ABSTRACT

Linking Plasticity in Goldenrod Anti-herbivore Defense to Population, Community, and

Ecosystem Processes

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Nutrient cycling plays a critical role in maintaining biodiversity and ecosystem services in agricultural, urban, and natural lands. However, across landscapes there is substantial unexplained heterogeneity in nutrient cycling. Classic thinking holds that abiotic factors are the source of this spatial heterogeneity with a secondary role of plant biomass. However, recent work suggests that higher trophic levels or variation in traits at the level of plant genotype may also play an important role in structuring nutrient environments. For instance, herbivores may indirectly create heterogeneity in cycling through the induction of chemical and structural changes in plants traits. Phenotypic plasticity due to anti-herbivore defense may then alter nutrient cycling rates by changing the microbial breakdown of plant litter inputs. Alternatively, variation among plant genotypes in the expression of these same traits may overwhelm the influence of phenotypic plasticity on soil processes. Both genetic and environmentally based changes in plant traits have separately been demonstrated to alter soil processes, but their interaction and the relative importance of these sources of variation across local landscapes is unknown.

I address this question by developing a plant trait-mediated, conceptual framework of nutrient cycling. I then evaluate this framework within an old-field ecosystem by focusing

on the dominant plant species, *Solidago altissima*, and its dominant grasshopper herbivore, *Melanoplus femurrubrum,* using a combination of lab assays, a greenhouse pot experiment, a field mesocosm experiment, and field surveys. First, I demonstrate that goldenrod individuals exhibit both genotypic variation and phenotypic plasticity in plant defensive trait responses across a nutrient and herbivory gradient in the greenhouse. At low nutrient supply, genotypes tolerate herbivory (inducing plant physiological changes that decrease the negative impact on fitness) while at high nutrient supply, the same genotypes induce a resistance response detectable through lower herbivore growth rates. These environmentally mediated changes in plant trait expression then altered the ability of a common microbial community to decompose senesced litter harvested from the same plants. Induced resistance in the population of genotypes grown at high nutrient levels led to decreased litter decomposition of herbivore legacy litter. In contrast, at low nutrient supply, herbivore legacy litter decomposed more efficiently compared to control litter. This suggests that the interaction between herbivory and nutrient supply could cause context-dependent acceleration or deceleration of nutrient cycling. As a result, trait plasticity may mediate effects of multiple environmental conditions on ecosystem processes in this system.

I tested this hypothesis using a three-year, raised bed, field experiment examining the effect of plasticity and locally relevant genetic variation on ecosystem processes in a naturalistic setting. Genotype clone clusters were planted in homogenized soil in enclosed cages with varying nutrient supply and grasshopper herbivory. Again, I documented strong genetically and environmentally-based trait variation in plant allocation, growth, and leaf traits. I next explicitly linked these genetic and plastic functional trait changes to concurrent changes in a variety of soil processes (microbially available carbon, plant available nitrogen, nitrogen mineralization potential, and microbial biomass) and litter decomposition rates.

Importantly, partitioning functional trait variation into genetic and environmental components improved explanatory power. I also documented potential differences in herbivore effects on "slow" vs. "fast" cycling in soil microbially available C pools. Within both experiments the magnitude of trait variation measured was similar to the variation expressed by individuals across a focal field.

Taken together, this dissertation demonstrates that plant genotype, herbivores, and nutrients can all modify litter decomposition and other soil processes within ecosystems through differential expression of plant functional traits. Due to the spatially clumped, clonal, and dominant nature of goldenrod, the genetic and herbivory-driven changes documented here could lead to a predictable mosaic of soil process rates across a single old field landscape. This work also highlights the complex interplay between genetically and environmentally-based trait variation in determining population and ecosystem processes within landscapes and improves our understanding of the often-overlooked indirect effects of plant/herbivore interactions on nutrient cycling. It suggests that herbivores may shape not only the evolution of plant populations, but also the soil nutrient environment and microbial community in which plants live. This sets up the potential for eco-evolutionary feedbacks between plant defense expression and soil nutrient availability. More broadly, it suggests that biotic factors, in addition to abiotic ones, play a key role in determining local-scale soil nutrient availability patterns and should potentially be accounted for within ecosystem models. These results are particularly salient in a world where anthropogenic nitrogen inputs continue to rise and climate change is predicted to increase herbivory and thus plant defensive trait induction on landscapes.

Linking Plasticity in Goldenrod Anti-herbivore Defense to Population, Community,

and Ecosystem Processes

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CHAPTER 1

INTRODUCTION

Ecologists and evolutionary biologists have long been fascinated by morphological and phenotypic diversity in the traits produced across species. Researchers link these trait differences to wide array of evolutionary and functional processes such as niche differentiation, community composition, functional diversity, and ecosystem process rates (May & Macarthu.Rh 1972; Hobbie 1992; Hodgson *et al.* 1999; Hooper *et al.* 2005; Kraft *et al.* 2015; Laughlin & Messier 2015). Attention has now turned to the potentially important role that intraspecific variation—the phenotypic variation found within a species—may play in determining community composition and ecosystem process rates, especially in species that are dominant within their communities (Post & Palkovacs 2009; Bolnick *et al.* 2011; Violle *et al.* 2012).

Intraspecific variation can arise through genetic differences in trait expression, environmentally-based difference in trait expression (phenotypic plasticity), and the interaction between the two (genetic variation for plasticity) (Whitman & Agrawal 2009). The community genetics paradigm (Antonovics 1992) focuses on how genetic variation of a dominant species influences the community composition of such disparate groups as arthropods and soil microbes as well as ecosystem processes such as biomass production, litter decomposition, and nutrient availability (Wimp *et al.* 2005; Whitham *et al.* 2006; Bangert *et al.* 2008; Schweitzer *et al.* 2008). While the origins of functional trait research lie in interspecific comparisons of trait expression across gradients (Grime 1977), there is a current push to more closely examine and quantify plastic responses within species (intraspecific variation) to environmental gradients and then link this variation to ecosystem processes (Wright & Sutton-Grier 2012).

If dominant species do indeed have large impacts on ecosystem processes (Smith & Knapp 2003), then understanding the relative role of genetically and environmentally based variation in determining that impact is important. This is because the source of the variation determines how the mean and variance of trait expression shift both within and across important environmental gradients ("natural" and anthropogenic). Further there may be another driver of trait variation within these systems whereby the developmental environment of an individual alters that individual's capacity for responding plastically to a later environment.

An important suite of plant functional traits are those associated with anti-herbivore defense 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 (Schweitzer *et al.* 2008). Herbivores can alter ecosystem processes by altering 1) the direct impact of herbivores on fast cycle soil processes through changes in frass, greenfall, canopy leaching, and carbon inputs or 2) indirectly through herbivore-mediated changes in plant tissue and expression patterns which are often slow cycle (e.g. changes in the chemical and structural components of plant litter inputs to ecosystems) (Hunter 2001; Bradford *et al.* 2008). At the intraspecific level, plant anti-herbivore defensive trait variation may have both genetic and plastic (inducible) components, making it a useful trait for examining the relative and interactive effects of genetic and environmental sources of trait variation on landscapes. These genetic and plastic trait expression patterns may in turn shape the local-scale spatial structure of ecosystem process rates and nutrient cycling within old-fields.

My dissertation work examines these and related question through the lens of a New England (USA) old-field ecosystem. After agricultural fields, which often have fairly

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homogenous soil environments due to years of tillage, are abandoned from cultivation, they are colonized by a succession of annual and perennial species before ultimately transitioning to forest. A dominant species within this temporal succession is *Solidago altissima (L)*, or tall goldenrod. This species is a rhizomatous perennial that spreads primarily through the clonal growth of deciduous ramets after colonization of a disturbed field. This growth pattern produces a genetic mosaic within a single old-field due to the spatial clumping of genotypes (Maddox *et al.* 1989). This species can also represent up to 95% cover of old-field system particularly within early to mid successional periods (Maddox & Root 1990). If genetic variation does indeed play a role in determining ecosystem process within this species than the genetic mosaic of genotypes should result in a corresponding mosaic of ecosystem process rates on the landscape.

However, this species is also known to exhibit plasticity in response to both herbivory (through changes in plant defense expression and tolerance) and nutrient environments (Meyer & Root 1993; Meyer 1998b, a; Heath *et al.* 2014), both of which may be variable across old-field landscapes. This system, therefore, provides an ideal setting to partition the relative effects of genetic and environmentally-based trait expression and determine how they interact to produce spatial variation in ecosystem process rates across old-field landscapes. I answer this question using a combination of literature synthesis, lab-based assays, a greenhouse pot experiment, a field mesocosm experiment, and field surveys.

Beginning in chapter 2, I synthesize literature on plant defense expression patterns in terrestrial and aquatic systems in response to resource gradients. I then develop a conceptual framework to predict how nutrient supply may alter the magnitude and direction of herbivore impacts on ecosystem process both 1.) directly through greenfall, canopy leaching,

frass and carcass inputs and 2.) indirectly through herbivore-mediated changes in plant trait expression patterns.

Motivated by insights from the synthesis, I report in Chapter 3 on experiments that quantify tolerance and resistance defensive trait expression within nine *S. altissima* genotypes grown across a nutrient gradient within the greenhouse. I found both genotypic variation and phenotypic plasticity in plant defensive trait responses in genotypes of goldenrod grown across a nutrient and herbivory gradient. At low nutrient supply, genotypes tolerated herbivory (induced plant physiological changes that decreases the negative impact on fitness) while at high nutrient supply, the same genotypes induced a resistance response detectable through lower herbivore growth rates. However, these responses occurred through many correlated changes in whole plant expression patterns (e.g. leaf nutrient content, structural traits, allocation patterns, and growth).

Chapter 4 reports on a closer examination of whether these herbivore-mediated trait changes in leaf tissue can alter rates of plant litter decomposition by soil microbial communities. I performed a lab-based decomposition assay using litter from plants grown in the greenhouse experiment. I seeded this litter with a common microbial community inoculum and then measured carbon mineralization rates over a 100-day assay to estimate decomposition efficiency by the microbial community. I found that induced resistance in the population of genotypes grown at high nutrient levels led to decreased litter decomposition efficiency of herbivore legacy litter. In contrast, at low nutrient supply, herbivore legacy litter decomposed more efficiently compared to control litter. An increase in decomposition at low nutrient levels and a decrease at high nutrient levels essentially canceled out the pattern in the control litter of increasing decomposition with a legacy of high nutrient supply.

Chapter 5 explores how well the greenhouse and lab microcosm results explain plant trait expression and soil processes in field mesocosms. I specifically examine the question of whether genetic or plastic variability in plant-trait expression plays a larger role in structuring soil nutrient availability using a three-year raised bed, field mesocosm experiment. I planted genotypes within spatially clumped clusters of three individuals apiece and then manipulated nutrient supply and grasshopper herbivory for two years. In the 3rd year, treatments were abated to examine at how quickly the signature of environmental treatment effects faded within the genotype clusters. I found that genetic variation shaped plant growth, allocation, and leaf traits but that these traits also exhibited strong plasticity to herbivory (and nutrient supply to a lesser degree). These plant trait changes were correlated with changes in a variety of soil processes (carbon mineralization potential, plant available nitrogen, nitrogen mineralization potential, and microbial biomass) and could be partitioned into genetic and environmental components. Within the raised beds themselves, I could not separate the indirect effects of herbivores through plant trait changes from direct effects on soil processes due to potential plant-soil feedbacks. Therefore in order to examine herbivore-mediated indirect effects more closely, I performed a companion lab-based litter decomposition assay and an herbivore feeding trial on leaf tissue from each of the genotype clusters. Here, as in Chapter 4, high nutrient levels promoted genetic determination of litter decomposition rates, which were then consistently lower on leaf litter coming from herbivory treatments.

Taken together, this dissertation integrates concepts and experimental approaches from plant defense theory, community genetics, ecosystem ecology, with functional trait based approaches to tackle questions about the development of local-scale spatial heterogeneity in trait expression and nutrient cycling within landscapes. These four chapters

provide a comprehensive look at the relative effects and potential interaction of genetic and environmental sources of variation in plant functional trait expression of *S. altissima* in old-field communities. I then trace the genetic and environmental sources of trait variation through to correlated changed in ecosystem processes. This work resolves how genetic and plastic trait variation together shape ecosystem process variation within and old-field landscape and conceptually advances approaches that attempt to integrate whole plant phenotypes and a wide range of types of traits into analytic frameworks.

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CHAPTER 2

INFLUENCE OF PLANT DEFENSES AND NUTRIENTS ON THE TROPHIC

CONTROL OF ECOSYSTEMS[*]

Introduction

Ecological systems are extraordinarily complex. Thus classical approaches to resolve ecosystem functioning have simplified analyses by conceptualizing ecosystems as being organized into trophic level compartments that contain organisms with similar feeding dependencies (*e.g.,* producers, herbivores, carnivores) (Elton 1927; Lindeman 1942). Two competing worldviews on the regulation of ecosystem productivity emanated from such a conceptualization of ecosystem structure. The bottom-up view posits that the productivity of each trophic level is essentially limited by the one immediately below it (Lindeman 1942; Feeny 1968), while the top-down view recognizes that resource levels influence production, but contends that herbivore populations are mostly limited by predators rather than producer biomass (Hairston *et al.* 1960). Accordingly, predators can indirectly increase the productivity of a given system by reducing the negative effects of herbivores on plant biomass, resulting in a world that is green with plant material, rather than denuded by herbivory (Paine 1969; Oksanen *et al.* 1981). Bottom-up theory countered that the world is green not because of predators, but instead due to variation in plant quality as a result of anti-herbivore defenses or weather patterns (Murdoch 1966; Ehrlich & Birch 1967; Scriber & Feeny 1975; White 1978; Feeny 1991; Polis & Strong 1996). This variation causes much of the "green" world to be inedible to herbivores; thus herbivores are still resource-limited.

The recognition of context-dependence in the degree of top-down or bottom-up control of ecosystems has resulted in gradual changes in how ecosystem functioning is envisioned. For instance, the "exploitation ecosystems" hypothesis (EEH) addresses context-dependence by combining elements of top-down and bottom-up concepts (Oksanen *et al.* 1981; Oksanen *et al.* 2000). At low levels of soil resource availability, plants are not productive enough to support herbivore populations and are thus bottom-up controlled (see Figure 2.1). At medium levels of soil resources, an ecosystem can support herbivore populations, which in turn control plant productivity, while carnivores enter the ecosystem and control the herbivore population at the highest resource availability, thus releasing plant productivity from herbivore control. As a result, there is now a general consensus that both top-down and bottom-up control can occur within the same ecosystem, but that their relative magnitude is context specific (Hunter & Price 1992; Power 1992; Chase *et al.* 2000b). Understanding of the basis for this context dependence in strength remains incomplete: while explanations for cross ecosystem differences have been offered (Shurin *et al.* 2002), explanations for spatial differences within ecosystems remain elusive. This chapter aims to begin resolving the basis for within ecosystem context-dependency in the strength of trophic control by focusing on one of the important mediating factors identified in early debates about top-down and bottom-up forcing within ecosystems: the expression of plant defensive traits. This focus is a natural extension of classic theory because the expression of plant defensive traits is also intimately tied to resource availability. I review here the interplay between resources, plant defenses and top-down and bottom-up control strength in an effort to offer generalizable principles that extend to explain differences across terrestrial and aquatic ecosystems.

Strong (1992) suggested that aquatic and terrestrial ecosystems are controlled in fundamentally different ways, with top-down control more prevalent in aquatic ecosystems, due in part to differences in primary productivity. However, a recent meta-analysis of experimental evidence concludes that net primary productivity does not differ between aquatic and terrestrial habitats, and instead producer nutritional quality is a consistently better indicator of the importance of consumers for top-down control (Cebrian & Lartigue 2004). This makes sense in light of the fact that plant defensive strategies directly interact with nutritional quality to determine plant palatability (Raubenheimer 1992). Subsequent theory (Vos *et al.* 2004) and experimental work (Verschoor *et al.* 2004b) have demonstrated that defensive traits that limit the efficacy of consumers to impact plants can be an important determinant of the relative strength of top down and bottom-up effects, ultimately mediating the presence of trophic cascades in ecosystems. That is, this integrative view of trophic control of ecosystems is beginning to be one of "control from the middle out", *sensu* (Trussell & Schmitz 2012), rather than from the top-down or bottom up.

In this chapter, I introduce and elaborate when defensive traits may play a key role in moderating trophic control of ecosystems from the middle out. I begin by clarifying the terminology used throughout the chapter to refer to defensive traits and then introduce a trait-based framework for thinking about how plant defenses may impact trophic control. Next, I highlight the dominant defensive traits found within aquatic versus terrestrial systems and review how nutrient availability may impact the strength of individual plant defenses within a species through phenotypic plasticity to nutrient availability (Cipollini *et al.* 2003) or through changes in mean community traits by the filtering of species that perform well in particular nutrient environments (Uriarte 2000) across terrestrial, freshwater, and marine ecosystems. I propose here that a trait-based approach offers greater opportunity for

understanding context dependency in the way defenses mediate trophic control than approaches that focus merely at the species level or lump all species into trophic groups. I then end with an exploration of the link between the expressed plant defense traits and ensuing food web interactions and ecosystem functioning.

Primary producer anti-herbivore defenses

Most plants lack the capability to actively move away from potential herbivores. Vascular plants in terrestrial or littoral systems are rooted in place and floating phytoplankton species in marine and pelagic systems lack directional escape from their consumers. However, none of these organisms are passive in their interactions with consumers. Thousands of plant species reduce herbivory by producing an arsenal of anti-herbivore defenses (Karban & Baldwin 1997). These include structural defenses, such as thorns, spines, or tough tissues that are difficult to chew, as well as chemical defenses, such as toxic compounds. Chemical defenses can be qualitative, where the mode of action is to poison a herbivore, or quantitative, such as leaf toughness or digestion inhibitors that force an herbivore to consume a larger quantity of food in order to extract the same nutrients, thereby prolonging their exposure to potential predation or parasitism (Feeny 1976). Defensive traits that decrease plant damage from herbivores or lower herbivore performance are collectively known as *resistance* traits.

A second general defensive strategy, known as *tolerance*, minimizes the negative impact of herbivory by enabling a plant to regrow quickly and thus regain lost photosynthetic capacity (Strauss & Agrawal 1999). This strategy may include an increase in growth rate, utilization of stored reserves, activation of dormant meristems, or a decrease in allocation to structural tissue, which lowers leaf toughness and leaf mass per area (LMA)

(Tiffin 2000). These traits would seem to increase the palatability of plant tissue, thereby rendering them ineffective as a defense. But, if a plant is able to produce tissue faster than the herbivore can remove it, or if the herbivore completes its life cycle and leaves the plant, then tolerance can overcome herbivore impacts.

In addition, these defenses can be described as being either *constitutive* or *induced*. If the defenses are always produced within a plant regardless of the presence of an herbivore, they are constitutive. Defenses are considered induced if they are expressed after an herbivore begins to inflict damage (Agrawal & Karban 1998). Inducible tolerance or resistance responses are a form of phenotypic (trait) plasticity that may be adaptive (Agrawal 2001) and could impact community dynamics through increasing trait variation within populations (Schmitz *et al.* 2003). The focus of this chapter will be on direct defenses, such as those described above; however, many plants also utilize indirect defenses, such as the release of plant volatiles that attract parasitoids and predators of the herbivore to the attacked plant (Arimura *et al.* 2005; Pohnert *et al.* 2007). Indirect defenses merit independent treatment and are described in more detail in Chapter 14 of this volume. Moreover, the efficacy of a plant defense is inherently tied to the environmental context in which it is expressed. A putative defense may not decrease an herbivore attack when it is expressed within a milieu of plants all expressing defense, but may work quite well if better quality, less defended plants are in the surrounding environment (Belovsky & Schmitz 1994).

Historically plant defenses have been measured in isolation. However, terrestrial and aquatic plants may respond to herbivores through the simultaneous expression of several commonly co-occurring traits or "plant defense syndromes" (Agrawal & Fishbein 2006; Ruehl & Trexler 2013). Structural and chemical defense expression and tissue allocation are individual traits that cumulatively determine the overall tolerance or resistance of a particular

plant. As such, I consider tolerance and resistance strategies (albeit not mutually exclusive; (Mauricio *et al.* 1997) to represent two common "plant defense syndromes" with distinct trait expression levels that are nonetheless useful for exploring potentially different effects of plant defense on trophic cascades.

Conceptual Framework

Mechanism Switching Hypothesis

The impact of an herbivore on plants will depend on the nature of herbivore resource limitation. Herbivores could be limited by *relative* resource supply if their per capita uptake rate of edible plant biomass is limited by the amount of time available to feed (Schmitz, 2008). In this case, there may be a surfeit of plants that herbivores cannot eat due to daily limitations on feeding imposed by the abiotic environment. Alternatively, herbivores could be limited by *absolute* resource supply if their per capita uptake of plant biomass is limited by the availability of total edible plant biomass (Schmitz, 2008). In this case, herbivores increase their per capita intake rate of edible plant biomass in direct proportion to the abundance of edible plant biomass. The nature of herbivore resource limitation also determines the extent to which predators can indirectly alleviate plant damage via direct interactions with herbivore prey. These ideas are encapsulated in the *Mechanism Switching Hypothesis* (MSH) of trophic control of ecosystems (Schmitz, 2008).

For example, consider a simple system of three trophic levels comprised of plants, herbivores and predators. In the absence of predators, plant abundance is limited by consumption from herbivores. Predators can reduce herbivore abundances, and thereby have an indirect effect on plants through cascading effects that alleviate plant damage— called a trophic cascade (Figure 2.2a). However, this response will only occur if the

herbivores that remain do not compensate and consume a larger per capita share of the plant biomass (*i.e.*, herbivores experience relative resource limitation). If instead, herbivores experience absolute resource limitation, any remaining herbivores are able to increase their per capita uptake of plants, such that predators have no net indirect effect on plant damage. In this conceptualization, the interaction between resource limitation and predators determines whether top-down control emerges.

I suggest that the MSH offers the means to extend the consideration of trophic control of ecosystems to include plant defenses. In essence, plant defenses can determine whether herbivores become relative or absolute resource limited. For example, the presence of a structural "resistance" defensive trait may increase the amount of foraging time an herbivore requires to gain the same nutritional pay-off (Moran & Hamilton 1980; Raubenheimer 1992). This strategy also has the advantage of increasing the amount of time that an herbivore is exposed to predation. In addition, when a predator consumes an herbivore, the remaining herbivores on the plant cannot increase their per capita feeding rate because spines and structural defenses inhibit feeding rate. These herbivores are foraging time limited and experience relative resource limitation that leads to a trophic cascade and top-down control (Figure 2.2b). If the resistance defense is a toxin rather than structural the same qualitative outcome occurs, but the mechanism differs. The herbivores experience toxin-limitation upon feeding. Despite perhaps having ample time to feed, herbivores can nonetheless only process a limited quantity of any toxin containing tissue per unit time. Therefore, when a predator removes an herbivore from the plant, other herbivores cannot increase their per capita feeding rate, resulting again in a trophic cascade. This case of an herbivore experiencing relative resource limitation created by a toxin, rather than by time, is not a scenario included in the undefended world originally assumed by MSH.

If the herbivores were originally absolute resource limited before plant induction, the presence of resistance causes a switch in the nature of trophic control, relative to undefended plants, leading to a trophic cascade. If the herbivores were originally relative resource limited, then there is no switch in trophic control; however, through inducing a defense (bottom-up effect), a plant is able to exacerbate the positive direct effects of the defense through the help of predators (top-down effect) that prey on herbivores.

In contrast, the induction of tolerance traits (increased growth rate, thinner leaves) may lead to an overall increase in herbivory through absolute resource limitation of herbivores (Figure 2.2c). If a predator removes an herbivore from a plant with tolerance traits, all other herbivores will increase their per capita feeding rate due to a lack of defended tissue. This will result in bottom-up control of primary production. If herbivores were relative resource limited in the presence of undefended tissue, the induction of tolerance traits would then shift them to absolute resource limitation removing top-down control.

Because plant defensive traits or herbivore behavior mediate the strength of trophic control over productivity, trophic control is from the middle out, rather than from the top-down or bottom-up. Moreover, the framework leads to an interesting new insight. While plants with resistance traits certainly derive a direct benefit by reducing herbivore feeding, plants expressing such traits gain a greater indirect benefit from predators through trophic cascades than would similar plant species that did not express such traits. While predators have been invoked before to explain low nutritive defenses that cause more damage to the plant through increased feeding requirements of the herbivore (Moran & Hamilton 1980), the result here is more general and applies to toxin-based qualitative defenses as well as structural ones. In addition, while the quantity of primary production shifts in response to herbivores and plant defensive syndrome response (resistance versus tolerance), the traits of

uneaten plant material are also impacted by these same factors. For example, plant litter in the absence of herbivores will be qualitatively different due to the lack of expressed defensive traits. Accordingly, the MSH can be extended to consider how these shifts in quality have the potential to impact community dynamics through nutrient cycling (see section on nutrient cycling below).

Functional trait-based approach

The MSH does not attempt to predict which plants will express which defensive traits in what environment (as do the plant defense or tolerance theories). Instead, given a defensive plant syndrome (resistance or tolerance), it predicts qualitatively whether bottom-up and top-down effects will prevail to impact community processes. Since it does not assume all individuals within a trophic level (or even species) have identical responses and traits, it has the components of a trait-mediated approach for determining what regulates community processes, (Schmitz *et al.* 2003; Schmitz *et al.* 2004; Duffy 2009). This functional trait approach of resistance versus tolerance can be applied within communities, species, or genotypes. I propose this framework as a way to predict when plant defensive traits will impact top-down and bottom-up control in ecosystems. This approach may also be useful for better understanding the basis for the purported contingency in trophic control observed between and within ecosystem types, such as between aquatic and terrestrial ecosystems.

The dominant defensive strategies in aquatic and terrestrial systems

Much previous work elucidating the differences between terrestrial and aquatic systems focused on the differences between the dominant primary producers in each system (Strong 1992; Chase 2000). Below, I summarize the known defenses of the primary

producers within pelagic (open water), terrestrial, and littoral (near-shore) ecosystems to explore whether there are systematic differences among ecosystem types in defense expression. I do not provide an exhaustive treatment here, as recent reviews have already been completed for most systems (Pohnert 2004; Hanley *et al.* 2007; Toth & Pavia 2007; Van Donk *et al.* 2011).

Pelagic autotrophs

The dominant players in aquatic pelagic systems are unicellular and multicellular phytoplankton that allocate little to structural tissue, resulting in highly edible tissues due to low C:N ratios (Sardans *et al.* 2012). Phytoplankton must be small enough to remain suspended in the water column, yet can escape predation if they exceed an herbivore's gape limitation (Fogg 1991). As a result, one common defense strategy is for groups of unicellular phytoplankton to join into colonies called coenobia, at the cost of an increased risk of sinking out of resource rich surface waters and potential decreases in nutrient uptake due to lower surface area (Lürling & Beekman 1999; Verschoor *et al.* 2004a). In contrast to terrestrial systems, phytoplankton are small relative to the zooplankton and other herbivores that eat them; an encounter with an herbivore often means a complete loss of fitness. Thus traditional tolerance strategies are not likely to be effective; instead, some phytoplankton and diatoms exude activated chemical defenses (secondary metabolites) into the water to deter herbivores from attacking or produce morphological structures, such as spines (Leibold 1989, 1999; Van Donk *et al.* 2011), in the presence of herbivores. Another strategy expressed at low resource availability in the green algae *Scenedesmus spp.* is a tough morphology that allows some individuals to pass through the zooplankton digestive system unharmed (Van Donk 1997).

Often plant defenses are induced, not by direct contact with the herbivore, but by the detection of chemical cues in the water column (kairomones) released by the herbivore (Pohnert *et al.* 2007). At high resource availability and in the presence of herbivores, some species are also able to induce changes in life history traits to speed up growth rates and generation times to outgrow herbivore species (Agrawal 1998). While not referred to as such in the literature, I argue that changing life history traits in the presence of herbivores can be thought of as belonging to a "tolerance" defensive strategy, because the effect is that different induced plant traits are expressed within the system. Defense induction is a more ubiquitous response within freshwater pelagic systems than in marine systems (Lass *et al.* 2003). In marine systems, induction is rare, but a few species of algal phytoplankton produce constitutive chemical resistance traits that can lead to toxic algal blooms and corresponding consumer die-offs (Pohnert 2004).

Terrestrial autotrophs

Terrestrial plants tend to be vascular, relatively long-lived, and allocate more resources to plant structure than most aquatic plants. Overall, plant tissue quality is lower than in aquatic systems due to the increased presence of lignin and cellulose (Sardans *et al.* 2012). In addition, terrestrial plants produce a cornucopia of chemical defenses (Harborne *et al.* 1999; Kaplan *et al.* 2008; Arnason & Bernards 2010). Some of these defenses, such as digestion-inhibitors and structural defenses, force herbivores to consume more tissue to attain the same nutrition. These defenses are common in terrestrial plants, in part, because herbivores do not consume an entire plant at one time and can choose to move to a more palatable plant before causing plant mortality (Moran & Hamilton 1980; Hanley *et al.* 2007). In contrast to pelagic systems, one encounter with an herbivore does not usually cause

vascular plant mortality. Direct contact with an herbivore's salivary chemicals or characteristic damage patterns are usually required for induction in vascular plants, although recent evidence also points to neighbor induction by leaf volatiles through airborne plant/plant communication (Karban *et al.* 1999; Karban *et al.* 2000). The ability of terrestrial plants to avoid mortality when attacked enables tolerance to be a more viable strategy for them to deal with herbivores (Rosenthal & Kotanen 1994). Some of the best examples of tolerance come from terrestrial systems with grazing herbivores; for instance, grasslands can be more productive in the presence of herbivory than without due to compensatory growth strategies (McNaughton 1985).

Resistance traits also vary by plant functional group. The resistance traits of the closely related grasses are dominated by phenolics, nitrogen containing defenses, toughness, and silica deposits in leaf tissue. While herbaceous and woody plants are derived from across the vascular plant phylogeny and express a wide range of resistance traits, there is a general pattern of greater inducibility and N-based defensive chemistry in herbaceous plants compared to woody species (Massad *et al.* 2011). Differences in functional group defense expression are manifest through succession, as perennial plants and then woody plants replace annual, herbaceous colonizers. As a result, resource rich early-successional systems are often dominated by tolerance responses and N-based defenses that shift toward toxic C-based defenses in late-successional, slow growing species (Davidson 1993).

Littoral and benthic autotrophs

Littoral and benthic autotrophs possess size, life history traits, and stoichiometric properties that are often intermediate between pelagic and terrestrial systems (Shurin *et al.* 2006). Communities consist of periphyton and macrophytes, including macroalgal species as

well as vascular macrophytes (derived from terrestrial lineages), which root and access light in the photic zone. Often these systems are characterized by resource subsidy inputs from the terrestrial community (Nowlin *et al.* 2008).

Marine systems contain a diverse array of non-vascular macroalgae that are both free-living and part of benthic periphyton communities. Their tissue can become calcified which confers both structural and chemical defense (Hay *et al.* 1994). Many toxic resistance compounds (primarily phlorotannins in brown algae) are expressed as well (Hay *et al.* 1988). However, few of these putative resistance compounds have been shown to provide effective defense against herbivores (*sensu* Karban and Baldwin, 1997). In addition, the lack of a vascular system in these plants would suggest a limited capacity for induction; however, recent work has demonstrated widespread induced resistance in response to small crustaceans and gastropods within this plant group, particularly in brown and green algae (Toth & Pavia 2007). There is also within-plant variation in chemical defense expression (Cronin & Hay 1996).

Historically, herbivores were considered unimportant to freshwater macroalgae, as herbivory rates were thought to be very low (Hutchinson 1975). However, meta-analysis has shown that herbivory rates are higher on macrophytes than terrestrial plants (Cyr & Pace 1993), suggesting that selection should favor defense expression in these plants. Although there is evidence of chemical resistance in macroalgae (Prusak *et al.* 2005), evidence of induction is rare (Camacho 2008). While unusual in marine systems, vascular macrophytes dominate littoral zones in freshwater communities. They produce chemical defenses, such as alkaloids, that are also common in terrestrial plants due to derived ancestry from many terrestrial vascular plant lineages (Ostrofsky & Zettler 1986; Chambers *et al.* 2008). In

addition they produce structural defenses that lower plant palatability (Cronin & Lodge 2003; Lamberti-Raverot & Puijalon 2012). Tolerance traits are not very well studied in either freshwater littoral or marine benthic systems, but they have the potential to be quite important, particularly in systems dominated by large grazers (Nolet & Nolet 2004; Burkepile *et al.* 2006; Burkepile 2013).

Grouping plant defense response by habitat or relatedness?

Most syntheses of trophic control in terrestrial and aquatic systems look for broad-brush similarities and differences and thus treat all species within a shared habitat type (*e.g.,* pelagic) as though they are selected for and capable of expressing the same convergent, adaptive traits. This may not be appropriate to do. For example, macrophytes are found within seven plant divisions, resulting in Chlorophyta (green algae) macrophytes that are more closely related to green algal phytoplankton species than to any vascular macrophyte (only found within Pteridophyta and Spermatophyta divisions; (Chambers *et al.* 2008). A result of macrophytes being spread across most of the plant phylogeny is that their trait expression may be constrained by the evolutionary history of the group from which they are derived.

For example, the molecular machinery necessary to produce many polyphenolic chemical defenses in terrestrial plants, such as tannins, flavonoids, and lignins, is thought to be a relic of evolutionary history, originally deployed to protect aquatic plants from damaging UV light as they gradually evolved to live on land (Rozema *et al.* 2002). These UV-activated defenses are therefore less prevalent in algal species that remained in aquatic environments, because water is much more effective at filtering UV rays. Therefore, chemical defenses (at least UV-activated ones) are predicted to be of greater importance in

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terrestrial than aquatic systems. However, closely related vascular macrophytes that reinvaded aquatic environments from many terrestrial vascular lineages (at least 211 independent recolonization events (Cook 1999)) should have molecular machinery more similar to terrestrial plants and thus produce these defenses (Rozema *et al.* 2002). Therefore, I argue for more finely resolved comparisons when exploring contingency among ecosystems, such as considering vascular land plants and littoral zone vascular macrophytes as equivalent and pelagic phytoplankton as being different. While rarely implemented in the aquatic literature, this approach would respect phylogenetic constraints on trait evolution in response to herbivores that may determine which potential plant defense strategies are available to an organism and perhaps explain some of the contingency in the outcomes across distantly related species.

Influence of nutrient availability on expressed defense strategies

MSH is incomplete in that it excludes a factor known to be important to plant defense expression: resource availability to plants. A shift in nutrient availability can change the absolute and relative costs of constitutive and induced defenses and potentially the outcome of plant competitive interactions (Cipollini *et al.* 2003). Thus the efficacy and selection for the plant defensive traits outlined above are influenced by the environmental context in which they are expressed (Belovsky & Schmitz 1994). Classical ways of thinking about the interaction of resource availability and trophic control depict a static pool of resources (Oksanen *et al.* 1981). Another approach is to take a dynamic perspective of nutrient pools in ecosystems that allows for consideration of feedbacks between the abiotic nutrient pool and biotic responses such as plant defense traits and trophic interactions (Loreau 2010; DeAngelis *et al.* 2012). In this section, I review a number of ways to approach

how plant defense expression interacts with nutrient availability and then propose a more dynamic way of viewing interactions between primary producers and their environment.

