez 2011). For example, individuals shifting their defence allocation strategy to flexibly match environmental contexts would result in deterministically changing mean trait values based on environment (Agrawal 2001; Glynn *et al.* 2007). Further, genetic variation for plasticity among genotypes ($G \times E$) or environmental interactions ($E \times E$) could lead to different trait variances across environmental gradients. On the other hand, widespread genetic variation in defence (G) with no plasticity would lead to large trait variance regardless of environmental context. As a result, partitioning these sources of variation may explain often cited context dependence in community and ecosystem experimental results (Schmitz *et al.* 2015).

Here, I report on a glasshouse experiment that quantified intraspecific trait variation (G, E, $E \times E$ and $G \times E$) of nine genotypes of a clonal, dominant species grown across a nutrient supply and herbivory gradient. First I examine whether plant defensive outcomes (resistance or tolerance) change depending on the nutrient environment. Then, I ask whether plant stoichiometry – the consumption ratio of nutrients by herbivores – can explain the observed patterns in resistance (Sterner & Elser 2002). Next, I examine whether trade-offs between tolerance and resistance occur across the nutrient gradient. However, changes in these plant defence outcomes co-occur with changes in many traits, often rendering bivariate approaches inadequate (Forsman 2015). Thus, I also quantify changes in whole-plant trait variation, as herbivores feed on (and selection operates on) whole-plant multidimensional phenotypes (Walsh & Blows 2009; Laughlin & Messier 2015), Lastly, I assess whether individual genotypes express equal whole-plant trait plasticity in response to environmental changes and whether collectively genotypes express equal trait variance within all levels of an environment.

Materials and methods

STUDY SPECIES

I focus on the interaction between tall goldenrod [*Solidago altissima* (L.)], a rhizomatous perennial that dominates abandoned agricultural fields in eastern North America and a common leaf-chewing herbivore, the red-legged grasshopper [*Melanoplus femur-rubrum* (De Geer)]. *S. altissima* is an obligate out-crosser; once established in fields, it spreads primarily through clonal growth of deciduous ramets that remain within 0·5 m of the previous year's parental ramet (Cain 1990). Rhizome material can be propagated to establish clonal lines. This species is known to exhibit tolerance (Meyer 1998; Cronin, Tonsor & Carson 2010), constitutive resistance and induced resistance (Abrahamson & Weis 1997; Bode, Halitschke & Kessler 2013).

SOURCE POPULATION

I obtained rhizomes from an old-field in Wallingford, CT, with 15–90% *S. altissma* cover that was abandoned from agriculture in 2001. I excavated nine genets (hereafter genotypes) at least 15 m apart to ensure unique genotypes (Cain 1990), and propagated them in a glasshouse for one generation to remove carryover effects. I used one source population to avoid a spatial-scale mismatch that might artificially inflate intraspecific variation (Tack, Johnson & Roslin 2012).

PROPAGATION

In April 2012, I planted 2 mL volume rhizome pieces (Abrahamson & Weis 1997), in 50% sterilized potting soil (Pro-Mix BX, New Rochelle, NY, USA) and 50% clay medium mixture (Turface MVP; PROFILE Products, Buffalo Grove, IL, USA). Planting was done concurrently with ramet expansion in the field, allowing the glasshouse to be matched with outdoor conditions (photoperiod and temperature levels). In early June, I transplanted the ramets to 4-L pots and randomly assigned each to a nutrient treatment (Fig. 1a). Biweekly, plants were exposed to one of four nutrient treatments (100 mL of water with fertilizer at either 0, 100, 200 or 400 p.p.m.) for the remainder of the growing season (Peters Excel fertilizer 15-5-15 N : P : K Cal-Mg special, Everris). These levels bracket those measured in plant tissue in the field (Horner & Abrahamson 1992). A total fertilizer was used to avoid nutrient co-limitation artefacts. Leaf number was higher for fertilized plants by the time herbivores were added (Fig. S1, Supporting information). Water was applied in equal quantities by drip irrigation. Within each nutrient treatment, individuals were assigned to herbivore treatment in a stratified random manner by assigning plants of similar size as pairs for the resistance and tolerance assay (Fig. 1b).

