Ecological and evolutionary consequences of genotypic variation and indirect genetic effects on plant-neighbor interactions

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Mark Alan Genung

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Abstract

Most explanations for community structure exclude the effects of genes above the population level, but recent research suggests that distinct genotypes of dominant plant species can also play important roles in structuring associated communities and influencing ecosystem processes. However, very little is understood about how the outcomes of plant-neighbor interactions are determined by intraspecific genotypic variation and indirect genetic effects (IGEs), which are influences on the phenotype of a focal individual due to the expression of genes in an interacting individual. Using clones of both *Solidago altissima* and *Solidago gigantea*, I established two common garden experiments and a decomposition experiment to determine how genotypic variation and neighbor genotype (IGEs) affected a range of population, community, and ecosystem level responses. These included above- and belowground plant productivity and biomass allocation, plant chemistry, pollinator visitation, decomposition rate, and nutrient cycling. Combined, the results from the first common garden and decomposition experiment showed that IGEs changed belowground plant traits, and these changes also affected litter quality at the time of plant senescence. These shifts in litter quality extended to affect ecosystem processes, specifically decomposition rate and nitrogen (N) immobilization. This result shows that IGEs can initiate “afterlife effects”, linking aboveground-belowground interactions with evolutionary processes. Because IGEs strongly affected belowground plant traits in my first common garden, I established a second common garden which manipulated the presence of belowground interactions between neighboring plants. The goal of this garden was to test the hypothesis that IGEs are most important for traits related to acquiring limiting resources, which for my species was the supply of belowground nutrients. I found that IGEs explained over 20 times as much variation in focal plant belowground biomass than did focal plant genotype, but only in pots which allowed belowground interactions. To explore the importance of IGEs to questions at the interface of ecology and evolution, I also conducted a primary literature review which indicated that IGEs link ecological and evolutionary dynamics and that the consequences of this ecological-evolutionary linkage begin with the phenotype of an individual within a population and extend to the associated community and ecosystem.

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INTRODUCTION.

While most explanations for community structure exclude the effects of genes above the population level, recent research suggests that distinct genotypes of dominant plant species can also play important roles in structuring associated communities (Whitham *et al.* 2003, 2006; Bailey *et al.* 2006; Crutsinger *et al.* 2006, 2008; Johnson *et al.* 2006; Mooney & Agrawal 2008; Genung *et al.* 2010, 2012; others) and influencing ecosystem processes (Whitham *et al.* 2003, 2006; Schweitzer *et al.* 2004, 2005, 2008; Crutsinger *et al.* 2006, 2009; Madritch et al. 2006, 2009, 2011; Genung *et al.* 2012; others). This research is part of the field of community and ecosystem genetics, which focuses on the idea that heritable genetic variation within species, especially dominant species, has effects on communities and ecosystems (Whitham *et al.* 2003, 2006). These effects are due to the extended phenotypes of genes; that is, phenotypes which are expressed levels above the population (i.e., pollinator community composition, rates of nutrient cycling) (Dawkins 1982; Whitham *et al.* 2003, 2006). However, the effects of genes need not be direct, or limited to the phenotype of the individual in which they are expressed. Indirect genetic effects (IGEs) allow for the possibility that the expression of genes in one species may affect the phenotype of a neighboring species (Wolf *et al.* 1998). However, the importance of IGEs relative to the direct effects of genotypic variation has received very little attention. To my knowledge, those studies which have explicitly considered “neighbor genotype” effects in plants (e.g., Cahill *et al.* 2005; Fridley *et al.* 2007; Bossdorf *et al.* 2009) have not explored the evolutionary implications which result from recognizing “neighbor genotype” effects as IGEs. The goal of my dissertation is to investigate the relative importance of direct and indirect genetic effects on a range of community and ecosystem processes, to develop hypotheses and mechanisms which explain why the relative importance of direct and indirect genetic effects may vary depending on the question being addressed, and to demonstrate that considering IGEs in ecological research provides a fuller and more accurate view of ecological and evolutionary patterns and processes.

The work presented in this dissertation bridges the fields of community and ecosystem genetics, plant-neighbor interactions, aboveground-belowground interactions, and eco-evo feedbacks. In Chapter 1, I examine how plant-neighbor interactions are influenced by genotypic variation, with consequences for plant productivity, resource allocation, and associated pollinator communities. This chapter aims to improve our understanding of how genotypic variation affects ecological processes by recognizing that neighbor genotypic variation (through IGEs) has the potential to drive population and community processes, either independently or in concert with focal plant genotypic variation. This work was originally published in *Ecology Letters* in 2012, and shows that neighbors had particularly strong effects on belowground biomass, while genotype by genotype (G x G) interactions affected pollinator visitation.

In Chapter 2, I extend the above results by testing whether “afterlife” effects of plant-neighbor interactions can alter ecosystem processes after plant senescence. I approached this question by establishing a litter mixing experiment to examine if plant traits which had been affected by IGEs during the growing season affected mass loss and nutrient dynamics. I found that coarse root biomass, rhizome biomass, and aboveground biomass were correlated with decomposition rate and nitrogen dynamics, and that growing season IGEs on plant chemistry could provide a mechanism for these correlations. These results show that plant-neighbor interactions can trigger a series of ecological relationships which culminate to affect ecosystem processes, and further highlight the importance of considering plant-neighbor interactions in community and ecosystem genetics. Chapter 2 will be submitted for publication in the near future.

Chapter 3 also extends Chapter 1, but in a different direction, by explicitly testing the hypothesis that belowground competition is responsible for the “neighbor genotype” effects. To test this hypothesis, I used an experiment in which half of the pots were divided belowground by an airtight, watertight barrier which prevented neighboring genotypes from interacting. Excluding belowground interactions reduced the relative importance of neighbor genotype identity (i.e., IGEs), and increased the relative importance of focal plant genotype identity, as determinants of plant traits (i.e., phenotypes). Neighbor genotype always explained more variation in focal plant belowground productivity than focal plant aboveground productivity. Because the plants used in the experiment were limited by nutrient availability, this result suggests that neighbor genotype variation is most important for traits related to acquiring limiting nutrients. I also found that, in some cases, the expression of genotypic variation and the transmission of IGEs was dependent on the presence or absence of belowground interactions between neighboring plants. I am pursuing additional analyses of soil chemistry and microbial activity in an attempt to explain the mechanisms behind these effects.

Chapter 4 was originally published in *Functional Ecology* in 2011 and explores the big-picture implications of plant-neighbor interactions by asking "what happens when focal plant genotypic variation affects associated communities, but neighbor plant genotypic variation (IGEs) affects focal plant fitness?" I review the literature on feedbacks between ecological and evolutionary processes. Along with my collaborators, I also present a model for understanding extended phenotypes and eco-evo feedbacks based on the classic model of phenotype and genotype space proposed by Lewontin (1974), and present a model of multi-selection based on the Price equation (Price 1972). This review suggests that IGEs and feedbacks link evolutionary and ecological dynamics, and that the consequences of this ecological-evolutionary linkage begin with the phenotype of an individual within a population and extend to the associated community and ecosystem.

REFERENCES

Bailey, J.K., Wooley, S.C., Lindroth, R.L. & Whitham, T.G. (2006). Importance of species interactions to community heritability: a genetic basis to trophic-level interactions. *Ecol. Lett.*, 9, 78-85.

Bossdorf, O., Shuja, Z. & Banta, J.A. (2009). Genotype and maternal environment affect belowground interactions between *Arabidopsis thaliana* and its competitors. *Oikos*, 118, 1541-1551.

Cahill, J.F., Kembel, S.W., Gustafson, D.J. (2005). Differential genetic influences on competitive effect and response in *Arabidopsis thaliana*. *J. Ecol.*, 93, 958-967.

Crutsinger, G.M., Collins, M.D., Fordyce, J.A., Gompert, Z., Nice, C.C. & Sanders, N.J. (2006). Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science*, 313, 966-968.

Crutsinger, G.M., Collins, M.D., Fordyce, J.A. & Sanders, N.J. (2008). Temporal dynamics in non-additive responses of arthropods to host-plant genotypic diversity. *Oikos*, 117, 255-264.

Crutsinger, G.M., Sanders, N.J. & Classen, A.T. (2009). Comparing intra- and inter-specific effects on litter decomposition in an old-field ecosystem. *Bas. Appl. Ecol.*, 10, 535-543.

Dawkins, R. (1982). *The extended phenotype: the gene as the unit of selection*. Oxford University Press, Oxford, UK.