Interspecific variation and community shifts

Environments with particular resource conditions may favor communities comprised of species with particular plant traits. Within the MSH framework previously outlined, at an interspecific level, defensive response can be thought of as an aggregate expression of functional traits of all members of a community—a so-called interspecific defense perspective. The growth/defense tradeoff hypothesis posits that at high nutrient levels, adapted plants grow so rapidly as to preclude investment in defense. At low nutrient levels, however, species are favored that grow slowly and have time to invest in defenses for their longer-lived more valuable leaves (Coley *et al.* 1985). In theory, therefore, if low-nutrient availability filters out species that express tolerance traits and over-represents species with resistance traits, then we may expect to see trophic cascades in those systems.

While there are many evaluations of this interspecific defense theory for terrestrial systems (Fine *et al.* 2006), few tests have been performed in aquatic systems particularly within littoral habitats or between macroalgal species (Pavia & Toth 2008). Because the goal of this chapter is to compare ecosystems on an equal footing, I will not focus on interspecific plant defense theory. Nevertheless, it is noteworthy that in planktonic algal systems, an interspecific growth/defense tradeoff is often invoked to explain community shifts due to herbivory or nutrients (Grover 1995). Here edible phytoplankton with high growth rates are replaced by defended, but slow growing species at low nutrient levels or high herbivory rates. The existence of such a growth-defense trade-off was supported by meta-analysis, but size-selective grazing by zooplankton species complicates the effect on

trophic cascades, with edible species still able to bloom in the presence of herbivores (Agrawal 1998).

Intraspecific variation and phenotypic plasticity

While interspecific species turnover is more often invoked in aquatic systems, possibly due to the short lifespans of phytoplankton, plants can also exhibit genotypic and phenotypic variation in defense allocation to resistance or tolerance within a species or over a single individual's lifespan (Glynn *et al.* 2007). A recent meta-analysis of ontogenetic changes in plant defense allocation in terrestrial plants showed little influence of ontogeny on tolerance. However, herbaceous plants shifted from relying on induced chemical defenses when young to constitutive chemical defenses when old. Woody plants also exhibited an increase in constitutive defenses over time, with an initial reliance on chemical defenses in the seedling stage shifting to physical defenses during the juvenile stage, and then an overall decrease in defense allocation when mature (Kasey E. Barton & Julia Koricheva 2010). While untested, according to the MSH hypothesis extended in this chapter, these life-cycle stage shifts in defense expression in response to ontogenetically-staged herbivory may result in different likelihoods of trophic cascades occurring throughout a growing season or plant's lifetime.

Resistance models

Plants show the bottom-up effect of nutrient gradients even in the absence of herbivores through variation in quality (nutrient content) and the level of constitutive defense allocation. For resistance traits, these relationships have been extensively investigated and formalized as plant defense theories, particularly for terrestrial systems

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(Herms & Mattson 1992; Stamp 2003; Wise & Abrahamson 2007). There are competing views about how plant defense allocation is related to nutrient and other abiotic resource levels. According to these different views, peak defense allocation could happen at high (for nitrogenous-based defenses) (Bryant *et al.,* 1987), low (Coley *et al.* 1985), or intermediate (Herms and Mattson, 1992) nutrient levels. Detailed treatment of resistance-based defense theory lies outside of the scope of this chapter and has been reviewed recently elsewhere (Koricheva 2002; Stamp 2003; Pavia & Toth 2008). However, a review of recent studies that manipulated nutrients and measured constitutive defensive traits found increasing, decreasing, and no effect of nutrient supply on resistance trait expression across ecosystems (Table 8.1). This supports the view that no clear theory has yet emerged as a leading contender to explain resistance defense expression in terrestrial or aquatic systems (Stamp 2003; Toth & Pavia 2007).

Tolerance models

While many intraspecific theories of tolerance have been proposed and tested (*e.g.,* the compensatory continuum hypothesis or the growth rate model), one recent approach integrates previous models to explain tolerance across resource conditions and may help predict where one might expect to see either tolerance or resistance traits dominating in ecosystems. The limiting resource model of tolerance (LRM), developed in terrestrial systems for vascular plants, uses a multistep dichotomous key to predict how changing the availability of a focal resource will impact tolerance by accounting for 1) whether the focal abiotic resource is limiting plant fitness in the low-focal resource environment; 2) if the herbivore damage affects the use/acquisition of the focal resource or of an alternative

resource; and 3) whether the herbivore damage causes the alternative resource to limit plant fitness (Wise & Abrahamson 2005).

While complex, these three factors offer the flexibility needed to explain whether tolerance would be higher, lower, or equal at different nutrient levels. For example, imagine that nitrogen is the focal limiting resource for a plant species and a foliar herbivore primarily impacts carbon acquisition. If the addition of nitrogen does not cause carbon to become limiting, then the model predicts that the plant should exhibit equal tolerance in both high and low nitrogen environments (Wise & Abrahamson 2005). When tested, the model accurately predicted the level of tolerance in 22 out of 24 cases of varying nutrient availability in terrestrial plants; 17 of these showed higher tolerance at lower nutrient availability (Wise & Abrahamson 2007). This result may be generalizable to most terrestrial species. I know of only one study to apply the LRM to aquatic plants—which measured brown seaweed response to herbivory across different N environments (Hay *et al.* 2011)—and the prediction of the LRM of equal tolerance between high and low nitrogen environments in this system was supported. Clearly, further examination of this idea (and possible expansion to include herbivore-mediated linkages between resources; (Bagchi & Ritchie 2011), especially in non-terrestrial ecosystems, is needed.

While tolerance is rarely investigated under that terminology in aquatic systems, aquatic ecologists have thoroughly tested the Growth Rate Hypothesis (GRH), which links N and P usage within an individual via protein synthesis. Fast growth strategies require high P-allocation to synthesize ribosomal RNA (Sterner & Elser 2002), thus environments with low N:P ratios favor species with fast growth rates. There is considerable empirical support for GRH from aquatic pelagic environments, but the model is rarely tested in terrestrial systems, where support is weak (Sardans *et al.* 2012). While not explicitly presented as an

intraspecific tolerance model, the GRH meets the criteria for tolerance if a mitigation of fitness impact is produced within a species in response to herbivory and available resources, and is therefore complementary to the LRM, outlined above. The GRH and LRM represent an example where terrestrial and aquatic ecologists are wrestling with similar concepts, but with different jargon, leading to the incorrect perception that aquatic and terrestrial systems operate differently.

Induced defenses

Studies rarely explicitly investigate whether resource availability influences whether plants induce or continuously express anti-herbivore defenses. An intriguing recent study that quantified this with the phytoplankton *Scenedesmus acutus* showed that low P availability resulted in the induction of colony formation in the presence of herbivores, whereas under high P colony formation was constitutive (O'Donnell *et al.* 2013). In terrestrial systems, a similar kind of experiment found that the constitutive expression of protein-based trypsin inhibitors and the ability to induce them increased with nutrient availability (Cipollini & Bergelson 2001). Future studies that manipulate both nutrient availability and herbivore presence are needed to resolve the general patterns among herbivory, nutrient availability, and defense induction across aquatic and terrestrial ecosystems.

Nutrient cycling links top-down and bottom-up effects

All classical plant defense theories (including EEH), view soil nutrient conditions as static and homogeneous. However, this may not be an accurate representation of nutrient dynamics. There is increasing recognition that species, especially consumers in higher trophic levels, play an important role in structuring nutrient environments through resource

consumption, nutrient cycling and translocation (Kitchell *et al.* 1979; Vanni 2002; Pringle *et al.* 2010; Schmitz *et al.* 2010). Moreover, phenotypic variation in species traits may determine spatial heterogeneity in the nutrient environment as well (Norberg *et al.* 2001; Cornwell *et al.* 2008). Thus, while the nutrient environment certainly impacts the degree to which plant resources express tolerance and resistance traits, their expression may also feed-back to influence nutrient cycling and hence change nutrient conditions. Whether a plant species utilizes a resistance or tolerance strategy against herbivores may thus have implications at both the community (Chase *et al.* 2000a) and ecosystem level by mediating bottom-up and top-down effects on nutrient cycling.

How defensive phenotypes (resistance versus tolerance) may alter ecosystem processes can be examined by expanding the linear trophic interaction chain perspective to include both above- and belowground linkages through nutrient cycling (Figure 2.3). Nutrient cycling broadly encompasses several ecosystem processes, including production following nutrient uptake and decomposition leading to nutrient release (DeAngelis 1980; DeAngelis *et al.* 1989; Moore *et al.* 2004). Nutrients create a common currency for all trophic levels (Andersen *et al.* 2004). Moreover, linking above- and belowground processes reveals interesting reciprocal feedbacks between herbivores and the nutrient base through direct and indirect interactions (Van der Putten *et al.* 2001; Bardgett & Wardle 2003; Schmitz 2010).

This conception facilitates consideration of a dynamic nature of plant-herbivore interactions. For instance, herbivores not only influence productivity through direct consumption of plants, but also indirectly by influencing the way nutrient availability becomes altered via induced plant responses that can decrease or increase plant palatability (nutrient content) and thereby alter decomposition of organic matter by microbes or the release of inorganic waste by animals (Schmitz 2010). Herbivore induced responses by plants

may impact slow-cycle inputs from uneaten organic plant litter (termed "after-life" effects), as well as fast-cycle inputs, such as inorganic materials from herbivore fecal output and canopy leaching (Hunter 2001). These indirect effects on cycling (Figure 2.3) are rarely quantified, particularly in terrestrial systems (Choudhury 1988; Bardgett & Wardle 2003; but see Frost & Hunter 2008), but point to the potential importance of a plastic plant trait (defense allocation) for mediating the relative magnitudes of nutrients entering the slow- and fast-cycle pathways of ecosystems.

Can plant defenses affect how nutrients move through aquatic and terrestrial systems?

A classic idea of herbivore-mediated nutrient cycling is the acceleration hypothesis (McNaughton *et al.* 1989; Belovsky & Slade 2000; Chapman *et al.* 2003), which proposes a positive feedback between herbivory and nutrient cycling. Herbivores consume a dominant species with highly nutritious leaf litter. These plants tolerate herbivory and by producing highly nutritious leaf regrowth cause herbivores to release large quantities of high quantity egesta, as well as facilitate plant canopy leaching and greenfall inputs. These factors collectively act to increase decomposition rates and ultimately increase the rate of nutrient supply to plants. In subsequent years, high resource supply favors the same dominant, nutritious plant species. In contrast, the deceleration hypothesis (Ritchie *et al.* 1998) posits that herbivores consume palatable plants selectively, thus shifting community composition toward less palatable species (Figure 2.4). Litter from a community of unpalatable species decomposes more slowly than one from a palatable community because of a positive relationship between palatability and decomposability (Grime *et al.* 1996; Ohgushi 2008; but see Palkova & Leps 2008).

The acceleration hypothesis uses intraspecific changes in plant tolerance traits to predict an increase in nutrient cycling through herbivory, while the deceleration hypothesis relies on interspecific trait changes within a community. I propose that both deceleration and acceleration of nutrient cycling are viable outcomes at both the inter- or intraspecific levels depending on 1) the degree of intraspecific variation in plant traits (genotypic and phenotypic plasticity) and 2) the degree to which the plant community is dominated by a single plant defense syndrome. For example, uneaten litter from a plant (or plant community) that expresses structural or quantitative resistance defenses may be broken down more slowly by the microbial community than plants expressing tolerance traits, thereby impacting available nitrogen in the system (Schweitzer *et al.* 2008). Qualitative resistance defenses that persist in the environment may have a similar effect (Figure 2.4). In contrast, plants that express tolerance traits produce high quality litter that may be broken down rapidly by the microbial community, resulting in a larger available nitrogen pool (Figure 2.4).

Few studies have looked for evidence of the impact of plant defense traits on nutrient cycling. However, it is clear that herbivores do have the potential to affect cycling rates across all systems. For example, in benthic kelp beds or pelagic lakes, consumers can increase net primary productivity (NPP) through increased nutrient cycling (Sterner *et al.* 1992; Steinberg 1995; Vanni 2002). Experiments also demonstrate that herbivores and plant traits can influence nutrient cycling in terrestrial systems. For example, pulses of cicada cadavers in Northern temperate forests increase plant growth rates the following year (Yang 2004). In addition, intraspecific variation in oak leaf phenotype influences fast- and slow-cycle litter decomposition (Madritch & Hunter 2005), and recent meta-analyses indicated plant traits (*e.g.,* LMA, lignin, and nutrient content) are the most important drivers of litter

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decomposition across global ecosystems (Cornelissen 1996; Cornwell *et al.* 2008). Moreover, there is evidence that resource pulses move more quickly through aquatic than terrestrial systems (Nowlin *et al.* 2008). Whether this is due in part to differential expression of defensive traits, while plausible given our synthesis above, remains unknown.

Differences in herbivore feeding guilds

Aquatic algae (phytoplankton and reef periphyton) experience greater herbivory than vascular macrophytes, which experience greater herbivory than terrestrial plants, with median annual primary productivity removed of 79%, 30%, and 18%, respectively (Cyr & Pace 1993). These differences in herbivory rates have often been cited as reasons for differences between top-down and bottom-up effects among ecosystems (Strong 1992). However, plant responses may also be impacted by the functional group of the herbivores that consume them (Gruner & Mooney 2013). Plant responses to herbivory in the grazing systems of the Serengeti may be more similar to marine kelp forests with extensive grazing by marine mammals than to other terrestrial ecosystems types (Burkepile 2013). It is often assumed that herbivores are more specialized on land (insects) than in pelagic or littoral ecosystems (Newman & Rotjan 2013). Specialized herbivores are likely to induce different plant defense responses than generalists (Feeny 1976; Bernays 2001); see also Chapter 14, this volume). Herbivore feeding guild and specialization is not currently explicitly incorporated into the MSH, but it is another trait-based approach that may be worthwhile to pursue in an examination of contingency in the interplay between plant defense and nutrients on trophic control of ecosystems.

Conclusions

Plants can produce both tolerance and resistance responses to herbivory and one see examples of each of these strategies across terrestrial and aquatic ecosystems. Chemical and structural resistance defenses tend to dominate terrestrial ecosystems, but play a smaller role in aquatic systems. The exception to this is terrestrial grazing ecosystems that are clearly dominated by plant tolerance responses to herbivory. In terrestrial systems, there is evidence that defense allocation is constrained to some degree by phylogenetic relationships (Armbruster 1997; Ronsted *et al.* 2012; but seeHaak *et al.* 2013), however this subject remains ripe for investigation within aquatic ecosystems. In particular, I suggest that a phylogenetic approach would be useful for understanding patterns within the phylogenetically diverse functional group of macrophytes. While tolerance responses are not often studied in aquatic systems under that terminology, I argue that induced changes in life history attributes that increase fitness in the presence of herbivory should be considered a tolerance trait and that tolerance traits may be very common yet overlooked in pelagic, benthic, and littoral communities. Plant defense theories are more refined and well tested in terrestrial systems than aquatic systems. In aquatic systems the stoichiometrically-based GRH accurately predicts higher growth rates in low N:P ratio environments. Which plant defense strategy (tolerance or resistance) a plant induces in response to herbivory has different ramifications for nutrient cycling, the coevolution of herbivores and plants, and community dynamics (Chase *et al.* 2000a).

Plant defense theory could advance through empirical tests among a broader range of ecosystem types, as well as benefit from contextualizing a system not in terms merely of a plant-herbivore linkage, but instead in terms of a trophic chain with direct and indirect effects among soil nutrients, plants, herbivores, and predators. Tests could also benefit from more emphasis on the role of tolerance as a defensive trait, because it helps to unify thinking

across ecosystem types once a common conceptual jargon is used. In general tolerance has been overlooked as an explanatory plant functional trait. For example, in Korcheiva's extensive meta-analysis on the cost of defensive traits, chemical, mechanical, and induced defenses were examined, but not tolerance traits (Koricheva 2002). A recently proposed terrestrial-based model, LRM (Wise & Abrahamson 2005), holds great promise for predicting tolerance traits across resource environments. I suggest that this model be tested broadly across ecosystems to determine whether it is generalizable.

The unresolved basis for wide variation in expression of resistance traits may stem from an incomplete conceptualization of the "system" and the context-dependent feedbacks that determine their expression. I suggest that taking a trait-based approach in the context of a food chain may help to resolve when and where these traits are expressed and how they impact trophic control of ecosystems. The MSH of trophic control may provide the basis for including plant defense traits (Schmitz 2008). I predict that "resistance" traits (both structural and qualitative) will result in a trophic cascade through relative resource limitation of herbivores, while "tolerance" traits will invoke absolute resource limitation of herbivores, resulting in herbivore control of primary productivity. I realize that this framework does not yet consider important additional factors, such as plant volatiles, herbivore feeding guild, and ontological shifts in plant defense, but nonetheless view it as a useful starting point.

This conception may also help offer a complementary explanation for variation in the strength of top-down control across nutrient supply or productivity gradients implicit in the classic EEH of trophic control of ecosystems. This theory predicts that top-down control should be strongest at intermediate levels of productivity, which is attributed to predator satiation (Oksanen *et al.* 2000). This result, as well as the finding that herbivore and predator efficiency are important explanatory factors, was supported by meta-analysis (Borer

et al. 2005). The MSH framework developed here, suggests that plant defense traits may also account for the weakening of top-down control. The expression of tolerance regrowth traits at high nutrient levels could cause herbivores that were relative resource limited at lower nutrient levels to become absolute resource limited. In turn, predators would no longer have an indirect positive effect on productivity. At high nutrient levels, these tolerance traits may allow plants to escape their herbivores by outgrowing them. This outcome is not formalized within the EEH, but is consistent with the outcomes presented there.

The induction of resistance and tolerance traits in plant communities may also have important effects on nutrient cycling and future resource availability through "after-life" effects of plant defense or tolerance traits that remain in uneaten plant litter entering the detrital food web. While different rates of nutrient cycling have been predicted and recorded within aquatic and terrestrial systems (Nowlin *et al.* 2008), it remains to be seen whether taking this functional trait approach may explain some of the contingency found within and between aquatic and terrestrial ecosystems.

The lack of empirical investigation into these topics makes generalization difficult. However, as anthropogenic nitrogen inputs increase (Vitousek *et al.* 1997) and climate change increases herbivory and the potential induction of plant defenses (Ayres 1993), it is increasingly important to understand how herbivory and nutrient context influence plant and herbivore populations across ecosystems. Tackling this question of whether and when plant defensive traits and nutrient availability modify trophic cascades within many ecosystem types is only the first step. Then will we be able to adequately address the question of whether defensive traits map to similar community responses in both aquatic and terrestrial ecosystems. Generalizable predictions of how soil nutrient environment changes the expression of plant defensive traits and productivity is essential in the current era of global

change and may also be useful to agriculturists interested in lowering pesticide use while maximizing yield.

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Figures
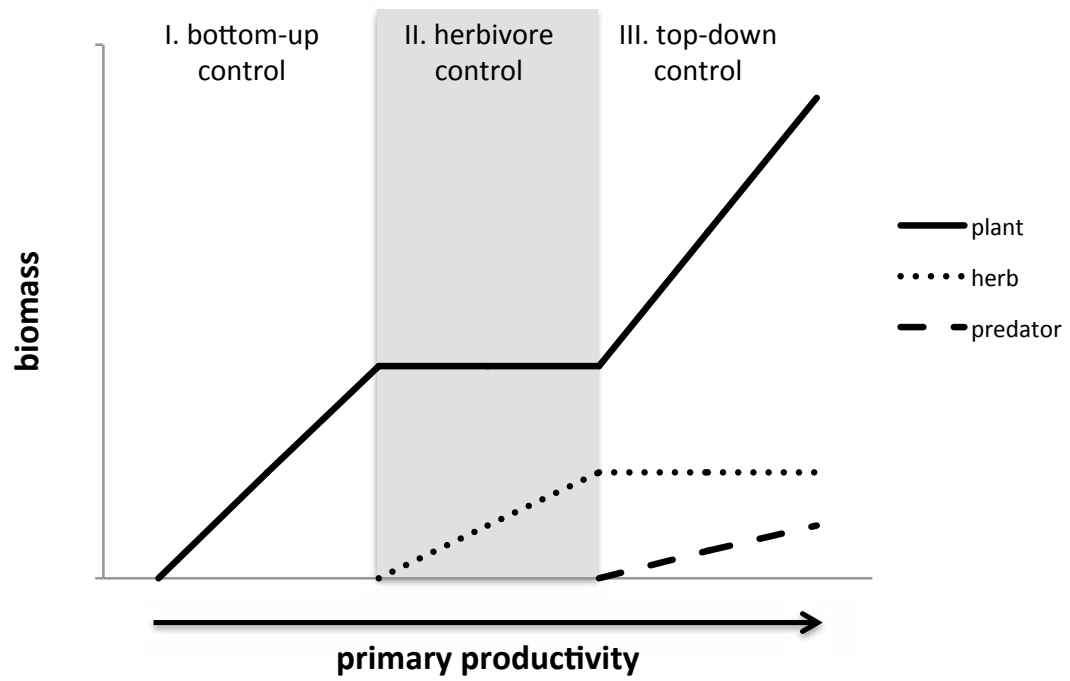
Figure 2.1: Conceptual figure of the Exploitation Ecosystems Hypothesis (EEH). Adapted from Fig 1 in (Oksanen *et al.* 2000)
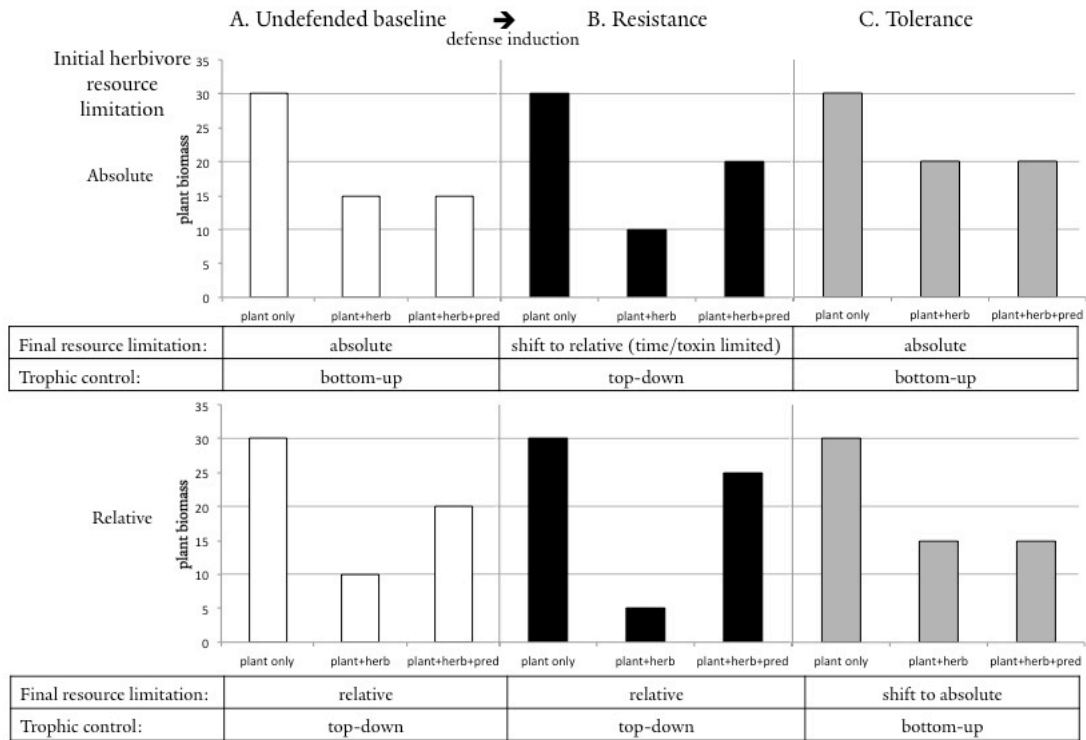
Figure 2.2: A conceptual framework for extending the Mechanism Switching Hypothesis of trophic control (Schmitz 2008) to include plant defenses and their impact on herbivore resource limitation. Bars represent predicted outcomes of herbivore resource limitation and plant defense on plant biomass. A) Undefended leaf tissue can be eaten by herbivores experiencing either absolute resource limitation leading to bottom-up control or relative resource limitation (*e.g.,* temperature limitations on feeding time) leading to top-down control of plant biomass. B) If plants induce a resistance response to herbivory (toxin or structural), the defenses impose relative resource limitation on herbivores because herbivores cannot increase feeding rate when a predator removes an herbivore (time of toxin limited feeding). C) In contrast, induced tolerance traits impose absolute resource limitation on herbivores due to high quality regrowth tissue. If a predator removes an herbivore, other herbivores will consume more, preventing a trophic cascade.

A. Aquatic food web

carnivores

microbes/
decomposers

Quality of
carcass and
litter
inputs shift

excretion

detritus

soluble nutrients

allochthonous inputs:
(terrestrial origin)

B. Terrestrial food web

exuvia, carcass

honeydew

labile greenfall leaf tissue, frass

change in litter quality, endophytes

canopy leaching

organic matter

leaching

detritivores:
microbes, arthropods

available nutrients

Figure 2.3: Potential pathways through which herbivores can influence nutrient cycling in (A) a generalized aquatic food web (adapted from Moore *et al.* 2004) (B) a generalized terrestrial food web. Dashed lines indicate a fast-cycle pathway that has within season/generation effects on nutrient cycling. Solid lines represent slow-cycle pathways with primarily between season or generation effects. Induced plant defensive trait responses to herbivory have the potential to alter the relative magnitude of these pathways resulting in differential cycling rates. Clip art from Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/).

TRAIT- MEDIATED HERBIVORE IMPACT ON NUTRIENT CYCLING
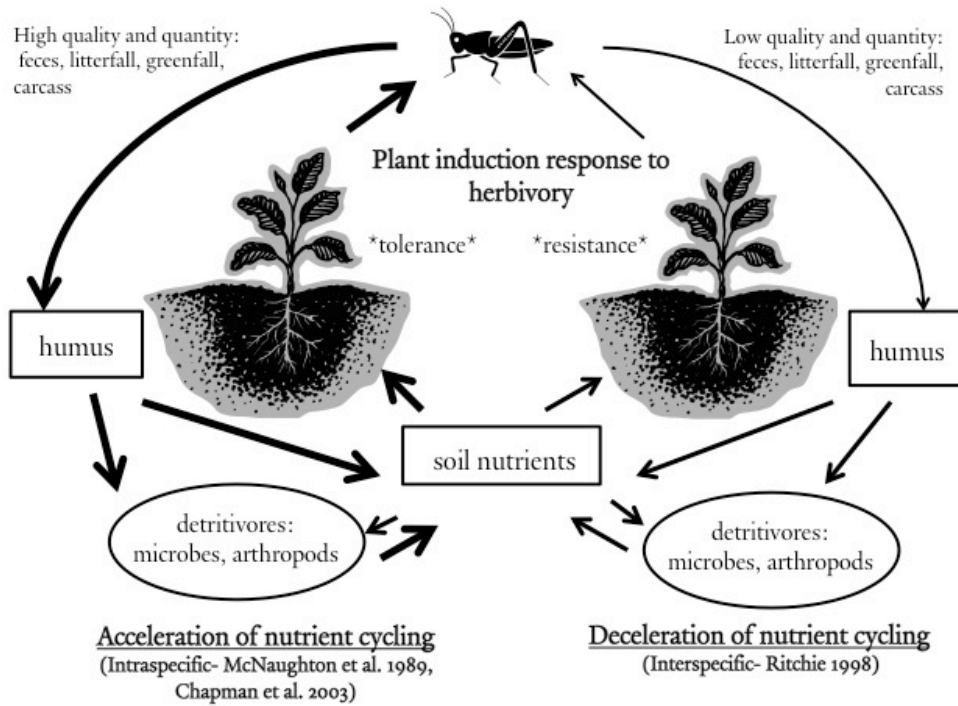
Figure 2.4: The defensive response trait (resistance versus tolerance) a plant produces in the face of herbivory may change the rate of nutrient cycling in a given system. Arrow line width represents the magnitude of nutrients moving through the pathway. Tolerance traits may result in an increase in herbivore egestion and high quality litter entering the detrital food web. Resistance responses may decrease nutrient return to the soil through herbivory, as well as provide low quality recalcitrant leaf tissue that is slowly broken down by the detrital food web, thus decreasing cycling rates.

Table 2.1: Studies that manipulated a focal nutrient and measured the effect on constitutive plant defense expression

Reference	Ecosystem	Zone	Primary producer	Species	Focal nutrient (FN)	Type of defense	Trait measured	Effect of ↑ in FN on trait
(Lundgren 2010)	marine	pelagic	phytoplankton	*Phaeocystis globosa*	N, P, N&P	structural	colony formation	↑
(O'Donnell *et al.* 2013)	freshwater	pelagic	phytoplankton	*Scenedesmus acutus*	P	structural	colony formation	↑
(Gavis *et al.* 1979)	freshwater	pelagic	phytoplankton	*Scenedesmus quadricauda*	nitrate (N)	structural	colony formation	↑
(Trainor & Siver 1983)	freshwater	pelagic	phytoplankton	*Scenedesmus quadricauda*	ammonium (N)	structural	colony formation	↑
(Lampert *et al.* 1994)	freshwater	pelagic	phytoplankton	*Scenedesmus acutus*	urea (N)	structural	colony formation	=
---	---	---	---	---	ammonium (N)	structural	colony formation	=
(Wiltshire & Lampert 1999)	freshwater	pelagic	phytoplankton	*Scenedesmus obliquus*	urea (N)	structural	colony formation	↑
(Van Donk 1997)	freshwater	pelagic	phytoplankton	*Scenedesmus spp.*	mult. nutrients	structural	cell wall thickness	↓
---	---	---	---	---	---	structural	size	↓
(Cronin & Lodge 2003)	freshwater	littoral	vascular macrophyte	*Potamogeton amplifolius; Nuphar advena*	mult. nutrients	chemical	phenols	↑
---	---	---	---	---	---	growth	growth rate	↑
(Lamberti-Raverot & Puijalon 2012)	freshwater	littoral	vascular macrophyte	*Myosotis scorpioides; Mentha aquatica*	mult. nutrients	structural	breaking force	↓
---	---	---	---	---	---	structural	density	↓
(Cronin & Hay 1996)	marine		macroalgae	*Dictyota ciliolata; Sargassum filipendula*	mult. nutrients	chemical	terpenoids	=
---	---	---	---	---	---	growth	growth rate	↑
(Van Alstyne 2000)	marine		macroalgae	*Fucus gardneri*	P	chemical	phlorotannin	↓
---	---	---	---	---	---	growth	growth rate	↓
(Arnold 1995)	marine		macroalgae	*Lobophora variegata*	N	chemical	phlorotannin	↓
(Ilvessalo *et al.* 1989)	marine	littoral	macroalgae	*Fucus vesiculosus*	N	chemical	phlorotannin	↓
(Hemmi & Jormalainen 2002)	marine	littoral	macroalgae	*Fucus vesiculosus*	mult. nutrients	chemical	phlorotannin	=
---	---	---	---	---	---	structural	toughness	↓
(Gowda *et al.* 2003)	terrestrial	forest	woody	*Acacia tortilis*	mult. nutrients	structural	spine mass	↑
(Cash *et al.* 2005)	terrestrial	forest	woody	*Acacia spp.*	mult. nutrients	structural	spine mass	=
(Bazely *et al.* 1991)	terrestrial	grassland	herb	*Rubus fruticosus*	mult. nutrients	structural	spine density	↓
(Hoffland *et al.* 2000)	terrestrial	grassland	herb	*Lycopersicon esculentum*	mult. nutrients	structural	trichome density	↓

(Forkner & Hunter 2000)	terrestrial	forest	woody	*Quercus spp.*	mult. nutrients	chemical	tannins, phenols	↓
(Osier & Lindroth 2001)	terrestrial	forest	woody	*Populus spp.*	mult. nutrients	chemical	tannins	↓
(Cornelissen & Stiling 2006)	terrestrial	forest	woody	*Quercus spp.*	mult. nutrients	chemical	tannin	=
---	---	---	---	---	---	structural	toughness	=
---	---	---	---	---	---	growth	N content	↕
(Wallace 1989)	terrestrial	grassland	herb	Var. monocots	N	structural	silica	↓
(Osier & Lindroth 2004)	terrestrial	forest	woody	*Populus spp.*	mult. nutrients	chemical	phenolic glycosides, condensed tannins	=
(Cipollini & Bergelson 2001)	terrestrial	greenhouse	herb	*Brassica napus*	mult. nutrients	chemical	protein-based trypsin inhibitors	↑

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CHAPTER 3

NUTRIENT SUPPLY ALTERS THE NATURE OF GOLDENROD'S PLASTIC

RESPONSE TO HERBIVORY THROUGH CHANGES IN RESISTANCE,

TOLERANCE, AND WHOLE-PLANT EXPRESSION PATTERNS[†]

Summary

1. Recent interest in using trait-based approaches to understand and predict ecosystem processes and evolutionary responses to environmental change (both biotic and abiotic), 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 defense 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 defense strategy from tolerance to induced resistance. Induced resistance was detected through decreased herbivore growth rates and a behavioral feeding shift of grasshoppers to older leaf tissue. This could not be fully accounted for through stoichiometric changes in leaf tissue quality.

4. A multi-dimensional 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 (ExE).

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.

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; McGill *et al.* 2006; 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 community and ecosystem processes (Smith & Knapp 2003; Whitham *et al.* 2006).

An important suite of plant functional traits is anti-herbivore defense expression because it 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 *et al.* 2006; Frost & Hunter 2008). Plant defense theory is steeped in a rich history of quantifying variation in defensive 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 *et al.* 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 may either be constitutive (always present in the plant) or induced (only produced after a plant is attacked) (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 depends on soil nutrient availability (Coley *et al.* 1985; Darrow & Bowers 1999). Many experiments focus on between species variation in defense expression across nutrient availability (Stamp 2003). However,

individual 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 (i.e. phenotypic variance) in tolerance and resistance traits may arise from genetic differences in expressed plant phenotypes (G), environmentally-based differences (E), as well as genetic variation in the capacity to respond to environments (GxE) (Whitman & Agrawal 2009). Throughout this paper, I define phenotypic plasticity as the capacity of a single genotype to exhibit a range of phenotypes across environments (i.e. an individual level trait), regardless of whether that variation also differs between genotypes (i.e. GxE, a population level trait) (Whitman & Agrawal 2009). Typically (E) is used to refer to variation attributable to all environments. However, ExE interactions may occur whereby the developmental environment (such as nutrients, light, or water) alters the direction or magnitude of plasticity 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 *et al.* 2013).

Often functional trait studies lump 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 defense 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 (GxE) or environmental interactions (ExE) could lead to different trait variances across environmental

gradients. On the other hand, widespread genetic variation in defense (G) with no plasticity would lead to large trait variance regardless of environmental context. As a result partitioning the sources of variation may explain often cited context dependence in community and ecosystem experimental results (Schmitz *et al.* 2015).

Here, I report on a greenhouse experiment that quantified phenotypic variation within nine genotypes of a clonal, dominant species collected from one old-field population to the same nutrient and herbivory gradients. I combine plant defense theory and trait-based approaches to examine plasticity both in emergent defensive outcomes (tolerance and resistance) as well as correlated changes in whole-plant trait expression. The study was designed to partition the relative contribution of genetic and environmental factors to intraspecific variation (G, E, and GxE) and specifically to evaluate how herbivores influence trait expression under different levels of soil nutrient supply (ExE). Further I examine whether plant stoichiometry— the consumption ratio of nutrients by herbivores— can explain the observed patterns in resistance (Sterner & Elser 2002).