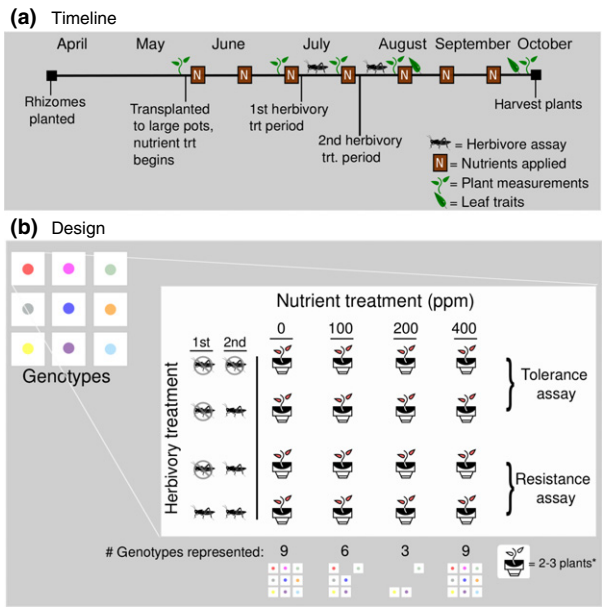

Fig. 1. The experimental (a) timeline and (b) design. *Solidago altissima* clones of nine genotypes were grown at four nutrient levels crossed with three herbivory levels and used to quantify tolerance and resistance. Trait and plant measurement data were used for a multivariate trait analysis. Early plant mortality before treatment assignment resulted in incomplete genotype replication in the middle two nutrient treatments (100 and 200 p.p.m.). *Later mortality led to some treatments with fewer than three plants.

RESISTANCE ASSAY

I collected grasshoppers from the source field. They were fed a common diet for 48 h, food-deprived for 12 h, then weighed and placed onto plants housed within individual screen mesh cages. First, 'induced' treatment plants were exposed to a 7-day period of herbivory by two third-instar individuals (5·1 ± 0·6% removal of leaf tissue). A second 'constitutive' group was not exposed to herbivory (Fig. 1b). After one more week of growth, all plants were exposed to 7 days of herbivory from two pre-weighed fourth-instar individuals (an additional 9·7 ± 1·8% leaf damage). I weighed them 12 h after removal and calculated a common index of plant resistance as $-1 \times$ grasshopper relative growth rate (where RGR = final mass-initial mass/initial mass) (Kempel *et al.* 2011). Plant damage was estimated by counting the number of damaged leaves on the plant, noting which section of the plant was damaged and then randomly selecting eight leaves on which to visually estimate per cent removed.

TOLERANCE ASSAY

Half of the remaining plants were exposed to one round of the herbivory treatment described for the resistance assay and the other half served as a control never exposed to herbivores (Fig. 1b). Tolerance – the ability to reduce the negative impact of herbivory on fitness – was calculated as a response ratio (RR): fitness of a damaged plant/fitness of an undamaged plant. A value of 1 would indicate a plant genotype is fully tolerant of herbivory within that nutrient level, with the proportional measure allowing for comparison across gradients (Strauss & Agrawal 1999). Tolerance was calculated using three common fitness measures: floral biomass, rhizomes produced, above-ground biomass and additionally root biomass as it is often associated with tolerance responses (Strauss & Agrawal 1999).

BIVARIATE TRADE-OFFS

Induction (resistance or susceptibility) was calculated as the difference between herbivore growth rate on a previously exposed (induced) plant vs. a control plant (constitutive) (Morris *et al.* 2006). To avoid spurious negative correlations in the analysis, constitutive resistance was calculated from an independent estimate of herbivore growth rates on the damaged set of the 'tolerance' assay plants. There was no correlation between tolerance and plant damage (Fig. S2).

TRAIT MEASUREMENTS

Trait data were collected on June 6th, July 11th, July 26th, August 14th and September 25th (Fig. 1a). I calculated RGR for both height and leaf number by placing metal rings around the top of the ramet on each sample date and recording subsequent growth. Seven days after herbivore removal, I harvested the two most recent fully expanded leaves without damage and measured leaf toughness as the force needed to puncture a leaf next to the midvein (penetrometer), leaf area [a scanner and IMAGEJ software (Schneider *et al.* 2012)] and leaf chlorophyll content (OptiSciences CCM-300, Hudson, NH, USA). Leaves were rehydrated, weighed wet and then dried at 50 °C and reweighed, and then, leaf mass per area (LMA), leaf dry matter content (LDMC) and leaf thickness were calculated. Dry leaf tissue was ground and analysed for C and N contents. Phenological status was noted every 5 days. On October 2nd, I harvested whole plants and sorted them into leaf, stem, root, rhizome, lateral stem and flower portions. These were dried at 60 °C and weighed for absolute and proportional allocation. I noted the number of rhizomes (each will produce

~one new ramet), adventitious buds, new lateral stems produced at the base of the plant and average rhizome length (a metric of plant spreading potential).