Fridley, J.D., Grime, J.P. & Bilton, M. (2007). Genotypic identity of interspecific neighbours mediates plant responses to competition and environmental variation in a species rich grassland. *J. Ecol.*, 95, 908-915.

Genung, M.A., Lessard, J.P., Brown, C.B., Bunn, W.A., Cregger, M.A., Reynolds, W.N. *et al.* (2010). Non-additive effects of genotypic diversity increase floral abundance and abundance of floral visitors. *PLoS ONE*, 5, e8711.

Genung, M.A., Schweitzer, J.A., Ubeda, F., Fitzpatrick, B.M., Pregitzer, C.C., Felker-Quinn, E. *et al.* (2011). Genetic variation and community change – selection, evolution, and feedbacks, *Func. Ecol.*, 25, 408-419.

Genung, M.A., Bailey, J.K. & Schweitzer, J.A. (2012). Welcome to the neighbourhood: interspecific genotype by genotype interactions in *Solidago* influence above- and belowground biomass and associated communities. *Ecol. Lett.*, 15, 65-73.

Johnson, M.T.J., Lajeunesse, M.J. & Agrawal, A.A. (2006). Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecol. Lett.*, 9, 24-34.

Madritch, M., Donaldson, J.R. & Lindroth, R.L. (2006). Genetic identity of *Populus tremuloides* litter influences decomposition and nutrient release in a mixed forest stand. *Ecosystems*, 9, 528-537.

Mooney, K.A. & Agrawal, A.A. (2008). Plant genotype shapes ant-aphid interactions: Implications for community structure and indirect plant defense. *Am. Nat.*, 171, E195-E205.

Price, G.R. (1972). Fisher's "fundamental theorem" made clear. *Annals of Human Genetics*, 36, 129-140.

Schweitzer, J.A., Bailey, J.K., Rehill, B.J., Martinsen, G.D., Hart, S.C. & Lindroth, R.L. *et al.* (2004). Genetically based trait in a dominant tree affects ecosystem processes. *Ecol. Lett.*, 7, 127-134.

Schweitzer, J.A., Bailey, J.K., Hart, S.C. & Whitham, T.G. (2005). Non-additive effects of mixing cottonwood genotypes on litter decomposition and nutrient dynamics. *Ecology*, 86, 2834-2840.

Schweitzer, J.A., Bailey, J.K., Fischer, D.G., LeRoy, C.J., Lonsdorf, E.V., Whitham, T.G. *et al.* (2008). Plant-soil microorganism interactions: Heritable relationship between plant genotype and associated soil microorganisms. *Ecology*, 89, 773-781.

Whitham, T.G., Young, W.P., Martinsen, G.D., Gehring, C.A., Schweitzer, J.A., Shuster, S.M. *et al.* (2003). Community and ecosystem genetics: A consequence of the extended phenotype. *Ecology*, 84, 559-573.

Whitham, T.G., Bailey, J.K., Schweitzer, J.A., Shuster SM, Bangert RK, Leroy CJ *et al.* (2006). A framework for community and ecosystem genetics: from genes to ecosystems. *Nat. Rev. Genet.*, 7, 510-523.

Wolf, J.B., Brodie, E.D., Cheverud, J.M., Moore, A.J. & Wade, M.J. (1998). Evolutionary consequences of indirect genetic effects. *Trends Ecol. Evol.*, 13, 64-69.

CHAPTER 1.

Welcome to the neighborhood: interspecific genotype by genotype interactions in Solidago influence above- and belowground biomass and associated communities

ABSTRACT

Intra- and interspecific plant–plant interactions are fundamental to patterns of community assembly and to the mixture effects observed in biodiversity studies. Although much research has been conducted at the species level, very little is understood about how genetic variation within and among interacting species may drive these processes. Using clones of both *Solidago altissima* and *Solidago gigantea*, we found that genotypic variation in a plant's neighbors affected both above- and belowground plant traits, and that genotype by genotype interactions between neighboring plants impacted associated pollinator communities. The traits for which focal plant genotypic variation explained the most variation varied by plant species, whereas neighbor genotypic variation explained the most variation in coarse root biomass. Our results provide new insight into genotypic and species diversity effects in plant–neighbor interactions, the extended consequences of diversity effects, and the potential for evolution in response to competitive or to facilitative plant–neighbor interactions.

INTRODUCTION

Biodiversity provides key ecosystem services and yet, in over 50 years of ecological research on the causes, mechanisms and consequences of biodiversity, few generalizations can actually be made (see Hooper *et al.* 2005 for review). For example, although it is increasingly understood that variation among and within species can have extended consequences for the diversity of communities and ecosystems in which these species are embedded (e.g., Johnson & Agrawal 2005; Bailey *et al.* 2006; Crutsinger *et al.* 2006; Johnson *et al.* 2006; Whitham *et al.* 2006; Mooney & Agrawal 2008) mechanisms for understanding the community and ecosystem effects of genetic variation remain elusive. Recent research suggests that genotypic diversity (i.e., the number of unique genotypes present in a given area) in dominant plant species can structure the diversity of associated communities (e.g., Booth & Grime 2003; Crutsinger *et al.* 2006, 2008; Johnson *et al.* 2006) and influence ecosystem processes (e.g., Hughes & Stachowicz 2004; Schweitzer *et al.* 2005; Crutsinger *et al.* 2006; Madritch *et al.* 2006). The mechanism invoked for diversity effects involves either species or genotype interactions (i.e., a genotype's traits change in the presence of certain neighbor genotypes) that occur in mixture and affect community and ecosystem processes. Therefore, simple studies to understand these interactions and how they influence patterns of biodiversity can be conducted with experimental designs which are more commonly associated with research into plant–neighbor interactions (Turkington & Harper 1979; Aarssen & Turkington 1985; Cahill *et al.* 2005; Fridley *et al.* 2007; Bossdorf *et al.* 2009).

Although most work in the field of community and ecosystem genetics has been conducted within a single site or population, species are embedded in a matrix with many other species in variable environments (Whitham *et al.* 2006). Because of the constant interactions between species and their abiotic and biotic environments, understanding the community and ecosystem consequences of genotype by environment (G × E) interactions is a rapidly emerging area of research. When G × E interactions have been examined, studies clearly indicate that abiotic factors such as site differences (Johnson & Agrawal 2005; Tack *et al.* 2010) and nutrient addition (Madritch *et al.* 2006; Rowntree *et al.* 2010), as well as biotic factors such as genotypic diversity (Schweitzer *et al.* 2005; Crutsinger *et al.* 2006; Johnson *et al.*2006; Madritch *et al.* 2006) and herbivory (Schweitzer *et al.* 2005) can all influence the community and ecosystem level impacts of plant intraspecific genetic variation. Investigating the role of G × E interactions is essential to understanding the effects of species and genotypic diversity in plants, because plant–neighbor interactions are a common type of G × E interaction in which the 'biotic environment' (e.g., neighbor plant) contains genes (i.e., genotype × genotype interactions or G × G) and both participants are fixed in space and forced to interact for resources (Turkington & Harper 1979). Studies of plant–neighbor interactions have previously shown that intraspecific genetic variation in an individual's neighbors (1) can have important consequences for overall plant fitness and performance (Turkington & Harper 1979; Aarssen & Turkington 1985; Cahill *et al.* 2005; Fridley *et al.* 2007; Bossdorf *et al.* 2009; Collins *et al.* 2010) and (2) is thought to be an important part of the maintenance of species and genetic variation in plant communities (e.g., Fridley *et al.* 2007; Lankau & Strauss 2007). Our study builds on the work of plant–neighbor interactions, genotypic diversity and coevolutionary theory to investigate how genetic variation in two naturally occurring, dominant old field plants may interact to influence above- and belowground plant traits, and associated pollinator communities.