I also explore bivariate trade-offs between tolerance, constitutive resistance, and induced resistance across the nutrient gradient. However, changes in tolerance and resistance occur in concert with many other phenotypic changes (Forsman 2015). After all, herbivores feed on (and selection operates on) whole-plant multi-dimensional phenotypes (Walsh & Blows 2009; Laughlin & Messier). I therefore complement the bivariate approach with one that considers whole-plant variation in response to the treatments. In doing so, I also evaluate whether individual genotypes express equal whole-plant trait variation in response to the environmental variation and whether collectively genotypes express equal total variance within all levels of an environment.

Overall, I ask: 1) Do plant defensive outcomes (resistance or tolerance) change depending on the nutrient environment? 2) Is resistance correlated with plant stoichiometry? 3) Are there tradeoffs between tolerance and resistance across the nutrient gradient? 4) What whole-plant phenotypic changes occur in concert with plant defensive changes? 5) Are there differences in the amount of variation expressed between genotypes or within each environmental treatment? And lastly, 6) do genotypes respond differently?

Materials and Methods

Study species: I focused 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 insect herbivore, the red-legged grasshopper (*Melanoplus femurrubrum* (De Geer 1773)). *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 lines of genetically identical plants. *S. altissima* exhibits a tolerance response through increased relative growth rate and photosynthetic rate (Meyer 1998; Cronin *et al.* 2010), background levels of chemical and structural defense (constitutive resistance), and heightened expression of chemical defense through induction of phenolics and diterpenoids in response to herbivory (Cooper-Driver & Le Quesne 1986; Abrahamson & Weis 1997; Bode *et al.* 2013).

Source population: Rhizomes were obtained from an old-field site in Wallingford, CT. After agricultural use of the field ceased in 2001, the field has been mowed yearly in the fall to prevent woody encroachment. The density of S. *altissma* cover ranges from 15-90% and it co-occurs with forbs and grasses. I excavated rhizomes from nine genets (hereafter

genotypes) at least 25m apart. This distance ensured that each collected rhizome was a unique genotype as even if rhizomes spread at the maximum recorded rate for the species they would still not spread 25m in the 11 years since colonization began (Cain 1990). I deliberately used one source population in order to avoid a spatial-scale mismatch that might artificially inflate intraspecific variation (Tack *et al.* 2012). Genotypes were propagated within the greenhouse for one generation to remove carryover effects.

Propagation: On April 1st 2012, I cut rhizomes into 2ml volume sections determined by water displacement in a graduated cylinder (Abrahamson & Weis 1997). Sections were planted in 9 cm pots in a mixture of 50% sterilized potting soil (Pro-Mix BX, Premier Brands, New Rochelle, NY) and 50% clay medium (Turface MVP, PROFILE Products LLC, Buffalo Grove, Il). On April 17 2012, plants had sprouted and were initially supplied with 100 mL solution of a total fertilizer (Peters Excel fertilizer 15-5-15 N:P:K Cal-Mg special, Everris) dissolved in water to yield a nitrogen (N) concentration of 400 ppm. I applied a total fertilizer, rather than simply N, to prevent experimental artifacts arising from plant nutrient co-limitation. The plants began growing at the same time as ramet expansion in the field, allowing the greenhouse to be matched with outdoor conditions (photoperiod and temperature levels; humidity was not controlled). On June 6th 2012, I transplanted the ramets to 4L pots and randomly assigned each to a nutrient supply treatment group within each genotype (see Figure 3.1a for detailed experimental timeline). Biweekly, plants were exposed to one of four nutrient treatments (100 ml of water with fertilizer at either 0, 100, 200, or 400 ppm) for the remainder of the growing season. These levels bracket those measured in plant tissue in the field, with the highest nutrient treatment equivalent to 1.3X nitrogen content in the above and belowground biomass of an average field ramet (Horner & Abrahamson 1992). Plants in each treatment group did not differ in total leaves before

nutrient supply treatments, but were different by the time herbivores were added (Figure S3.1 in Supporting Information). 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 (Figure 3.1b). Water was applied in equal quantities to all plants by drip irrigation twice daily.

Resistance assay: I collected juvenile *M. femurrubrum* grasshoppers from the same source field as the *S. altissima*. Collected grasshoppers were fed a common diet of lettuce and bran for 48 hours, food-deprived for 12 hrs, then weighed and placed onto plants housed within individual screen mesh cages (Figure 3.1). First, I exposed plants belonging to the "induced" treatment to a seven-day period of herbivory by two 3rd instar *M. femurrubrum* individuals (5.1±0.6% removal of leaf tissue). A second "constitutive" group was not exposed to herbivory. After one more week of growth, all plants were exposed to seven days of herbivory from two pre-weighed 4th instar individuals (an additional 9.7±1.8%) leaf damage. I weighed them 12 hours after removal and calculated a common index of individual plant resistance as -1 x average grasshopper relative growth rate (where relative growth rate = final mass-initial mass/initial mass) (Kempel *et al.* 2011). Multiplying the growth rate by -1 makes the index more intuitive because higher resistance corresponds to lower herbivore growth rates. Plant damage was estimated by counting the number of damaged leaves on the plant, randomly selecting eight leaves on which to visually estimate percent removed, and noting on which section of the plant leaves were damaged.

Tolerance assay: The remaining plants were also divided into two groups (Figure 3.1b). Half were exposed to one round of the herbivory treatment described for the resistance assay and the other half served as a control group that was never exposed to herbivory. Tolerance—the proportional reduction in fitness of each goldenrod genotype as a result of

herbivory—was calculated as a response ratio: fitness of a damaged plant/fitness of an undamaged plant. A value of 1 would indicate a plant genotype is fully tolerant of herbivory. These measures were calculated separately for each genotype at each nutrient level. Proportional representation allows for comparison across gradients, because it is not biased by differential size between nutrient treatments (Strauss & Agrawal 1999). I used three common end of season *Solidago* species fitness attributes to calculate tolerance: floral biomass, rhizomes produced, and aboveground biomass. I also compared root biomass because belowground allocation 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 from the growth rates of the herbivores applied to the damaged set of the "tolerance" assay plants (Morris *et al.* 2006). Further, tolerance may become spuriously correlated with resistance through lower damage levels on more "resistant" plants (Morris *et al.* 2006). However, within this experiment there is no correlation between tolerance and plant damage (Figure S3.2 in Supporting Information).

Trait measurements: Growing season trait data were collected on June 6th, July 11th, July 26th, August 14th, and September 25th (see Figure 3.1a for timeline). I calculated relative growth rate for both height and leaf number by placing metal rings around the top of the ramet on each sample date and recording subsequent growth. Leaf chlorophyll content was measured using a handheld OptiSciences CCM-300 chlorophyll content meter. Seven days after herbivore removal, I harvested the two most recent fully expanded leaves without

damage from each plant for leaf trait measurements. I used a penetrometer to measure leaf toughness as the force needed to puncture a leaf next to but not including the mid-vein. I measured leaf area using a leaf scanner and ImageJ software. Leaves were rehydrated, weighed wet, and then dried at 50°C and reweighed. These measurements were used to calculate LMA [leaf mass per area], LDMC [leaf dry matter content], and leaf thickness. Dry leaf tissue was then ground and analyzed for C and N content analysis using a CHN analyzer. The phenological status of each plant (i.e. growing, bolting, flowering, or in seed) was noted every 5 days after the first plant began bolting until harvest. On Oct 2nd I harvested whole plants and separated them into leaf, stem, root, rhizome, lateral stem, and flower portions. Each portion was oven dried at 60°C, and weighed to calculate both absolute and proportional plant allocation. I also noted the number of individual rhizomes produced by each plant (each will produce ~one new ramet the next year), average rhizome length (as a metric of plant spreading potential), and the number of adventitious buds and new within season lateral ramets produced at the base of the stem, as a measure of alternative reproductive strategies.

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 *et al.* 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 that included nutrient x genotype and herbivory x genotype effects were also originally tested, but were not significantly better than a model without them for any of the univariate response variables and so were not included in the final

model. The random effect of 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 *et al.* 2014). A significant nutrient supply x 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 ppm and lowest: 0 ppm nutrient levels).

Effect sizes of the herbivory treatment were calculated as the mean of genotype response ratios (separate for each nutrient level). These were calculated as induced genotype x/control genotype x. The same technique was used to assess the effect size of nutrient addition on genotypes between high and low nutrient treatments (separate for induced and constitutive plants).

I also determined whether tolerance differed across the nutrient gradient using linear mixed effects analysis with nutrient level as fixed effect and genotype as a random effect. Herbivory is implicit within this model because the response variable (tolerance) is an integrative measure. I further tested whether or not the tolerance reaction norm line was lower than a line with an intercept of one, indicating that herbivory had a significant negative impact on that fitness measure. Pearson correlation coefficients were calculated to determine if there were tradeoffs 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 visualize how trait values of plants responded to

herbivory along the nutrient gradient. This method is essentially a multivariate linear regression followed by a PCA of the fitted values. A permutation analysis is used to determine the significance of the explanatory factors on the multivariate trait data observed (analogous to non-parametric 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 in Supporting Information) 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 redundancy analysis, 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 that could be attributed to genotype versus the environmental treatments (nutrients and herbivory) using the function *varpart* (within the vegan package).

Lastly, I used the function *betadisper* (also within the vegan package) to test whether trait variation differed among groups. This command implements a permutational test of the homogeneity of multivariate dispersions similar to Levene's test in univariate statistics (Anderson *et al.* 2006) and has been used as an estimate of intraspecific variation (de Bello *et al.* 2011). I used Euclidian distance among individuals, adjusting for potential bias due to unequal number of individuals within groups. First, by testing whether genotypes exhibit different dispersion from their respective genotypic means, I determined whether total plasticity (dispersion from mean genotype value) in multivariate trait expression in response to the treatments differed among genotypes. Second, I used the same command to test whether each treatment group within an environmental gradient exhibited the same

population-level trait variation (nutrients and herbivory separately). In other words, whether cumulatively genotypes are occupying the same amount of trait morphospace within each treatment across the gradient.

Results

Does developmental resource environment alter plant responses to herbivory?

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 (Figure 3.2a, $F_{1,41}$=7.5, p=0.009, Table S3.1 in Supporting Information). At low nutrient levels 8 genotypes exhibited induced susceptibility to herbivores (RR=1.25±0.11, herbivory effect at 0ppm: $F_{1,14}$=5.54, p=0.03), while at high nutrient levels 7 of 9 genotypes exhibited induced resistance (RR=0.72±0.14; herbivory effect at 400ppm: $F_{1,17}$=12.42, p=0.002; see Figure 3.2b). At high nutrient levels (but not low) there was a shift toward grasshoppers feeding on the lower leaves of induced plants (Figure 3.2c).

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, Figure 3.3b) across the entire nutrient gradient. However, total aboveground biomass was reduced with herbivory (~16% reduction, intercept is significantly different than one: t=3.4, p=0.003, Figure 3.3c), 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 to undamaged plants (RR=1.31±0.16), while at high nutrient levels, herbivory reduced the number of rhizomes produced 18% compared to undamaged plants (RR=0.82±0.09) (negative slope, $F_{1,23}$=6.75, p=0.015, Figure 3.3a). Root biomass was negatively affected by herbivory across all nutrient treatments although to a smaller degree at high nutrient levels (0 ppm RR =0.69±0.09; 400 ppm RR =0.85±0.09; intercept is significantly different than one: t=3.6, p=0.002, Figure 3.3d).

Are changes in resistance correlated with plant 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; LMM nutrient: $F_{1,39}$=20.11, p<0.0001; see Figure 3.4a). 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 x nutrient: $F_{1,39}$=7.08, p=0.01, Figure 3.4b, 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 induced plants (RR=0.92±0.10) (herbivory x nutrient: $F_{1,39}$=4.46, p=0.04, Figure 3.4c, and Table S1). However, none of these measurements was able to directly explain the variation in resistance found across the genotypes and treatments (Figure S3.3 in Supporting Information).

Are there tradeoffs between tolerance and resistance across the nutrient gradient?

No significant trade-offs between tolerance and resistance were detected within genotypes (Fig. S3.4 in Supporting Information). However, high constitutive resistance in a

genotype consistently predicted a lower level of induced resistance within that particular genotype at low nutrient levels (Figure S3.4e).

What whole-plant changes occur with the plant defense changes?

A redundancy analysis quantifying how multidimensional plant phenotypes responded to nutrient supply and herbivory identified two significant canonical axes and accounted for 67% of the variation in the suite of plant traits (Figure 3.5). 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 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. Increases in herbivory led to plants with a higher proportional allocation to tougher leaves with a higher leaf dry matter content (LDMC), leaf mass per area (LMA), and lower post–treatment leaf and plant height relative growth rate.

Herbivory (F_1= 9.85, $p<0.001$) and nutrient (F_1= 47.0, $p<0.001$) treatments were both significant predictors of multivariate plant traits (Table S2 in Supporting Information). However, there was also a significant herbivore x nutrient interaction on plant traits (F_1= 2.14, $p=0.04$). 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 belowground adventitious buds than control plants, indicating an altered reproductive strategy.

Are there overall differences in individual genotype plasticity or in the population-level variation expressed within each environment?

Genotypes exhibited significantly different amounts of multivariate trait plasticity to combined stressors (*betadisper*: df=8 F=2.69 *p*=0.009, Figure 3.5c size of genotype ellipses), with some responding more strongly to herbivory and others to nutrient treatment (Figure 3.5c shape of genotype ellipses). The breadth of population-level trait variation within an environment was not different between herbivory treatments (*betadisper*: df=2 F=0.02 *p*=0.97; i.e. similar ellipse sizes between treatment groups, Figure 3.5b). Overall, trait dispersion was different between nutrient treatments (*betadisper*: df=3 F=4.38 *p*=0.006, Figure 3.5a), but post hoc paired comparisons (TukeyHSD) showed that this was entirely due to the larger dispersion of the incompletely sampled 200ppm nutrient treatment. The paired comparison of the lowest (0ppm) and highest (400ppm) nutrient levels of interest showed no difference trait dispersion between these nutrient levels (*p*=0.76).

Do genotypes respond differently?

Few G and GxE effects were detected within the univariate analysis. The exception was a significant effect of genotype on leaf carbon content (χ_1^2=76.03, *p*<0.001). It is worth noting that this failure to detect an effect may be due to low power. Genotypes do have a strong effect when included as a fixed effect within the multivariate analysis (this is done at the individual plant level hence more replication) (genotype, $F_{1,8}$=17.88, *p*=0.001). However, including genotype in the RDA results in environmentally-based trait changes that are harder

to interpret as there are 9 significant RDA axes rather than 2 and many significant interactions (e.g herbivory x genotype, $F_{1,8}=1.41$, $p=0.04$; nutrient x genotype, $F_{1,8}=3.27$, $p=0.001$; see Table S3.4 and Fig. S3.5 in Supporting Information).

Discussion

An impressive body of comparative and experimental studies examines the evolution and ecology of plant defense expression (Feeny 1976; Bowers & Stamp 1993; Stamp 2003; Fornoni 2011). While many of these studies quantify intraspecific trait variation in the form of phenotypic plasticity (i.e. any study which quantifies induced resistance, tolerance, or defense across environments) there has been little integration of plant defense results with functional trait or whole-plant approaches that seek to quantify landscape-level trait variation in order to predict ecosystem and community processes. This study explicitly integrates these approaches to provide a more nuanced understanding of the intraspecific trait variation found across biotic and abiotic gradients within a dominant species that can account for up to 90% of plant biomass in old-fields. It demonstrates that even within one source population the resource environment that a plant experiences influences constitutive and induced plant resistance and tolerance patterns. These shifts occur in concert with changes in whole-plant allocation patterns and trait expression. I will first place these results within the context of plant defense theoretic models, then explore what added insight can be gained from understanding correlated whole plant expression, and finally demonstrate how this approach may provide useful estimates of intraspecific variation patterns and predictions of context dependence that can be integrated into trait-based models and predictions.

Support for common plant defense models

At low nutrient levels, plants exhibited increased tolerance of herbivore damage, manifest as higher asexual reproduction relative to control plants (Figure 3.3). However, for the same genotypes at high nutrient levels, previous herbivory resulted in lower herbivore growth rates (i.e. higher resistance, Figure 3.2) in spite of the fact that leaf N content remained higher at high nutrient levels (Figure 3.4).

The resistance results provide equivocal support for the growth differentiation balance hypothesis (GDBH) (Herms & Mattson 1992). This framework predicts a peak in constitutive defense at mid-range nutrient levels and induced defense at high nutrient levels. While I found more 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 the byproduct 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 *et al.* 1985; Stamp 2003), 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 levels 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 (Wise & Abrahamson 2007).

Plant stoichiometry and resistance patterns

Nutrient supply can also alter leaf carbon or nitrogen content through primary metabolism changes, 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. I found that while leaf nutrient content was not able to directly explain the documented resistance changes (see Figure S3.5), plant stoichiometry did partially mirror the induced resistance patterns (Figure 3.4). 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 defense, is driving the trend. While such defenses—known to contribute to anti-herbivore defense in this species (Bode *et al.* 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 leaf dry matter content, see Figure 3.5c).

Integrating plant defense with whole-plant trait changes

Herbivores interact with whole organisms that express suites of co-varying growth, structural, and defense traits simultaneously rather than each in isolation (Núñez-Farfán *et al.* 2007; Forsman 2015). Thus while plant defense theory provides an important predictive framework for when intraspecific variation through phenotypic plasticity will occur in

response to resource conditions, these shifts occur within the context of many other physiological changes. For example 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 (Figure S3.4), failing to support the commonly posited (though often unsupported) tolerance/resistance trade-off (Leimu & Koricheva 2006; Núñez-Farfán *et al.* 2007). One reason may be that other traits and allocation patterns within an individual 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 defense, 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 multi-dimensional phenotype approach (Walsh & Blows 2009).

Quantification of whole-plant trait variation revealed both genetic effects on traits (~36% of the variation) as well as differential effects of nutrients and herbivory on plant expression patterns (~31%). While it is perhaps not surprising that a species that has been used to demonstrate large genetic diversity effects on community processes (Crutsinger *et al.* 2006) would exhibit a large amount of genotypic variation, the additional and nearly equivalent magnitude of environmental context on traits is notable. This suggests that ecosystem and community models that currently incorporate species or genotype mean trait values may have improved accuracy if plasticity to environmental context was explicitly considered (Wright & Sutton-Grier 2012).

Comparison of trait variation expressed within genotypes and between environments

Moreover, while the magnitude of multivariate trait plasticity to environmental context (i.e. multivariate dispersion) exhibited by individual genotypes differed (Figure 3.5c), 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 morpho-space 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 of intraspecific variation within and across environments 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. Such mean and variance estimates could then be applied to models to predict trait expression of the dominant species across old-fields of varying nutrient availabilities and herbivore densities. While whole-plant patterns are illustrated here, the approach might be particularly useful if ecosystem or community modelers have already identified one or a few traits that are key control points in their models (Bassar *et al.* 2012; DeAngelis *et al.* 2012). For example, in the univariate analysis, I document increased resistance (mean and also variance) at high nutrient levels. If resistance is a key factor affecting decomposition (for example) than incorporating the shifting means and variance of resistance into the model may improve predications across and within resource environments.

Implications for experimental design

Previous work on plant defense within this species has produced conflicting results, noting both induced resistance and induced susceptibility (Brown & Weis 1995; Cronin *et al.* 2010; Hakes & Cronin 2011; Bode *et al.* 2013; Uesugi *et al.* 2013; Heath *et al.* 2014). This study suggests a potential mechanism for this pattern. First, genotypes vary in their inducibility. Second, genotypes alter their defense strategy based on nutrient availability. Many experiments may be inadvertently impacting induction by utilizing experimental plants grown within small pots (<1L soil). The size of the pot naturally limits the potential size of the plant because root-bound plants are more resource limited (water, nutrients) even with equal fertilizer applications (Poorter *et al.* 2012). Using larger pots that each hold over 4L of soil in this experiment, resulted in a large differential in plant size across the nutrient supply gradient, fully spanning the range of ramet sizes found within the source population (<1m-2m). Perhaps differential stress due to root-bound status across experimental designs may be contributing to differential measured defense expression.

Conclusions

Few studies explicitly link plant defense emergent outcomes (such as changes in tolerance and resistance) 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 (Post & Palkovacs 2009; 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 *et al.* 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 sub-populations (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; Schweitzer *et al.* 2008).

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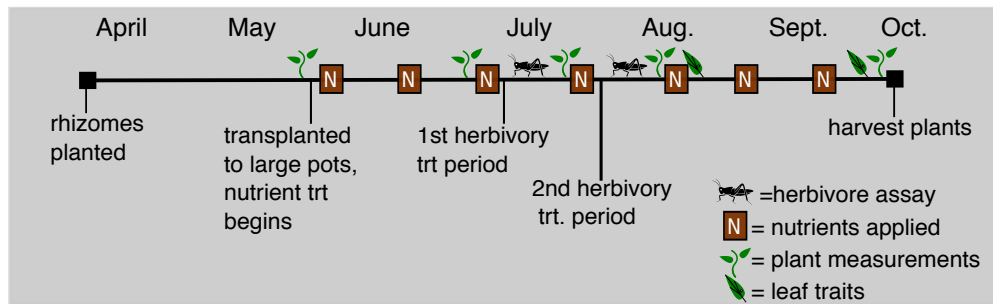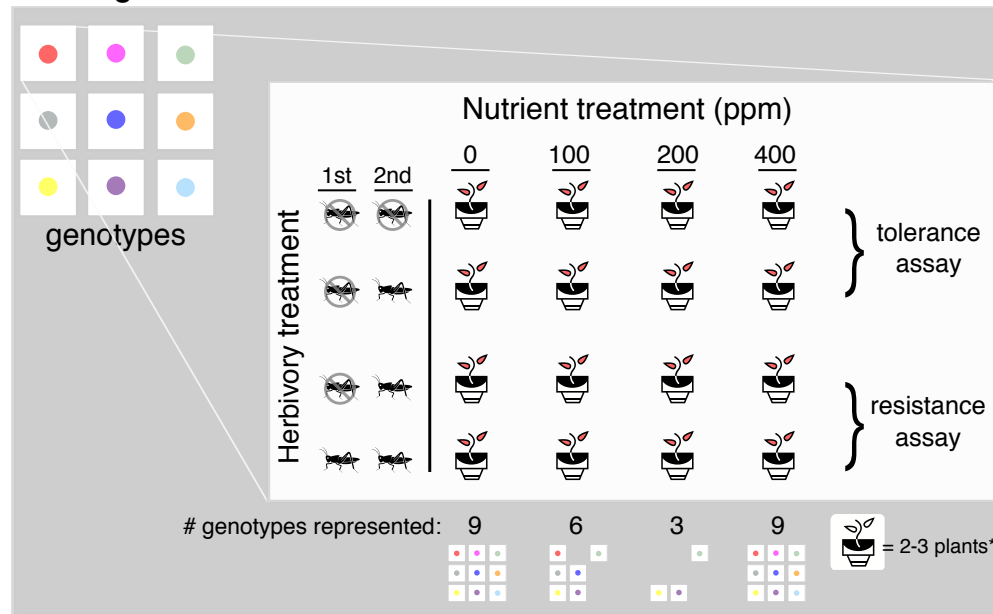
a. timeline

b. design

Figure 3.1: The experimental (a.) timeline and (b.) design. Three plant clones were grown at 4 nutrient levels crossed with 3 herbivory levels within each of 9 genotypes. They were used to quantify tolerance and resistance. Trait and plant measurement data were used for a multivariate trait analysis. Early plant mortality before assignment to treatment resulted in the absence of some genotypes in the middle two nutrient treatments (100 and 200ppm). *Later mortality events led to some treatments having fewer than 3 plants.
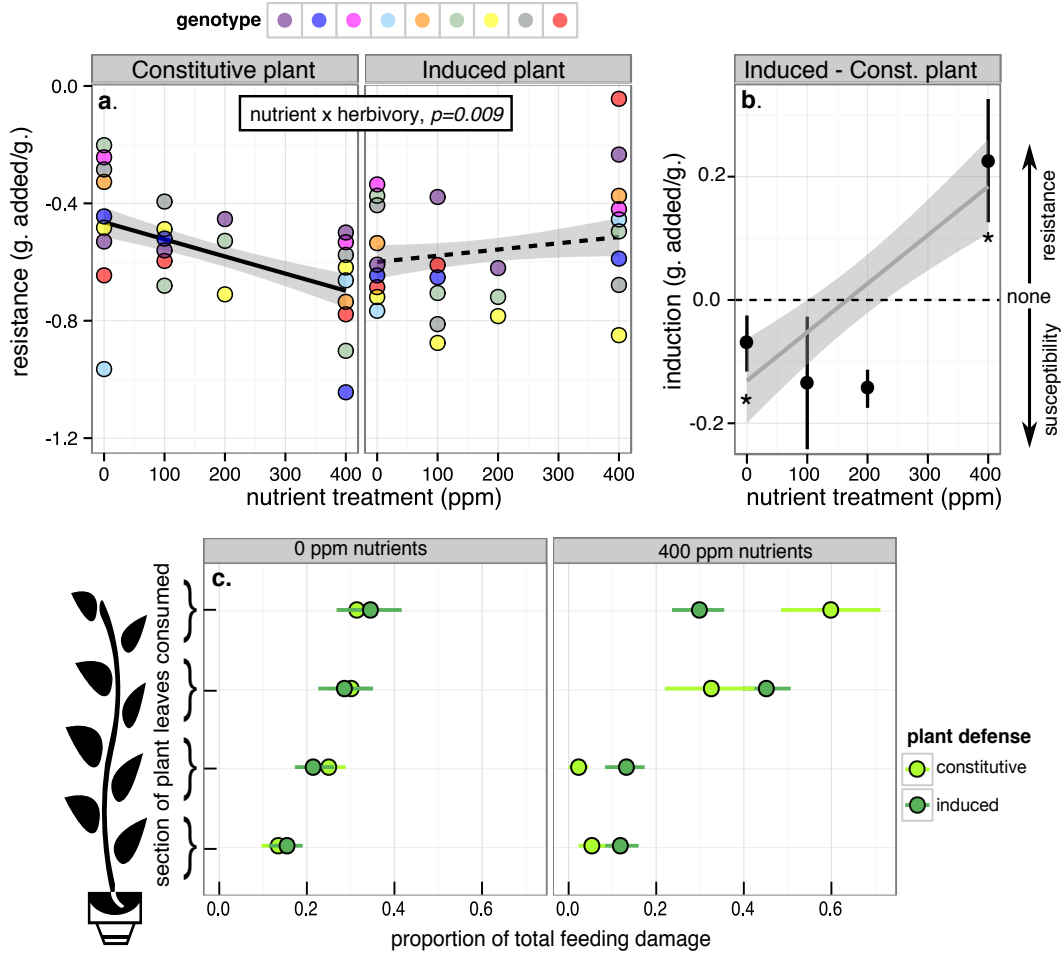
Figure 3.2: Genotypic means of a plant resistance index (-1 x herbivore relative growth rate) of individuals previously fed on by herbivores (induced plant) versus control plants (constitutive) across the nutrient treatment gradient (a.). The line±SE (shaded area) is a linear model relating y~ nutrient treatment (each herbivore treatment separately). Control plants had lower resistance as nutrients increased while induced plants had higher resistance as nutrient levels increased resulting in a significant herbivory x nutrient level interaction (Table S1 in Supporting Information). All genotypes were measured at the 0 and 400 nutrient level and qualitative results did not change if only those two treatments were included in the model. (b.) Induction was estimated as the difference within a genotype between constitutive and induced plant resistance. Plants at low nutrient levels exhibited induced susceptibility (higher herbivore growth rates on induced plants), while high nutrient plants exhibited induced resistance (lower herbivore growth rates). *Indicates significant difference in resistance between constitutive and induced plants within a given nutrient treatment using a LMM (see text). (c.) Behaviorally, herbivores shift to feeding on lower leaves of induced plants at high nutrient levels (demonstrated by changes in proportion of total feeding damage by herbivores (mean± 95%CI) .
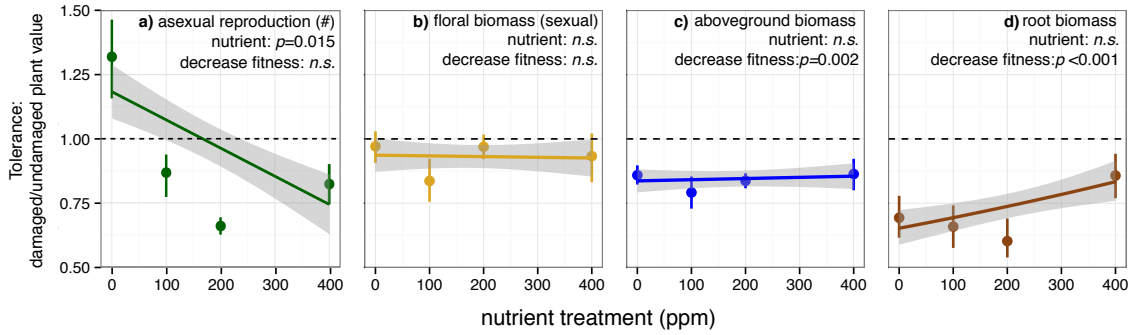
Figure 3.3: Plant tolerance (mean of genotypes ±SE, n=9) of herbivory across the nutrient gradient as measured by fitness related traits: (a) rhizome number, (b) floral biomass, (c) aboveground biomass, and (d) root allocation. The dashed line represents equal fitness between damaged and undamaged individuals 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 high. 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 impact of herbivory on aboveground and root biomass across the nutrient gradient (intercept of line different from 1).
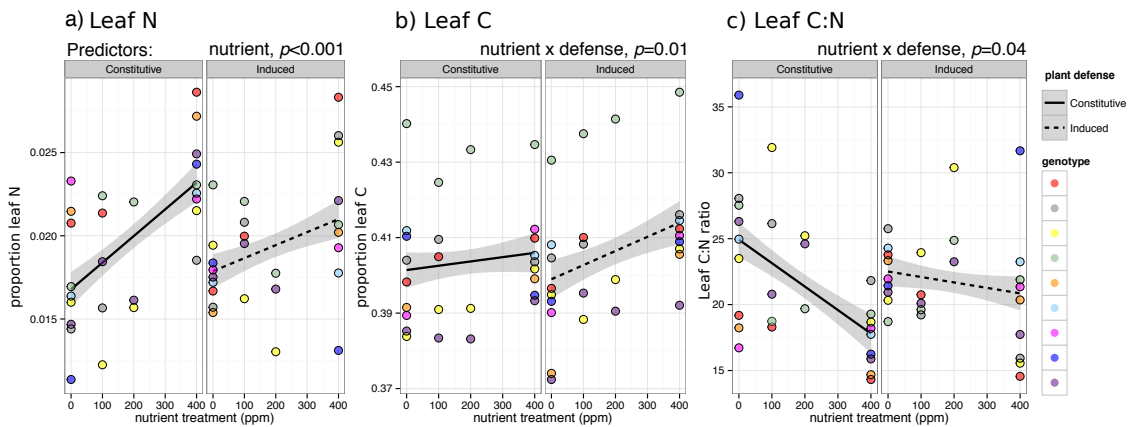
Figure 3.4: Herbivory and nutrient effects on genotypic means of leaf nutrient content. Fertilization (a.) increased N content regardless of herbivory treatment, (b.) but leaf C content and (c.) Leaf C:N response to nutrients depended on herbivory treatment (significant herbivory x nutrient interaction, 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 to show color.
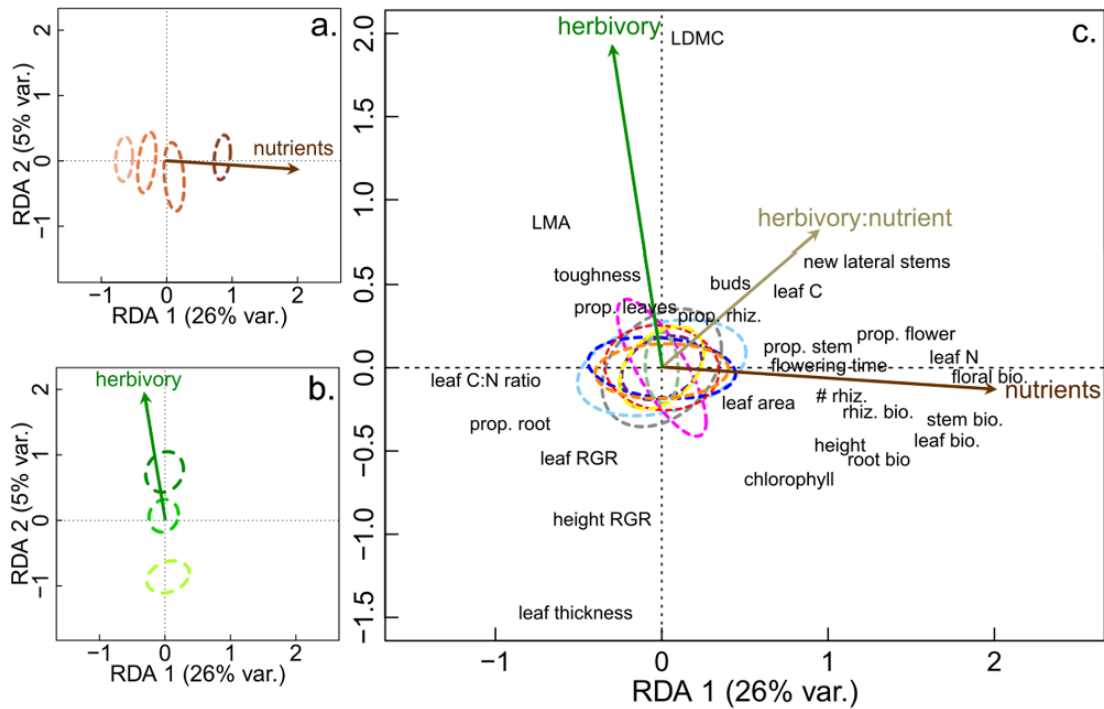
Figure 3.5: Whole plant trait and allocation patterns change in response to nutrient supply and herbivory. This is visualized using the first two axes (both significant and the same across all figures) of a partial redundancy analysis (RDA) representing the multivariate plant response to (a.) nutrients and (b.) herbivory. Genetic effects on trait variation (36%) were removed (conditioned out) which is why genotype ellipses are centered at the origin (c.). However each genotype's trait response to environments is shown by the dispersion around the origin (ellipses represents the 95% CI of a genotype's morphospace; same colors as Figs 1, 2, and 3; also see Fig. S5 for genotype variation). An additional 31% of the variation in plant traits was explained by the fixed environmental factors. 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). 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 (a.) nutrient or (b.) herbivory environment did not differ across environments. Abbreviations: LMA=Leaf Mass per Area. LDMC= Leaf Dry Matter Content.
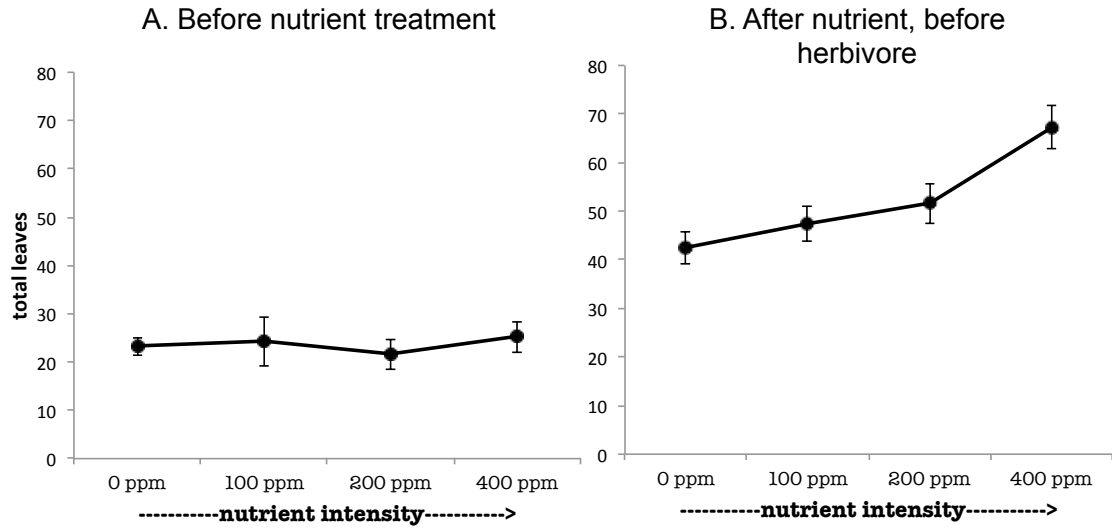
Figure S3.1: Nutrient treatment had a significant effect on plant growth before herbivores were added.
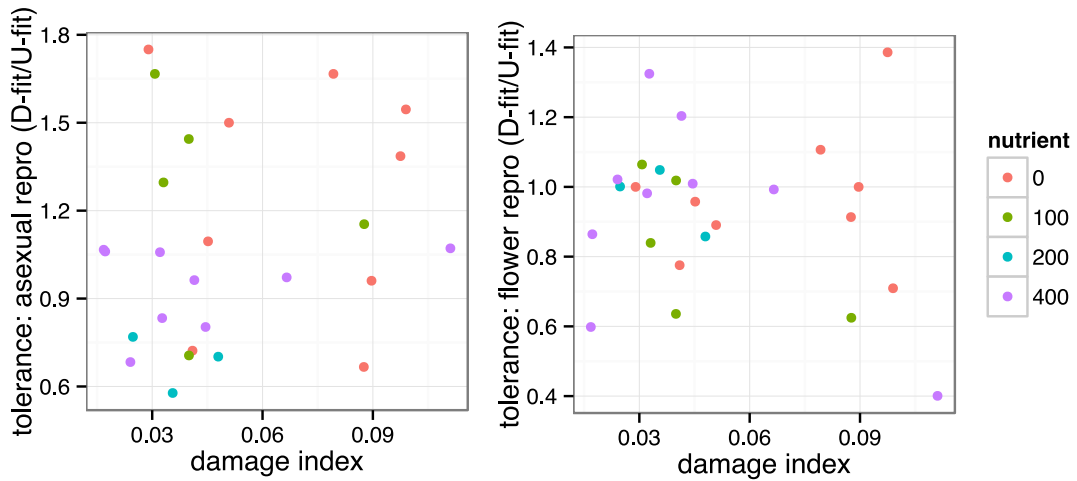
Figure S3.2: Genotypic means of herbivore damage vs. tolerance measures. No significant correlation between the two was observed.
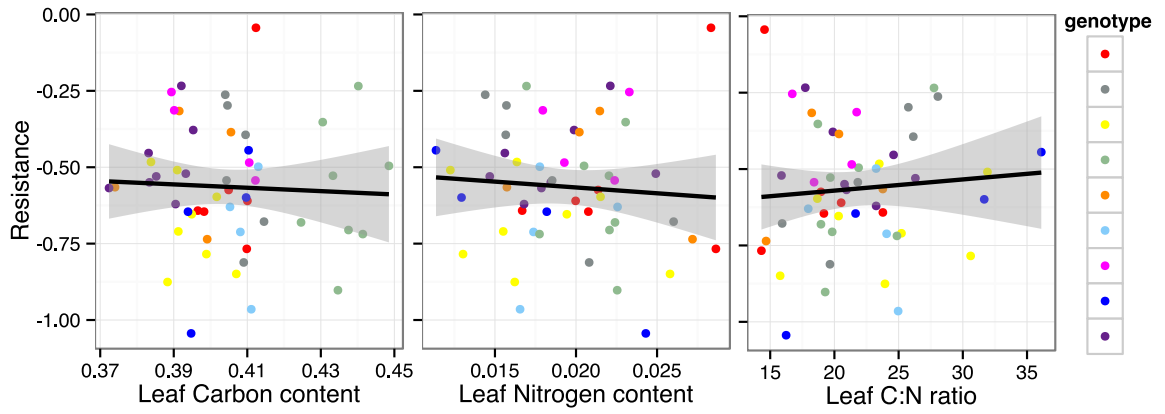
Figure S3.2: Direct relationship between leaf stoichiometry and plant resistance to herbivores (calculated as -1 x herbivore growth rate). The black line is a linear model relating x~y with 95% CI as the shaded region. None of these relationships are significant.
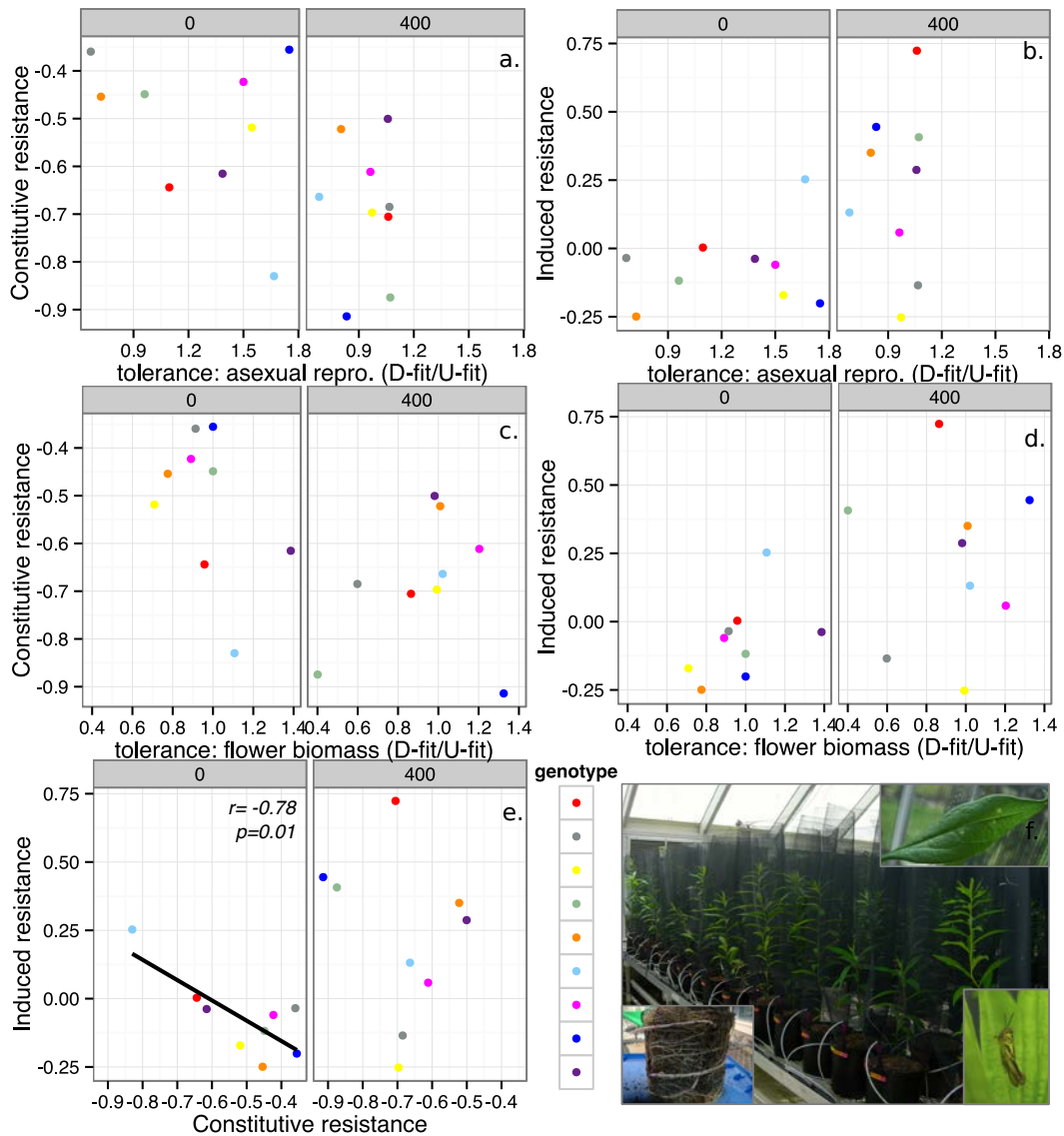
Figure S3.4: Trade-offs between tolerance and resistance at the genotype level at both low (0) and high (400) nutrient levels (a-e). If no line is drawn then the relationship is not significant at the p<0.05 level. Tolerance is quantified as the fitness of an herbivore-damaged plant (D-fit) / fitness of an undamaged plant (U-fit). *Solidago altissima* plants were grown in a greenhouse at different nutrient levels (f.) and exposed to herbivory from *Melanoplus femurrubrum* grasshoppers (f. inset). Feeding damage from herbivores could be selective as some grasshoppers "tasted" leaves of induced plants (see holes in leaf- f. inset upper right) and decided to feed on different tissue.
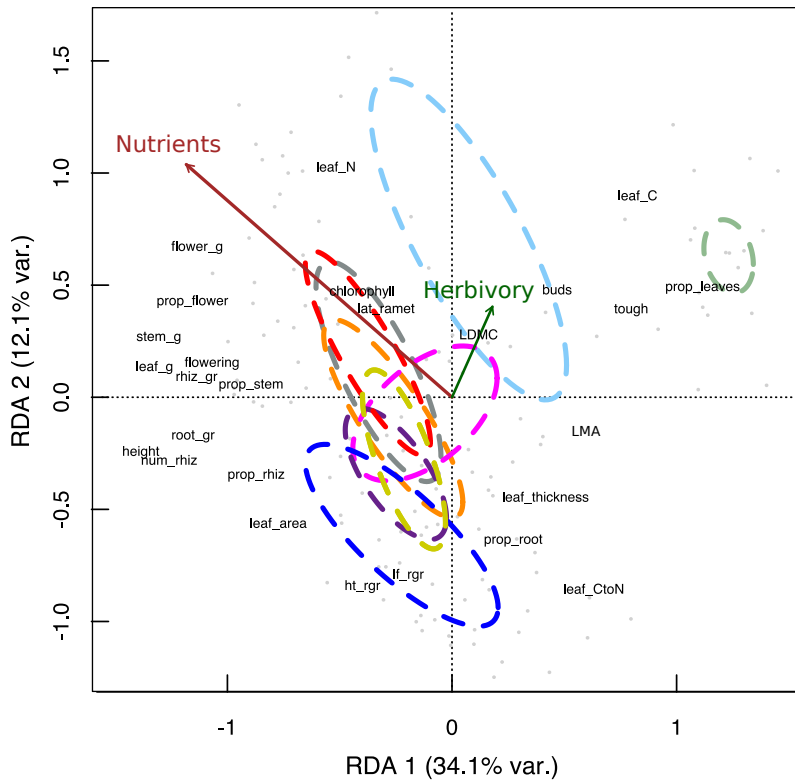
Figure S3.5: Redundancy analysis without genotype variation conditioned out (nutrients, herbivory and genotype as predictors) explains ~67% of variation across all of the constrained axes with nutrients, herbivory, and genotype as significant predictors (Table S1). Genotype vectors are not included so as not to clutter the figure, but colored genotype ellipses show 95% morphospace intervals for each genotype.