STATISTICAL ANALYSIS

All analyses were completed in R (R Development Core Team 2009). First, I performed a linear mixed-effects analysis using the *lmer* function in the package LME4 (Bates, Maechler & Bolker 2012) assigning resistance index and plant nutrient content as response variables; herbivore history (induced vs. constitutive plant) and nutrient supply as fixed effects; and plant genotype as a random effect. *F*-tests were used to test the significance of fixed effects while random factors were assessed using a likelihood ratio test (Zuur *et al.* 2009). Random effect structures including $G \times E$ effects were tested, but did not improve the fit and thus were not included in the final model. Genotype only had a significant effect in the leaf carbon content model, but was kept within all final models to account for the unbalanced nature of the experiment. Degrees of freedom (Satterthwaite approximation), type III SS and *P*-values were calculated using *lmertest* (Kuznetsova, Brockhoff & Christensen 2014). A significant nutrient supply × herbivory interaction indicates that the effect of herbivory on induced resistance or leaf nutrient composition differed across nutrient environments. Where this occurred, I ran two additional models comparing the induced and constitutive plants within each of the two nutrient treatments with full replication (the highest: 400 p.p.m. and lowest: 0 p.p.m. nutrient levels).

Effect sizes of the herbivory treatment were calculated separately for each nutrient level as the mean of genotype response ratios (RR: induced genotype X/control genotype X). The effect of nutrient addition was similarly calculated (separately for induced and control plants). A RR of 1 would indicate no difference with treatment, while >1 indicates a proportional increase and <1 a proportional decrease. RR are symmetric about 1 (e.g. 0·5 indicates the same magnitude of decrease as 1·5 indicates of increase).

I determined whether tolerance differed using linear mixed-effects analysis with nutrient level as a fixed effect and genotype as a random effect. I further tested whether herbivory had a negative impact on fitness by determining whether the tolerance reaction norm line was significantly lower than a line with an intercept of one. Pearson correlation coefficients were calculated to determine whether there were trade-offs among mean genotype levels of constitutive resistance, induced resistance and tolerance.

Next, I used a constrained multivariate approach, redundancy analysis (RDA) within the VEGAN package (Oksanen *et al.* 2012) to quantify how traits simultaneously change in response to herbivory along the nutrient gradient. RDA is a multivariate linear regression followed by a PCA of the fitted values. A permutation analysis determines the significance of the explanatory factors on the multivariate trait data observed (analogous to PERMANOVA). Visualization is similar to PCA, but the first canonical axes are constrained to only represent the variation explained by the linear predictors in the model (here, herbivory and nutrient supply). The 26 measured traits (see Table S3) were transformed as necessary to conform to the assumption of multivariate normality and standardized by scaling to a variance of 1. I ran the model first with the variance associated with genotype conditioned out (i.e. a partial RDA, analogous to treating genotype as a random effect) to allow better visual interpretation of the effect of environmental factors and then with genotype included as an additional fixed effect. I also partitioned the variance attributed to genotype vs. the environment using the function *varpart* (VEGAN).

Lastly, I used the function *betadisper* (VEGAN) adjusting for potential bias due to unequal number of individuals within groups to test whether trait variation differed among groups. This function implements permutational test of the homogeneity of multivariate dispersions similar to Levene's test in univariate statistics (Anderson, Ellingsen & McArdle 2006) and has been used as an estimate of intraspecific variation (de Bello *et al.* 2011). First, by testing whether genotypes exhibit differential dispersion from their respective genotypic means, I determined whether plasticity in multivariate trait expression in response to the treatments differed among genotypes. Secondly, I used the same command to test whether cumulatively genotypes occupy the same amount of trait morphospace within each treatment.

Results

RESISTANCE

Induced and constitutive plants responded differently based on nutrient supply. Plants in the constitutive (control) group decreased resistance to herbivory with increasing nutrient supply (RR = 1·86 ± 0·34; i.e. herbivore growth rates increased); however, nutrient additions to previously induced plants had a negligible effect on resistance (RR = 1·03 ± 0·23; 1 = no change), resulting in a significant interaction between herbivory and nutrients (Fig. 2a, $F_{1,41} = 7{\cdot}5$, $P = 0{\cdot}009$, Table S1). At low nutrient levels, eight genotypes exhibited induced susceptibility to herbivores (RR = 1·25 ± 0·11, herbivory effect at 0 p.p.m.: $F_{1,14} = 5{\cdot}54$, $P = 0{\cdot}03$), while at high nutrient levels seven of nine genotypes exhibited induced resistance (RR = 0·72 ± 0·14; herbivory effect at 400 p.p.m.: $F_{1,17} = 12{\cdot}42$, $P = 0{\cdot}002$; see Fig. 2b). At high nutrient levels (but not low), there was a shift towards grasshoppers feeding on the lower leaves of induced plants (Fig. 2c).