Studying how neighboring plants compete for pollinators or facilitate each other's pollination involves considering a system in which plant–pollinator interactions are mutualistic, but neighboring plants can have either an antagonistic or synergistic effect on each other's sexual reproduction (reviewed in Mitchell *et al.* 2009). Exploitative competition for pollinators between neighboring plants may reduce plant fitness when the quantity of visits to a given plant is reduced because pollinators are attracted to its co-flowering neighbors instead (e.g., Macior 1971; Pleasants 1980). Plant fitness may also be reduced due to deposition of incompatible pollen, pollen wastage, or stigma clogging; these effects may be especially important for close congeners. However, plant fitness may be increased due to facilitative interactions between neighboring plants if pollinators are attracted to the increased floral density of species mixtures (e.g., Thomson 1982; Ghazoul 2006; Lazaro *et al.* 2009). Mitchell *et al.* (2009) highlights that both ecological and evolutionary context can affect how competition and facilitation between neighboring plants affects plant–pollinator interactions. The ecological context includes environmental variation (i.e., variation in the presence or number of particular neighboring species), whereas the evolutionary context includes the heritable phenotypic variation within each of the interacting plant species which scales up to determine trait variation (Mitchell *et al.* 2009). This perspective is directly relatable to the goals of G × G studies, which manipulate both the evolutionary context of focal plant phenotypic variation by including distinct genotypes of plants, and the ecological context of biotic environmental variation by manipulation of neighbor plant genotype identity.

We used *Solidago* spp. as a model system to examine the role of G × G interactions in affecting above- and belowground plant traits and arthropod pollinator visitation. We hypothesized that G × G interactions would affect plant–plant interactions and provide a mechanism for understanding effects that occur when species and genotypes co-occur in natural systems. We established a common garden experiment using clonally replicated individuals (i.e., genotypes) of both *Solidago altissima* and *Solidago gigantea*, with each genotype planted alone in monoculture treatments and with all possible interspecific combination of genotypes. We examined how intraspecific genotypic variation (i.e., 'focal genotype') and genotypically based biotic environmental variation (i.e., 'neighbor genotype') affected a range of population and community traits. Specifically, we addressed the following questions: (1) Does focal plant genotypic variation influence above- and belowground productivity, floral biomass, and pollinator visitation? (2) Does the biotic environment affect these same traits, either through the effects of neighbor plant genotype or G × G interactions? Our results indicate that the genotype identity of a plant's neighbor affected both above- and belowground biomass, and that interspecific indirect genetic effects can drive patterns of pollinator visitation. Across species, the genotype identity of a plant's neighbor had more consistent effects on coarse root biomass than did the focal plant's genotype identity, which suggests that genetic variation in a plant's neighbors may be an important, but less frequently considered mechanism explaining population and community trait variation in ecological communities.

METHODS

Study species

Solidago altissima is a dominant species in abandoned agricultural fields where it can have large impacts on biodiversity and ecosystem function (Maddox & Root 1987; Crutsinger *et al.* 2006). Intraspecific genetic variation in *S. altissima* has been shown to affect herbivores (e.g., Maddox & Root 1987; Crutsinger *et al.* 2006), flowering phenology (e.g., Gross & Werner 1983), as well as floral visitor abundance (Genung *et al.* 2010). *Solidago gigantea* is less common than *S. altissima* in southeastern old fields, but these two species are among the most frequently co-occurring species pairs in the genus *Solidago* (Abrahamson *et al.* 2005). Although the two species are ecologically similar, they differ in life-history traits (Abrahamson & Weis 1997), allocation of resources to different growth forms, and tolerance for variation in soil moisture (Abrahamson *et al.* 2005). Specifically, *S. altissima* allocates relatively more biomass belowground and is more capable of tolerating broad variation in soil moisture than is *S. gigantea* whereas *S. gigantea* allocates relatively more biomass into flowers (Abrahamson *et al.* 2005). Both *S. altissima* and *S. gigantea* are gynomonoecious, self-incompatible, and rely on insect pollination for fertilization (Wise *et al.* 2008). Each capitulum contains 10–15 pistillate ray flowers surrounding 3–7 hermaphroditic disc flowers (Abrahamson & Weis 1997); the ray flowers mature and become receptive to pollen before the disc flowers (Gross & Werner 1983). Each ray and disc flower produces a single seed (Wise *et al.* 2008).

Garden description

In April 2008, a common garden experiment was established at Freels Bend on the reservation of Oak Ridge National Laboratory to examine the community and ecosystem level impacts of genotype-based plant–neighbor interactions in *Solidago*. This common garden includes three locally collected genotypes (i.e., clonal families) of both *S. altissima* and *S. gigantea*. The *S. altissima* and *S. gigantea* clones we utilized were originally propagated by G.M. Crutsinger and clones were maintained at the University of Tennessee and Freels Bend. The genotypes were collected from random locations around the study site at Freels Bend; sampled individuals from both species were carefully collected from unique connected genets that were at least 50–150 m apart (Crutsinger *et al.* 2006; Supplementary Material). Rhizomes were collected from connected ramets to ensure they were from the same genet. The three *S. altissima* genotypes were originally collected and determined as unique genotypes using AFLP (Crutsinger *et al.* 2006, Supplementary Material); however, molecular data is unavailable for the *S. gigantea* genotypes.

The experimental treatments included genotype monocultures as well as all possible interspecific combinations of *S. altissima* and *S. gigantea* genotypes, planted together in 95 L pots (n = three replicates per genotype-neighbor genotype combination, six monoculture treatments and nine genotype mixture combinations, a total of 45 pots). All plants were propagated from cloned stocks of genotypes. A 3-cm rhizome of each species and genotype were grown in a greenhouse in flats in standard potting media for 8 weeks; the plants received regular water. Rooting hormones (Hormodin, OHP Inc., Mainland, PA, USA) were applied to each rhizome. When the plants were *c.* 10 cm in height they were transplanted into the pots at the field site. Each pot initially included four individuals, but variation in plant density occurred due to clonal production of new ramets beginning during the initial growing season (2008) that continued throughout the experiment. In monocultures, all four individuals were clones of the same genotype. In genotype mixtures, each pot initially contained two individuals of each genotype (four plants total/pot). The pots were randomly placed in a grid formation within an old field with *c.* 3 m separating each pot from its neighbors. The field was not mown during the course of the experiment, creating an aboveground environment which closely mimicked that which the plants would experience in natural systems. Supplementary water was pumped to each pot in equal amounts when conditions required. The pots were filled with Fafard Growing Mix #1 (Conrad Fafard Inc, Agawam, MA, USA), and invading plants were removed throughout the experiment. Approximately 10 g of fertilizer (24/8/16, Miracle-Gro, Marysville, OH, USA) was applied once to each pot in April 2008.

Plant trait measurements

We measured a series of plant and community traits over the course of a growing season; these included rhizome biomass, coarse root biomass, vegetative biomass, floral biomass, and arthropod pollinator visitation. To determine vegetative biomass, we measured the aboveground height of the plants during peak productivity in early August and used an allometric equation to estimate aboveground biomass (Appendix 1). To estimate floral biomass, we used a representative panicle of *S. altissima* with known floral biomass as a unit of measurement. We chose a panicle which was smaller than average, *c.* ½ the size of an average panicle, and estimated floral biomass as the number of replicates of the representative panicle required to equal the floral abundance of the pot (*sensu* Genung *et al.* 2010). We measured floral biomass for each plant at the time when floral abundance was at its peak; this ranged from late August through late October 2009.

Belowground plant structures were sampled after the plants had senesced in December 2009, by carefully removing all plant structures intact from the soil. Aboveground differences allowed us to differentiate *S. altissima* and *S. gigantea* belowground structures in species mixture pots. We did not assess fine roots (< 2 mm in diameter) which became disconnected from the larger root structure during the excavation process, because we could not identify which *Solidago* species had produced the roots. We separated all rhizomes (i.e., horizontal underground stems) from coarse roots (> 2 mm in diameter) by hand in the laboratory. All belowground structures were air-dried for 2 weeks before weighing; a subsample was dried (70 °C for 48 h) such that all final biomass is presented on a dry mass basis. Although we were careful to excavate entire rhizome systems, in some cases rhizomes were severed during the excavation process; these pots were excluded from our analysis of belowground traits because we could no longer be certain which genotype had produced the rhizomes in question. One rhizome biomass sample and one coarse root biomass sample were excluded due to labeling errors.

Pollinator visitation was assessed using visual surveys of the pots, performed ten times beginning in early August and continuing through mid-November (approximately every 10 days). We summed pollinator visitation across all 10 surveys for our analyses; this sum is referred to as 'pollinator visitation'. During each survey, each pot was observed for five min from a distance of *c.* 3 m and the abundance of insects which visited flowers was counted (*sensu* Genung *et al.*

2010 and references therein). A pollinator visit was recorded if an insect contacted a reproductive portion of the plant (Lazaro *et al.* 2009). Pollinators were grouped into taxa based on differences the surveyor (MAG) could consistently identify from a range of 3 m. The most common pollinator taxa on *S. gigantea* were halictid bees, especially *Agapostemon* species, and the most common pollinator taxa on *S. altissima* were *Apis* species and *Bombus* species (see Appendix 2 for a complete list of observed pollinator taxa). Following the visual survey, flowering panicles were shaken onto a sheet of white paper to assess pollinators which had not moved between plants during the survey time; by far the most common pollinator recorded in this way was *Chauliognathus pennsylvanicus*. We also estimated pollinator visitation per unit floral biomass per unit time (hereafter 'per-flower visitation'), where time included the total length of surveys during which plants were flowering.