Table S3.1: Linear mixed effects model results for plant resistance index (see Fig 2) and leaf nutrient content (see fig 3: C, N, and C:N ratio). Genotype is included as a random effect.

fixed effect	Type III SS	NumDF	DenDF	F	p
Resistance Index					
feeding	0.0005	1	41	3.616	0.064
nutrient	0.0542	1	41	1.672	0.203
feeding:nutrient	0.2430	1	40	7.486	**0.009**
Leaf N					
feeding	0.000002	1	39	0.67	0.42
nutrient	0.000218	1	39	20.11	**<.0001**
feeding:nutrient	0.000026	1	39	2.36	0.13
Leaf C					
feeding	0.00007	1	39	1.00	0.32
nutrient	0.00099	1	39	27.08	**<.0001**
feeding:nutrient	0.00026	1	39	7.08	**0.01**
Leaf C:N					
feeding	0.038	1	39	2.25	0.14
nutrient	185.167	1	39	11.64	**<.0001**
feeding:nutrient	70.926	1	39	4.46	**0.04**

Table S3.2: Model results of the partial RDA analysis on multivariate trait data with genotype variation conditioned out (similar to genotype as a random effect). Pseudo F-values are generated using a permutational test (n=999) and these are the marginal effects of terms (e.g. Type III SS). See Fig 5 for visualization).

	Df	Var	Pseudo-F	p
herbivore	1	0.842	9.84	**0.001**
nutrient	1	4.021	46.99	**0.001**
herbivore:nutrient	1	0.183	2.14	**0.022**

Table S3.3: Trait loadings on the first two constrained axes of the partial RDA analysis (genotype –based trait variance removed). These are the only two significant constrained axes.

	RDA1	RDA2
LMA	-0.38198	0.276246
leaf_thickness	-0.34588	-0.666876
LDMC	0.07365	0.964153
flowering	0.51851	0.042578
lat_ramet	0.37374	0.111192
num_rhiz	0.54286	-0.037533
stem_g	1.06242	-0.08706
flower_g	1.06196	-0.004064
leaf_g	1.04561	-0.134189
root_gr	0.66448	-0.162904
rhiz_gr	0.73273	-0.074286
height	0.6168	-0.159156
buds	0.28932	0.156052
leaf_N	0.92496	0.013127
leaf_C	0.3157	0.106417
leaf_CtoN	-0.83932	-0.022128
chlorophyll	0.43387	-0.229479
tough	-0.23169	0.136407
prop_root	-0.61603	-0.167221
prop_leaves	-0.07868	0.100633
lf_rgr	-0.28457	-0.214999
ht_rgr	-0.19405	-0.394039
prop_flower	0.761	0.015949
prop_rhiz	0.13548	0.079075
prop_stem	0.50846	0.078301
leaf_area	0.36073	-0.04198

Table S3.4: RDA analysis results for explanatory factors using a permutational test with Euclidean distance (see Figure 5 for visualization). Simplifying the model by removing the insignificant three-way interaction does not change the qualitative results of the model.

Fixed effect	Df	Var	Pseudo-F	p
herbivore	1	0.8311	11.6903	**0.001**
nutrient	1	4.4164	62.1174	**0.001**
genotype	8	10.1706	17.8815	**0.001**
herbivore:nutrient	1	0.1843	2.5929	**0.021**
herbivore:genotype	8	0.8047	1.4147	**0.043**
nutrient:genotype	8	1.8603	3.2707	**0.001**
herbivore:nutrient:genotype	8	0.7651	1.3452	0.085

CHAPTER 4

LEGACY EFFECTS OF HERBIVORY AND NUTRIENT SUPPLY ON GOLDENROD LEAF TRAITS EXPLAIN TEMPORAL AND CUMULATIVE PATTERNS IN LITTER DECAY

1. Genetically based differences in mean trait values within a species can have wide-ranging and important effects on community and ecosystem processes. It is less well understood how plasticity within a genotype to environmental conditions alters that genotype's effect on ecosystem processes and whether biotic and abiotic environments can interact (ExE) to modify ecosystem processes. If so, this may provide a mechanistic link between living plant defensive strategies and decomposition.

2. This study addressed these questions using a microcosm decomposition experiment using litter from nine goldenrod (*Solidago altissima*) genotypes grown across 4 levels of nutrient supply (abiotic nutrient legacy) and two levels of herbivore feeding (biotic herbivore legacy). Senesced litter was combined with a small quantity of a common soil inoculum from the source field and decomposed over 100 days during which microbial respiration was periodically assayed.

3. Environmental (biotic and abiotic) legacy effects significantly predicted all decomposition metrics. However, the relative explanatory power of genetic vs. environmental legacy varied based on the particular metric.

4. Low nutrient legacy litter exhibited the highest cumulative carbon mineralization rates which were tied to increases in leaf C:N ratios and leaf mass area. The leaf traits and legacy effects that were dominant determinants of carbon mineralization varied over time in a 100-day decomposition assay. Herbivory interacted with nutrient treatment

such that litter mass loss and decomposition efficiency was highest for high nutrient litter only when herbivores were not present. Litter mass loss was closely associated with high chlorophyll content and leaf area of litter, while leaf N content played the largest role in determining decomposition efficiency.

5. Herbivore growth rates on the living tissue of these same plants was positively related to decomposition efficiency and negatively related to cumulative carbon mineralization.

6. Here, environmental context (herbivores, fertilization, and their interaction) alters decomposition dynamics through differing intraspecific plant trait expression patterns that in turn alter the microbial processing of plant litter. These may be important local-scale determinants of ecosystem processes such as decomposition within and across landscapes.

Introduction

Spatial heterogeneity in soil fertility influences the outcome of species interactions and often influences community assembly and diversity across landscapes (May & Arthur 1972; Tilman 1988; Lovett & Ruesink 1995). How local-scale soil heterogeneity arises in the first place remains an open question. Classic thinking holds that in terrestrial systems abiotic factors such as landscape topology, climatic patterns, and geological weathering are primary responsible for creating heterogeneity through differential release of nutrients into the soil (Hunter *et al.* 1988). While the effects of plant biomass have long been considered important (if subordinate) in determining soil heterogeneity, the effects of higher trophic levels are less well understood and often ignored within ecosystem models (Tansley 1935; Pastor & Naiman 1992; Wardle *et al.* 2000; Coupe & Cahill 2003; Schmitz *et al.* 2013). Yet recent work suggests that these higher trophic levels, can play an important and sometimes dominant role

in structuring local nutrient environments across landscapes through spatially selective resource consumption that drives nutrient cycling, nutrient translocation, and changes in trait expression patterns (Kitchell *et al.* 1979; Pastor & Naiman 1992; Bardgett & Wardle 2003; Pringle *et al.* 2010; Schmitz *et al.* 2010).

Herbivores in particular alter ecosystem processes through numerous direct and indirect nutrient pathways that link above and belowground communities (DeAngelis 1980; DeAngelis *et al.* 1989; Moore *et al.* 2004). One indirect pathway is through genetically or environmentally based changes in plant trait expression in response to herbivory. In many plant species, herbivore feeding may induce the plastic expression of plant defense traits (in the form of anti-herbivore chemistry or structures) or growth trait changes that can cause spatial variation in plant indivisduals nutrient quality across landscapes (Agrawal 1998; Andrew *et al.* 2007; Hakes & Cronin 2011). If plant anti-herbivore defensive traits influence plant-litter decomposition rates (Hättenschwiler & Vitousek 2000; Schweitzer *et al.* 2008), then any spatial differences in phenotypic defense expression should become an important determinant of spatial heterogeneity in plant litter decomposition and hence nutrient release to soil.

Such phenotypic variation (i.e. intraspecific trait variability-ITV) in plant litter traits may arise from genetic differences in expressed plant phenotypes (G), environmentally-based differences (E), as well as genetic variation in the capacity to respond to environments (GxE) (Whitman & Agrawal 2009). Recent work has shown conclusively that genetically based sources of variation contribute to explaining how a dominant species alters a host of community and ecosystem processes (Schweitzer *et al.* 2004; Wimp *et al.* 2005; Whitham *et al.* 2006; Hughes *et al.* 2008; Fischer *et al.* 2013). However, many of these studies are done within common gardens that minimize environmental variation, with hybrids or genotypes

collected from across wide geographic areas, potentially overemphasizing the genetic contribution to trait variation compared to that which would exist in a singular field setting (Tack *et al.* 2012).

At these local scales, understanding the interplay of individual genotypes with different environmental contexts to determine plasticity in trait expression may be key to predicting spatial variation in plant responses that create heterogeneity in soil nutrients. This is because genetic variation that is evident in one environment may become effectively "hidden" in another environment (i.e. if genotypes that are variable in one environment converge on the same trait value within an alternative environment) (Albert *et al.* 2011). This has important implications when accounting for environmental effects (E) on plant phenotypic expression. Further, if different environmental contexts have an interactive effect on trait expression, then it would be inappropriate to subsume all environmental sources of variation under one term because it could mask confounding or conflating effects of multiple environments. For example, plant developmental environment (such as nutrients, light, or water) might alter the direction or magnitude of plasticity expressed by a genotype in response to a later environment (such as herbivory). Thus, herbivore-induced differences may only be expressed within certain developmental environments, thereby altering the population level trait variance between environments (Cipollini & Bergelson 2001; O'Donnell *et al.* 2013). Such legacy effects of multiple environmental factors may be a key source of context-dependence in plant litter decomposition. These scenarios highlight the importance of incorporating the sources of intraspecific trait variability (ITV) into evolutionary and ecological models (Bolnick *et al.* 2011; Violle *et al.* 2012). Few studies, however, mechanistically link multiple environmental effects to community or ecosystem processes through changes in plant trait expression (an exception being Johnson *et al.* 2009).

Here I explore these dynamics through a study that quantifies the relative magnitude of the legacy effects of genotype in relation to two environmental factors (nutrient supply gradient; and insect herbivory) on leaf litter decomposition of a dominant old-field plant species, tall goldenrod (*Solidago altissima*). Litter decomposition is a key ecosystem process that contributes to nutrient cycling in ecosystems through the breakdown of recalcitrant plant-derived compounds by the microbial community into plant-available forms of nutrients such as ammonium (NH_4^+) and nitrate (NO_3^-). Within the ecosystem literature, decomposition rates are often tied to differences in leaf litter traits such as nutrient content, structural components, and the constitutive defensive traits of litter. Past work with these same genotypes in a greenhouse experiment demonstrated that each of these classes of traits plastically responded to both nutrient supply and herbivory environments with a unique emergent multivariate trait phenotype expressed in the presence of both herbivory and high nutrient supply (see Chapter 3). Within this study I test the hypothesis that the legacy of these plastic trait expression changes alters decomposition dynamics of litter from these plants. If so, it suggests that genetic or environmental mediated changes in a dominant species' plant traits at the scale of one field can have potential effects on a subsequent season's nutrient supply through heterogeneity in decomposition dynamics. The study was designed to answer the following specific questions:

1. What is the relative contribution of genetic vs. environmental legacy (herbivory and nutrient supply) of a living plant on decomposition of that individual's leaf litter?

2. What are the environment-based changes in growing season leaf traits that are most important to litter decomposition?

3. Are there temporal dynamics in which of the environmental legacy effects or plant traits dominate soil microbial action as measured by carbon mineralization patterns?

4. Can decomposition rate be connected to the palatability of a living plant to herbivores?

Materials and Methods

Overview: To measure genetic and multiple environmental legacy effects of a plant litter on an ecosystem process (decomposition), I used an assay that seeds a litter substrate with a small quantity of a common microbial soil inoculum to determine how the microbial community "perceives" the litter quality of that plant. I mechanistically link legacy effects on decomposition through changes in leaf litter traits using mixed models and a multivariate analytic approach. I then explored potential interactions between plant developmental nutrient environments and herbivory to determine if legacy effects of nutrients, herbivores, and their interaction on different genotypes result in similar effects on decomposition, mediated by plasticity of each plant genotype. Using a non-destructive measurement of carbon mineralization, I further evaluated how the legacy effects and plant traits determine microbial respiration over a 100-day assay. Finally as herbivore growth rates were also measured on living plant tissue, the different facets of the study also permit an explicit test of the palatability/decomposability hypothesis (Grime *et al.* 1996; Chapman *et al.* 2003; Palkova & Leps 2008; Kagata & Ohgushi 2011).

Study species: I examined genetic and environmental legacy effects on leaf litter decomposition of tall goldenrod (*Solidago altissima* (L.)), a rhizomatous perennial that dominates abandoned

agricultural fields in eastern North America. Nine genotypes were grown in a greenhouse across a nutrient supply gradient and then exposed to herbivory from a common leaf-chewing insect herbivore, the red-legged grasshopper (*Melanoplus femurrubrum* (De Geer 1773)). *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 lines of genetically identical plants. *S. altissima* exhibits a tolerance response through increased relative growth rate and photosynthetic rate (Meyer 1998; Cronin *et al.* 2010) particularly at low nutrient levels (see Chapter 3), background levels of chemical and structural defense (constitutive resistance), and heightened expression of chemical defense through induction of phenolics and diterpenoids in response to herbivory (Cooper-Driver & Le Quesne 1986; Abrahamson & Weis 1997; Bode *et al.* 2013). Induced resistance was highest within the studied genotypes at high nutrient supply (Chapter 3).

Source population: Rhizomes were obtained from one old-field site in Wallingford, CT. The density of S. *altissma* cover within the field ranges from 15-90% and it co-occurs with forbs and grasses. I excavated rhizomes from nine genets (hereafter genotypes) at least 15 m apart. I deliberately used one source population in order to avoid a spatial-scale mismatch that might artificially inflate intraspecific variation (Tack *et al.* 2012). Genotypes were propagated within the greenhouse for one generation to remove maternal carryover effects.

Propagation: On April 1st 2012, I cut rhizomes into 2ml volume sections determined by water displacement in a graduated cylinder (Abrahamson & Weis 1997). Sections were planted in 9 cm pots in a mixture of 50% sterilized potting soil (Pro-Mix BX, Premier Brands, New

Rochelle, NY) and 50% clay medium (Turface MVP, PROFILE Products LLC, Buffalo Grove, Il). On April 17 2012, plants had sprouted and were initially supplied with a 100 mL solution of total fertilizer (Peters Excel fertilizer 15-5-15 N:P:K Cal-Mg special, Everris) dissolved in water to yield a nitrogen (N) concentration of 400 ppm. I applied a total fertilizer, rather than simply N, to prevent experimental artifacts arising from plant nutrient co-limitation. The plants began growing when ramet expansion occurred in the field, allowing the greenhouse to be matched with outdoor conditions (photoperiod and temperature levels; humidity was not controlled).

Nutrient legacy treatment: On June 6th 2012, I transplanted the ramets to 4L pots and randomly assigned each to a nutrient supply treatment group within each genotype (see Figure 4.1a for detailed experimental timeline). Biweekly, plants were exposed to one of four nutrient treatments (100 ml of water with fertilizer at either 0, 100, 200, or 400 ppm) for the remainder of the growing season. These levels bracket those measured in plant tissue in the field, with the highest nutrient treatment equivalent to 1.3X nitrogen content in the above and belowground biomass of an average field ramet (Horner & Abrahamson 1992). Plants in each treatment group did not differ in total leaves before nutrient supply treatments, but were different by the time herbivores were added (Figure S3.1 in Supporting Information). Within each nutrient treatment, individuals were assigned to herbivore treatment in a stratified random manner by assigning plants of similar size as pairs (Figure 4.1b). Water was applied in equal quantities to all plants by drip irrigation twice daily.

Herbivory legacy treatment: I collected juvenile *M. femurrubrum* grasshoppers from the same source field as the *S. altissima*. Collected grasshoppers were fed a common diet of lettuce and

bran for 48 hours, food-deprived for 12 hrs, then weighed and placed onto plants housed within individual screen mesh cages (Figure 3.1). Control plants were not exposed to herbivores. "Herbivory" plants were exposed to two seven-day periods of herbivory by two 3rd instar *M. femurrubrum* individuals resulting in 9.7±1.8% leaf damage. Grasshoppers from the second round of herbivory were weighed 12 hours after removal and used to calculate a common index of individual plant resistance as -1 × average grasshopper relative growth rate [where relative growth rate = (final mass-initial mass)/initial mass] (Kempel *et al.* 2011). The resistance of control plants was estimated by calculating the growth rate of herbivores over a one-week interval on a separate group of plants that had not previously been exposed to herbivores (see Chapter 3 for details).

Leaf trait measurements: Growing season trait data were collected on August 14th, and September 25th (see Figure 4.1a for timeline). I calculated the relative growth rate for leaf number (the rate at which leaves were added to the plant), I placed metal rings around the top of the ramet after herbivores were removed and recorded subsequent leaf accrual. Seven days after herbivore removal, I harvested the two most recent fully expanded, undamaged leaves from each plant for leaf trait measurements. I used a penetrometer to measure leaf toughness as the force needed to puncture a leaf at a position next to but not including the midvein. Leaf chlorophyll content was measured using a handheld OptiSciences CCM-300 chlorophyll content meter. I measured leaf area using a leaf scanner and ImageJ software. Leaves were rehydrated, weighed wet, and then dried at 50°C and reweighed. These measurements were used to calculate LMA [leaf mass per area], LDMC [leaf dry matter content], and leaf thickness (Vile *et al.* 2005). Dry leaf tissue was then ground and analyzed for C and N content analysis using an elemental analyzer (Thermo DeltaPlus Advantage

coupled to a Costech ECS 4010 Elemental Analyzer via a Conflo III interface). On Oct 2nd I harvested whole plants and separated them into leaf, stem, root, rhizome, lateral stem, and flower portions. Each portion was oven dried at 60°C, and weighed to calculate proportional plant allocation. The only proportional measure used in this study is allocation to leaf tissue.

Soil inoculum and litter: Litter from each individual plant was homogenized and then milled to pass through a 2mm sieve. Four samples of the top 7 cm of surface soil below the litter layer were collected from the same source field as the plants and grasshoppers, transported to the lab, homogenized, sieved to 2mm, and then frozen at -20°C (to kill invertebrates but not microbes) prior to use in the decomposition assay.

Microcosms: 50 mL centrifuge microcosms held a subsample of litter substrate (1 g dry weight equivalent), which was then seeded with a smaller quantity (0.5 g dry weight equivalent) of soil inoculum to provide a common initial microbial community. The inoculum represented only 10% of the volume of the litter and contributes little C and N to the microbes (microbial respiration in soil-only microcosms was 0.3-1.2% of the respiration rate of soil+litter microcosms of identical weight). This is an adaptation of a standard method (Bradford *et al.* 2008; Strickland *et al.* 2009; Keiser *et al.* 2011) where a common microbial community is used to decompose litter of varying sources to assess the relative quality of the litter.

Treatment groups: Two full replicate runs of decomposition were completed. Replicate 2 was started 20 days after replicate 1. Each consisted of litter from 1-2 individual plants of 9 genotypes grown under four levels of previous nutrient supply (0, 100, 200, 400 ppm) and

two levels of herbivory history (control or herbivory). Due to lower propagation success within some genotypes, not every genotype was represented at the middle nutrient levels. For overall decomposition models, cumulative metrics from the two replicate runs were averaged.

Decomposition assay: Litter and soil were mixed together within the microcosms, adjusted to 65% water holding capacity, and then incubated within a 20°C dark growth chamber. For ten, 24-hour windows across the 100-day assay (rep 1: 5, 8, 11, 16, 24, 31, 41, 55, 75, 100 and rep 2: 2, 4, 6, 9, 14, 20, 30, 50, 75, 100) microbial respiration (carbon mineralization) rates were measured within each microcosm. This was accomplished by capping each microcosm, flushing the headspace with CO_2 free air, incubating for 24 hours, and then measuring the CO_2 content of the air from the headspace over the litter sample using an infrared gas analysis technique (IRGA- Li-COR model LI-7000, Lincoln, NE, USA). Cumulative carbon respiration rates were calculated by integrating rate values across the 100 days. At the end of the 100 days, the litter remaining was oven-dried at 60°C and weighed to calculate litter mass loss. I calculated a decomposition efficiency metric by dividing the litter mass loss over the course of the study by the cumulative carbon mineralization over the course of the study. This mass loss per unit of carbon respired metric represents decomposition efficiency, as it indicates that the microbial community must respire less carbon for a given amount of decomposition.

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 *et al.* 2012) assigning decomposition as response variables; herbivore history (herbivory vs.

control plant) and nutrient supply as fixed effects; and plant genotype as a random effect. The significance of fixed effects was assessed using F-tests, whereas significance of random factors was assessed using a likelihood ratio test (Zuur *et al.* 2009). The random effect of genotype was kept within all final models to account for the unbalanced nature of the experimental design. Degrees of freedom (Satterthwaite approximation), type III SS, and p-values were calculated using *lmertest* (Kuznetsova *et al.* 2014). The r^2 values of the fixed vs. random components in the model provide a measure of the quality of the fit for each model (Nakagawa & Schielzeth 2013). A significant nutrient supply x herbivory interaction indicates that the effect of herbivory on the decomposition metric differed across nutrient environments.

I also used redundancy analysis (RDA), a constrained multivariate approach within the vegan package in R (Oksanen *et al.* 2012) to quantify and visualize how trait values of plants responded to herbivory along the nutrient gradient. RDA is essentially a multivariate linear regression followed by a PCA of the fitted values to create constrained RDA axes that only display variation associated with the predictors. A permutation analysis was then used to determine the significance of the predictors on the observed multivariate trait data (analogous to non-parametric PERMANOVA). Visualization is similar to PCA, but the first canonical axes are constrained only to represent the variation explained by the linear predictors in the model (here, herbivory, nutrient supply, and genotype). The 11 measured leaf traits 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 included as a fixed effect. I then conditioned out the variation associated with genotype (i.e. a partial redundancy analysis, analogous to treating

genotype as a random effect) to understand whether genotypes responded similarly to environmental factors even if genotypic means differed.

Post-hoc decomposition response vectors were fit to the RDA axes using the command *envfit* to quantify how decomposition metrics (decomposition efficiency, cumulative carbon mineralization, litter mass loss) relate to the leaf trait changes caused by the herbivory and nutrient treatments. Vectors are fit to point in the direction of the ordination where the response variable is changing most rapidly and is most correlated with the trait values. The lengths of the vectors are scaled to the strength of the relationship (r^2) and are calculated using a permutation test with 5000 replications. Vectors are only retained and plotted if significant at the $p<0.05$ level. While the implementation is different, these tests (and the r^2 values) are analogous to performing a regression relating the response variable to the RDA1+RDA2 axes. Further I employed the same technique to examine temporal dynamics across the 100-day experiment in microbial respiration levels (cumulative carbon mineralization- the only response variable that does not require destructive sampling). Lastly, I investigated whether herbivore growth rates (measured in August on a living plant) was predictive of any of the subsequent decomposition metrics using a mixed model with herbivore growth rate as a continuous fixed factor and genotype as a random effect in the model.

Results

Legacy effects on litter decomposition

Total cumulative carbon mineralization was highest on litter with a low nutrient supply legacy within both herbivory treatments ($F_{1,56}= 5.0$, $p=0.02$, Fig. 4.2b). Litter mass loss increased across the nutrient gradient on control plants, but decreased across the

gradient on herbivory plants resulting in a significant herbivory x nutrient interaction ($F_{1,56}$=

12.5, p=0.0008, Fig 4.2c). Decomposition efficiency of microbes on the plant litter increased

across the nutrient gradient on control plants (nutrient, $F_{1,58}$= 12.4, p=0.0008) but remained

unchanged across the gradient when litter came from plants with a history of herbivory

($F_{1,58}$= 7.4, p=0.008). This resulted in a significant interaction between herbivory and

nutrient legacies on decomposition efficiency ($F_{1,58}$= 9.98, p=0.002, Fig. 4.2a). The random

effect of genotype explained the most variation within the cumulative carbon mineralization

model (r^2=0.58). Random effects of genotype explained less variation in the decomposition

efficiency model (r^2=0.15), and an intermediate amount in litter mass loss model (r^2=0.49).