TOLERANCE

Whether tolerance changed over the nutrient gradient depended on which measure was used as a fitness proxy. Plants were fully and equally tolerant of herbivory in terms of flower biomass produced (no reduction with herbivory, Fig. 3b) across the entire nutrient gradient. However, total above-ground biomass was reduced with herbivory (~16% reduction, intercept ≠ one: $t = 3{\cdot}4$, $P = 0{\cdot}003$, Fig. 3c), but the slope was not different than zero indicating equally reduced tolerance across the nutrient gradient. In contrast, asexual reproduction differed across the nutrient supply gradient. Here, at low nutrient levels, herbivory resulted in a 31% proportional increase in the number of rhizomes produced compared with undamaged plants (RR = 1·31 ± 0·16), while at high nutrient levels, herbivory reduced the number of rhizomes produced 18% compared with undamaged plants (RR = 0·82 ± 0·09) (negative slope, $F_{1,23} = 6{\cdot}75$, $P = 0{\cdot}015$, Fig. 3a). Root biomass was negatively affected by herbivory across all nutrient treatments although to a smaller degree at high nutrient levels (0 p.p.m. RR = 0·69 ± 0·09; 400 p.p.m. RR = 0·85 ± 0·09; intercept is significantly different than one: $t = 3{\cdot}6$, $P = 0{\cdot}002$, Fig. 3d).

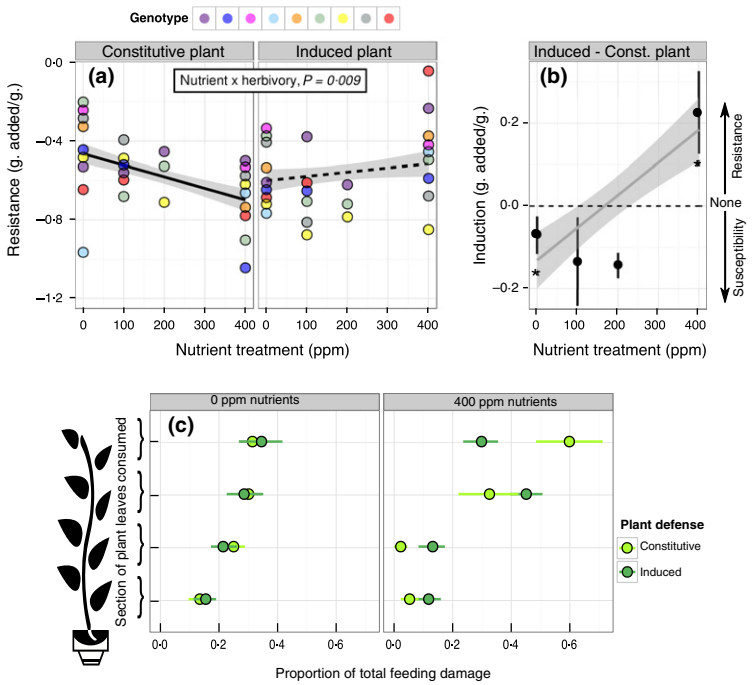

Fig. 2. Genotypic means of a plant resistance index (−1 × herbivore relative growth rate) of individuals previously fed on by herbivores (induced plant) vs. control plants (constitutive) across the nutrient treatment gradient (a). The line ± SE (shaded area) is a linear model relating y~ nutrient treatment. Control plants had lower resistance as nutrients increased while induced plants had higher resistance as nutrient levels increased resulting in a significant herbivory × nutrient level interaction (Table S1). Qualitative results did not change if only the fully replicated 0 and 400 nutrient levels were included in the model. (b) Plants at low nutrient levels exhibited induced susceptibility (lower resistance on induced plants), while high nutrient plants exhibited induced resistance (higher resistance on induced plants). * indicates significant difference in resistance between constitutive and induced plants within a given nutrient treatment using a LMM (see text). (c) Behaviourally, herbivores shift to feeding on lower leaves of induced plants at high nutrient levels (mean ± 95% CI; proportion feeding damage).