Statistical analyses

To determine the effects of focal plant genotype, neighbor plant genotype, and the interaction of focal genotype and neighbor genotype, we selected only the pots which contained one genotype of both *S. altissima* and *S. gigantea* and tested which factors explained variation in our measured plant and community traits. We used a generalized linear model with a normal distribution and an identity link function to test for the effects of plant and neighbor genotypic variation. This approach excluded monoculture pots because including these pots would confound the effects of neighbor species identity and pot-level species and genotypic diversity with neighbor genotype identity effects. We separated our analyses by species such that our data points remained independent within each analysis. However, the two analyses themselves are not independent. For each species, our analysis included the following terms: focal genotype, neighbor genotype, and focal genotype × neighbor genotype. Focal genotype and neighbor genotype were entered as fixed factors. To determine the percentage of variation accounted for by each experimental factor, we repeated this analysis, with the same factors and responses, as a general linear model. Variances were calculated with the following equation: (treatment sum of squares)/ (total sum of squares) × 100%) (Johnson 2008).

To determine whether neighbor trait values, as opposed to neighbor genotype identity, could explain variation in focal plant traits, we repeated the above analysis and substituted the neighbor plant's trait value in place of neighbor genotype identity. The trait value (i.e., rhizome biomass, coarse root biomass, aboveground vegetative biomass, floral biomass, pollinator visitation, per-flower visitation) used was always the same as the response variable in the focal plant. Again, we separated our analyses by species such that our data points remained independent. Our analysis included the following terms: species identity, focal genotype, neighbor trait value, and focal genotype × neighbor trait value. Focal genotype was entered as a fixed factor. Variances were calculated for each factor as described in the preceding paragraph.

To determine which plant traits were most important to pollinator visitation, we used generalized linear models to examine how pollinator visitation and per-flower visitation were affected by plant traits. Again, we separated our analyses by species and neighbor species such that our data points remained independent. Focal genotype was entered as a fixed factor. Floral biomass was not used as a predictor for per-flower visitation. Although factorial combinations of plant traits may be important for predicting pollinator visitation, we were unable to include these factors because of sample size limitations.

RESULTS

Consistent with the hypothesis that genotypic variation in a plant's neighbor would affect plant traits, we found that neighbor genotype affected both above- and belowground biomass (**Table 1**, **Figure 1**). In addition, consistent with the hypothesis that G × G interactions would affect the response of plants in mixture, we detected a G × G interaction affecting pollinator visitation (Table 1, Fig. 1). The traits for which focal genotype explained the most variation varied by plant species (Table 1). The per cent of variation explained by focal plant genotype ranged between 6 and 21% for *S. altissima* focal plants, and 12 and 65% for *S. gigantea* focal plants. We detected weaker effects (i.e., not consistently significant across focal species, and less proportion variance explained) of focal genotype on per-flower pollinator visitation.

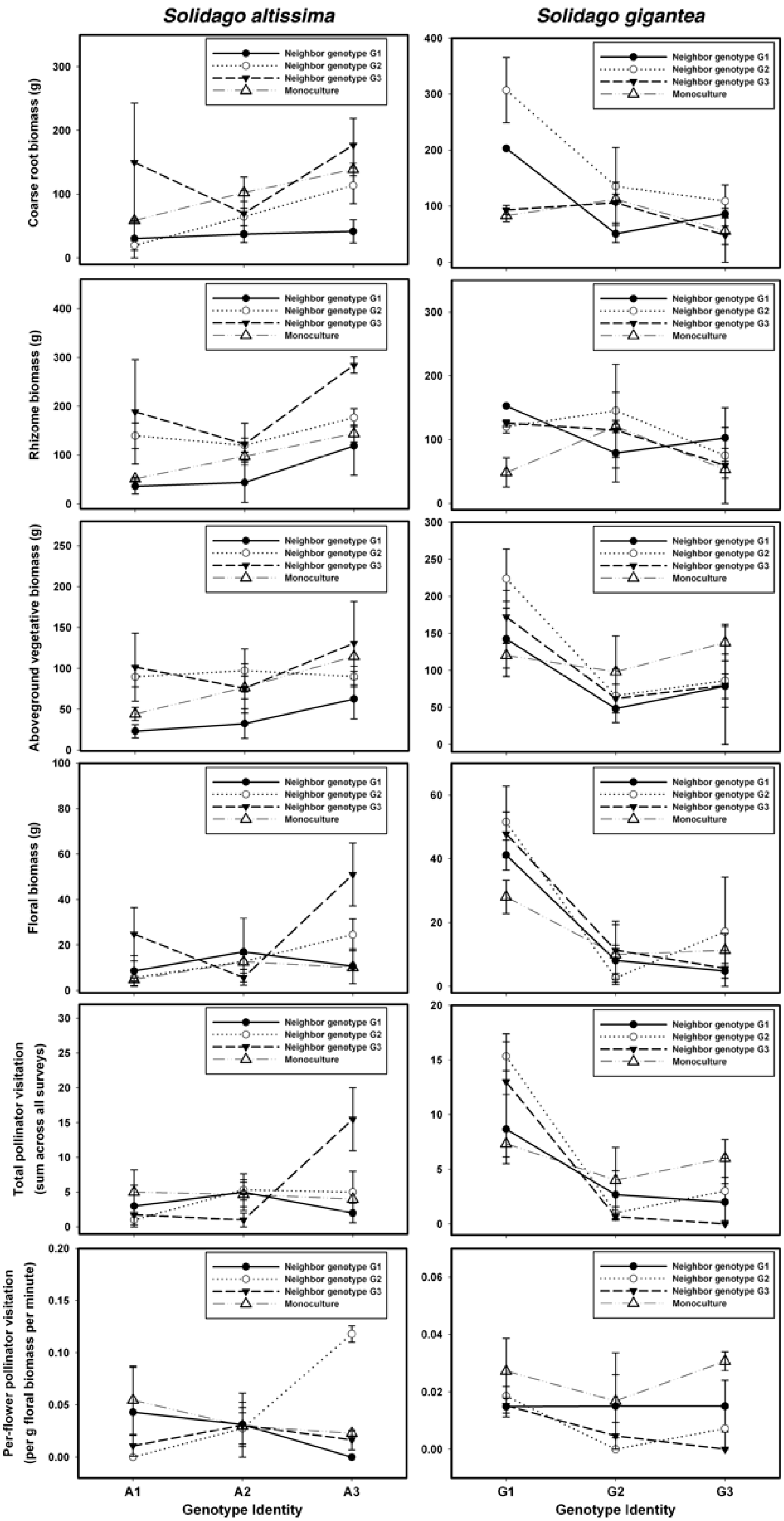
Although focal plant genotype was a significant factor affecting above- and belowground productivity, floral biomass, pollinator visitation and per-flower visitation when in mixture, the genotype of the interacting neighbor plant was a consistent predictor of coarse root biomass and also affected *S. altissima* vegetative biomass (Table 1). The per cent of variation explained by the genotype of the neighboring plant ranged between 1 and 36% for *S. altissima* focal plants, and 0 and 14% for *S. gigantea* focal plants. The traits for which neighbor genotype explained the

Table 1. Growing season genotype by neighbor genotype interactions. The results of generalized linear models testing the effects of focal genotype, neighbor genotype, and focal genotype by neighbor genotype interactions are shown for six traits. Per flower visitation refers to the number of pollinators visits a plant received, per unit floral biomass, per unit time. To ensure independence within each analysis, data were only analyzed for pots containing one genotype of both *Solidago altissima* and *Solidago gigantea*, and the analysis was run separately for each species. The most significant effects of focal genotype are found for *S. gigantea* focal plants and the pollinator visitation trait. The most significant effects of neighbor genotype were found for *S. altissima* focal plants and the coarse root biomass trait. A significant G × G interaction was detected for pollinator visitation to *S. altissima*. Bold values are significant at $\alpha = 0.05$.