Genetic effects on multivariate trait expression:

The RDA analysis of leaf trait variation among individual plants revealed that

genotype ($F_{8,32}$= 6.24, p=0.001), herbivory ($F_{1,32}$= 11.63, p=0.001) and nutrients ($F_{1,32}$= 8.88,

p=0.001) were significant predictors and accounted for 76% of the total leaf trait variation

with 9 significant RDA axes. Genotype accounted for the largest proportion of variation

(37%), manifest through differences in the genotypic mean trait values (ellipses in Fig 4.3).

High nutrient supply was associated with high leaf N, chlorophyll content, thin leaves and

low C:N ratios (Fig. 4.3). Herbivory was associated with high leaf dry matter content

(LDMC) and leaf carbon content. Post-hoc vector fitting of cumulative carbon

mineralization rates revealed that higher mineralization occurred on leaf litter that was

tougher, had higher LMA and leaf C, and on plants that allocated a larger proportion of

biomass to leaf tissue (Fig. 4.3). Carbon mineralization was highest on litter with a low

nutrient, herbivory legacy. However, the different genotypic means confound the

interpretation of the environmental legacy effects in this analysis (see Fig. 4.3). Therefore, it

is difficult to determine whether higher carbon mineralization is associated with herbivory or just with the traits of the green genotype, which has significantly different genotypic mean trait values.

Environmental legacy effects on multivariate trait expression and decomposition

Upon conditioning out the variation in the trait data attributable to genotype (37%) essentially centering the ellipses, I was able to resolve whether different genotypes exhibited similar patterns in trait plasticity in response to nutrients and herbivory (Fig. 4.4a). Nutrient ($F_{1,56}$= 8.05, p=0.001) and herbivory ($F_{1,56}$= 10.4, p=0.001) treatments explained a further 27% of the leaf trait variation and I found a significant interaction between nutrients and herbivory ($F_{1,56}$= 3.4, p=0.009). There were two significant RDA axes that quantified this trait variation with herbivory (accounting for 66%) and nutrients (accounting for 34%) of the leaf variation accounted for by these two axes. The post-hoc vector analysis, examining how decomposition rates were related to legacy effects through leaf trait changes, showed that cumulative carbon mineralization was highly related to a low nutrient legacy (see arrows in Fig. 4.4a and Table 4.1). This is consistent with the results of the linear mixed model relating mineralization to legacy effects (shown in Fig 4.2b). The RDA approach reveals the leaf litter trait changes related to that effect. The patterns occur through higher cumulative carbon mineralization from tissue with high leaf litter C:N ratios and leaf mass area (LMA). Litter mass loss was highest on high nutrient legacy litter without a history of herbivores and was associated with chlorophyll content, and leaf area. Decomposition efficiency was also highest on high nutrient legacy litter without a history of herbivores but was more strongly correlated with high leaf N and leaf area and negatively correlated with leaf C:N and LMA (Fig 4.4a and Table 4.1).

Temporal dynamics

There were clear temporal trends in which leaf traits explained microbial respiration (carbon mineralization) over the 100-day assay (Fig 4.4b and Table 4.1). Microbial respiration could be explained by variation in leaf traits attributable to the legacy effects of herbivory and nutrients for 9 of 10 sample time points in replicate 1 and 8 of 10 in replicate 2 (only significant vectors are shown in the figure and the vector length is scaled to r^2 which ranged from 0.10-0.47, Table 4.1). Initial respiration rates (day 4 and 5) were highest on the litter of low nutrient plants without herbivores. This was associated with leaf tissue that exhibited higher leaf relative growth rates and low toughness. By the 10th day, respiration was positively correlated with leaf N, leaf C content, chlorophyll, and leaf area; which describe litter with a high nutrient, low herbivory legacy. By day 20-30, microbial respiration shifted toward being highest on leaf litter from low nutrient plants which was correlated with high leaf C:N. By day 75 this shifted toward higher respiration of low nutrient, herbivory litter with high LMA, but by day 100 respiration was again dominated by leaf C:N ratio which was correlated with the low nutrient treatment.

Living plant palatability to herbivores and decomposition rates

Grasshopper growth rates on living plant tissue were positively related to the decomposition efficiency of a plant's litter ($F_{1,65}$= 6.8, p=0.01, and Fig. 4.5) and negatively related to cumulative carbon mineralization ($F_{1,61}$= 4.4, p=0.04). Seven of the nine genotypes exhibited this pattern. Herbivore growth rates did not predict litter mass loss ($F_{1,61}$= 1.7, p=0.19) which was instead dominated by genotype effects (random effect r^2 =0.52).

Discussion

This study demonstrates that the legacy effects of plant genotype, nutrient supply, and herbivory on litter decomposition can be explained by leaf trait changes and a dominant interaction between herbivory and nutrient supply. In other words, the interaction between the legacy effect of the herbivore environment and the developmental nutrient environment overrides the positive relationship between increasing nutrient supply and litter decomposition seen in the absence of herbivory. I found no evidence that either genetic or environmental legacy played a more important role in determining decomposition rates. Instead they both contributed to overall intraspecific variation in litter decomposition, with their relative importance dependent on the decomposition response metric measured.

Legacy effects on litter decomposition

Legacy effects were linked to characteristic trait changes in response to abiotic and biotic environments that were qualitatively similar within each genotype even though genotypic trait means differed. Litter mass loss was highest when litter came from high nutrient, no herbivore plants with a large leaf area and chlorophyll content. Interestingly, with genotypic variation removed, mass loss became uncoupled from cumulative carbon mineralization rates (microbial respiration, see Fig 4.4a) which was highest on low nutrient litter with high C:N ratios and LMA. The uncoupling of these two common decomposition measures may seem counter-intuitive, but the disparity can be explained by the different mechanism of action implied by the two metrics. Litter mass loss represents the loss of carbon, hydrogen, oxygen, and nitrogen atoms through respiration of CO_2 by microbes, evaporation of H_2O, and loss of N through denitrification pathways (Schlesinger & Bernhardt 2012), while carbon mineralization measurements only include the C respired by

microbes. If decomposition by microbes is differentially efficient across the treatments (e.g. the respiration of one unit of carbon results in a different amount of litter mass loss between treatments) then the results are reasonable.

Two broad categories' of mechanisms may lead to such a result. First, differences in carbon use efficiency (CUE) of the microbial biomass within the mesocosms (i.e. the ratio of microbial growth to C uptake) may account for the observed differences(Manzoni *et al.* 2012). This could occur through the greater carbon cost to microbes of a larger number of enzymatic steps required to break down a unit of recalcitrant or chemically protected litter tissue per unit carbon assimilated (Ågren & Bosatta 1987) or through switching to differentially efficient metabolic pathways for induced vs. constitutive litter (Manzoni *et al.* 2012). Secondly, when microbes encounter low quality tissue with stoichiometric ratios that don't match their metabolic needs (high C:N), they may engage in a potentially beneficially process called "energy spilling" or "overflow respiration" where more carbon (energy) is emitted in order to break down the same mass of litter tissue and gain access to potentially limiting N (Russell & Cook 1995; Russell 2007; Bradford 2013). Here, through stoichiometric imbalance, anabolic and catabolic pathways are uncoupled with the energy produced lost through heat rather than contributing to the production of exoenzymes or any other microbial product or biomass(Russell 2007). An increase in this type of respiration on lower quality litter would produce the observed patterns.

While this study is not able to differentiate between these alternative mechanisms it is clear that higher cumulative carbon respiration of microbes on low nutrient legacy or "induced" litter is perceived by microbes as a lower quality substrate and leads to lower relative litter mass loss through a less efficient decomposition process. In contrast, when tissue quality is high (high nutrient or constitutive litter), microbes may more be more

efficiently incorporating C into their biomass (lower carbon mineralization), through breaking organic tissue into component parts of oxygen and hydrogen and nitrogen more efficiently. Overall these results fit within the theoretical predictions of the Schimel and Weintraub (2003) model of decomposition which links carbon and nitrogen use while simultaneous accounting for potential changes in exoenzyme production and overflow respiration.

I also found temporal succession in which traits control carbon mineralization rates over the 100 day assay, suggesting a temporal succession of microbial communities. Initial decomposition was based on labile available resources driven by nitrogen availability and high quality C (chorophyll) on litter from leaves with a large leaf area. Later in the assay, carbon mineralization was highest on litter with high C:N and LMA (leaf mass per area), suggesting that mineralization is highest later in the assay when there is a large quantity of recalcitrant C for fungi to decompose. This pattern raises the possibility that on high nutrient, no herbivory litter, microbes may have better carbon use efficiency early in the assay, turning more C into microbial biomass. That leaves less recalcitrant carbon for fungi to process later in the assay at lower CUE. While speculative, this would explain the pattern of higher decomposition efficiency in this type of litter legacy early in the assay coupled with lower relative cumulative mineralization toward the end.

Implications for nutrient cycling rates

Taken together it appears that both deceleration and acceleration of nutrient cycling in response to herbivory are viable outcomes within this system, with the critical determinant of the direction of the trend being nutrient availability (Hobbie 1992; Burghardt & Schmitz 2015; Schmitz *et al.* 2015). The acceleration hypothesis predicts a positive intraspecific

feedback between herbivory and nutrient cycling due to high quality regrowth of the grazed species (McNaughton *et al.* 1989; Belovsky & Slade 2000). In contrast, the deceleration hypothesis (Ritchie *et al.* 1998) is an interspecific hypothesis that posits that herbivores consume palatable plants selectively, thus shifting community composition toward less palatable (and decomposable) species (Grime *et al.* 1996; Ohgushi 2008; but see Palkova & Leps 2008). Plant traits play a critical role in both these hypotheses. As such, herbivores may not only influence plant productivity by directly consuming plants, but also indirectly by changing soil nutrient availability (Choudhury 1988; Bardgett & Wardle 2003; but see Frost & Hunter 2008). Here, we see the acceleration hypothesis potentially operating at low nutrient levels (where I measured higher decomposition rates of herbivory legacy litter vs. control litter) and the deceleration hypothesis operating at high nutrient levels (lower decomposition rates of herbivory legacy litter vs. control litter) Acting together, these mechanisms should cause nutrient cycling to remain constant across variable nutrient environments in the presence of herbivores (see 4.2).

Connecting litter decomposition with plasticity in defensive strategies expressed by living plants

These decomposition patterns can also be tied to shifts in defensive strategy employed by the living individuals of these genotypes in response the nutrient supply gradient. In a previous study, I documented induced resistance of herbivore-damaged plants at high (but not low) nutrient supply (see Chapter 3)(Burghardt 2016). Within this study I document lower relative decomposition rates for the litter from this treatment combination (high nutrient, herbivory litter). This suggests that at high nutrient levels, induced defensive responses to herbivory may have a negative effect on plant available nutrients through lower decomposition rates. This may manifest as a possible "penalty of induction" in terms of later

nutrient availability. Indeed, I only demonstrate induced resistance in high nutrient supply plants where costs may be lower. In contrast, at low nutrient supply I documented the opposite pattern. Here, I found "induced susceptibility" of genotypes after they were fed on by herbivores, which was associated with a higher collective tolerance of herbivory in terms of rhizome production (see Chapter 3). Tolerance encompasses a plant strategy whereby plants change plant traits in response to herbivores in an attempt to regain lost photosynthetic capacity in order to minimize the negative effects of herbivores on fitness (Strauss & Agrawal 1999; Tiffin 2000). The plant traits associated with this response may involve producing larger leaves with low mass per area (LMA) and higher relative N content (Meyer 1998; Chase *et al.* 2000). Collectively within this study these changes were associated with increased decomposition rates of litter from herbivory legacy litter relative to control at low nutrients. This suggests that at low nutrient levels plant trait shifts associated with herbivory may ameliorate (or at least not exacerbate) local plant nutrient limitation as herbivory increases decomposition relative to undamaged plants. This may increase nutrient availability the following growing season.

Does higher measured palatability of living tissue to herbivores predict higher decomposition rates?

Further, I document a positive relationship between herbivore growth rates and litter decomposition efficiency (Fig. 5) and a negative relationship with cumulative carbon mineralization associated with low quality tissue (Fig 4.4a) providing support for the palatability/decomposability hypothesis. While both relationships were significant, the explained variance was low (although including genotype as a random effect improved explained variance significantly). In general, support for this hypothesis has been mixed but is typically measured at the interspecific rather than intraspecific level (Grime *et al.* 1996;

Schadler *et al.* 2003; Palkova & Leps 2008; Kagata & Ohgushi 2011). It may be easier to document such relationships through intraspecific comparisons where phylogenetic correlations do not play a role.

Advantages of the analytic approach

The combination of mixed model and multivariate approaches that I utilized within this study has distinct advantages over exclusively univariate approaches, especially when attempting to link genetic or environmental factors to community or ecosystem processes through trait-based mechanisms. For example, in order to determine which traits are altered by treatments and then predict decomposition, a univariate approach involves first determining, one by one, which individual traits are significantly altered by the treatments, and then relating those traits that are deemed significant to decomposition using multiple regression. However, plant traits are highly correlated. Thus one quickly runs into problems of multicollinearity during the process of model selection. Multivariate approaches are designed to deal well with correlated predictors like the leaf traits studied here (Ramette 2007). In addition, RDA allows visual representation of explicit predictive linkages and tracking of temporal dynamics over time (Fig. 4.4b). Most importantly for experimental approaches, it allows the examination of only the trait variation attributable to experimental treatments and removal of variation due to blocking factors.

Conclusions

Overall these results suggest that abiotic and biotic environmental contexts (and their interaction) play a large and often underappreciated role in determining the mean and variance of plant trait expression in ways that determine the level of subsequent ecosystem

processes such as decomposition (Schmitz *et al.* 2015). In fact, I found that the legacy effects of the biotic environment- herbivory- could mask the variation in decomposition rates created by the abiotic developmental environment-nutrient supply. Genotype also played an important role in determining decomposition rates, confirming previous studies (Schweitzer *et al.* 2005; Fischer *et al.* 2013). However, the documentation of the interaction and relative importance these three locally relevant sources of trait variation (herbivory, nutrient supply, and genotype) considered together is novel and important for predicting local scale dynamics (Tack *et al.* 2012). Given that goldenrod is a dominant species that spreads spatially primarily through clonal growth, there is often considerable spatial clumping of genotypes within old-field landscapes (Cain *et al.* 1991). The differences in environmental conditions across space may then interact with these genotypes allowing predications and testing of how context dependence in decomposition rate may arise to create landscape scale heterogeneity in soil nutrient supply. This is an intraspecific expression of the so-called Zinke effect, whereby the identity of a plant species present may result in soil processes or nutrient availability up to 41% different than that of an conspecific neighbor (Zinke 1962; Waring *et al.* 2015). While field testing such an intraspecific expression of this effect is beyond the scope of this paper, linking plant defense expression to nitrogen availability through decomposition differences may provide a biotic mechanism to explain the sources of heterogeneity in nutrient cycling and pools within single fields and across landscapes.

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Figures

a. timeline
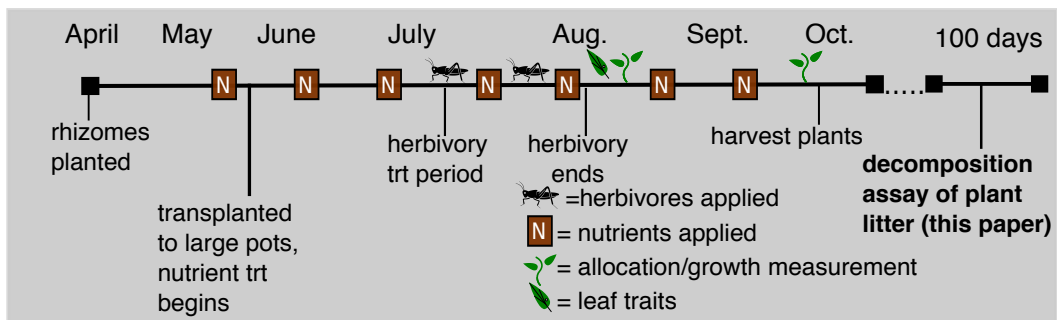
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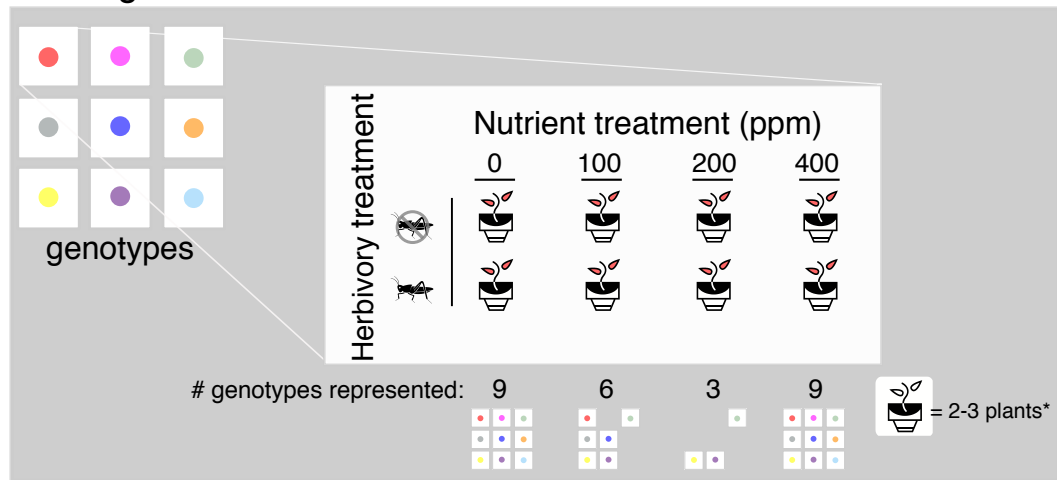
Figure 4.1: Experimental design that produced the litter used within this study for a decomposition assay. Litter had legacy effects of 4 levels of nutrient treatments applied throughout the growing season and two herbivory treatments (a no herbivore control or 2 weeks of feeding by 2 grasshoppers). 2-3 plants were grown within each treatment and the litter for each decomposed in separate assays. Most leaf trait measurements were taken 7 days after herbivores were removed from the plant, but total proportional allocation to leaf tissue was determined at harvest.
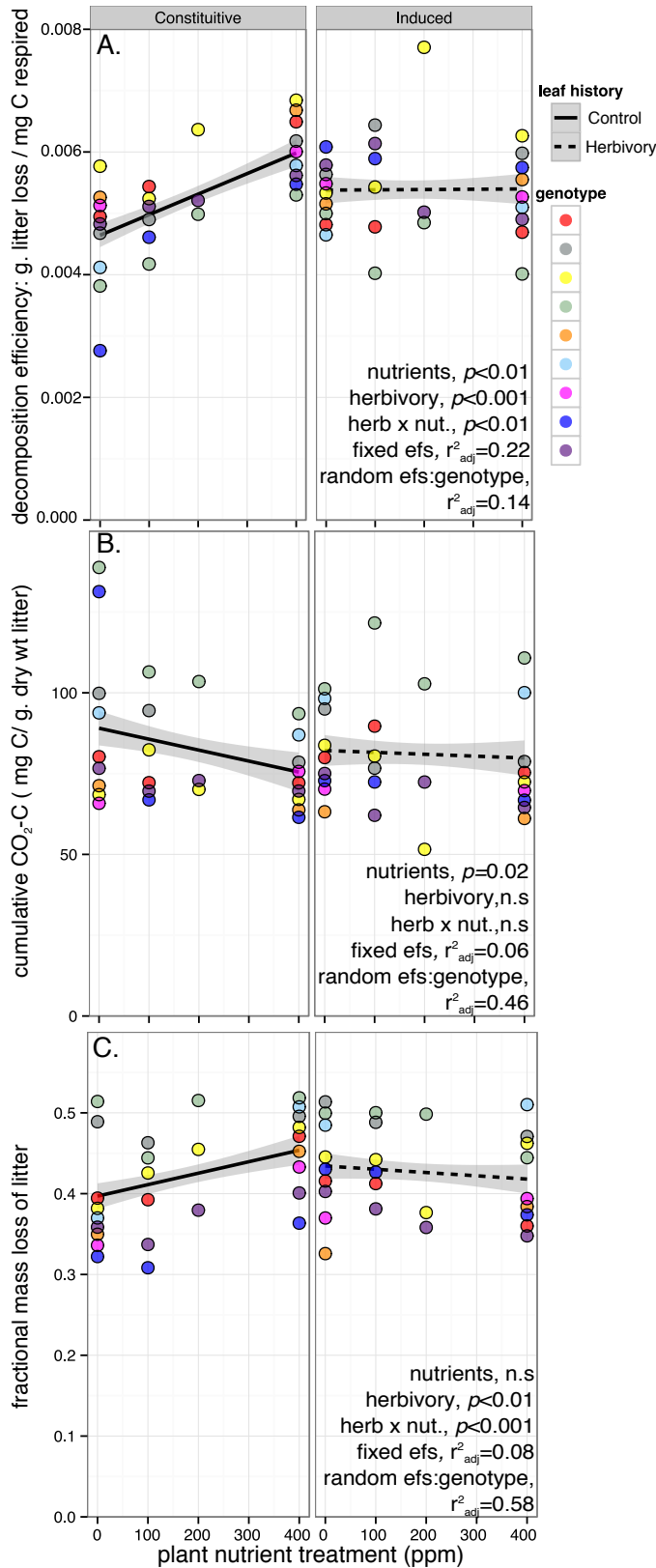
Figure 4.2: Legacy effects of nutrient and herbivory treatments on the decomposition of litter by a common microbial community as measured by A.) a decomposition efficiency index (litter mass loss/cumulative carbon mineralization). Previous nutrient supply and herbivory both alter decomposition efficiency with a significant herbivore x nutrient interaction. This occurred through B.) nutrient –based changes in cumulative carbon mineralization, and C. herbivory and herbivory x nutrient-based differences in litter mass loss. Circles are genotypic means and if points completely overlapped they have been jittered slightly to show color. The black line±SE (shaded area) is a linear model relating y~nutrient treatment (each panel separate).
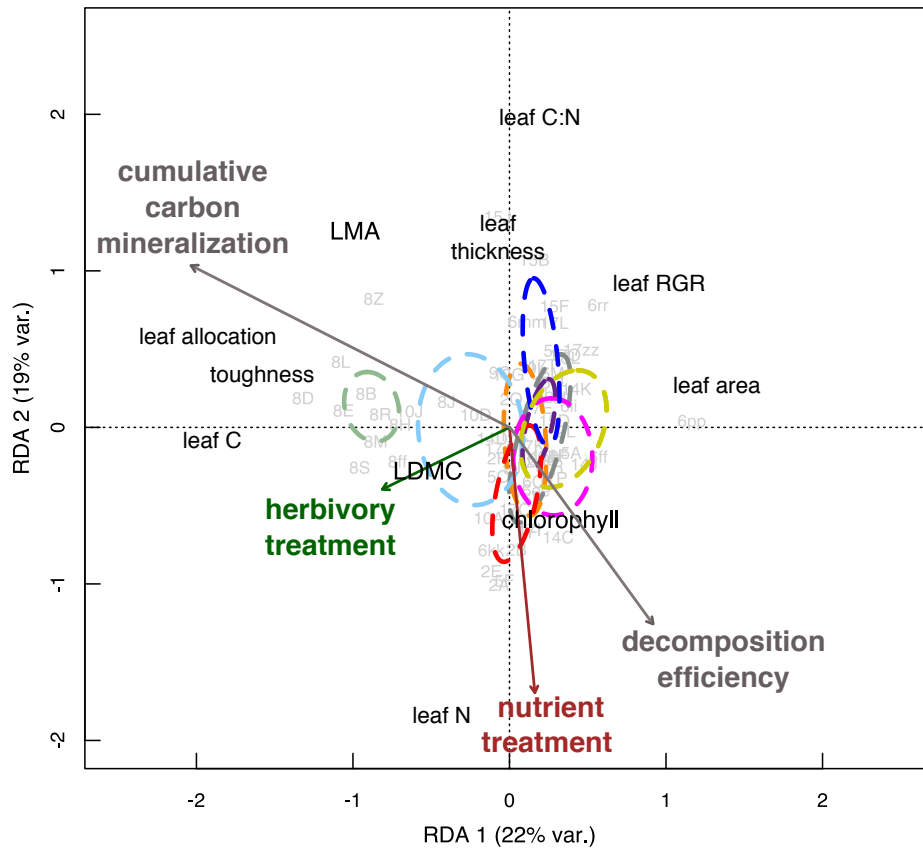
Figure 4.3: An RDA analysis constrained by nutrients, herbivory, and plant genotype explained 76% of the variation in leaf traits (r^2_{adj}=0.50). The first two RDA axes which only explain variation associated with those predictors is shown here. An individual plant's weighted average ("wa") score are plotted in gray with 95% CI ellipses plotted around the individuals of each genotype (colors as in Fig 4.1). Leaf traits in black are plotted at the end of their respective vectors (but arrows not shown). Vectors represent the direction in the RDA trait space which is most correlated with how plants respond to 1) the two treatments imposed on the plants (herbivores and nutrients) and predict 2) the cumulative carbon mineralization produced from the deposition of that individual's leaf tissue or the decomposition efficiency of the microbial community. The length of the vector is scaled on the r^2 from the regression of the environmental factor with the trait variation explained by the two axes. For example, nutrient treatments result in thin leaves with high leaf N and low C:N ratios. Herbivory resulted in leaf tissue with higher LDMC (leaf dry matter content) and leaf C content. Cumulative carbon respiration is highest on tough, high C litters, which are also general characteristics of the sage green and light blue genotype. All vectors displayed are significant at the p<0.05 level using a permutation test (cumulative carbon mineralization: r^2=0.55, p=0.003, decomposition efficiency r^2=0.38, p=0.002). These trait axes did not predict litter mass loss.
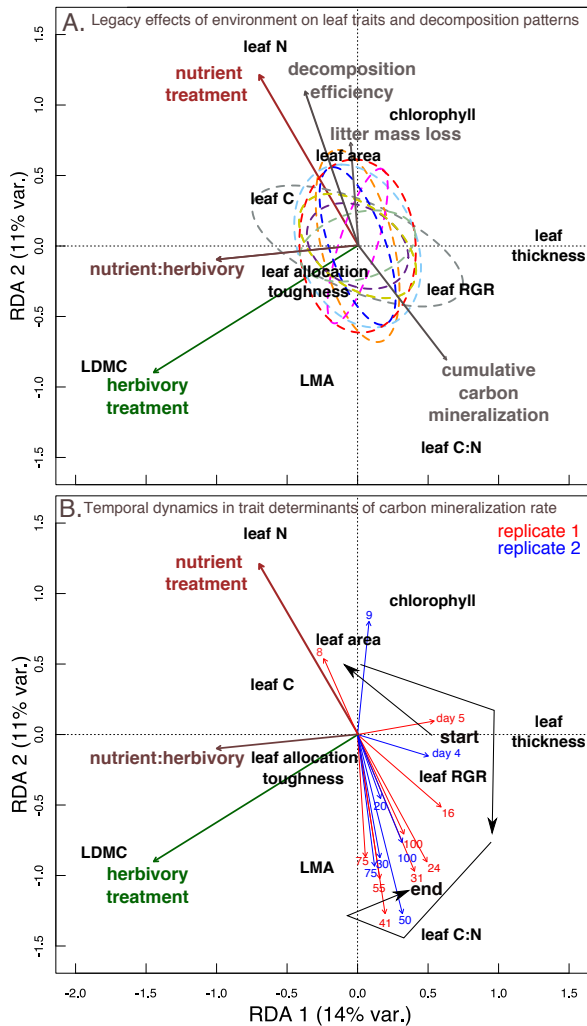
Figure 4.4: Both plots represent an RDA of the same trait data associated with individual plants as Fig. 4.2 but with variations associated with genotype (37%) removed first (i.e. conditioned out- all genotype ellipses are now centered at the origin) so that these RDA axes explain only the trait variation associated with nutrient and herbivory treatments. A.) shows genotype trait dispersion (ellipses), the correlations between leaf trait changes in response to treatment (black leaf trait names), which trait changes are associated with herbivory (green) and increased nutrients (brown) vectors, and how traits correlate with subsequent overall decomposition patterns (gray vectors). The vector length denotes the relationship strength. Only significantly associated vectors are shown. B.) The temporal dynamics of carbon mineralization across time point measurements in the assay. The vectors point toward the leaf trait most correlated with C-mineralization rates on that day. Vectors are only shown for days where trait variation associated with the treatments were significantly correlated with carbon respiration (3 out of 20 days were not explained by variation attributable to nutrient or herbivory treatments).
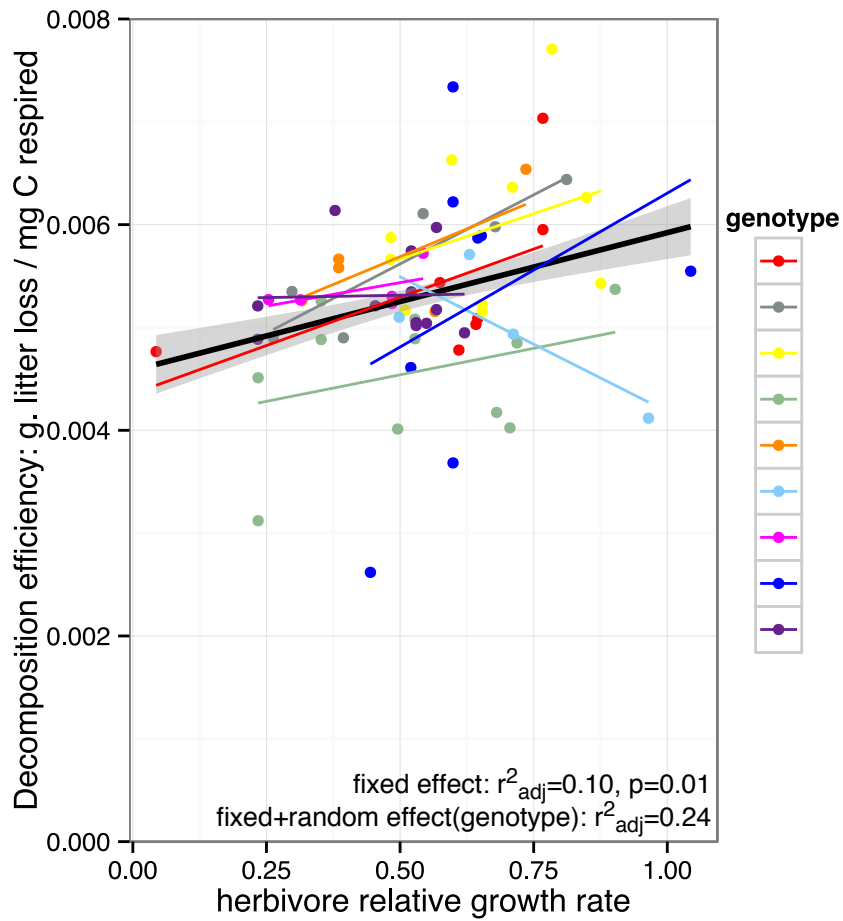
Figure 4.5: Relationship between living plant palatability to herbivores as measured by the growth rate of grasshoppers and the decomposition efficiency of the microbial community in breaking down leaf litter from the same plant.

Table 4.1: Vector fit results from Fig 4.4

Vectors	r2	*p*
Cumulative carbon mineralization	**0.07**	**0.003**
Litter mass loss	**0.05**	**0.012**
Decomposition efficiency	**0.23**	**0.000**
Replicate 1	r2	*p*
Day 5	**0.09**	**0.027**
Day 8	**0.10**	**0.007**
Day 11	0.03	0.274
Day 16	**0.18**	**0.001**
Day 24	**0.31**	**0.001**
Day 31	**0.32**	**0.001**
Day 41	**0.48**	**0.001**
Day 55	**0.31**	**0.001**
Day 75	**0.21**	**0.001**
Day 100	**0.16**	**0.001**
Replicate 2	r2	*p*
D2	0.02	0.337
D4	**0.07**	**0.026**
D6	**0.06**	**0.070**
D9	**0.16**	**0.001**
D14	0.00	0.798
D20	**0.06**	**0.020**
D30	**0.20**	**0.001**
D50	**0.43**	**0.001**
D75.2	**0.22**	**0.001**
D100.2	**0.17**	**0.001**

CHAPTER 5

HERBIVORE-INDUCED PHENOTYPIC PLASTICITY AND GENETIC

VARIATION IN GOLDENROD DETERMINE DECOMPOSITION AND SOIL

PROCESSES AT TWO NUTRIENT SUPPLY LEVELS IN A 3 YEAR MESOCOSM

EXPERIMENT

Summary

1. Community genetics and functional trait paradigms have both linked components of intraspecific variation to ecosystem processes. While this work has documented that dominant plant species can alter soil and decomposition processes through both genetic and environmental effects, respectively, these processes are rarely examined 1) concurrently 2) through the lens of both abiotic and biotic environmental changes or 3) linked to whole plant multivariate trait expression patterns.

2. Within a 3-year raised bed field mesocosm experiment I manipulated both the abiotic (nutrient supply) and biotic (herbivory) environments experienced by spatial clusters of four goldenrod (*Solidago altissima*) genotypes reflective of field-relevant levels of genetic variation. Environmental treatments were imposed for 2 years and then removed in the final year to determine how long the signature of environmental treatments remained detectable in plant traits. I followed the colonization and clonal spread of these genotypes, as the initial densities of 3 individual plants per genotype cluster in spring 2013 increased to up to 76 individuals by fall 2015. Throughout, I assessed 1) the relative impacts of genetic variation and environment on plant growth, allocation, and leaf traits; 2) soil processes in the raised beds beneath each

genotype; 3) herbivore performance through feeding trials; and 4) decomposition of senesced leaf tissue harvested from each genotype population.

3. I found a strong signal of genotype-based plant trait variation at the outset of the experiment. Environmental treatment effects could be detected in plant trait measurements within one month of each treatment and by the end of year two explained a larger relative proportion of trait variation than genotype. After the cessation of treatments, within a year, plant traits reverted to genotype based.

4. In general, genotype effects were the dominant predictor of plant allocation and demography traits (5 of 6), while plant leaf trait variation was dominated by herbivory effects (7 of 9) and often combined with genetic variation to determine plant traits (6 of 9). Nutrients played a role in determining leaf N and C:N ratios and mediating expression in other traits.

5. Treatments also predicted ecosystem processes. Genotype and herbivory treatments predicted litter decomposition efficiency and carbon mineralization patterns with a 9% reduction in each due to herbivory. Litter mass loss was 5% higher in litter from high nutrient beds. In soil sampled from beneath the genotype clusters, herbivory resulted in an 8% reduction in early season microbially available (labile) C. Interestingly, this pattern reversed by the end of the season with 12% higher labile C in herbivory treatment soils than controls. The genotype of a population was the primary determinant of spring plant available N and fall N mineralization potential.

6. A multivariate approach used to link genetic and treatment-based plant functional trait expression to ecosystem processes revealed that both genetic trait variation and plastic trait changes caused by herbivory (increased toughness, leaf mass area, C:N content etc.) explained or were correlated with the magnitude of a number of soil

processes with their relative importance process dependent. Litter decomposition patterns, which could be directly tied to leaf trait variation, were described better by plastic trait variation than genetic variation. Often models of trait variation that were partitioned into separate genetic and plastic components were better able to explain ecosystem process measurements than combined models, highlighting the importance of partitioning intraspecific variation into its component parts.