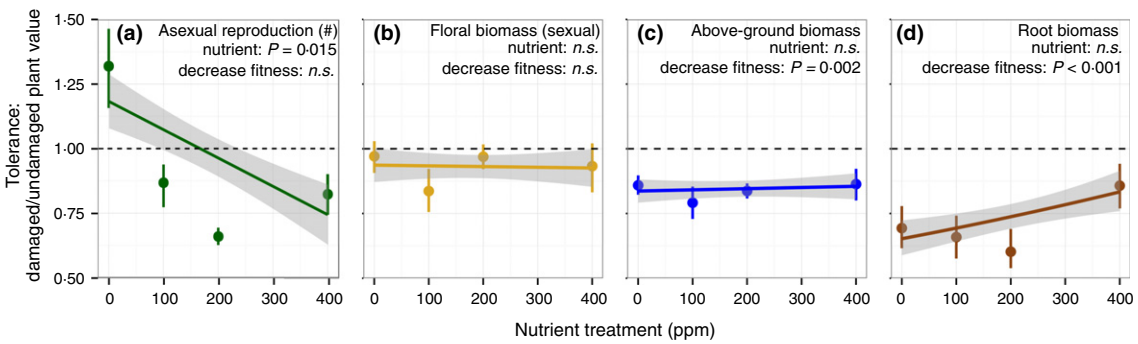

Fig. 3. Plant tolerance (mean of genotypes ±SE, n = 9) of herbivory across the nutrient gradient as measured by fitness-related traits: (a) rhizome number, (b) floral biomass, (c) above-ground biomass and (d) root allocation. The dashed line represents equal fitness between damaged and undamaged individuals of a genotype. Shaded area is the SE of a linear model relating y~ nutrient treatment. Plants were more tolerant of herbivory in terms of asexual reproduction at low nutrient levels than at high nutrient levels. While nutrient level did not have a significant effect on the slope of above-ground tissue allocation (c) and root allocation (d), there is significant negative impact of herbivory on above-ground and root biomass across the nutrient gradient.

LEAF NUTRIENT CONTENT

Nutrient addition resulted in higher leaf N content regardless of previous herbivory (control plant RR = 1·42 ± 0·12; induced plant RR = 1·21 ± 0·11; Linear mixed model nutrient: $F_{1,39}$ = 20·11, P < 0·0001; see Fig. 4a). In general, nutrients also increased leaf C content in plants but did so to a larger degree on induced plants (RR = 1·04 ± 0·007) than on control plants (RR = 1·01 ± 0·01) (herbivory × nutrient: $F_{1,39}$ = 7·08, P = 0·01, Fig. 4b, and Table S1). Taken together, this resulted in a larger overall decrease in plant C : N ratio at high nutrient levels on control plants (RR = 0·74 ± 0·06) than on induced plants (RR = 0·92 ± 0·10) (herbivory × nutrient: $F_{1,39}$ = 4·46, P = 0·04, Fig. 4c, and Table S1). However, none of these measurements was able to directly

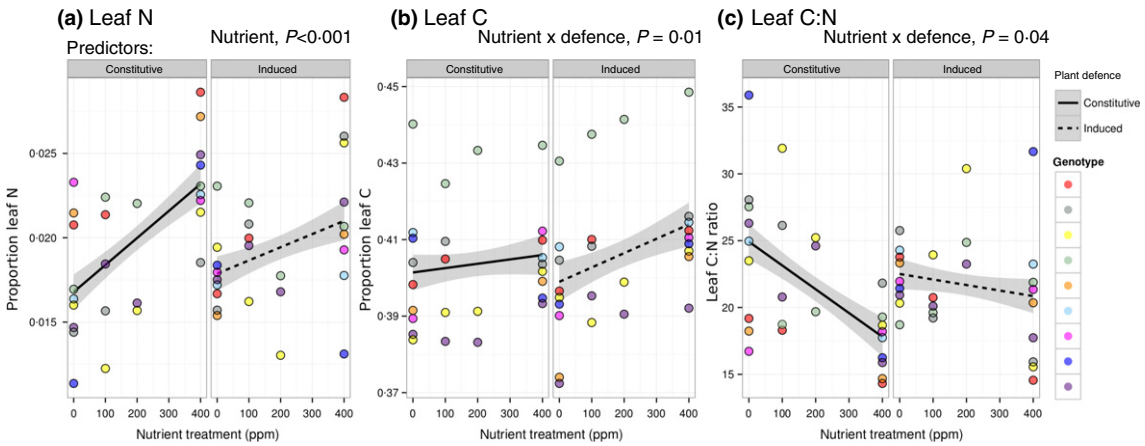

Fig. 4. Herbivory and nutrient effects on genotypic means of leaf nutrient content. Fertilization (a) increased N content regardless of herbivory treatment, (b) but leaf C content and (c) leaf C : N response to nutrients depended on herbivory treatment (significant herbivory × nutrient interaction, see Table S1). The line ± SE (shaded area) is a linear model relating y ~ nutrient treatment (each panel separate). If points completely overlapped, they have been jittered slightly.

explain the variation in resistance found across the genotypes and treatments (Fig. S3).

BIVARIATE TRADE-OFFS

No significant trade-offs between tolerance and resistance were detected within genotypes (Fig. S4). However, high constitutive resistance in a genotype consistently predicted a lower level of induced resistance within that particular genotype at low nutrient levels (Fig. S4e).