Trait	**Focal genotype**			**Neighbor genotype**			**G × G**		
	d.f.	***p***	**$*r^2$**	**d.f.**	***p***	**$*r^2$**	**d.f**	***p***	**$*r^2$**
(a) *Solidago altissima*									
Rhizome biomass	2, 25	**0.026**	**0.170**	2, 25	**0.001**	**0.354**	4, 25	0.902	0.022
Coarse root biomass	2, 24	0.060	0.137	2, 24	**0.005**	**0.285**	4, 24	0.413	0.093
Vegetative biomass	2, 27	0.290	0.055	2, 27	**0.002**	**0.358**	4, 27	0.782	0.039
Floral biomass	2, 27	0.076	0.127	2, 27	0.066	0.135	4, 27	0.152	0.170
Pollinator visitation	2, 27	**0.012**	**0.210**	2, 27	0.492	0.029	4, 27	**0.027**	**0.272**
Per flower visitation	2, 27	0.124	0.106	2, 27	0.841	0.008	4, 27	0.104	0.209
(b) *Solidago gigantea*									
Rhizome biomass	2, 24	0.206	0.116	2, 24	0.842	0.012	4, 24	0.784	0.062
Coarse root biomass	2, 25	**0.003**	**0.286**	2, 25	**0.042**	**0.136**	4, 25	0.161	0.141
Vegetative biomass	2, 27	**< 0.001**	**0.421**	2, 27	0.663	0.016	4, 27	0.676	0.047
Floral biomass	2, 27	**< 0.001**	**0.645**	2, 27	0.926	0.002	4, 27	0.732	0.023
Pollinator visitation	2, 27	**< 0.001**	**0.616**	2, 27	0.379	0.023	4, 27	0.483	0.043
Per flower visitation	2, 27	**0.045**	**0.186**	2, 27	0.212	0.089	4, 27	0.737	0.056

$*r^2$ estimate obtained from ordinary least square analysis, and represents the percentage of variation explained by a given factor in the full model.

Figure 1. Growing season genotype by neighbor interactions affect plant traits. The relative importance of focal genotype, neighbor genotype, and G × G interactions varies across plant traits and by focal species. Per flower visitation refers to the number of pollinators visits a plant received, per unit floral biomass, per unit time. Mean trait values for plant traits and pollinator visitation are presented for each focal genotype of each species depending on the neighbor genotype with which they were grown. *Solidago altissima* (left panels) or *Solidago gigantea* (right panels) focal genotype identity is listed along the *x*-axis and each connected set of points represents the genotype identity of the neighboring plants.


Solidago altissima
Solidago gigantea
Neighbor genotype G1
Neighbor genotype G2
Neighbor genotype G3
Monoculture
Coarse root biomass (g)
Rhizome biomass (g)
Aboveground vegetative biomass (g)
Floral biomass (g)
Total pollinator visitation (sum across all surveys)
Per-flower pollinator visitation (per g floral biomass per minute)
A1
A2
A3
G1
G2
G3
Genotype Identity
Genotype Identity

most variation varied by plant species, but for both species the most consistently explanatory effects of neighbor genotype were on belowground traits (Table 1). Importantly, we also detected an interspecific G × G interaction affecting pollinator visitation to *S. altissima* (Table 1). This result demonstrates that the extended effects on pollinator visitation found in plant–plant mixtures are a consequence of interactions amongst individual genotypes. In other words, the pollinators which visited an individual depended upon the genotypically based variation in the individual's biotic environment. We found no neighbor genotype effects or G × G interactions for per flower pollinator visitation. We also found that neighbor plant traits (as opposed to neighbor genotype) could explain variation in focal plant traits. We found that neighbor biomass affected focal plant biomass for the following traits: rhizome biomass, coarse root biomass and aboveground vegetative biomass (Table 2). For floral biomass, pollinator visitation, and per flower visitation we found no effect of neighbor traits on the focal plant (Table 2).

We also found species level effects with respect to which plant and community traits were influenced by each of the model factors. In general, focal genotype was the most significant predictor of plant traits in *S. gigantea*, whereas neighbor genotype was the most significant predictor of plant traits in *S. altissima* (Table 1). These results demonstrate that there is little phenotypic plasticity in the response of *S. gigantea* to the genetic environment of *S. altissima*. In contrast, *S. altissima* demonstrated significant plasticity in their response to *S. gigantea*, particularly in belowground traits (Conner & Hartl 2004). For both species, the only plant trait which consistently predicted pollinator visitation was floral biomass, whereas coarse root biomass predicted per flower visitation to *S. gigantea*, but not *S. altissima* (Table 3).

Averaging across species, focal genotype explained the most variation in aboveground plant biomass and pollinator visitation, and the least variation in belowground plant biomass, whereas the opposite pattern was observed for neighbor genotype. However, the proportion of variation explained by focal genotype and neighbor genotype varied by plant species (**Figure 2**). Focal genotype and neighbor genotype explained roughly the same amount of variation in belowground plant traits, whereas focal genotype explained more variation in aboveground plant and pollinator visitation than did neighbor genotype. This result suggests that processes (which may be competitive or facilitative) related to coarse root and rhizome biomass may be strongly influenced by genetically based biotic environmental variation.

Table 2. Growing season genotype by neighbor trait interactions. The results of an ANCOVA generalized linear model testing the effects of focal genotype, neighbor trait, and focal genotype by neighbor trait interactions are shown for six plant traits. 'Neighbor trait' was always the same as the response variable for the focal plant. To ensure independence of data points within each analysis, data were only analyzed for pots containing one genotype of both *Solidago altissima* and *Solidago gigantea*, and the analysis was run separately for each species. For rhizome biomass, coarse root biomass, and aboveground vegetative biomass, the biomass produced by a plant's neighbor influenced the biomass of the focal plant. For floral biomass, pollinator visitation and per flower visitation, we found no effect of neighbor traits on the focal plant. Bold values are significant at $\alpha = 0.05$.