7. Taken together, this study demonstrates the complex interplay between genetic and environmentally based trait variation in determining population and ecosystem processes within landscapes. Understanding how these sources of intraspecific variation interact to determine ecosystem and community processes should increase the predictive power of functional trait-based approaches deployed to understand trait responses to changing environments.

Introduction

Intraspecific variation encompasses both genetic and environmental (plastic) sources of phenotypic variation along with their interaction (GxE)(Whitman & Agrawal 2009). Both genetic and plastic trait variation have been independently found to influence ecosystem processes. For example, the community genetics paradigm in plant ecology (Antonovics 1992) relates genetic variation within dominant or foundation species to ecosystem processes (Wimp *et al.* 2005; Whitham *et al.* 2006). This paradigm holds that differences in the level of ecosystem processes come from within-species genotypic differences in trait expression. In particular, genetic variation in the expression of anti-herbivore defense traits has been found to influence plant litter chemistry and decomposition (Schweitzer *et al.* 2004; Schweitzer *et al.* 2008). Orthogonal to this is the functional traits paradigm in plant ecology, which measures

how trait expression of different plant species varies across environmental gradients, and, more recently, compares the plastic expression of plant traits across environmental gradients (Wright *et al.* 2004; Violle *et al.* 2007; Wright & Sutton-Grier 2012; Kraft *et al.* 2015). Plastic functional trait variation (such as anti-herbivore defense) is then linked to ecosystem process rates (Hunter 2001; Madritch & Hunter 2005; Frost & Hunter 2008a).

There have, however, been growing calls to combine the salient components of both paradigms in order to understand the role of different components of intraspecific variation (genetic and plastic) in determining functional trait expression and ecosystem processes (Albert *et al.* 2011; Violle *et al.* 2012; Siefert *et al.* 2015; Crutsinger 2016). Whether intraspecific variation in plant traits that determine ecosystem processes arises through genetically based sources, as investigated by the community genetics paradigm, or plastic responses to environments as investigated by the functional traits paradigm, is important for predicting process rates across gradients and in response to anthropogenic environmental changes. Such integration is timely. Genetically based plant trait expression is known to be dependent on the environmental context in which an organism lives through phenotypic plasticity in trait expression patterns (Agrawal 2001; Johnson & Agrawal 2005; Schweitzer *et al.* 2005). Thus, across environmentally heterogeneous landscapes (ubiquitous in real ecosystems (Hakes & Cronin 2011)), phenotypic plasticity can either mask or accentuate genetically based effects on ecosystem processes (Chapman *et al.* 2003; Schweitzer *et al.* 2005). Further, developmental environment (such as nutrient supply) may alter the ability of an organism to respond plastically to later environments (such as herbivory) altering the mean and variance of trait expression patterns found across environments, even potentially for the same group of genotypes.

Analyses of genotypic and plastic effects have typically focused on one or few traits at a time. Yet plant resistance to herbivores is often correlated with changes in plant structure, allocation, and growth strategies, in addition to secondary defense chemistry (Agrawal & Fishbein 2006; Carmona *et al.* 2011; Carmona & Fornoni 2013; Heath *et al.* 2014). This suggests that a multivariate approach to understanding the genetic and plastic effects of plant traits on ecosystems processes may be more appropriate than single trait approaches (Walsh & Blows 2009; Kraft *et al.* 2015). This may be especially crucial when examining plant trait expression along multiple environmental gradients (Chapter 4).

Two key environments gradients that may alter plant defense expression are nutrient supply and the presence of herbivores. Plants exhibit plastic responses in trait expression to both herbivory (through induced chemical or physical defense and physiological changes) and nutrient supply (Coley *et al.* 1985). Further these two environmental factors may be linked though feedback mechanisms (Hobbie 1992; Bardgett & Wardle 2003; Baxendale *et al.* 2014). For example, herbivores can influence ecosystem processes either directly through greenfall, canopy leaching, excretion, or carcass inputs (Hunter 2001; Frost & Hunter 2008b; Hawlena *et al.* 2012) or indirectly through changes in plant trait expression within the uneaten plant tissue that then alter ecosystem processes through so-called "after-life" effects (Choudhury 1988; Grime *et al.* 1996; Genung *et al.* 2013). Theoretical frameworks posit that herbivores can either decelerate or accelerate nutrient cycling within ecosystems. Deceleration would occur through selective feeding of herbivores on palatable species or genotypes resulting in in decreases in community or population level tissue quality with subsequent decreases in nutrient cycling due to low quality litter inputs (Ritchie *et al.* 1998; Schadler *et al.* 2003). Alternately, herbivory may accelerate nutrient cycling through high quality frass and carcass inputs coupled with high quality plant regrowth tissue (McNaughton

et al. 1989; Belovsky & Slade 2000; Bardgett & Wardle 2003; Chapman *et al.* 2003) which may feedback to increase nutrient cycling (Baxendale *et al.* 2014). Numerous studies have then tried to predict in which ecosystems and species you might expect to see either pattern (Schweitzer *et al.* 2005; Chapman *et al.* 2006).

A recent greenhouse experiment with several genotypes of tall goldenrod (*Solidago altissima (L)*), a species dominant in eastern USA old-field communities, suggests an intriguing alternative (see Chapter 4). The experiment revealed that a plant genotype's plastic response to herbivores depends on nutrient supply in ways that alter the magnitude and direction of ecosystem processes. Genotypes grown at high nutrient levels induced resistance with the corresponding trait changes leading to decreased litter decomposition efficiency of herbivore legacy litter relative to control litter. In contrast, at low nutrient supply, plants did not induce a resistance response (instead becoming more susceptible to herbivores) and subsequently the litter from those plants decomposed more efficiently compared to control litter. This suggests that the interaction between herbivory and nutrient supply should cause context-dependent acceleration or deceleration of nutrient cycling such that plant genotype and trait plasticity mediate effects of multiple environmental conditions on ecosystem process in this system.

Here, I examine this possibility by tracking genetic and plastic trait expression of goldenrod within a 3-year field mesocosm experiment. Genotypes of goldenrod were from one source field population and thus represent a realistic range of genetic variation found within a typical locally interacting field population. This was paired with experimentally manipulated field-relevant levels of environmental variation (herbivory and nutrient supply). First I investigate how quickly plant trait plasticity is detectable after environmental treatments are applied and how long the signature of environment persists after treatments

are removed. Next using a univariate mixed model approach, I examine how genotype, nutrient supply, and herbivory shape plant trait expression and ecosystem processes including plant litter decomposition, soil N build-up and soil C release. While previous work has already documented that dominant plant species can alter soil and decomposition processes through both genetic and environmental effects ((Whitham *et al.* 2006; Frost & Hunter 2008a; Schweitzer *et al.* 2008)), these processes are rarely examined concurrently, examined through the lens of both abiotic and biotic environmental gradients, or linked to whole plant multivariate trait expression patterns (Walsh & Blows 2009; Kraft *et al.* 2015; Crutsinger 2016). To examine the last question, I go an additional step and partition the genetic and treatment-based multivariate variation in plant traits within each experimental population and then link each component to a suite of soil and decomposition processes. This elucidates the correlated phenotypic trait changes that underlie genetic and environmental effects on ecosystem processes.

Specifically I address the following questions:

1. What is the relative magnitude of genetic vs. environmental (nutrient supply and herbivory) determinants on plant traits across the experiment? How quickly do environmental treatments cause measurable plant trait changes and how long do the changes last after the cessation of treatments?

2. What are the direct effects of genotype, herbivory, and nutrient treatments on plant trait expression, plant population metrics, litter decomposition, soil processes, and herbivore performance?

3. Can differences in soil processes, leaf litter decomposition, and herbivore performance be directly tied to genetic and environmental sources of trait variation?

Which traits are key determinants of these processes and are they genetically-based, plastic, or both?

4. Taken together, do the measured patterns support the hypothesis of nutrient-dependent effects of herbivory on deceleration or acceleration of ecosystem processes (herbivory x nutrient interactions)?

Materials and Methods

Overview

The 3-year experiment reported here resolves the linkage between trait plasticity of different tall goldenrod (*Solidago altissima*) genotypes and ecosystem processes. Using a split-plot design in raised-bed mesocosms, I manipulated nutrient supply (abiotic environment) and grasshopper herbivory (biotic environment) within spatially clumped, enclosed groups of 4 different genotypes collected from a single old-field population (with starting densities of 3 clonal individuals). Experimental treatments were imposed for two years and then removed in the final year to determine how quickly plant trait variation returns to being entirely genetically defined. Plant growth, population parameters, and leaf trait measurements were taken in all years. In the second year, I also measured patterns of plant genotype allocation to flowers, stems and leaves, and measured genetic and environmental treatment effects on soil processes (soil carbon mineralization potential, build-up of plant available N, net nitrogen mineralization potential, and microbial biomass using substrate induced respiration). Measurements taken from soil within the beds potentially include both direct and indirect effects of herbivores. To isolate the effect of herbivore indirect effects through induced plant trait changes, I also deployed two, companion lab-based assays. The first was an herbivore feeding trial using leaf tissue collected from each population to

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understand how herbivores might respond to leaf trait variation induced by the mesocosm treatments. Secondly, I measured the implications of this leaf trait variation for plant litter decomposition using a lab microcosm assay where litter from each genotype population was seeded with a small quantity of a common soil innocula and decomposition was tracked for 100 days using microbial respiration measurements.

Study species and system

Tall goldenrod (*Solidago altissima*), is a rhizotomous perennial that can exist in dense stands with up to 95% cover (Maddox & Root 1990). Once established in fields, *S. altissima* spreads primarily through clonal growth of deciduous ramets that remain within 0.5 m of the parental ramet and result in dense clumps of genetically identical individuals within fields (Cain 1990). *S. altissima* faces herbivory from a dominant generalist leaf-chewing insect herbivore, the red-legged grasshopper (*Melanoplus femurrubrum* which can result in important effects on old-field plant community structure and ecosystem functioning (Schmitz 2006). *S. altissima* exhibits a variety of plastic responses to herbivores through both tolerance and resistance traits. Tolerance manifests as increased relative growth rate and photosynthetic rate (Meyer 1998). All *S. altissima* plants contain background levels of chemical and structural defense (constitutive resistance) and they are capable of heightening chemical defense through induction of protease inhibitors, phenols, and diterpenoids in response to herbivore foraging (Cooper-Driver & Le Quesne 1986a; Abrahamson & Weis 1997; Johnson *et al.* 2007; Bode & Kessler 2012). Previous experimentation with *S. altissima* genotypes collected from the same source field demonstrated substantial genetic variation for plant traits, defense induction, and tolerance of herbivory that differed with level of soil nutrient supply (Chapter 3). Moreover, the mass ratio hypothesis (Grime 2001) posits that plant species like

S. altissima, that dominate fields should have a strong influence on ecosystem nutrient cycling, especially as succession progresses (Uriarte 2000). Hence, the extended temporal and numeric dominance of *Solidago* in combination with clustering of related individuals with similar defensive trait expression (through either genetic or environmental sources) across the landscape, make this an ideal system for investigating how genotypic-dependent variation in plant defense trait expression might influence community interactions and heterogeneity in soil nutrient pools.

Raised bed mesocosms

In fall 2012, I constructed 24, 1 m x 2 m wooden raised-bed mesocosms that were lined with landscape cloth at the bottom to allow drainage but not root encroachment. Each mesocosm was filled with a homogenized 1:1 mixture of sand and local old-field topsoil. Soil was mixed within each bed using a gas-powered tiller (MacKissic Mid-Tine MT4H) and then partitioned in the middle to physically create a split-plot that prevented belowground interactions between treatments on each side of the mesocosm (Fig. 1b) Soil was conditioned over the winter to allow a return to natural soil processes after the disturbance. In June 2013, 72 clones each of four genotypes that had been started in the greenhouse (see plant propagation section) were transplanted into the raised beds in groups of 3 spatially clumped clonal individuals within each treatment unit (Fig. 5.1b). The traits and plastic responses to herbivory and nutrient supply of these specific genotypes were previously characterized in a greenhouse experiment (see Chapter 3). After transplant, 2 m tall cages constructed of Lumite mesh were placed over the entire bed over a PVC frame (see Fig 5.1b) with an additional mesh partition splitting herbivory treatments.

Environmental treatments:

Nutrient treatments (either high or low) were applied at the bed level 3 times over the growing season in year 1 and 2 (Year 1: applications occurred on June 16, July 12, August 12; Year 2: applications occurred on May 27, June 21, July 18). Within each "high nutrient" bed, 10 L of a 400 ppm solution of a total fertilizer (Peters Excel fertilizer 15-5-15 N:P:K Cal-Mg special, Everris) dissolved in water was applied evenly using a backpack sprayer to each side of the bed (herbivory and control). "Low nutrient" beds received simply an equivalent quantity of water. As nutrients were applied in dissolved form, application dates were adjusted to coincide with dry periods to avoid excessive nutrient leaching. In year 1 on July 27, 3rd instar *M. femurrubrum* grasshopper nymphs were collected from the same source field as the goldenrod rhizomes and placed within the randomly assigned herbivory treatment side of the raised bed. Grasshoppers were stocked at a density of one nymph per two ramets (3 grasshoppers total per bed). Densities were kept low in year one to allow plant establishment and this resulted in average damage of ~5-6% (cf. 11% average damage measured on individuals of the same genotypes exposed to natural herbivory in the adjacent field). In year 2, on June 22, 3rd instar nymphs were again collected and placed within the mesocosms (application dates changed based on yearly variation in grasshopper emergence dates). Production of new rosettes from each of the original clumps of 3 parental clones ranged between 17-72. The applied grasshopper density was accordingly adjusted to the density of ramets in each bed, to produce a density of one grasshopper per 4 ramets. Due to early emergence of grasshoppers in 2014 there was a longer period of herbivory than in 2013 and this number of herbivores resulted in 7-12% leaf tissue removal, which falls within field estimates that year (Burghardt, unpublished data).

Plant propagation

Genotypes were grown in a common greenhouse environment in large pots for two generations before they were used within this experiment. Doing this removed carry-over effects from the maternal environment. In April 2013, rhizomes were harvested from the pots and cut into 2 mL volume sections, determined by water displacement in a graduated cylinder (Abrahamson & Weis 1997). Sections were planted into individual 9 cm pots in a mixture of 50% sterilized potting soil (Pro-Mix BX, Premier Brands, New Rochelle, NY) and 50% clay medium (Turface MVP, PROFILE Products LLC, Buffalo Grove, Il) and sprouted. When plants were 15 cm tall they were supplied with a 100 mL solution of total fertilizer (Peters Excel fertilizer 15-5-15 N:P:K Cal-Mg special, Everris) dissolved in water to yield a nitrogen (N) concentration of 400 ppm.

Plant growth, performance, and allocation

Clonal fitness was tracked over the experiment by nondestructively monitoring individuals' production of new clones each spring, and survivorship of ramets in the fall. Clonal reproduction is a more appropriate measure of fitness than sexual reproduction once *S. altissima* plants become established in a field (Cain 1990). At the end of year 2 on September 29 all aboveground plant biomass was harvested, separated into flower, stem, and leaf portions, dried at 50°C and then weighed to determine proportion allocation to plant parts between genotype clusters. A small portion of the senesced leaf litter was reserved for a decomposition assay and then the rest of the litter was replaced to the location in the raised bed from which it originated.

Plant leaf traits and nutrient content

Plant leaf trait measurements were collected in year 1 on July 25 (after nutrient treatment but before herbivore introductions) and September 9 (after both treatments), in year 2 on August 22, and in year 3 on August 16 (see Fig 1a). Each time, I harvested one of the most recent fully expanded, undamaged leaves from each plant for leaf trait measurements (3 per experimental unit). Only 3 ramets were present in each experimental unit in the first year and all were sampled. The genotype clusters in year 2, which ranged from 17-72 individual ramets, made leaf trait assessment of each impractical. Instead 3 ramets were selected to sample leaf traits in a stratified random manner (3 individuals across the clump as evenly spaced as possible). I measured toughness of each leaf using a penetrometer that revealed the force needed to puncture a leaf at a position next to but not including the midvein. I measured leaf area using a leaf scanner and ImageJ software. Leaves were rehydrated, weighed wet, and then dried at 50°C and reweighed. These weights were used to calculate LMA [leaf mass per area], LDMC [leaf dry matter content], and leaf thickness (Vile *et al.* 2005). Dry leaf tissue was ground and analyzed for C and N content analysis using an elemental analyzer (Thermo DeltaPlus Advantage coupled to a Costech ECS 4010 Elemental Analyzer via a Conflo III interface). Leaf nutrient content was only assessed once in year 1 (September 20) and once in year two (September 1). Mean experimental unit averages were calculated for each trait where multiple measurements were made for use in analysis.

Soil process measurement

In year 2 (2014) I also sampled the soil below the genotype population within each experimental unit on May 22 (before treatments began for the season) and again on October 1st at the end of the season (n=96). I took 2 samples to a depth of 10cm within each

treatment, homogenized them, sieved to 4 mm, placed a subsample in a 50 mL prewieghed centrifuge tube with 25 mL of 2 M KCl solution for nitrogen extractions. The soil solutions were packed on ice and transported to the lab where they were kept at 5°C until assays were performed. Once back at the lab I placed the nitrogen extraction tubes in a shaker for 1 hour, then capped and refrigerated them overnight before extracting 10mL of solution and freezing the samples until analysis (Robertson *et al.* 1999).

Net nitrogen mineralization potential was calculated by weighing out 5 g dry weight equivalent of additionally sampled soil into a centrifuge tube, adjusting water holding capacity to 65%, covering the tubes, and then incubating at 20°C at 100% humidity (to preventing drying). After 28 days, I performed another KCl extraction on the incubated soil and compared it to the initial extraction to measure net nitrogen mineralization and nitrification rates. I used a FlowAnalyzer to measure plant available forms of nitrogen- nitrate (NO_3^-) and ammonium (NH_4^+).

I used a soil carbon mineralization assay to measure microbially available soil carbon (Bradford *et al.* 2008a). Two sub-samples of 6 g equivalent dry weight soil were placed in individual 50 mL centrifuge tube microcosms. Soil was maintained at 65% water holding capacity and incubated in a 20°C dark growth chamber maintained at 100% humidity to prevent drying. For 5, 24-hour windows across the 55-day assay (day 1, 5, 14, 30, 55) microbial respiration (carbon mineralization) rates were measured within each microcosm. This was accomplished by capping each microcosm, flushing the headspace with CO_2 free air, incubating for 24 hours, and then measuring the CO_2 content of the air from the headspace over the litter sample with an infrared gas analysis technique (IRGA- Li-COR model LI-7000, Lincoln, NE, USA). Cumulative carbon respiration rates were calculated by integrating rate values across the 55 days.

I estimated relative soil microbial biomass using substrate induced respiration (SIR) (Fierer *et al.* 2003). A solution of autolyzed yeast extract (a labile C source) was mixed with 4 g dry weight equivalent soil that was conditioned in an incubator overnight, then shaken for one hour, flushed with CO_2 free air and capped. After a 4 hour incubation at 20°C, microbial respiration rates were estimated as described above for the carbon mineralization assay. Here, however, microbes should not be carbon limited, thus giving a relative estimate of microbial biomass.

Litter decomposition assay

Litter from each genotype cluster collected in at the end of year 2 (fall 2014) was homogenized and then milled to pass through a 4 mm sieve. Four samples of the top 7 cm of surface soil below the litter layer were collected from the same source field as the plants, transported to the lab, homogenized, sieved to 2mm, and then frozen at -20°C (to kill invertebrates but not microbes) prior to use in the decomposition assay. I prepared 50 mL centrifuge microcosms with a subsample of litter substrate (1 g dry weight equivalent), and then seeded it with a 0.5 g dry weight equivalent of soil inoculum to provide a common initial microbial community. The inoculum represented only 10% of the volume of the litter and thus contributed little C and N to the microbes. (Microbial respiration in soil-only microcosms was 0.3-1.2% of the respiration rate of soil+litter microcosms of identical weight.)

This method is an adaptation of a standard method (Bradford *et al.* 2008a; Strickland *et al.* 2009; Keiser *et al.* 2011) where a common microbial community is used to decompose litter of varying sources to assess the relative quality of the litter. Litter and soil were mixed together within the microcosms, adjusted to 65% water holding capacity, and then incubated

within a 20°C dark growth chamber. Microbial respiration (carbon mineralization) rates were measured within each microcosm for ten, 24-hour periods across the 100-day assay (Day 5, 8, 11, 16, 24, 31, 41, 55, 75, 100). This was accomplished by capping each microcosm, flushing the headspace with CO_2 free air, incubating for 24 hours, and then measuring the CO_2 content of the air from the headspace over the litter sample using an infrared gas analysis technique (IRGA- Li-COR model LI-7000, Lincoln, NE, USA). Cumulative carbon respiration rates were calculated by integrating rate values across the 100 days. At the end of the 100 days, the litter remaining was oven-dried at 60°C and weighed to calculate litter mass loss. A decomposition efficiency metric was calculated by dividing the litter mass loss by the cumulative carbon mineralization. The mass loss per unit of carbon respired is an index of decomposition efficiency, as it implys that the microbial community respires less carbon for a given amount of litter decomposition (see Chapter 4 discussion for a detailed treatment).

Herbivore performance assay

In year 2 I also collected juvenile *M. femurrubrum* grasshoppers from the same source field as the *S. altissima* a performance assay. Collected grasshoppers were fed a common diet of lettuce and bran for 48 hours, food-deprived for 12 hours, and then weighed. On August 22 I harvested one undamaged, recently expanded leaf from 3 plants within each experimental unit. Cut ends of the 3 leaves were placed in one water tube to maintain moisture content and transported back to the lab. Leaves were placed within a plastic microcosm, and covered with mesh. I then placed the previously food-deprived grasshoppers onto leaves housed within individual lab microcosms. After 48 hours of feeding, leaf tissue was removed from the microcosm, scanned for leaf area loss and weighed. Twelve hours after leaf removal grasshoppers were weighed and used to calculate

grasshopper relative growth rate [where relative growth rate = (final mass-initial mass)/initial mass] and weight of leaf tissue removed per gram grasshopper (Kempel *et al.* 2011).

Statistical analysis

   I calculated mean trait values of all genotypic clones within a treatment and the mean level of soil process associated with clones in each treatment. This yielded 96 experimental units (4 genotypes x 2 nutrient levels x 2 herbivory levels x 6 replicates) available for analysis. The split plot aspect of the design was accounted for using a random effects structure within linear mixed models and conditioning in RDA. All analyses were completed in R (R Development Core Team 2009).

   I first performed separate univariate linear mixed effects analyses using the *lmer* function in the package lme4 (Bates *et al.* 2012) to assess treatment effects on the response variables of plant trait expression, soil processes, and litter decomposition respectively. The models used nutrient treatment, herbivory treatment, and genotype and their interactions as fixed effects. Response variables were transformed as necessary to fit model assumptions of normality and homogeneity of variance. Random effects for block, raised bed, and genotype combination in a bed were included in all models to account for the experiment split plot design. The significance of fixed effects was judged using F-tests (Zuur *et al.* 2009). Degrees of freedom (Satterthwaite approximation), type III SS, and p-values were calculated using *lmertest* (Kuznetsova *et al.* 2014). The r^2 values of the fixed and random components in each model were calculated to provide a relative measure of the quality of the fit for each model (Nakagawa & Schielzeth 2013). The *standardize* function within the *arm* package was used to calculate and compare effect sizes among significant factors (Gelman 2008).

The responses of plant and leaf traits to treatments are often highly correlated. I therefore used redundancy analysis (RDA), a constrained multivariate approach within the *vegan* package in R (Oksanen *et al.* 2012), to quantify and visualize how the entire suite of measured plant traits changed in response to treatments. I first examined the trait responses to treatments across the 3-year experiment by building a RDA trait space of trait variation attributable to genotype, herbivory and nutrient treatment for each sample date. I then examined the 2014 season data more closely using individual partial RDAs to partition out trait expression changes attributable to genetic vs. environmental treatments. To do this I built a series of models that systematically examined the variation attributable to one treatment and excluding variation attributable to other treatments. I then did a post-hoc analysis that fit concurrently collected ecosystem process measurement vectors to each RDA trait space to test whether the trait changes attributable to the treatments could explain variation in the measured ecosystem and decomposition processes (approach described in detail below). This approach allows testing for the causal relationship between leaf trait expression and subsequent decomposition rates, as well as effects of herbivore feeding rates during lab-based assay. While it cannot address causality in the raised bed experiment, because feedbacks may be present among traits and soil processes, the analysis nevertheless illuminates correlations between treatment-induced plant trait variation and ecosystem processes. This approach has an advantage over typical multiple regression model selection procedures because it removes effects of multicollinearity of the explanatory variables (plant traits).

More specifically, RDA is a multivariate linear regression, followed by a PCA of the fitted values to create constrained RDA axes. These axes display variation associated with the predictors. I used a permutation analysis to determine the significance of the predictors

on the observed multivariate trait data (analogous to non-parametric PERMANOVA). Visualization and interpretation of the axes is similar to PCA, but the first canonical axis is constrained only to represent the variation explained by the linear predictors in the model (here, herbivory, nutrient supply, and genotype). The response matrix (similar to the "species" matrix in the community analyses where this approach is often used) consisted of columns of mean plant allocation and leaf trait values with a row ("site") for each experimental unit (n=96). Trait values were transformed as necessary to conform to the assumption of multivariate normality and standardized by scaling to a variance of 1. All models were run with the variation associated with block effect removed to account for the experimental structure (similar to a random effect).

I examined temporal dynamics, by performing independent RDA analyses for each year's data on plant trait variation using plant genotype, herbivory, and nutrients as predictors. I then examined trends in the relative genetic and environmental components of plant trait variation measured at the end of the environmental treatments in 2014. I did this by running 3 RDA models. The first examined variation attributable to genotype (genotype as fixed effect with variation attributed to herbivory, nutrient treatment and block removed), the second considered only environment (herbivory and nutrient treatment as fixed effects with variation attributed to genotype and block effect removed), and the third model combined the two (genotype, nutrients, and herbivores as fixed effect and block effect removed).

Litter decomposition, soil process, and herbivore feeding response vectors were fit to the RDA axes, for post-hoc evaluation, using the command *envfit* to quantify how decomposition metrics (decomposition efficiency, cumulative carbon mineralization, litter mass loss) related to the leaf trait changes caused by herbivory and nutrient treatments.

Vectors represent the direction of the ordination along which the response variable is increasing most rapidly and is most correlated with the trait values. The lengths of the vectors reflect the strength of the relationship, as measured by r^2 and are calculated using a permutation test with 5000 replications. Vectors are only retained and plotted if significant at the $p<0.05$ level. These tests (and the r^2 values) are analogous to performing a regression relating the response variable to the RDA1+RDA2 axes.

Results

Temporal patterns in trait changes across the experiment

Genotype-based differences in plant traits (here, height and leaf number) were already detectable prior to implementation of treatments (Fig. 5.2a; herbivory: $F_{1,78}=0.07$, *n.s*, nutrient: $F_{1,78}=0.48$, *n.s*, genotype: $F_{3,78}=9.29$, $p=0.001$, $r^2=0.22$, $r^2_{adj}=0.13$). One month after nutrient treatments were initiated, genotype differences were still evident but all plant genotypes in the high nutrient treatment had increased leaf toughness, number of leaves, and lateral auxiliary stems sprouting from their bases (Fig 5.2b; herbivory: $F_{1,78}=0.549$, *n.s*, nutrient : $F_{1,78}=2.90$, $p=0.02$, genotype: $F_{3,78}=10.44$, $p=0.001$, $r^2=0.26$, $r^2_{adj}=0.16$). At this point herbivores were stocked within the cages. Herbivore effects on plant traits (LMA, leaf C content, and leaf toughness) became evident by the end of the growing season, and nutrient effects were no longer evident (Fig 5.2c; herbivory: $F_{1,78}=2.60$, $p=0.04$, nutrient: $F_{1,78}=1.16$, *n.s*, genotype: $F_{3,78}=9.04$, $p=0.001$, $r^2=0.31$, $r^2_{adj}=0.19$). After one more year of persistent treatment application, the nutrient and herbivore effects increased in importance to match the importance of genotype, with all being significant predictors of plant trait variation (Fig 5.2d; herbivory: $F_{1,78}=5.75$, $p=0.001$, nutrient: $F_{1,78}=4.06$, $p=0.001$, genotype: $F_{3,78}=7.99$, $p=0.001$, $r^2=0.33$, $r^2_{adj}=0.22$). Trait plasticity was ephemeral, however, as the

effects of nutrients and herbivory vanished by the end of the 2015 season, one year after treatments were removed (Fig 5.2e; herbivory: $F_{1,78} = 0.808$, *n.s.*, nutrient: $F_{1,78} = 1.15$, *n.s*, genotype: $F_{3,78} = 6.33$, *p*=0.001, r^2=0.33, r^2_{adj}=0.22). No interactions between the predictors were detected for any sample date.

Genetic and environmental effects on plant traits and ecosystem processes (univariate models)

Leaf trait expression: Seven of the nine measured leaf traits were differentially expressed in herbivore-exposed genotype clusters (Fig. 5.3 and Table 5.1a for full statistical tables). Standardized coefficients from the univariate mixed effects analyses revealed that herbivory caused a 14% increase in leaf toughness, a 10% increase in LMA, a 5% increase in LDMC, a 5% increase in leaf thickness (9% at high nutrient levels, herbivory x nutrient, see Fig. 5.3d), a 20% increase in leaf area (only at high nutrients, herbivory x nutrient, Fig. 5.3e), a 7% decrease in N, and 3% increase in C:N ratio. Genotypic variation was evident in six of nine leaf traits (leaf toughness, LDMC, leaf thickness (herbivory x genotype), leaf area, rust infection, leaf N, and leaf C:N ratio). Nutrient supply only had consistent effects on leaf N which increased 13%, and leaf C:N ratio which decreased 6%. Herbivory effects on leaf thickness and leaf area depended on nutrient supply (significant herbivory x nutrient interaction, see above). Leaf carbon content was the only plant trait not explained by any fixed effect.

Plant allocation and growth patterns: The quantity of aboveground biomass produced was significantly predicted by plant genotype, and ranged from an average of 200 g for the lowest producing genotype to over 500 g on average for highest producing genotype. However

there was also significant genotype x herbivory effects, indicating a difference in tolerance to herbivory (in terms of biomass) among all the genotypes (see Fig 5.4a and Table 5.1b for statistical models for all plant growth and allocation response variables). Further an herbivory x nutrient interaction revealed that the impact of herbivores on biomass differed according to level of nutrient supply. In contrast the season-long loss of the ramets produced in spring 2014 was clearly tied to herbivory treatment (13% lower survival in the herbivory treatment, see Fig 5.4b). The number of ramets that emerged in spring 2015 following cessation of the treatments was only explained by plant genotype and varied widely between genotypes (Fig 5.4c). Plant allocation to stem, leaf, and flower biomass were all strongly tied to genotype, which often resulted in large differences in allocation patterns (e.g. up to 38% higher flower allocation in the orange genotype) (see Fig 5.4f and Table 5.1b for statistical models).

Decomposition of leaf litter from the mesocosm genotype clusters: Microbes decomposing litter from plants subjected to herbivory treatment respired 8% more total carbon than microbes decomposing litter from control plants (cumulative carbon mineralization Fig. 5.5b, Table 5.1c). Fractional litter mass loss from the same decomposition assay was only explained by nutrient treatment (high nutrient legacy litter lost 5% more mass than low nutrient litter). I combined these two metrics to produce a decomposition efficiency metric that estimates the amount of carbon that microbes must respire for a given amount of mass loss. This metric revealed that microbes had 9% lower efficiency on leaf tissue from herbivore treatments than on litter from controls. Litter from different plant genotypes decomposed with different efficiencies (Fig. 5.5a, Table 5.1c).

Soil processes and nutrient availability: The presence of herbivores in Year 1 (2013) decreased Year 2 (2014) spring microbially available carbon (the labile C pool) by 8%, regardless of nutrient environment (Fig 5.5d, Table 5.1d for all statistical models). By fall 2014, the microbially available C pool in soils beneath plants with herbivores had only decreased 2% from spring levels while soils from plants in control conditions decreased 14% (Fig.5.5e). Both spring total plant available N levels (NO_3 and NH_4) and fall net nitrogen mineralization potential (Fig 5.5g and h) showed genotype effects on nitrogen dynamics. Microbial biomass (SIR) was not predicted by any fixed effect terms (Fig 5.5f).

Linking ecosystem effects to plant trait expression changes (multivariate models)

Treatment-based changes in plant allocation and leaf traits and soil processes: Partial RDA analyses, used to examine the relative explanatory power of genotype versus environment on variation in plant growth, allocation, and multiple leaf trait multivariate expression patterns revealed that trait expression patterns were significantly effected by 1) genotype (Fig 5.6b; genotype: $F_{3,78}$ =7.66, p=0.001, r^2=0.18), 2) biotic and abiotic environment (Fig 5.6c; herbivory: $F_{1,78}$ =6.0, p=0.001, nutrient : $F_{1,78}$ =3.9, p=0.001, r^2=0.09) as well as 3) and their combined influence (Fig 5.6d; herbivory: $F_{1,78}$ =5.8, p=0.001, nutrient : $F_{1,78}$ =3.72, p=0.003, genotype: $F_{3,78}$ =7.44, p=0.003 r^2=0.33). Post-hoc vector fitting showed that the treatment-based plant trait changes encompassed by these RDA axes were significantly correlated with shifts in soil processes and nutrient availability. However which soil processes were explained depended on whether the axes represented genetic or environmentally based variation (Table 5.2). Genetically-based plant trait variation was associated with changes in fall carbon mineralization potential, the seasonal change in C-mineralization potential (both associated

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with one genotype that had high aboveground biomass), fall net nitrogen mineralization potential (associated with leaf N), and litterbag mass loss (higher mass loss under the purple genotype) where leaf traits were tougher, with high C:N, and LMA (Fig 5.6b, Table 5.3). Trait variation related to herbivory and nutrient treatment was correlated with a larger range of soil processes. Here the seasonal change in carbon mineralization was higher under plants facing herbivory and this was correlated with increases in leaf traits such as LMA, thickness, and toughness of those plants (Fig 5.6c, Table 5.3). In contrast, spring carbon mineralization potential was correlated with spring plant available nitrogen levels and both were highest for plants not facing herbivory under high nutrient supply. Plants within these treatments tended to be less tough with lower LMA and later had higher ramet survival across the season. Microbial biomass (SIR), and fall and spring net nitrogen mineralization potential were all highest under high nutrient plants with no herbivores. Resolution of how trait variation predicted soil processes was lost when both genetic and environmental variation was included within one RDA analysis and then related to soil processes. Four of ten soil processes could be related to trait variation using a combined approach while separate analyses considered together explained eight of ten (four by genetic and six by environmental variation, Table 5.3).