WHOLE-PLANT TRAIT EXPRESSION

A RDA quantifying how multidimensional plant phenotypes responded to nutrient supply and herbivory identified three significant constrained axes and accounted for 67% of the variation in the suite of plant traits (Fig. 5). First, genotype, which accounted for 36% of the trait variation, was removed (conditioned out). The environmental factors (herbivory and nutrient treatment) then combined to account for another 31% of the trait variation. The first

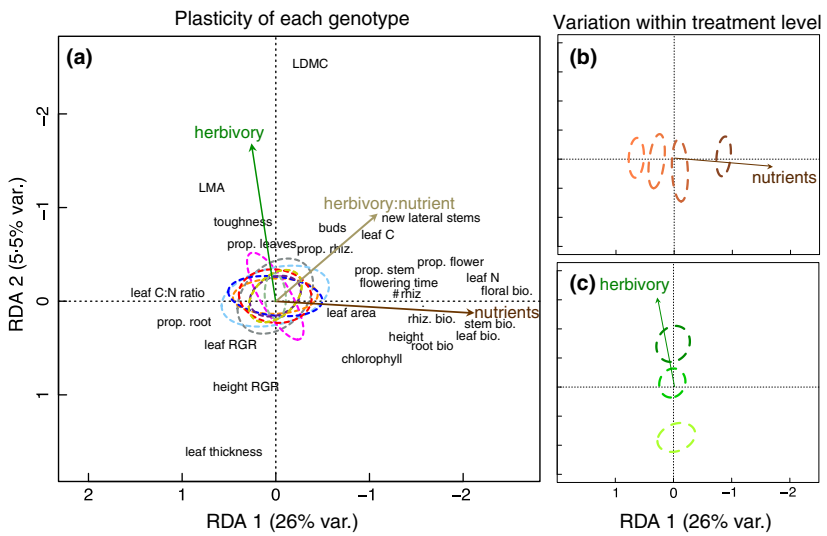

Fig. 5. Whole-plant trait and allocation patterns change in response to nutrient supply and herbivory. This is visualized using the first two axes (significant and the same across all panels) of a partial redundancy analysis (RDA) representing the multivariate plant response to nutrients and herbivory. Genotypic effects on trait variation (36%) were first removed so that genotype ellipses are centred at the origin. Environmental treatments then explained a further 31% of the variation in plant traits. Plant traits (black) are placed at the end of their respective vector (not shown) associated with that trait (e.g. higher leaf toughness is associated with increased herbivory), (a) highlighting the ability of each genotype (coloured ellipses = 95% CI of a genotype's morphospace) to respond plastically to each treatment (dispersion around the origin). Individual genotypes exhibit different amounts of multivariate trait plasticity to these combined stressors (i.e. individual genotype ellipses have significantly different multivariate dispersions). Population-level trait variation (i.e. the variation expressed across all genotypes within a given (b) nutrient or (c) herbivory environment) did not differ across environments. LMA, leaf mass per area; LDMC, leaf dry matter content.

RDA axis was associated with increased nutrients and accounted for 26% of the variation in plant traits. Many of the traits associated with this axis are related to increases in the size of plant parts (stems, flowers, leaves, rhizomes, roots). In addition, as nutrient availability increased, leaf N content increased and leaf C : N decreased while, proportionally, plants allocated more to sexual reproduction and less to root tissue. The second RDA axis was associated with herbivory and accounted for 5% of the variation. Herbivory led to plants with a higher proportional allocation to tougher leaves with a higher LDMC, LMA and lower post-treatment leaf and plant height RGR. Herbivory ($F_1 = 9{\cdot}85$, $P < 0{\cdot}001$) and nutrient ($F_1 = 47{\cdot}0$, $P < 0{\cdot}001$) treatments were both significant predictors of multivariate plant traits (Table S2) with a significant herbivore × nutrient interaction ($F_1 = 2{\cdot}14$, $P = 0{\cdot}04$). High nutrient plants exposed to herbivory exhibited increased leaf C and a higher proportional allocation to stems and rhizomes with a concomitant proportional decrease in allocation to roots. Over the course of the growing season, these plants produced relatively more lateral stems at the base of the plant and below-ground adventitious buds than control plants, indicating an altered reproductive strategy.

MULTIVARIATE PLASTICITY

Genotypes exhibited significantly different amounts of multivariate trait plasticity to combined stressors (*betadisper*: d.f. = 8, $F = 2{\cdot}69$, $P = 0{\cdot}009$, Fig. 5a size of genotype ellipses), with some responding more strongly to herbivory and others to nutrient treatment (Fig. 5a shape of genotype ellipses).

POPULATION-LEVEL VARIATION ACROSS ENVIRONMENTS

The breadth of population-level trait variation within an environment was not different between herbivory treatments (*betadisper*: d.f. = 2, $F = 0{\cdot}02$, $P = 0{\cdot}97$; i.e. similar ellipse sizes between treatment groups, Fig. 5c). Overall, trait dispersion was different between nutrient treatments (*betadisper*: d.f. = 3, $F = 4{\cdot}38$, $P = 0{\cdot}006$, Fig. 5b), but post hoc paired comparisons (Tukey HSD) showed that this was entirely due to the larger dispersion of the incompletely sampled 200 p.p.m. nutrient treatment. The paired comparison of the lowest (0 p.p.m.) and highest (400 p.p.m.) nutrient levels of interest showed no difference in trait dispersion ($P = 0{\cdot}76$).