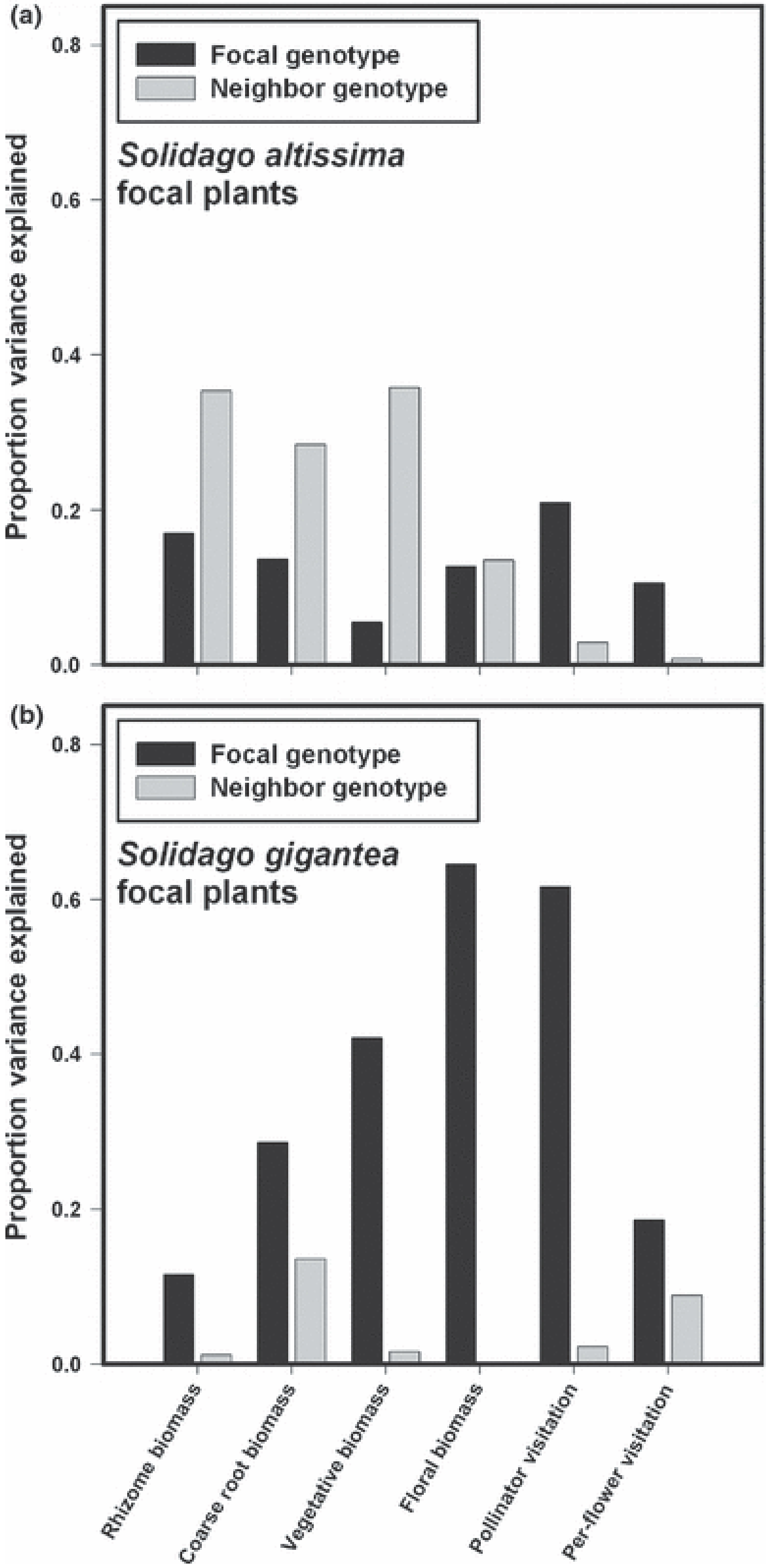
Trait	**Focal genotype**			**Neighbor trait**			**G × Neighbor trait**		
	d.f.	***p***	**$*r^2$**	**d.f.**	***p***	**$*r^2$**	**d.f**	***p***	**$*r^2$**
(a) *Solidago altissima*									
Rhizome biomass	2, 24	0.142	0.117	1, 24	**0.010**	**0.354**	2, 24	0.617	0.027
Coarse root biomass	2, 24	0.773	0.015	1, 24	**0.005**	**0.285**	2, 24	0.230	0.088
Vegetative biomass	2, 27	0.500	0.033	1, 27	**0.001**	**0.358**	2, 27	0.986	0.001
Floral biomass	2, 27	0.248	0.085	1, 27	0.106	0.135	2, 27	0.245	0.086
Pollinator visitation	2, 27	0.110	0.141	1, 27	0.534	0.029	2, 27	0.502	0.042
Per flower visitation	2, 27	0.077	0.150	1, 27	0.296	0.008	2, 27	0.101	0.132
(b) *Solidago gigantea*									
Rhizome biomass	2, 24	0.507	0.036	1, 24	**0.004**	**0.012**	2, 24	0.050	0.178
Coarse root biomass	2, 24	0.275	0.076	1, 24	0.219	0.136	2, 24	0.609	0.028
Vegetative biomass	2, 27	0.084	0.100	1, 27	**0.046**	**0.016**	2, 27	0.738	0.011
Floral biomass	2, 27	**< 0.001**	**0.469**	1, 27	0.420	0.002	2, 27	0.702	0.008
Pollinator visitation	2, 27	**< 0.001**	**0.637**	1, 27	0.935	0.023	2, 27	0.244	0.037
Per flower visitation	2, 27	0.079	0.169	1, 27	0.298	0.089	2, 27	0.656	0.026

$*r^2$ estimate obtained from ordinary least square analysis, and represents the percentage of variation explained by a given factor in the full model.

Table 3. Plant trait influences on pollinator visitation. The results of a generalized linear model analysis linking plant traits to pollinator visitation, grouped by species and neighbor species (represented by the first and second names, respectively, at the top of each column). Per flower visitation refers to the number of pollinator visits a plant received, per unit floral biomass, per unit time. All listed traits and response variables were measured on focal plants, and not neighboring plants. Only data from pots containing both species are presented here. Floral biomass is not used to predict per flower visitation. Floral biomass is the only trait which consistently predicts total pollinator visitation. In contrast, several traits predict per flower pollinator visitation, but only for *Solidago gigantea* focal plants with *Solidago altissima* neighbors. Bold values are significant at α = 0.05.

	Response = Total pollinator visitation		**Response = Per-flower visitation**	
Trait	***S. altissima/S. gigantea***	***S. gigantea/S. altissima***	***S. altissima/S. gigantea***	***S. gigantea/S. altissima***
	P	***P***	***P***	***P***
Rhizome biomass	0.070	0.506	0.734	0.253
Coarse root biomass	0.271	0.390	0.152	**0.009**
Vegetative biomass	0.675	0.897	0.100	0.418
Floral biomass	**< 0.001**	**< 0.001**	NA	NA

Figure 2. Effect size of genotype and neighbor genotype across plant traits. Per flower visitation refers to the number of pollinators visits a plant received, per unit floral biomass, per unit time. The proportion variance explained by focal genotype and neighbor genotype varies depending on whether the focal plant is an individual of *Solidago altissima* (a) or *Solidago gigantea* (b). Proportion variation in plant and community traits (along *x*-axis), as explained by focal plant genotype identity (black bars) and neighbor genotype identity (grey bars), is shown. Belowground traits (rhizome biomass, root biomass), aboveground traits (vegetative biomass, floral biomass) and community traits (pollinator visitation and per flower visitation) are included.


(a)
0.8
0.6
0.4
0.2
0.0
Proportion variance explained
Focal genotype
Neighbor genotype
Solidago altissima
focal plants
(b)
0.8
0.6
0.4
0.2
0.0
Proportion variance explained
Focal genotype
Neighbor genotype
Solidago gigantea
focal plants
Rhizome biomass
Coarse root biomass
Vegetative biomass
Floral biomass
Pollinator visitation
Per-flower visitation

DISCUSSION

We examined whether (1) genotypic variation in a plant's neighbors and (2) indirect genetic effects (i.e., effects on an individual's phenotype due to genes in an interacting individual; Wolf *et al.* 1998) between plants and their neighbors influenced above- and belowground productivity, floral biomass, and pollinator visitation. Our results indicate that genotypic variation in a plant's neighbors affected both above and belowground plant traits, and that G × G interactions between neighboring plants extended to associated communities (specifically pollinators visiting *S. altissima*). The neighbor genotype effects and G × G interactions we detected are both types of indirect genetic effects, because the focal plant's traits are altered due to the genotype identity of its neighbor; however, the indirect genetic effect in G × G interactions is contingent on the genotype identity of the focal plant. In addition, the strongest effects of focal genotype and neighbor genotype varied by plant species. For example, focal genotype explained the most variation in pollinator visitation and rhizome biomass for *S. altissima* focal plants, and the most variation in pollinator visitation and floral biomass for *S. gigantea* focal plants.

Indirect genetic effects in community and ecosystem genetics

We found that genotypic variation in a plant and its neighbor affected a wide range of plant traits, and we also found that G × G interactions between neighboring plants extended to affect pollinator communities (Table 1). These results align with those from across a wide range of plant systems which have shown that intraspecific genetic variation affects traits at the population and community level (Schweitzer *et al.* 2004, 2008; Crutsinger *et al.* 2006; Johnson *et al.* 2006; Whitham *et al.* 2006). Although a smaller collection of studies have examined how genetically based plant–neighbor interactions affect population-level responses (e.g., Aarssen & Turkington 1985; Fridley *et al.* 2007; Bossdorf *et al.* 2009), our data suggest that these interactions can also affect belowground biomass and associated communities. Although we did not detect an effect of species diversity (Appendix 3) our results emphasize the fact that species interactions (which may be genotype-based) can occur in mixture even without consistently increasing or decreasing a given trait value relative to monoculture. Importantly, the most consistent effects of genetically-based biotic environmental variation (i.e., 'neighbor genotype') were on coarse root biomass, suggesting that studies which solely examine aboveground biomass may not detect the effects of species or genotypic variation in a plant's neighbor.

The effects of focal genotype varied by species, and explained the most variation in floral biomass and pollinator visitation for *S. gigantea* focal plants, and pollinator visitation and rhizome biomass for *S. altissima* focal plants (Table 1). The effects of neighbor genotype also varied by plant species, and explained the most variation in coarse root biomass for *S. gigantea* focal plants, and rhizome biomass and aboveground biomass for *S. altissima* focal plants. The effects of neighbor genotype were largest on *S. altissima* focal plants, and this pattern appears to be driven by an especially vigorous *S. gigantea* genotype 'G1' which suppresses the production of rhizome, root, and aboveground biomass in its *S. altissima* neighbors (Fig. 1). As competition is likely to be more intense belowground in nitrogen limited environments (Tilman 1988; Wilson & Tilman 1993), the observation that neighbor genotype is important to belowground productivity suggests that intraspecific variation for traits related to nitrogen acquisition are responsible. This idea is also supported by data showing that, for *S. gigantea* focal plants, the only significant effect of neighbor genotype was on coarse root biomass (Table 1). As the total belowground biomass of focal plants was negatively correlated with neighbor plant belowground biomass, the mechanism for our observed neighbor effects probably involves neighboring plants directly competing for space and resources. Other pathways, such as chemical inhibition (i.e., allelopathy) between neighboring plants, or indirect interactions in which one genotype impacts its neighbor by altering associated communities cannot be ruled out as also contributing to neighbor effects.