Treatment-based changes in leaf traits linked to decomposition and herbivore feeding: Leaf trait expression patterns were significantly effected by 1) genotype (Fig 5.6e; genotype: $F_{3,78}$ =5.52, p=0.001, r^2=0.09), 2) biotic and abiotic environment (Fig 5.6f; herbivory: $F_{1,78}$ =7.18, p=0.001, nutrient : $F_{1,78}$ 5.47, p=0.001, r^2=0.12) as well as their combined influence (Fig 5.6g; herbivory: $F_{1,78}$ =6.91, p=0.001, nutrient : $F_{1,78}$ =5.27, p=0.002, genotype: $F_{3,78}$ =5.35, p=0.003 r^2=0.30). Environmental factors played a larger role in determining leaf traits than genotype. A post-

hoc vector fit analysis demonstrated all partial RDA models (genotype only, environmental only, and combine) were able to explain a significant portion of the variation in decomposition efficiency, microbially respired C, the growth rate of an herbivore feeding on that plant tissue, and how many grams of leaf tissue the grasshopper consumed per gram of grasshopper body weight (Table 5.3). However, the vector fit (r^2-variance explained) varied by model. Herbivore consumption rates and growth rate were best explained by genetically determined trait differences (Fig. 5.6e, Table 5.3). The environmental variation only model did the best job of explaining both decomposition efficiency (through changes in leaf N), and cumulative microbial respiration (through changes in C:N ratio)(Fig. 5.6f, Table 5.3). In three of four cases, a partitioned model of trait variance (either genotype or environment) did a better job of explaining the response variable than the combination model. The exception was decomposition efficiency, which was best explained by both genetic identity and environmental treatment in tandem.

Discussion

Genetic and plastic plant trait variation

Over a three year, mesocosm experiment I detected a strong (Fig. 5.3 and 5.4) and persistent (Fig. 5.2) genetic basis for plant growth, allocation, and leaf trait expression patterns, with each genotype expressing a characteristic suite of traits. Through the manipulation of both the abiotic (nutrient supply) and biotic (herbivory) environments that the genotypes experienced, I was also able to detect strong, phenotypically plastic responses of genotypes to both environmental treatments (Fig. 5.3 and 5.4). The relative importance of genetic vs. environmental factors in determining trait expression value depended on the trait measured. Plant growth and allocation patterns had a dominant genetic component and

appeared more stable across environments. Plant leaf traits were more phenotypically plastic in response to herbivory than plant growth traits with genetic variation still evident (Fig. 5.3). In general, nutrient supply altered expression patterns in more subtle ways by altering the magnitude or variance of expression patterns rather changing the direction of patterns. For some traits (aboveground biomass, leaf area, and leaf thickness) the nutrient environment that a plant experienced altered all the later response of all genotypes to herbivory (herbivory x nutrient interaction). Additionally, within a few traits (above-ground biomass and leaf thickness), genotypes exhibited varying abilities to respond plastically to herbivory (genotype x herbivory interaction). Clearly, within this system, trait expression patterns are the result of a complex interplay between top-down (herbivores), bottom-up (nutrients), and genetic effects. Such complexity is often not considered when examining intraspecific effects on ecosystem processes (Hunter & Price 1992; Schmitz 2008).

Genetic and environmental effects on soil processes and litter decomposition

Univariate mixed models detected genotypic identity and treatment-based changes in litter decomposition and soil processes. I found that up to 23% of variation in levels of soil processes (such as plant available N and microbially available C) was attributable to the identity of genotype clusters, and herbivory consistently altered these soil processes (Fig 5.5). I also documented clear legacy effects of treatments on litter decomposition by a common microbial community. Herbivory decreased decomposition efficiency 9% while increasing the quantity of carbon respired by microbes by 8%. Litter mass loss was determined by nutrient supply and was 5% higher on plants grown within the high nutrient treatment.

Plant responses as functional traits tying treatments to ecosystem responses

Within the community genetics paradigm the link between genotypes and ecosystem processes is often considered to be based on trait differences however this link is implicit rather than explicit (Crutsinger 2016). However in order to incorporate this approach with a functional traits approach this connect must be explicit. For example, the genetic and plastic trait responses documented with univariate models can only be considered to be functional "effect" traits (sensu Lavorel & Garnier 2002) if they have a direct role in determining the changes in ecosystem processes also documented in the univariate analysis. I therefore used a multivariate approach to partition multivariate trait expression patterns into genetic and environmental components, explicitly linking ecosystem process changes to genotype and treatment-based variation in plant trait expression (Fig. 5.6a). In doing so, I identified which traits were playing a dominant role in explaining the observed changes ecosystem processes and determined whether that variation is due to genetic or environmental influences. In doing so, I found that both genetic and plastic trait changes explained ecosystem processes to varying degrees, which were dependent on the ecosystem process being examined. For example, plastic trait changes overrode genetic variation to explain the observed decomposition efficiency and microbial respiration rates in a litter decomposition assay (Fig 5.6, Table 5.3). In contrast, while plastic trait changes were still significantly linked to soil microbially available carbon measures, plant trait variation related to genotype explained the observed variation best.

Genetic and plastic changes in plant multivariate trait expression govern litter decomposition

The multivariate analytic approach (Fig. 5.6a) reveals that insights from lab-based litter decomposition assays and herbivore feeding trials can be causally linked to genetic and environmentally based legacy effects manifest trhough leaf trait expression changes (Fig.

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5.6e-g). The revealed interplay between genetics and environment ultimately determines litter decomposition efficiency. For example, univariate mixed model analyses (Fig. 5.5b) showed that cumulative carbon mineralization was higher when a common microbial community was subjected to plant litter with a legacy of herbivory (5.5a-b). Decomposition efficiency was also lower on this litter indicating that microbes are less efficient at decomposing the same quantity of litter, indicative of a lower resource quality as perceived by microbes (see Chapter 4 for detailed discussion). The trait partitioning approach revealed that, in general, microbial respiration rates were higher and decomposition efficiency lower when litter had high C:N ratios. This increase in carbon mineralization rate could be predicted by both genetic and plastic components of leaf trait variation. The genetic variation occurred because one genotype in particular (Fig. 4.6e, purple) has traits which are similar to those plastically expressed by the other genotypes in response to herbivory (Fig. 4.6f; higher C:N ratios, LMA, and leaf toughness) that are positively related to microbial respiration and negatively related to decomposition efficiency. Here, both genetics and the environment are playing a joint role in determining litter decomposition rates, but those effects are mediated by trait expression patterns, which this approach can document.

Genetic and plastic multivariate whole plant trait expression and changes in soil processes

I also documented intriguing, but causally elusive, links between treatment-based plant growth and leaf trait patterns and soil processes. This stems from the potential for within season plant-soil feedbacks within the mesocosms (Bezemer *et al.* 2013; Baxendale *et al.* 2014). Herbivores can have indirect (plant-mediated) effects on soil processes through mechanism such as production of legacy litter documented above, but they also may have direct effects on soil processes through frass or carcass inputs (Lovett & Ruesink 1995; Frost

& Hunter 2008b). Nutrient cycling is also a temporally dynamic process and, in temperate systems, herbivores may effect cycling both through fast cycle (within season: frass, greenfall, canopy leaching, carcass decomposition, root exudates) or slow cycle (between seasons: litter inputs) effects (Lovett & Ruesink 1995; Hunter 2001; Bradford *et al.* 2008b) (Fig. 2.3).

I found some evidence for a differential effect of herbivores on "fast" vs. "slow" microbially available C cycling. For example, in the spring following the first year of treatments, microbially available carbon pools were lower beneath genotype clusters that were exposed to herbivores the previous year, suggesting slower cycling due to the documented lower decomposition rates of herbivore treated litter. However, by the fall, this trend had reversed so that higher C mineralization potential occurred in soils beneath herbivory treatment plants relative to controls (a 14% decrease in C in control soils vs. a 2% decrease in herbivory soils). There are a number of potential mechanisms that could explain this pattern. Possibly, herbivore related soil inputs over the course of the summer (frass, greenfall, canopy leaching, and carcass inputs) may have directly increased labile carbon in the soil beneath herbivory populations (Hunter 2001). Alternatively, plants may have responded to herbivory by increasing belowground carbon-based root exudates to stimulate microbial activity increasing the plant available forms of nutrients in the soil that then could alter trait expression (Strickland *et al.* 2015). Or, both could be occurring at once. Research examining the directionality in these relationships may shed light on interesting patterns that are typically masked within old-field systems.

Relative explanatory power of genetic vs. plastic sources of trait variation

Importantly, the explanatory power of treatment-based variation on soil processes often improved when genetic and environmental components of trait variation were modeled individually rather than being combined within one RDA that encompassed all intraspecific trait variation. This is due to some processes being more closely tied to genotype identity and others to environmental treatment. For example, the combined model could link trait expression to all three carbon mineralization metrics and fall net nitrogen mineralization potential. It could not explain the percent mass loss from a common litterbag (explained by genotype), spring N mineralization potential, fall plant available N, or fall relative microbial biomass (explained by plastic trait variation- Fig 5.6b-d, Table 5.3). This suggests that separating sources of intraspecific variation into component parts may add the nuance necessary to predict patterns and provide additional insight to models that include unspecified intraspecific variation. Lastly, this study clearly demonstrates that phenotypically plastic trait changes may override genetic effects for some litter and soil processes, but not others.

Nutrient supply dependent effect of herbivores on ecosystem dynamics

I found no evidence for the general hypothesized pattern of differential directional effects of herbivory on ecosystem processes based on nutrient environment (herbivory x nutrient interaction). The only exception was aboveground biomass production. The predicted pattern (based on a greenhouse experiment and lab decomposition assay- Chapter 4) was that there would be 1) a relative decrease in decomposition and soil processes in the presence of herbivores compared to controls at high nutrient levels due to induced resistance and 2) an increased relative decomposition in herbivory treatments, relative to controls, at low nutrient levels, which was tied to higher herbivore growth rates on

previously damaged plants (induced susceptibility). I failed to detect this pattern in the field potentially because nutrient availability within all the raised-bed mesocosms may have been near the high end of the range used within the greenhouse experiment. Even though field soil was mixed with sand to lower baseline nutrient availability in all beds, plants did not show classic signs of nutrient limitation such as increased ramet number or biomass with nutrient application (although more subtle changes in trait expression were detected). Initially ramets were planted 30 cm apart within the raised beds and likely did not experience competition from the other two clones until the second year when densities increased by an order of magnitude. This lack of competition in combination with potential mycorrhizal associations that assist in gathering resources (absent in the greenhouse experiment) may explain the lack of nutrient limitation (Vannette & Hunter 2011). A lack of nutrient limitation has also been noted in other old-field nitrogen manipulations (Blue *et al.* 2011). As a result, while I did not document the expected deceleration to acceleration of soil N cycling with increased nutrient supply, the study nevertheless provides overall support for an intraspecific version of the deceleration hypothesis (Ritchie *et al.* 1998). The evidence for this is the decreased litter decomposition due to phenotypically plastic trait changes in response to herbivory. These results are similar to reductions in decomposition documented for galled leaf litter in the *Populus* model system (Schweitzer *et al.* 2005).

Are decomposition patterns from field and greenhouse litter complementary?

If plants in the field mesocosms were not nutrient limited, then the documented decomposition patterns match quite well the cumulative and temporal predictions of decomposition dynamics generated from plants grown at high nutrient levels in the greenhouse (Fig. 5.7, Table 4.4). In both cases at high nutrient levels, litter from plants that

faced herbivory decomposed less efficiently than control litter. This was driven by the same litter traits (higher C:N resulted in less efficient decomposition) as measured in the field, and followed the same general temporal trend in which leaf traits determined microbial respiration rates (Fig. 5.7, Table 4.4). Greenhouse experiments are often criticized as not similar enough to a field setting to capture a realistic picture of dynamics. The congruence in decomposition dynamics observed within this study suggests otherwise.

The role of plant chemical defense

As with all trait-based (and much genetic correlation) research, it is worth noting that the traits found to be linked to particular outcomes here may not be the actual causal agent driving the ecosystem processes. There is instead the possibility that the traits are correlated with another, underlying causal agent. Here a candidate for an underlying trait altering decomposition is chemical defense. Defense production within *S. altissima* is complex and multifaceted involving the production of a number of chemical compounds, primarily, C-based terpenoids and phenols and N-based protease inhibitors (anti-digestive proteins) (Cooper-Driver *et al.* 1985; Cooper-Driver & Le Quesne 1986b; Johnson *et al.* 2007; Bode *et al.* 2013; Heath *et al.* 2014). These compounds are known to be costly for plants to produce (Heath *et al.* 2014). What is not currently known is whether these compounds remain in leaf tissue after senescence or whether they are catabolized or translocated from foliar tissue belowground. What is clear is that structural changes in leaf tissue (LMA, toughness, LDMC etc.) within this species at least partially determine decomposition dynamics.

Landscape scale implications

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This study suggests that the colonization and subsequent spatial clumping of genetic variation due to the clonal growth pattern of *S. altissima* (Cain 1990) may result in a mosaic of differential soil process rates across a single old-field. Layered on top of these rates are plastic trait responses to herbivore damage which generally decrease litter decomposition rates, while lowering spring soil microbially available C, and raising fall levels of labile C. As herbivore damage of *Solidago* individuals across old fields is extremely spatially heterogeneous (Hakes & Cronin 2011), the attendant plastic trait expression in response to herbivory could be heterogeneous as well. But, in insect outbreak years, herbivore induced changes in trait expression occur more uniformly across the entire field, resulting in greater homogeneity of soil processes (Root 1996). *Solidago* species can account for up to 95% of the plant cover (particularly in early to mid successional) of old fields Hence, the interplay between genotypic and environmental drivers of intraspecific variation and ecosystem processes could play a particularly important and undocumented role in determining levels of ecosystem processes within this kind of ecosystem. These results also suggest the potential for homogeneity to arise from the converse, that in the absence of nutrient limitation, soil nutrient variability across the landscape will only produce subtle changes in trait expression of genotypes. This possibility is likely as evidence shows that *S. altissima* tends not to be nutrient limited in early succession but rather limited by water availability or light competition with neighbors (Abrahamson *et al.* 1988; Wise & Abrahamson 2007; Blue *et al.* 2011).

Conclusions

By manipulating locally relevant sources of genetic variation, herbivore pressure, and soil nutrient availability, I demonstrate the potential for a intraspecific variation in a

dominant species to structure (by plant genotype and herbivory), field-level mosaics of ecosystem process rates, at least over 3 year time scales. This suggests that biotic factors and intraspecific variation may play a larger role in terrestrial ecosystem processes than typically acknowledged (Schmitz *et al.* 2013). Further research should examine how these dynamics may change in later successional stages when nutrient limitation may resurface (Robertson *et al.* 1988).

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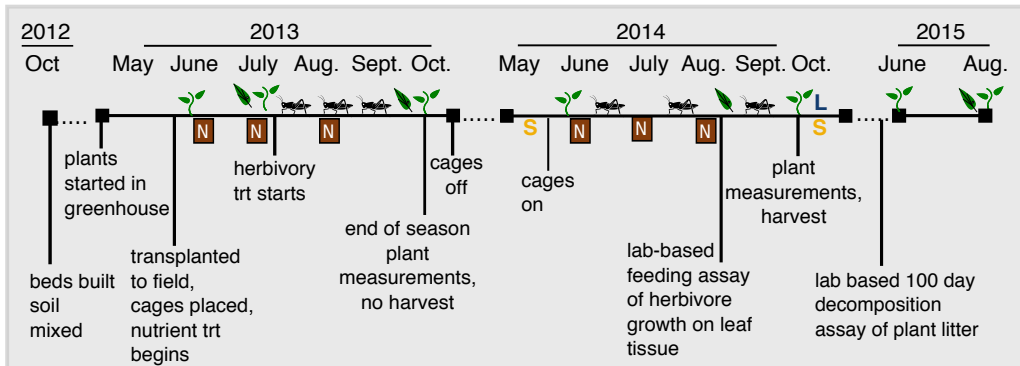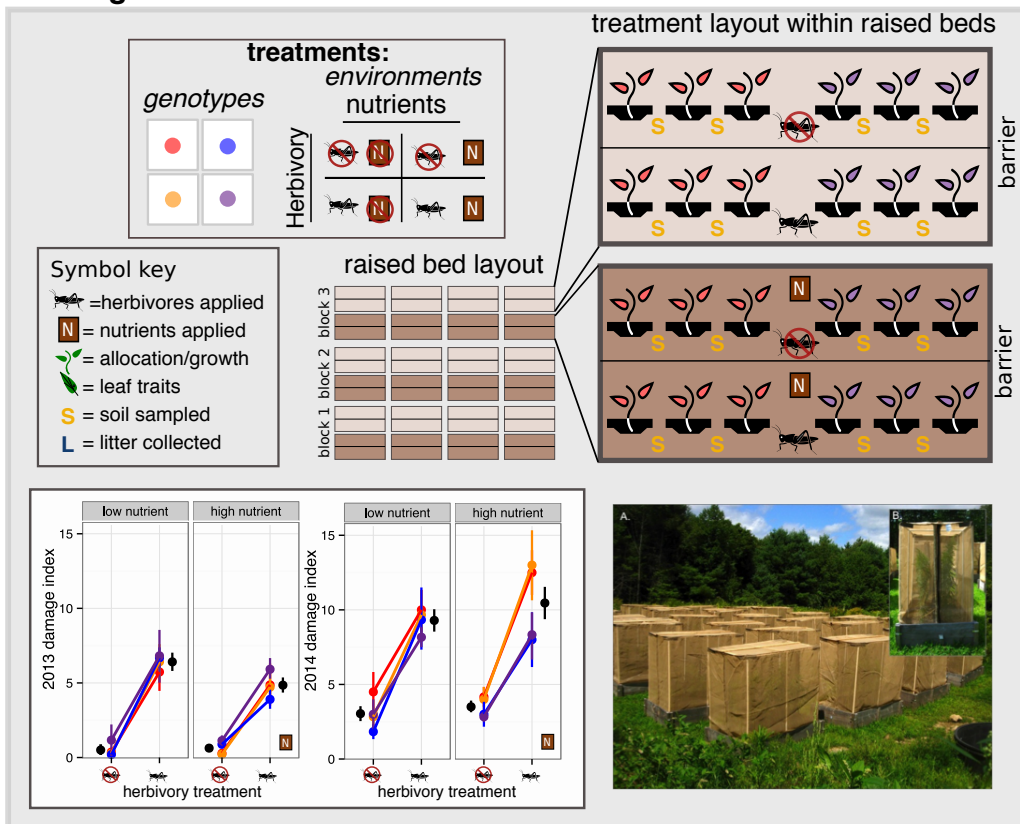
Figures

Figure 5.1: Experimental A). timeline and B). design of 3 year field mesocosm experiment examining genotype, nutrient supply, and herbivory (amount of damage each year- shown in inset), effects on plant traits and ecosystem processes. Spatial clusters of 4 genotypes (starting with n=3 individual clones) were grown in a split plot design with nutrients applied at the level of the raised bed. Beds were then partitioned in the middle (both above and belowground). Half of each bed was exposed to herbivores and the other was a control. All models were run with population averages of plant traits and soil processes (n=96, 2 genotypes and 2 herbivory treatments over 24 beds). Individual bed, block, and genotype pair were included as random effects in linear mixed models.
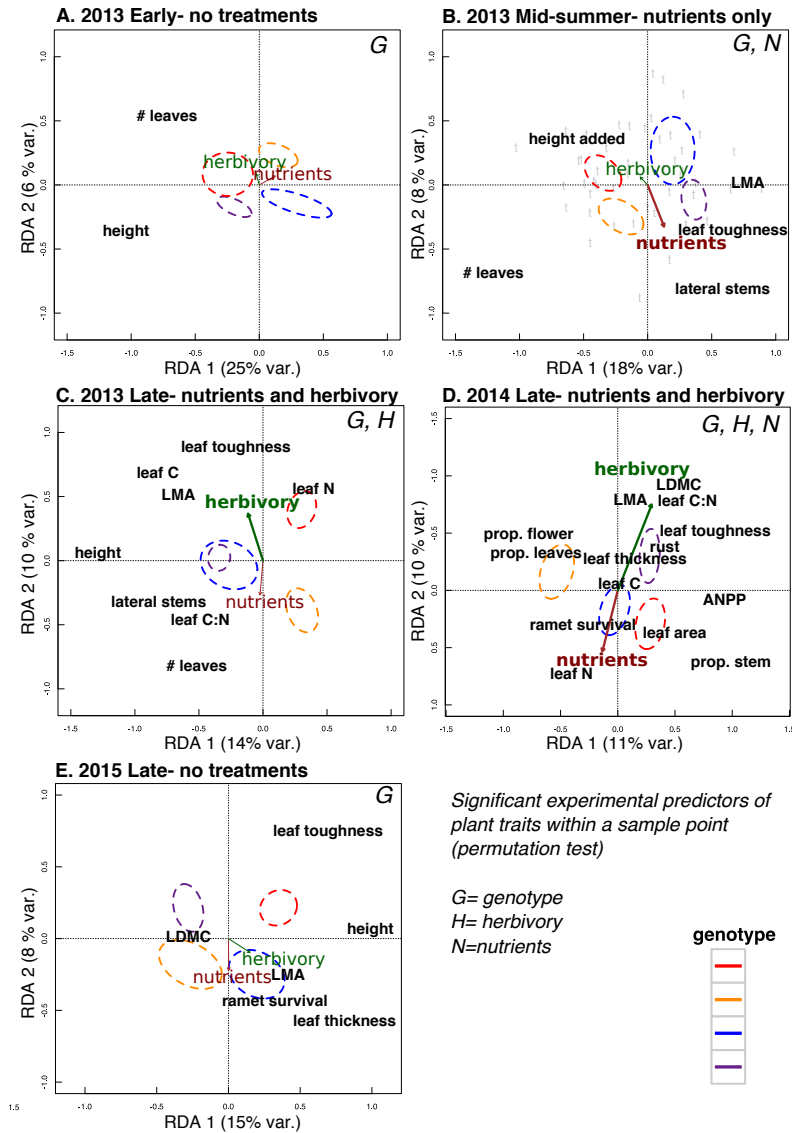
Figure 5.2: Changes in plant trait expression across 3 experimental years as nutrient and herbivore treatments were applied and then removed (2015). Multivariate trait changes in leaf traits are represented with an RDA trait space (essentially a constrained PCA which only represents the trait variation attributable to genotype, herbivory, and nutrient treatments). The 96 experimental unit scores representing one genotype population in one bed ("site scores") are not plotted to keep the plot readable, however a 95% confidence ellipse of the weighted average score is drawn around the each genotype mean trait value. The 0, 0 point represents an average plant with vectors (nutrients and herbivores) and ellipses drawn to show how the treatment changes the mean trait value. Each RDA trait space was produced independently from the leaf trait data collected at that time point. Not all traits were collected in each time point due to experimental constraints so the comparison is a qualitative one between the relative importance of genotype, nutrients, and herbivory in explaining population level trait variation at each time point. The significance of the predictors was tested using a permutation test.
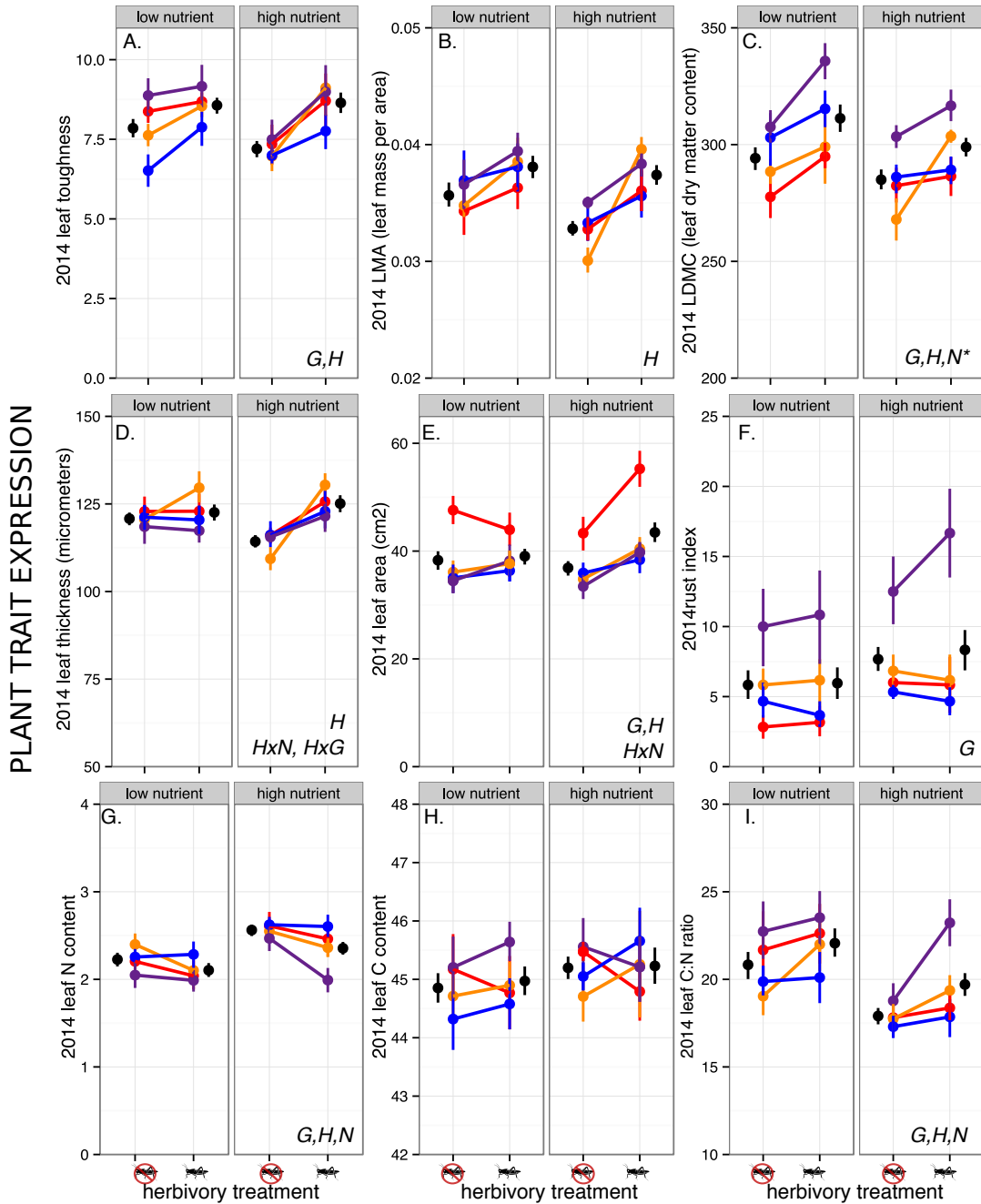
Figure 5.3: Experimental treatment effects of genotype (G) as well as herbivory (H) and nutrient supply (N) environments on mean plant trait expression patterns of four genotypes (n=96, 4G x 2N x 2H x 6 replicate genotype clusters) by the end of 2014 (second year of treatments). Inset letters indicate a significant effect of that predictor on trait expression patterns using a linear mixed model with random effects specified to account for the split plot design. Colors represent each genotype's population trait mean (±SE) at that treatment level; with black offset dots representing the overall mean. N* indicates a marginally significant effect of p=0.51.
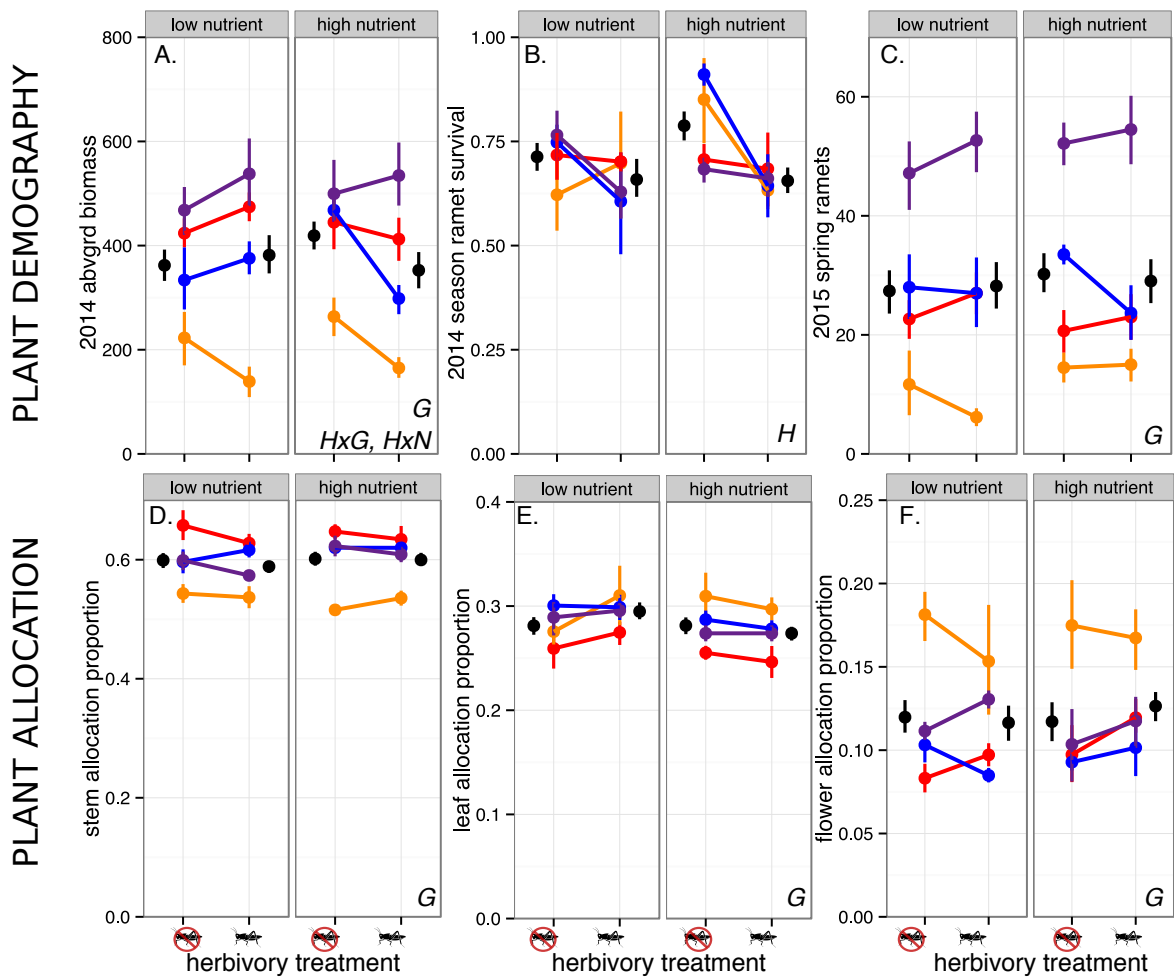
Figure 5.4: Experimental treatment effects of genotype (G) as well as herbivory (H) and nutrient supply (N) environments on the plant allocation and demographic patterns of four genotypes by the end of 2014 (second year of treatments). Inset italic letters indicate a significant effect of that predictor on trait expression patterns using a linear mixed model with random effects specified to account for the split plot design. Colors represent each genotype's population mean (±SE) at that treatment level; with black offset dots representing the overall mean.
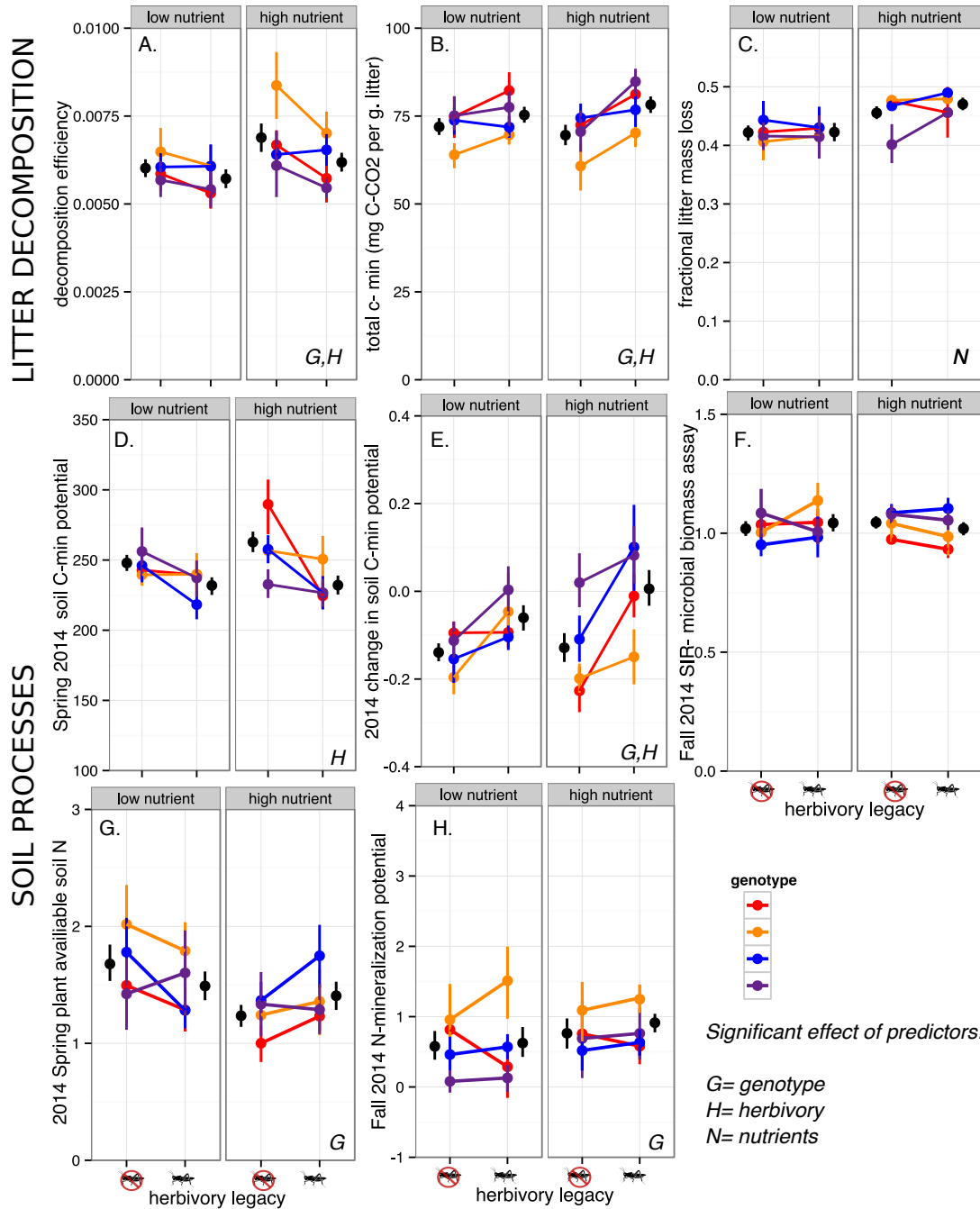
Figure 5.5: Experimental treatment effects of genotype (G) as well as herbivory (H) and nutrient supply (N) environments ecosystem processes in 2014 (second year of treatments). A lab microcosm assay quantified the decomposition patterns (relative quality) of litter harvested from the four genotypes by microbes in a common soil inoculum. Soil processes were measured from soil samples taken from the soil underneath the genotype clusters in each bed (n=96). Inset italic letters indicate a significant effect of that predictor on these ecosystem processes using a linear mixed model with random effects specified to account for the split plot design. Colors represent each genotype's population trait mean (±SE) at that treatment level; with black offset dots representing the overall mean.
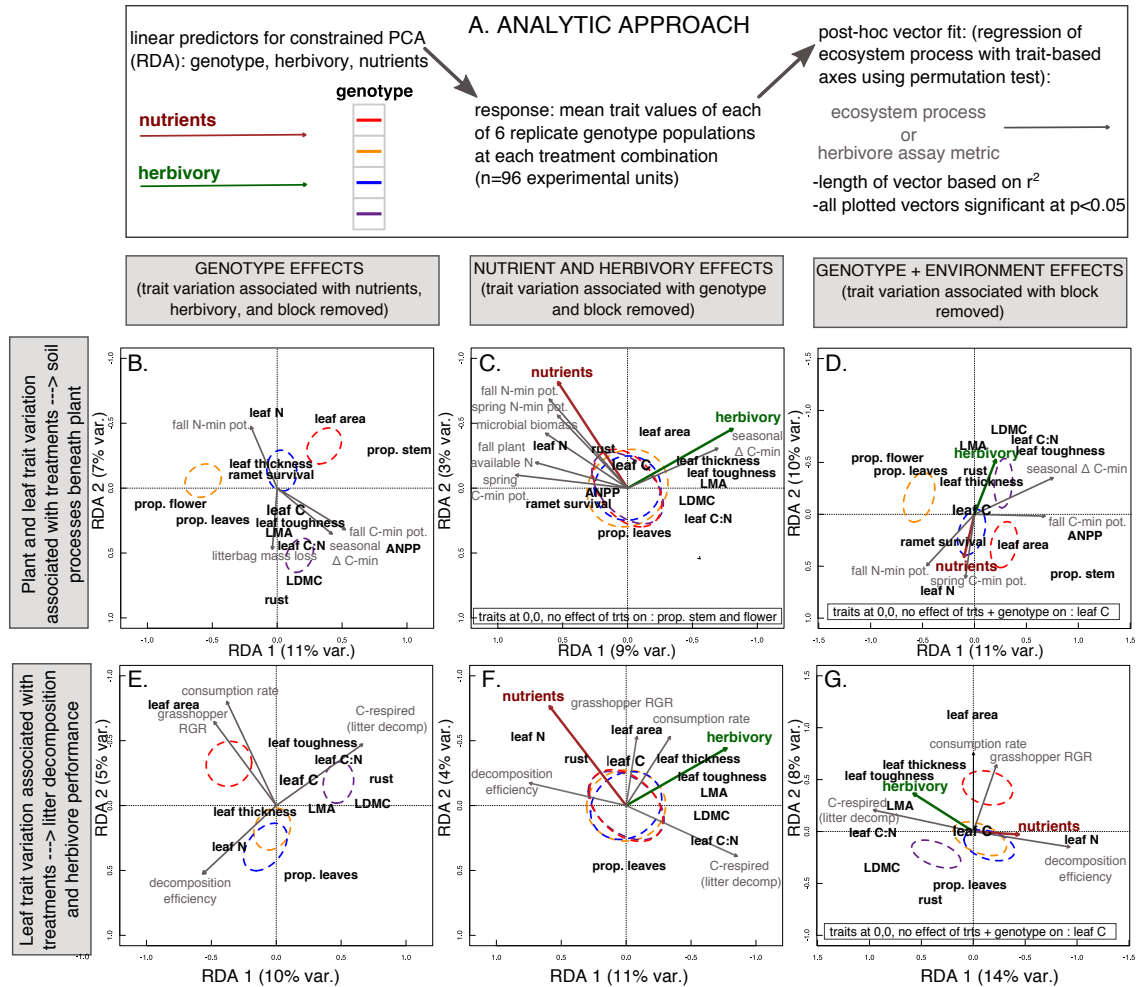
Figure 5.6: A redundancy analysis approach (RDA) was used to summarize and partition genetic and environmental treatment effects on plant trait variation. Plant trait space was plotted for each figure independently using combinations of predictors that isolated genetic effects (B and E), environmental effects (C and F), and combined (D and G). Ecosystem process vectors (gray) were then fit to the RDA axis within each figure using a permutation test (see A.). These vectors can be interpreted as which treatment-based trait changes explain variation in a particular ecosystem process. Plant allocation and leaf traits were related to soil processes (B,C,D), while only leaf traits were used to examine litter decomposition and herbivore consumption rates within the lab based assays using leaf tissue harvest from plants (E, F, G). Again, individual experimental unit values (site scores) are not displayed to avoid cluttering the figure, but 95% confidence ellipses around the genotype centroid and vectors indicating the direction and magnitude of the effect of nutrient supply and herbivory on trait values are drawn to indicate how treatments alter experimental unit traits from the mean value (at 0,0).
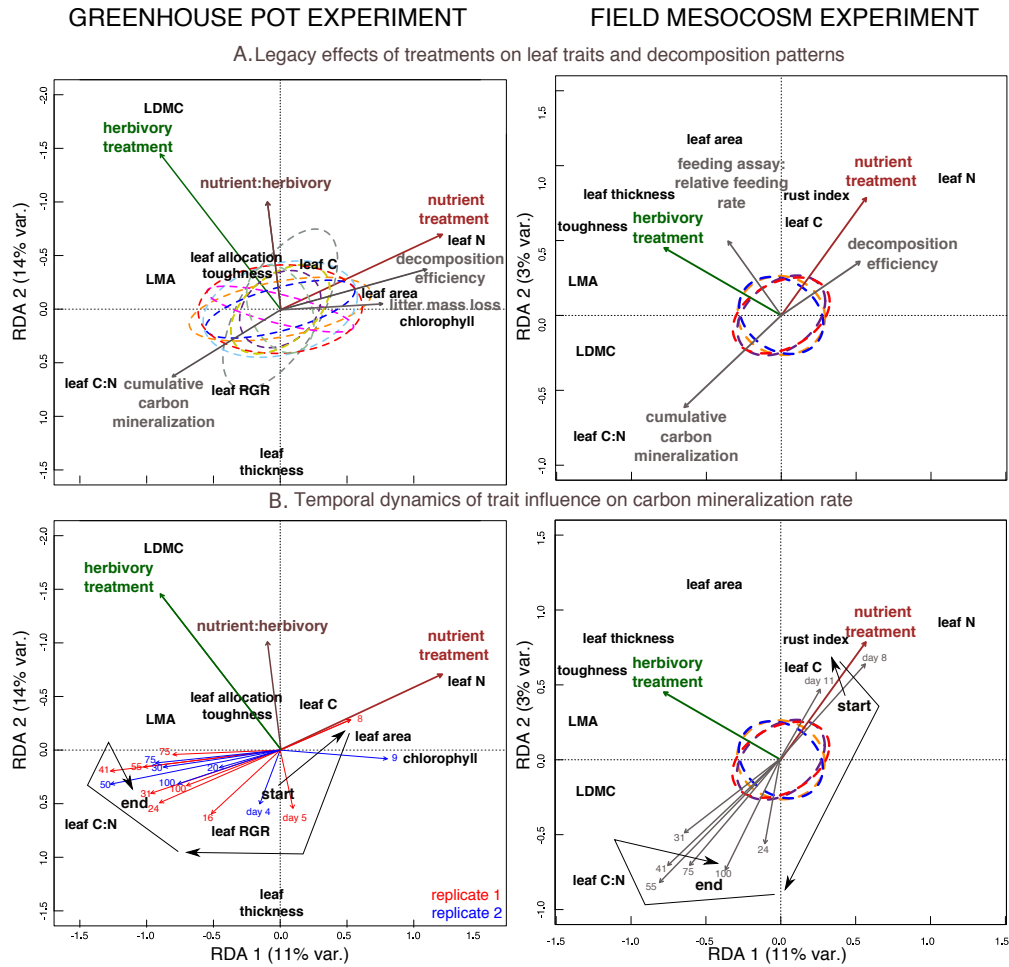
Figure 5.7: Comparison between temporal and cumulative dynamics of a litter decomposition lab assay from a 9 genotypes grown under different nutrient and herbivory regimes in the greenhouse and four of those same genotypes grown with the same treatments but within a field mesocosm experiment (this study). All panels are from RDAs produced from the leaf trait data associated with individual plants in each experiment Variation associated with genotype was removed first (i.e. conditioned out- all genotype ellipses are now centered at the origin) so that these RDA axes explain only the trait variation associated with nutrient and herbivory treatments. The top panels show genotype trait dispersion (ellipses), the correlations between leaf trait changes in response to treatment (black leaf trait names), which trait changes are associated with herbivory (green) and increased nutrients (brown) vectors, and how traits correlate with subsequent overall decomposition patterns (gray vectors). The vector length denotes the relationship strength. Only significantly associated vectors are shown. The lower panel shows the temporal dynamics of which leaf traits correlate with carbon mineralization across time point measurements in the assay. The vectors point toward the leaf trait most correlated with C-mineralization rates on that day. 3 out of 20 days in the pot experiment and 2 of 10 in the field experiment were not explained by variation attributable to nutrient or herbivory treatments. Note the lack of significant interaction between herbivore and nutrient treatment in the field experiment.