GENOTYPIC DIFFERENCES

Few G and $G \times E$ effects were detected within the univariate analyses (resistance, tolerance and leaf nutrient content). The exception was a significant effect of genotype on leaf carbon content ($\chi_1^2 = 76{\cdot}03$, $P < 0{\cdot}001$). It is worth noting that this failure to detect an effect may be due to low power and that many of the 23 other plant traits did exhibit strong G and $G \times E$ effects (K.T. Burghardt, unpublished data). This is probably what led to genotype having a strong effect when included as a fixed effect within the multivariate analysis (genotype, $F_{1,8} = 17{\cdot}88$, $P = 0{\cdot}001$). However, including genotype in the RDA results in environmentally based trait changes that are harder to interpret as there are nine significant RDA axes rather than 3 and many significant interactions (e.g. herbivory × genotype, $F_{1,8} = 1{\cdot}41$, $P = 0{\cdot}04$; nutrient × genotype, $F_{1,8} = 3{\cdot}27$, $P = 0{\cdot}001$; see Table S4 and Fig. S5).

Discussion

This study explicitly integrates plant defence and trait-based approaches to provide a nuanced understanding of the intraspecific trait variation found across biotic and abiotic gradients within a dominant species (up to 90% cover in old-fields). I found that developmental nutrient supply changes a genotype's plant defensive strategy when that genotype is subsequently exposed to herbivores. Specifically, at low nutrient levels, plants exhibited increased tolerance of herbivore damage, manifest as higher asexual reproduction relative to control plants (Fig. 3). However, for the same genotypes at high nutrient levels, previous herbivory resulted in lower herbivore growth rates (i.e. higher resistance, Fig. 2) in spite of the fact that leaf N content remained higher at high nutrient levels (Fig. 4). Changes in whole-plant allocation and trait expression occurred in concert with these strategy shifts, with genotypes being differentially plastic in response to nutrients and herbivores. These results underscore the multifaceted and integrative nature of plant defence strategies and highlight that plastic trait changes (in addition to genetically based responses) may have important effects on communities and ecosystem processes (Antonovics 1992) and explain context dependence in environmental effects on trait expression.

SUPPORT FOR COMMON PLANT DEFENCE MODELS

These results provide equivocal support for the growth differentiation balance hypothesis (GDBH) (Herms & Mattson 1992). This framework predicts a peak in constitutive defence at mid-range nutrient levels and induced defence at high nutrient levels. While I found elevated induced resistance at high nutrient levels, I saw no evidence of increased constitutive resistance at mid-range nutrient levels as predicted. However, I cannot rule out that this may be a by-product of incomplete genotype replication at mid-range nutrient levels. The presence of the highest constitutive resistance at low nutrient levels also provides intraspecific support for the Resource Availability Hypothesis (Coley, Bryant & Chapin 1985), which is usually evaluated at the interspecific level (Zandt 2007).

The documentation of a higher tolerance of herbivory at low nutrient levels is in accord with the growth rate model (GRM) (Hawkes & Sullivan 2001) and in opposition to

the Compensatory Continuum Hypothesis (CCH) (Maschinski & Whitham 1989). A more recent framework, the Limiting Resource Model of Tolerance (LRM), which integrates the GRM and CCH, also successfully predicts the results (Wise & Abrahamson 2007). In this case, plant growth is limited by nutrients at low nutrient supply, while herbivore damage negatively impacts an alternative resource (carbon acquisition) through the removal of leaf tissue. This alternate resource is limiting at high nutrient levels, which results in lower tolerance at high nutrient levels. Higher tolerance at low nutrient levels has been found to be the dominant pattern in experimental manipulations of nutrients.

In *S. altissima,* nutrient supply-dependent induction may help explain conflicting results found in defence expression across studies (Brown & Weis 1995; Uesugi, Poelman & Kessler 2013; Heath *et al.* 2014). Because genotypes vary in their inducibility and alter their defensive strategy based on nutrient availability, studies may be measuring defence on differentially resource-limited plants (e.g. root bound plants in small pots vs. field plants).

PLANT STOICHIOMETRY AND RESISTANCE PATTERNS

Increasing nutrient supply can also alter leaf carbon or nitrogen content through primary metabolism changes, thereby influencing herbivore growth rates (Behmer 2008). As leaf nutrient content changes occur simultaneously with resistance trait changes, I examined whether the former solely drove observed patterns. While leaf nutrient content was not able to directly explain the documented resistance changes (see Fig. S5), plant stoichiometry did partially mirror the induced resistance patterns (Fig. 4). Induced plants had static C : N ratios across the nutrient gradient, while control plants increased in quality (C : N decreased). However, given that herbivore growth rates were lower on high nutrient, induced plants than low nutrient, induced plants (same C : N), at least one additional resistance mechanism, perhaps carbon-based defence, is driving the trend. While such defences – known to contribute to anti-herbivore defence in this species (Bode, Halitschke & Kessler 2013) – were not directly measured, the multivariate trait analysis showed that induced resistance occurred in concert with increased leaf carbon content and structural allocation (toughness and LDMC, see Fig. 5a).