Most community genetics studies have focused on interactions across trophic levels, and those studies which have examined within trophic level interactions have focused on competition and allelopathy (Booth & Grime 2003; Fridley *et al.* 2007; Lankau & Strauss 2007; Bossdorf *et al.* 2009); however, interspecific genetic variation may also affect facilitative interactions within the same trophic level (Michalet *et al.* 2011). Facilitation within a trophic level has particular importance for plant–pollinator interactions, because co-flowering neighboring plants can compete for pollinators or facilitate each other's pollination (Thomson 1982; Callaway 1995). Both variation in the presence or number of particular neighboring species and the heritable phenotypic variation within the interacting plant species can affect pollinator visitation to neighboring plants (Mitchell *et al.* 2009). Our results indicate that pollinator visitation to *S. gigantea* was not affected by the genotype identity of (Table 1) or pollinator visitation to (Table 2) neighboring *S. altissima* plants, but rather by genotypic variation for the floral biomass trait (Tables 1 and 3). This result emphasizes the evolutionary context of plant–pollinator interactions by indicating that natural selection can act on genotypic variation for floral biomass in *S. gigantea* focal plants, regardless of the genotype identity of those plants' *S. altissima* neighbors. In contrast, pollinator visitation to *S. altissima* was affected by both focal genotype and an interspecific G × G interaction with a neighboring *S. gigantea* genotype (Table 1). This result appears to be due to an increase in the performance of genotype 'A3' when planted with 'G3' relative to other *S. gigantea* neighbors. This pattern is visible across all measured traits (Fig. 1), although it is especially pronounced for pollinator visitation. This G × G interaction emphasizes the ecological context of plant–neighbor interactions affecting pollinator visitation by showing that genotypic variation in an individual's biotic environment may exert fitness effects on a focal plant, and that these biotic environmental effects depend upon the genotype of the focal plant. As a G × G interaction was not detected for per flower visitation, floral abundance and flowering duration are likely to be at least partially responsible for the G × G interaction observed for total pollinator visitation. Although ecologists have spent over a century researching how neighboring plants of different species compete for pollinators or facilitate each other's pollination (Robertson 1895; Clements & Long 1923; Macior 1971; Pleasants 1980; Thomson 1982; Callaway 1995; review Mitchell *et al.* 2009), our results extend this perspective by including the effects of intraspecific genotypic variation and interspecific G × G interactions on pollinator visitation to neighboring plants. The significant effects of focal plant and neighbor plant genotypic variation show that plant–pollinator interaction studies conducted at the species level may overlook the importance of considering finer genetic scales. In addition, because natural selection operates on the genetic variation present in populations, the results of G × G studies have implications for whether plant–neighbor interactions affect the rate and direction of evolutionary change, given that the trait being measured has an impact on fitness. These G × G interactions create the opportunity for individuals to adapt to fine-scale genetic variation in their environment (Fridley *et al.* 2007) and may also support the idea that plant–neighbor interactions are responsible for the maintenance of high levels of genetic variation which are displayed over small areas by many plant populations (Linhart & Grant

1996) because of the fitness consequences of intransitive (i.e., rock-paper-scissors) competitive relationships between interacting genotypes (Fridley *et al.* 2007; Lankau & Strauss 2007). Similarly, neighboring conspecific plants can also influence each other's pollination success through the effects of genotypic variation and diversity (Genung *et al.* 2010), perhaps due to increased aboveground productivity in patches containing multiple genotypes (e.g., Crutsinger *et al.* 2006).

Plant response to the genetic environment of neighbors

Incorporating the effects of plant–neighbor interactions into the field of community and ecosystem genetics will lend more insight into our understanding of how genetic variation within species scales up to affect patterns and processes above the population level. Our results demonstrate that focal genotype and neighbor genotype can affect plant and community traits independently of each other, or in combination, and that neglecting to consider the influences of genotypic variation in a plant's neighbors can lead to an incomplete understanding of patterns and processes in natural systems. In particular, belowground plant traits may be especially affected by plant–neighbor interactions in the nitrogen-limited environments (Tilman 1988; Wilson & Tilman 1993) which occur world-wide (LeBauer & Treseder 2008). This suggests that a better understanding of how much carbon plants are investing into belowground biomass requires considering species and genetic variation in neighboring plants which are also competing for belowground resources. Most studies of plant–neighbor interactions in a G × G context may have underestimated the importance of biotic environmental variation (but see Collins *et al.* 2010) by not including belowground trait measurements. Although some studies have found that root biomass is not related to plant competitive ability (Cahill 2003), belowground productivity represents an important part of net primary production and contributes organic carbon which can be sequestered or used by soil microorganisms (Bessler *et al.* 2009), reinforcing the importance of including measurements of belowground biomass. As belowground biomass in *Solidago* species has a genotypic basis (Table 1), the genotype identity of a plant's neighbors will determine the belowground environment in which the plant has to compete.

It remains to be seen whether focal genotype and neighbor genotype influence associated communities in natural settings, when moisture, soil texture, and other factors which affect plant productivity and pollinator visitation may vary along gradients. In fact, quantifying the importance of intraspecific genetic variation relative to other ecological factors remains a major issue in the field of community and ecosystem genetics (Johnson *et al.* 2008; Bailey *et al.* 2009). Future studies should (1) investigate whether genotype-based plant–neighbor interactions scale up to affect associated communities in natural systems or (2) manipulate other ecological factors (i.e., density, nutrient availability, competition) alongside intraspecific genetic variation. These approaches will allow for a better determination of the importance of "community and ecosystem genetics" questions to broad ecological and evolutionary dynamics. Although the importance of genotype-based plant–neighbor interactions in natural systems remains unclear, results from our common garden experiment show that biotic environmental variation and G × G interactions can have important effects on belowground biomass production in plants and also extend to affect associated pollinator visitation. These findings reinforce the idea that organisms cannot be solely studied at the species level or as individuals, but rather a full understanding of ecological patterns must incorporate intraspecific genetic variation both within a focal species and the neighbors with which it interacts.

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REFERENCES

Aarssen, L.W. & Turkington, R. (1985). Biotic specialization between neighboring genotypes in *Lolium perenne* and *Trifolium repens* from a permanent pasture. *J. Ecol.,* 73, 605-614.

Abrahamson, W.G. & Weis, A.G. (1997). Evolutionary ecology across three trophic levels: goldenrods, gallmakers, and natural enemies. *Monographs in Population Ecology, Volume 29*. Princeton University Press, Princeton, New Jersey, USA.

Abrahamson, W.G., Dobley, K.B, Houseknecht, H.R. & Pecone, C.A. (2005). Ecological divergence among five co-occuring species of old-field goldenrods. *Plant Ecol.*, 177, 43-56.

Bailey, J.K., Wooley, S.C., Lindroth, R.L. & Whitham, T.G. (2006). Importance of species interactions to community heritability: a genetic basis to trophic-level interactions. *Ecol. Lett.*, 9, 78-85.

Bailey, J.K., Schweitzer, J.A., Ubeda, F., Koricheva, J., LeRoy, C.J., Madritch, M.D. *et al.* (2009). From genes to ecosystems: A synthesis of the effects of plant genetic factors across levels of organization. *Philos. T. Roy. Soc. B*, 364, 1607-1616.

Bessler, H., Temperton, V.M., Roscher, C., Buchmann, N., Schmid, B., Schulze, E.D. *et al.* (2009) Aboveground overyielding in grassland mixtures is associated with reduced biomass partitioning to belowground organs. *Ecology*, 90, 1520-1530.

Booth, R.E. & Grime, J.P. (2003). Effects of genetic impoverishment on plant community diversity. *J. Ecol.*, 91, 721-730.

Bossdorf, O., Shuja, Z. & Banta, J.A. (2009). Genotype and maternal environment affect belowground interactions between *Arabidopsis thaliana* and its competitors. *Oikos*, 118, 1541-1551.