Tables:

Table 5.1: Univariate linear mixed effects models of treatment on A. plant traits B. allocation and growth C. litter decomposition and D. soil processes. Type III tests with Satterthwaite's approximation for degrees of freedom.

A. PLANT TRAITS

Rust index (fungal pathogen)

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	0.0051	0.0051	1	58	0.01	0.905
nutrient (N)	0.8093	0.8093	1	19	2.27	0.148
genotype (G)	**15.0406**	**5.0135**	**3**	**54**	**14.09**	**0.000**
HxN	0.0036	0.0036	1	58	0.01	0.920
HxG	1.4399	0.4800	3	58	1.35	0.267
NxG	0.6774	0.2258	3	67	0.63	0.595
HxNxG	0.7693	0.2564	3	58	0.72	0.544
r^2_{adj}- fixed						0.27
r^2_{adj} - fixed+random effects						0.68

Leaf area

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	**322.45**	**322.45**	**1**	**58**	**7.47**	**0.008**
nutrient (N)	50.31	50.31	1	18	1.17	0.294
genotype (G)	**2060.14**	**686.71**	**3**	**11**	**15.91**	**0.000**
HxN	**205.34**	**205.34**	**1**	**58**	**4.76**	**0.033**
HxG	30.89	10.30	3	58	0.24	0.869
NxG	35.70	11.90	3	67	0.28	0.843
HxNxG	195.55	65.18	3	58	1.51	0.221
r^2_{adj} - fixed						0.42
r^2_{adj} - fixed+random effects						0.44

Leaf toughness

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	**28.08**	**28.08**	**1**	**58**	**24.58**	**0.000**
nutrient (N)	0.50	0.50	1	19	0.44	0.514
genotype (G)	**10.34**	**3.44**	**3**	**52**	**3.02**	**0.038**
HxN	3.14	3.14	1	58	2.75	0.102
HxG	1.81	0.60	3	58	0.53	0.665
NxG	1.58	0.52	3	70	0.46	0.709
HxNxG	3.51	1.17	3	58	1.03	0.388
r^2_{adj} - fixed						0.32
r2adj- fixed+random effects						0.40

LDMC

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	**5796.4**	**5796.4**	**1**	**59**	**16.27**	**0.000**
nutrient (N)*	1543.1	1543.1	1	18	4.33	0.052
genotype (G)	**11472**	**3824**	**3**	**54**	**10.73**	**0.000**
HxN	57.8	57.8	1	59	0.16	0.688
HxG	1021.6	340.5	3	59	0.96	0.420
NxG	993.2	331.1	3	72	0.93	0.431
HxNxG	1607.1	535.7	3	59	1.50	0.223
r^2_{adj} - fixed						0.35
r^2_{adj} - fixed+random effects						0.52

LMA

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	**0.0002980**	**0.0002980**	**1**	**59**	**26.14**	**0.000**
nutrient (N)	0.0000256	0.0000256	1	17	2.24	0.153
genotype (G)	0.0000540	0.0000180	3	56	1.58	0.205
HxN	0.0000282	0.0000282	1	59	2.47	0.122
HxG	0.0000834	0.0000278	3	59	2.44	0.074
NxG	0.0000111	0.0000037	3	71	0.32	0.808
HxNxG	0.0000268	0.0000089	3	59	0.78	0.509
r^2_{adj} - fixed						0.23
r^2_{adj} - fixed+random effects						0.57

Leaf thickness

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	**959.12**	**959.12**	**1**	**58**	**17.40**	**0.000**
nutrient (N)	30.78	30.78	1	17	0.56	0.465
genotype (G)	352.42	117.47	3	39	2.13	0.112
HxN	**491.87**	**491.87**	**1**	**58**	**8.92**	**0.004**
HxG	**630.48**	**210.16**	**3**	**58**	**3.81**	**0.015**
NxG	140.93	46.98	3	70	0.85	0.470
HxNxG	22.89	7.63	3	58	0.14	0.937
r^2_{adj} - fixed						0.58
r^2_{adj} - fixed+random effects						0.64

Leaf N

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	**0.658**	**0.658**	**1**	**59**	**11.52**	**0.001**
nutrient (N)	**0.395**	**0.395**	**1**	**19**	**6.93**	**0.017**
genotype (G)	**0.800**	**0.266**	**3**	**60**	**4.67**	**0.005**
HxN	0.043	0.043	1	59	0.77	0.385
HxG	0.273	0.090	3	59	1.60	0.200
NxG	0.232	0.077	3	69	1.36	0.263
HxNxG	0.238	0.079	3	59	1.39	0.255
r^2_{adj} - fixed						0.29
r^2_{adj} - fixed+random effects						0.69

Leaf C

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	0.1343	0.1342	1	59	0.16	0.694
nutrient (N)	0.4599	0.4598	1	22	0.54	0.472
genotype (G)	3.6334	1.2111	3	71	1.41	0.246
HxN	0.0455	0.0455	1	59	0.05	0.819
HxG	3.5825	1.1941	3	59	1.39	0.253
NxG	1.5725	0.5241	3	71	0.61	0.609
HxNxG	1.3673	0.4557	3	59	0.53	0.662
r^2_{adj} - fixed						0.08
r^2_{adj} - fixed+random effects						0.52

Leaf C:N

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	**0.671**	**0.671**	**1**	**59**	**11.12**	**0.001**
nutrient (N)	**0.369**	**0.369**	**1**	**19**	**6.11**	**0.023**
genotype (G)	**1.065**	**0.355**	**3**	**60**	**5.89**	**0.001**
HxN	0.028	0.028	1	59	0.46	0.501
HxG	0.263	0.088	3	59	1.45	0.237
NxG	0.220	0.073	3	69	1.22	0.310
HxNxG	0.246	0.082	3	59	1.36	0.263
r^2_{adj} - fixed						0.29
r^2_{adj} - fixed+random effects						0.69

B. PLANT GROWTH AND ALLOCATION PATTERNS

Proportion leaf tissue

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	0.0002	0.0002	1	59	0.21	0.650
nutrient (N)	0.0014	0.0014	1	18	1.26	0.277
genotype (G)	**0.0152**	**0.0051**	**3**	**73**	**4.63**	**0.005**
HxN	0.0027	0.0027	1	59	2.48	0.121
HxG	0.0008	0.0003	3	59	0.24	0.865
NxG	0.0042	0.0014	3	73	1.29	0.283
HxNxG	0.0016	0.0005	3	59	0.50	0.683
r^2_{adj} - fixed						0.18
r^2_{adj} - fixed+random effects						0.41

Proportion stem tissue

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	0.0009	0.0009	1	58	0.84	0.364
nutrient (N)	0.0004	0.0004	1	17	0.35	0.562
genotype (G)	**0.0697**	**0.0232**	**3**	**54**	**21.28**	**0.000**
HxN	0.0004	0.0004	1	58	0.40	0.531
HxG	0.0051	0.0017	3	58	1.54	0.213
NxG	0.0062	0.0021	3	71	1.90	0.137
HxNxG	0.0018	0.0006	3	58	0.56	0.646
r^2_{adj} - fixed						0.42
r^2_{adj} - fixed+random effects						0.66

Proportion flower tissue

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	0.0002	0.0002	1	57	0.13	0.720
nutrient (N)	0.0002	0.0002	1	17	0.13	0.725
genotype (G)	**0.0617**	**0.0206**	**3**	**17**	**12.38**	**0.000**
HxN	0.0010	0.0010	1	57	0.58	0.450
HxG	0.0054	0.0018	3	57	1.09	0.363
NxG	0.0025	0.0008	3	70	0.50	0.684
HxNxG	0.0009	0.0003	3	57	0.18	0.909
r^2_{adj} - fixed						0.32
r^2_{adj} - fixed+random effects						0.40

Aboveground biomass

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	13179	13179	1	59	1.59	0.212
nutrient (N)	3313	3313	1	18	0.40	0.535
genotype (G)	**1213384**	**404461**	**3**	**61**	**48.79**	**0.000**
HxN	**44385**	**44385**	**1**	**59**	**5.35**	**0.024**
HxG	**78221**	**26074**	**3**	**59**	**3.15**	**0.032**
NxG	7074	2358	3	70	0.28	0.836
HxNxG	35111	11704	3	59	1.41	0.248
r^2_{adj} - fixed						0.60
r^2_{adj} - fixed+random effects						0.78

Seasonal ramet survival

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	**0.210**	**0.210**	**1**	**75**	**6.05**	**0.016**
nutrient (N)	0.031	0.031	1	75	0.89	0.349
genotype (G)	0.021	0.007	3	46	0.20	0.894
HxN	0.036	0.036	1	75	1.05	0.309
HxG	0.111	0.037	3	75	1.07	0.367
NxG	0.074	0.025	3	75	0.72	0.546
HxNxG	0.136	0.045	3	75	1.31	0.277
r^2_{adj} - fixed						0.14
r^2_{adj} - fixed+random effects						0.22

2015 spring ramets

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	0.221	0.221	1	59	0.16	0.691
nutrient (N)	2.033	2.033	1	18	1.47	0.241
genotype (G)	**183.548**	**61.183**	**3**	**72**	**44.27**	**0.000**
HxN	0.071	0.071	1	59	0.05	0.822
HxG	4.664	1.555	3	59	1.13	0.346
NxG	6.567	2.189	3	72	1.58	0.201
HxNxG	1.379	0.460	3	59	0.33	0.802
r^2_{adj} - fixed						0.58
r^2_{adj} - fixed+random effects						0.64

C. DECOMPOSITION ASSAY

Decomposition efficiency

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	**0.00001**	**0.00001**	**1**	**58**	**7.11**	**0.010**
nutrient (N)	0.00000	0.00000	1	16	2.93	0.106
genotype (G)	**0.00001**	**0.00000**	**3**	**59**	**4.55**	**0.006**
HxN	0.00000	0.00000	1	58	1.15	0.288
HxG	0.00000	0.00000	3	58	1.28	0.291
NxG	0.00000	0.00000	3	70	1.14	0.339
HxNxG	0.00000	0.00000	3	58	0.34	0.798
r^2_{adj} - fixed						0.15
r^2_{adj} - fixed+random effects						0.70

Cumulative carbon mineralization from litter decomposition

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	872.17	872.17	1	59	14.89	**0.000**
nutrient (N)	0.45	0.45	1	17	0.01	0.931
genotype (G)	**873.37**	**291.12**	**3**	**61**	**4.97**	**0.004**
HxN	166.92	166.92	1	59	2.85	0.097
HxG	275.19	91.73	3	59	1.57	0.207
NxG	86.23	28.74	3	70	0.49	0.690
HxNxG	92.59	30.86	3	59	0.53	0.666
r^2_{adj} - fixed						0.16
r^2_{adj} - fixed+random effects						0.68

Litter mass loss

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	0.00145	0.00145	1	58	0.51	0.479
nutrient (N)	**0.01473**	**0.01473**	**1**	**18**	**5.15**	**0.036**
genotype (G)	0.00936	0.00312	3	73	1.09	0.358
HxN	0.00124	0.00124	1	58	0.43	0.512
HxG	0.00339	0.00113	3	58	0.40	0.757
NxG	0.00445	0.00148	3	73	0.52	0.671
HxNxG	0.00639	0.00213	3	58	0.75	0.529
r^2_{adj} - fixed						0.15
r^2_{adj} - fixed+random effects						0.44

D. SOIL PROCESSES

Change in carbon mineralization potential of soils from spring 2014 to fall 2014

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	**0.27**	**0.27**	**1**	**76**	**14.33**	**0.000**
nutrient (N)	0.04	0.04	1	76	1.84	0.179
genotype (G)	**0.27**	**0.09**	**3**	**54**	**4.73**	**0.005**
HxN	0.02	0.02	1	76	0.96	0.331
HxG	0.01	0.00	3	76	0.10	0.962
NxG	0.15	0.05	3	76	2.54	0.063
HxNxG	0.11	0.04	3	76	1.90	0.137
r^2_{adj} - fixed						0.29
r^2_{adj} - fixed+random effects						0.41

Spring 2014 carbon mineralization potential

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	**0.27**	**0.27**	**1**	**76**	**14.33**	**0.000**
nutrient (N)	0.04	0.04	1	76	1.84	0.179
genotype (G)	**0.27**	**0.09**	**3**	**54**	**4.73**	**0.005**
HxN	0.02	0.02	1	76	0.96	0.331
HxG	0.01	0.00	3	76	0.10	0.962
NxG	0.15	0.05	3	76	2.54	0.063
HxNxG	0.11	0.04	3	76	1.90	0.137
r^2_{adj} - fixed						0.26
r^2_{adj} - fixed+random effects						0.33

Fall 2014 SIR

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	0.00	0.00	1	57	0.07	0.799
nutrient (N)	0.00	0.00	1	19	0.17	0.686
genotype (G)	0.03	0.01	3	74	0.61	0.610
HxN	0.01	0.01	1	57	0.71	0.403
HxG	0.03	0.01	3	57	0.60	0.620
NxG	0.10	0.03	3	73	2.05	0.115
HxNxG	0.05	0.02	3	57	0.95	0.423
r^2_{adj} - fixed						0.16
r^2_{adj} - fixed+random effects						0.33

<u>Spring plant available N</u>

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	0.00	0.00	1	57	0.00	0.968
nutrient (N)	1.21	1.21	1	16	3.94	0.065
genotype (G)	**2.46**	**0.82**	**3**	**31**	**3.27**	**0.050**
HxN	0.68	0.68	1	76	2.20	0.143
HxG	0.73	0.73	1	57	2.36	0.130
NxG	0.12	0.04	3	57	0.13	0.942
HxNxG	1.82	0.61	3	68	1.98	0.126
r^2_{adj} - fixed						0.16
r^2_{adj} - fixed+random effects						0.33

<u>Fall N mineralization potential</u>

fixed effects	Sum Sq	Mean Sq	NumDF	DenDF	F	p
herbivory (H)	0.09	0.09	1	58	0.13	0.719
nutrient (N)	0.65	0.65	1	17	0.94	0.346
genotype (G)	**9.48**	**3.16**	**3**	**33**	**4.55**	**0.009**
HxN	0.00	0.00	1	58	0.00	0.965
HxG	1.22	0.41	3	58	0.58	0.628
NxG	1.29	0.43	3	69	0.62	0.604
HxNxG	0.43	0.14	3	58	0.21	0.893
r^2_{adj} - fixed						0.17
r^2_{adj} - fixed+random effects						0.38

Table 5.2 Vector fits of ecosystem process data to plant trait RDAs (see Fig 5.6)

Vectors	Genotype RDA		Environment RDA		Genotype + Environment RDA	
	r2	p	r2	p	r2	p
Spring soil C-min	0.01	0.618	**0.12**	**0.002**	**0.08**	**0.016**
Fall soil C-min	**0.13**	**0.003**	0.05	0.088	**0.10**	**0.009**
Change in C-min potential	**0.10**	**0.006**	**0.08**	**0.013**	**0.14**	**0.001**
Spring SIR	0.01	0.718	0.03	0.314	0.01	0.592
Fall SIR	0.01	0.567	**0.09**	**0.013**	0.03	0.264
Spring plant available N	0.04	0.139	0.04	0.137	0.03	0.199
Fall plant available N	0.01	0.507	**0.08**	**0.015**	0.05	0.084
Spring net N-min potential	0.05	0.103	**0.09**	**0.009**	0.07	0.037
Fall net N-min potential.	**0.08**	**0.015**	**0.13**	**0.002**	**0.09**	**0.011**
Spring litterbag mass loss	**0.07**	**0.031**	0.01	0.507	0.01	0.664

Table 5.3 Vector fits of decomposition data to leaf trait RDAs (see Fig 5.6)

Vectors	Genotype RDA		Environment RDA		Genotype + Environment RDA	
	r^2	p	r^2	p	r^2	p
Litter mass loss	0.02	0.4011	0.01	0.7932	0.01	0.5507
Decomposition efficiency	**0.12**	**0.0028**	**0.15**	**0.0006**	**0.18**	**0.0004**
Total C-mineralized	**0.13**	**0.0008**	**0.23**	**0.0002**	**0.20**	**0.0002**
g. eaten/g. grasshopper	**0.17**	**0.0002**	**0.10**	**0.0062**	**0.12**	**0.0026**
RGR of grasshopper	**0.14**	**0.0010**	**0.07**	**0.0272**	**0.08**	**0.0194**

Table 5.4 Vector fits of carbon mineralization rates by day to leaf trait RDAs (see Fig 5.7)

Vectors	Genotype RDA		Environment RDA		Genotype + Environment RDA	
	r2	p	r2	p	r2	p
Day 5	0.04	0.185	0.03	0.228	0.01	0.638
Day 8	**0.27**	**0.000**	**0.19**	**0.000**	**0.22**	**0.000**
Day 11	**0.12**	**0.004**	**0.07**	**0.029**	**0.09**	**0.015**
Day 16	**0.09**	**0.010**	0.03	0.179	**0.06**	**0.059**
Day 24	**0.17**	**0.000**	**0.10**	**0.011**	**0.11**	**0.004**
Day 31	**0.27**	**0.000**	**0.18**	**0.000**	**0.19**	**0.000**
Day 41	**0.31**	**0.000**	**0.24**	**0.000**	**0.23**	**0.000**
Day 55	**0.34**	**0.000**	**0.27**	**0.000**	**0.29**	**0.000**
Day 75	**0.25**	**0.000**	**0.20**	**0.000**	**0.21**	**0.000**
Day 100	**0.22**	**0.000**	**0.15**	**0.000**	**0.15**	**0.000**

CHAPTER 6

CONCLUSIONS

Overall, my dissertation research program focused on determining how intraspecific, phenotypic variation in functional traits predicts ecosystem level processes. I did so by partitioning intraspecific variation into genetic and environmentally determined (plastic) components to resolve context-dependency in trait expression across landscapes (Schmitz 2010; Schmitz *et al.* 2015). I explored this dynamic through the lens of anti-herbivore plant defense traits that result in variation in plant quality across landscapes (Agrawal 1998; Andrew *et al.* 2007; Hakes & Cronin 2011). Here, I define potential plant defensive responses broadly to encompass whole-plant phenotypic changes in plant growth, allocation and leaf traits to reflect recent work that demonstrates that other traits and mixed strategies may have more important impacts on plant resistance to herbivores than secondary plant chemistry (Carmona *et al.* 2011; Carmona & Fornoni 2013; Moles *et al.* 2013).

First, I developed a conceptual framework in Chapter 2 that combines elements of two theoretical constructs (plant defense and herbivore effects on nutrient cycling) to produce a novel model where intraspecific variation in trait plant defense expression could change the directionality of herbivore impacts on nutrient cycling rates. In Chapter 3, I tested this framework with a greenhouse pot experiment that demonstrated that goldenrod individuals exhibit both genotypic variation and phenotypic plasticity in plant defensive trait responses across a nutrient and herbivory gradient. In doing so, I documented a unique whole plant phenotype expressed in response to herbivory only at high nutrient levels. This corresponds to higher plant resistance to herbivores as measured through grasshopper relative growth rates. In contrast, at low nutrient levels, I detected induced susceptibility to herbivores in the same population of genotypes (higher growth rates of herbivores on

previously browsed plants). This was coupled with a shift toward a higher tolerance of herbivory (*i.e.*. a less severe negative impact of herbivore damage on relative fitness).

I used senesced litter harvested from these plants to show in Chapter 4 that litter decomposition rates can be shifted by plant strategy regardless of whether such trait changes are genetic or environmentally mediated. Litter from high nutrient plants that faced herbivores (a group with higher resistance to herbivores when living) exhibited lower mass loss and decomposition efficiency compared with high nutrient, control litter. This pattern reversed at low nutrient levels with decomposition efficiency higher on litter from plants that had faced herbivores. This suggests that the interaction between herbivory and nutrient supply could cause context-dependent acceleration or deceleration of nutrient cycling. Thus, plant genotype and trait plasticity shape ecosystem processes via multiple environmental drivers that may interact.

In Chapter 5, I further explored this dynamic with a naturalistic three-year raised bed experiment. As in the greenhouse, I documented strong genetically and environmentally-based trait variation in plant allocation, growth, and leaf traits. I explicitly linked these genetic and plastic functional trait changes to concurrent changes in litter decomposition rates and a variety of soil processes (carbon mineralization potential, plant available nitrogen, nitrogen mineralization potential, and microbial biomass). Importantly, partitioning functional trait variation into genetic and environmental component parts improved their explanatory power on ecosystem processes. However, I saw little evidence to support a nutrient-mediated shift in the effect of herbivores on nutrient cycling rates. This may have been due to both of the experimental nutrient supply levels being on the upper end of the nutrient gradient imposed in the greenhouse. If, this was the case (as suggested by growth patterns), than the results from the greenhouse and field concur that at high nutrient levels,

herbivores will decrease litter decomposition rates leading to a deceleration in nutrient cycling in the longer term (Ritchie *et al.* 1998; Schweitzer *et al.* 2005).

Nutrient cycling is a temporally dynamic process. Herbivores may alter cycling both through fast cycle (within season: frass, greenfall, canopy leaching, carcass decomposition, root exudates) or slow cycle effects (between seasons: litter decomposition)(Lovett & Ruesink 1995; Hunter 2001; Bradford *et al.* 2008)(Fig. 2.3). The soil beneath genotype clusters exhibited lower levels of spring microbially available C (slow cycle) after one year of treatments. This could have been due to the lower decomposition efficiency of herbivore legacy litter in the bed. In general, microbially available C decreases over the growing season and by the fall of that year I found a larger reduction in microbially available C under control genotype clusters compared to herbivory-exposed genotype clusters. This suggests that herbivory is increasing fast cycle carbon inputs over the course of the growing season. The exact source of the effect is unclear and probably reflects a combination of processes (frass, greenfall, and increased root exudates) that could not be experimentally separated. Further work should explore these pathways more closely.

Though this research program, I did indeed find that plant defensive responses form a suite of plant functional "effect" traits (sensu Lavorel & Garnier 2002) important in the determination of plant-litter decomposition and other soil processes within old field landscapes (Hättenschwiler & Vitousek 2000; Schweitzer *et al.* 2008). Further, the developmental nutrient environment that a plant experienced altered the later plastic response of that genotype to herbivory. Lastly, I found that both genetic and plastic sources of variation within a functional trait resulted in ecosystem process changes. Which of these two best explained a given ecosystem process and the relative magnitude of their importance varied by the process measured. Quantifying these relative components is key because they

are an important determinant of how the mean and variance of trait expression and therefore local process rates will change in response to anthropogenic or natural environmental change.

As such, plant defense expression should prove an important context-dependent determinant of decomposition rates. This work reveals that any spatial clumping of trait expression within this dominant, clonal species (due to genotype, herbivory, and to a lesser degree nutrient environment) should result in spatial heterogeneity in local soil nutrient pools beneath the plant. This represents a novel intraspecific and biotically mediated, induced example of "Zinke" effects whereby the identity of a plant species alters soil processes below that individual (Zinke 1962; Waring *et al.* 2015). These changes in soil processes have clear implications for below-ground organisms such as microbial communities and invertebrate microbivores (Bradford *et al.* 2008). For plant populations, these results suggest that defensive induction by genotypic clusters may lower subsequent nutrient availability in the soil beneath that cluster.

While not quantified explicitly within this dissertation, this dynamic may result in fitness costs of defense induction to the plants that are only evident over multiple years. Lastly, for insect herbivores, these results suggest that through selective feeding patterns, grasshoppers might shape soil nutrient availability, altering plant resistance encountered by subsequent generations of grasshoppers. While mobile generalist species such as *M. femurrubrum* can move away from poor quality food sources, many old-field insect herbivores are less mobile and thus may be strongly effected by leaf quality changes and trait heterogeneity in general (Maddox & Root 1990; Root & Cappuccino 1992).

While all of these potential effects are speculative, they suggest that intraspecific variation in genetic and plastic plant traits have cascading effects that spatially structure soil

processes, communities, and ultimately the diversity of organisms found within old-field landscapes. For example, within the community genetics paradigm, arthropod community composition and diversity is one of the response variables most closely tied to plant genetic identity and diversity (Johnson & Agrawal 2005; Wimp *et al.* 2005; Crutsinger *et al.* 2006). Presumably this is due to plant phenotypic variation and diversity (usually implicit but for a quantification of this see Johnson *et al.* 2009). The evidence presented in this dissertation suggest that such diversity promoting trait variation may arise in old fields not just through genetic effects but also through plastic responses to variable herbivory and nutrient availability promoting trait expression variation on the landscape.

Investigating causal mechanisms requires the experimental field and mesocosm methods utilized throughout this dissertation, however, one important question is whether the trait variation generated by the experimental treatments imposed captures a similar amount of variation as that seen between plant individuals within a field. If field survey trait variation is substantially larger than experimentally induced variation, it suggests potential additional and unmeasured factors increase trait variation on the landscape. On the other hand, if trait variation is substantially smaller across a field than within the experiments one might expect that the environmental treatments imposed in the experimental work represent a larger scale of environmental variation than typically exists within a single field. To briefly investigate that question, I compared experimental measurements of the coefficient of variation (CV: standard deviation divided by the mean) for two important plant functional traits, leaf mass area and leaf relative growth rate to field measurements of 155 individual plants from a gridded systematic field survey within the genotype source field. CV for the rate of leaf addition was 0.34 in the field survey compared to 0.33 in the greenhouse and a slightly lower 0.26 among individuals in the sandbox mesocosm experiment. The CV of

LMA was 0.14 between field survey individuals with a slightly higher CV of 0.18 measured in the greenhouse and slightly lower CV of 0.10 among sandbox individuals. These data suggest that the experiments presented within this dissertation are recapitulating a similar magnitude of trait variation to that measured within a single field. The slightly lower variation detected within the sandbox experiment in all likelihood reflects the smaller gradient of realized nutrient availability achieved by the treatments within that experiment and the smaller number of genotypes measured.

As a whole, this dissertation integrates concepts and experimental approaches from plant defense theory, community genetics, ecosystem ecology, and functional trait approaches. It uses these tools to tackle questions about the development of local-scale spatial heterogeneity in trait expression and nutrient cycling within landscapes. In doing so, it highlights often-overlooked indirect effects of plant/herbivore interactions on nutrient cycling. It also suggests that herbivores may shape not only the traits and evolutionary trajectories of the plant populations that they feed on, but also the soil nutrient environment and microbial community in which plants live. This sets up the potential for eco-evolutionary feedbacks (Post & Palkovacs 2009; Crutsinger 2016) between plant defense expression and soil nutrient availability mediated through phenotypic plasticity (Bardgett & Wardle 2003; Van der Putten *et al.* 2013; Baxendale *et al.* 2014). More broadly, it suggests that biotic factors, in addition to abiotic ones, play a key role in determining local-scale soil nutrient availability patterns and should potentially be taken into account within ecosystem models. These results are particularly relevant in a world where anthropogenic nitrogen inputs continue to rise and climate change is predicted to increase herbivory and thus plant defensive trait induction on landscapes.

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