INTEGRATING PLANT DEFENCE WITH WHOLE-PLANT TRAIT CHANGES

Herbivores interact with whole organisms that express suites of covarying growth, structural and defensive traits simultaneously rather than each in isolation (Forsman 2015). Thus, while plant defence theory provides an important predictive framework for when phenotypic plasticity will occur in response to resource conditions, these shifts occur within the context of many other physiological changes. In this study, while plant strategies were clearly changing across the nutrient gradient, a bivariate analysis detected no clear trade-off for individual genotypes between tolerance and either constitutive or induced resistance levels (Fig. S4), failing to support the commonly posited (though often unsupported) tolerance/resistance trade-off (Leimu & Koricheva 2006). However, other traits and allocation patterns within an individual may also change across the gradient obscuring trade-offs and integrating plant responses. For example, recent work suggests that secondary metabolites such as tannins, previously considered to play a primary role within anti-herbivore defence, may also allow a plant to better reallocate resources below-ground after herbivory, linking together tolerance and resistance processes (Madritch & Lindroth 2015). Such linked functions and whole-plant responses may be better captured with a multidimensional phenotype approach (Walsh & Blows 2009).

Whole-plant trait variation was both genetically (~36%) and environmentally based (~31%; plastic changes to nutrients and herbivory) with a unique multivariate phenotype expressed by plants that experienced herbivory at high nutrient supply. While it is not surprising that a species with documented strong genetic diversity effects on community processes (Crutsinger *et al.* 2006) would exhibit genotypic variation, the additional and nearly equivalent magnitude of environmentally-based changes is notable. This suggests that ecosystem and community models that currently incorporate species or genotype mean trait values may be improved if plasticity to environmental context was explicitly considered (Wright & Sutton-Grier 2012).

COMPARISON OF TRAIT VARIATION EXPRESSED WITHIN GENOTYPES AND BETWEEN ENVIRONMENTS

While the magnitude of multivariate trait plasticity to environmental context exhibited by individual genotypes differed (Fig. 5c), the size of the trait space occupied by the collective population of genotypes within each environment (nutrient level or herbivory) did not change across the gradients. This indicates that the same collection of genotypes occupy approximately equal volume of morphospace across a variety of environments. Therefore, extensive and approximately equal phenotypic variation (different means with the same variance) would be maintained on a landscape within quite permissive environments of high nutrients or no herbivory as well as highly stressful environments with few nutrients or many herbivores. Quantification of the mean and variance also allows predictions. For example, anthropogenic nitrogen deposition is a known phenomenon in the study area (Vitousek *et al.* 1997). These results suggest that while deposition may change mean trait expression on the landscape, it probably is not altering constitutive population-level variance present within this species. This approach might be particularly useful if modellers have already identified one

or a few traits that are key control points (Bassar *et al*. 2012; DeAngelis *et al*. 2012).

Conclusions

Few studies explicitly link plant defence emergent outcomes to concurrent changes in whole-plant trait expression. Quantifying the phenotypic structure and trait variance within a local population in response to two interacting stressors as I have done here may enable prediction of population-level effects and identification of potential feedbacks or strategy shifts across gradients (Vindenes & Langangen 2015). In addition, by focusing on locally co-occurring genotypes, this study quantified sources of plant phenotypic variation that are at a scale which is ecologically and evolutionarily relevant for local populations of herbivores, predators and microbes (Tack, Johnson & Roslin 2012). As a result, this study adds complementary insight to previous work that demonstrates high intraspecific trait variation when comparing or combining genotypes from populations widely distributed across species ranges or between hybridizing subpopulations (Crutsinger *et al*. 2006; Whitham *et al*. 2006). Ultimately, understanding the local structure of trait variation may help explain the contingent nature and strength of community interactions and ecosystem processes such as nutrient cycling rates (Hunter 2001; Schweitzer *et al*. 2005).

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Data accessibility

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.q1j71 (Burghardt 2016).

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Nutrient treatment effect on plant growth before herbivore treatment.
Fig. S2. Correlation between the genotypic means of herbivore damage and tolerance.
Fig. S3. Direct relationship between leaf stoichiometry and resistance.
Fig. S4. Trade-offs between tolerance and resistance.
Fig. S5. Redundancy analysis results that include genotype as an explanatory factor.
Table S1. Linear mixed model results for resistance and leaf nutrient response to treatments.
Table S2. Permutation tests of explanatory factors in the partial RDA.
Table S3. Trait loadings on partial RDA axes.
Table S4. Permutation tests of explanatory factors in RDA with genotype as a fixed effect.