Cahill, J.F. (2003). Lack of relationship between belowground competition and allocation to roots in 10 grassland species. *J. Ecol.*, 91, 532-540.

Cahill, J.F., Kembel, S.W., Gustafson, D.J. (2005). Differential genetic influences on competitive effect and response in *Arabidopsis thaliana*. *J. Ecol.*, 93, 958-967.

Callaway, R.M. (1995). Positive interactions among plants. *Bot. Rev.*, 61, 306-349.

Clements R.E., & Long, F.L. (1923). *Experimental pollination: An outline of the ecology of flowers and insects.* Carnegie Institute of Washington, Washington, DC, USA.

Collins, A., Hart, E.M., & Molovsky, J. (2010). Differential response to frequency-dependent interactions: an experimental test using genotypes of an invasive grass. *Oecologia*, 164, 965-969.

Conner, J.K. & Hartl, D.L. (2004). *A primer of ecological genetics*. Sinauer Associates, Sunderland, MA, USA.

Crutsinger, G.M., Collins, M.D., Fordyce, J.A., Gompert, Z., Nice, C.C. & Sanders, N.J. (2006). Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science*, 313, 966-968.

Crutsinger, G.M., Collins, M.D., Fordyce, J.A. & Sanders, N.J. (2008). Temporal dynamics in non-additive responses of arthropods to host-plant genotypic diversity. *Oikos*, 117, 255-264.

Fridley, J.D., Grime, J.P. & Bilton, M. (2007). Genotypic identity of interspecific neighbours mediates plant responses to competition and environmental variation in a species rich grassland. *J. Ecol.*, 95, 908-915.

Genung, M.A., Lessard, J.P., Brown, C.B., Bunn, W.A., Cregger, M.A., Reynolds, W.N. *et al.* (2010). Non-additive effects of genotypic diversity increase floral abundance and abundance of floral visitors. *PLoS ONE*, 5, e8711.

Ghazoul, J. (2006). Floral diversity and the facilitation of pollination. *J. Ecol.*, 94, 295-304.

Gross, R.S. & Werner, P.A. (1983). Relationships among flowering phenology, insect visitors, and seed set of individuals – experimental studies on 4 co-occurring species of goldenrod (*Solidago*, Compositae). *Ecol. Monogr.*, 53, 95-117.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S. *et al.* (2005). Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecol. Monogr.,* 75, 3-35.

Hughes, A.R. & Stachowicz, J.J. (2004). Genetic diversity enhances the resistance of a seagrass system to disturbance. *P. Natl. Acad. Sci. USA*, 101, 8998-9002.

Johnson, M.T.J. & Agrawal, A.A. (2005). Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). *Ecology*, 86, 874-885.

Johnson, M.T.J., Lajeunesse, M.J. & Agrawal, A.A. (2006). Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecol. Lett.*, 9, 24-34.

Johnson, M.T.J., Dinnage, R., Zhou, A.Y. & Hunter M.D. (2008). Environmental variation has stronger effects than plant genotype on competition among plant species. *J. Ecol.*, 96, 947-955.

Johnson, M.T.J. (2008). Bottom up effects of plant genotype on aphids, ants, and predators. *Ecology*, 89, 145-154.

Lankau, R.A. & Strauss, S.Y. (2007). Mutual feedbacks maintain both genetic and species diversity in a plant community. *Science*, 317, 1561-1563.

Lazaro, A., Lundgren. R. & Totland, Ø. (2009). Co-flowering neighbors influence the diversity and identity of pollinator groups visiting plant species. *Oikos*, 118, 691-702.

LeBauer, D.S. & Treseder, K.K. (2008). Nitrogen limitation of primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, 89, 371-379.

Linhart, Y.B. & Grant, M.C. (1996). Evolutionary significance of local genetic differentiation in plants. *Ann. Rev. Ecol. Syst.*, 27, 237-277.

Macior, L.W. (1971). Co-evolution of plants and animals. Systematic insights from plant-animal interactions. *Taxon*, 20, 17-28.

Maddox, G.D. & Root, R.B. (1987). Resistance to 16 diverse species of herbivorous insects within a population of goldenrod, *Solidago altissima*: genetic variation and heritability. *Oecologia*, 72. 8-14.

Madritch, M., Donaldson, J.R. & Lindroth, R.L. (2006). Genetic identity of *Populus tremuloides* litter influences decomposition and nutrient release in a mixed forest stand. *Ecosystems*, 9, 528-537.

Michalet, R., Xiao, S., Touzard, B., Smith, D.S., Cavieres, L.A., Callaway, R.M. *et al.* (2011). Phenotypic variation in nurse traits and community feedbacks define an alpine community. *Ecol. Lett.*, 14, 433-443.

Mitchell, R.J., Flanagan, R.J., Brown, B.J., Waser, N.M., Karron, J.D. (2009). New frontiers in competition for pollination. *Ann. Bot.*, 103, 1403-1413.

Mooney, K.A. & Agrawal, A.A. (2008). Plant genotype shapes ant-aphid interactions: Implications for community structure and indirect plant defense. *Am. Nat.*, 171, E195-E205.

Pleasants, J.M. (1980). Competition for pollinators in Rocky-Mountain plant-communities. *Ecology*, 61, 1446-1459.

Robertson, C. (1895). The philosophy of flower seasons, and the phaenological relations of the entomophilous flora and the anthophilous insect fauna. *Am. Nat.*, 29, 97-117.

Rowntree, J.K., McVennon, A. & Preziosi, R.F. (2010). Plant genotype mediates the effects of nutrients on aphids. *Oecologia*, 163, 675-679.

Schweitzer, J.A., Bailey, J.K., Rehill, B.J., Martinsen, G.D., Hart, S.C. & Lindroth, R.L. *et al.* (2004). Genetically based trait in a dominant tree affects ecosystem processes. *Ecol. Lett.*, 7, 127-134.

Schweitzer, J.A., Bailey, J.K., Hart, S.C. & Whitham, T.G. (2005). Non-additive effects of mixing cottonwood genotypes on litter decomposition and nutrient dynamics. *Ecology*, 86, 2834-2840.

Schweitzer, J.A., Bailey, J.K., Fischer, D.G., LeRoy, C.J., Lonsdorf, E.V., Whitham, T.G. *et al.* (2008). Plant-soil microorganism interactions: Heritable relationship between plant genotype and associated soil microorganisms. *Ecology*, 89, 773-781.

Tack, A.J.M., Ovaskainen, O., Pulkkinen, P. & Roslin, T. (2010) Spatial location dominates over host-plant genotype in structuring an herbivore community. *Ecology*, 91, 2660-2672.

Thomson, J.D. (1982). Patterns of visitation by animal pollinators. *Oikos*, 39, 241-250.

Tilman, D. (1988) Plant strategies and the dynamics and structure of plant communities. Princeton University Press, Princeton, NJ, USA.

Turkington, R. & Harper, J.L. (1979). Growth, distribution and neighbor relationships of *Trifolium repens* in a permanent pasture. 4. Fine-scale biotic differentiation. *J. Ecol.*, 67, 245-254.

Whitham, T.G., Bailey, J.K., Schweitzer, J.A., Shuster SM, Bangert RK, Leroy CJ *et al.* (2006). A framework for community and ecosystem genetics: from genes to ecosystems. *Nat. Rev. Genet.*, 7, 510-523.

Wilson, S.D. & Tilman, D. (1993). Plant competition and resource availability in response to disturbance and fertilization. *Ecology*, 74, 599-611.

Wise, M.J., Coffey, L.E., & Abrahamson, W.G. (2008). Nutrient stress and gall-flies interact to affect floral-sex ratio in gynomonoecious *Solidago altissima* (Asteraceae). *Am. J. Bot.* 95, 1233-1239.

Wolf, J.B., Brodie, E.D., Cheverud, J.M., Moore, A.J. & Wade, M.J. (1998). Evolutionary consequences of indirect genetic effects. *Trends Ecol. Evol.* 13, 64-69.

CHAPTER 2.

Indirect genetic effects drive ecosystem processes: Interspecific genotype interactions affect decomposition and nutrient dynamics after plant senescence

ABSTRACT

Aboveground-belowground linkages are recognized as divers of community dynamics and ecosystem processes, but the impacts of plant-neighbor interactions on these linkages are virtually unknown. Plant-neighbor interactions are a type of indirect genetic effect (IGE) if the focal plant's phenotype is altered by the expression of genes in a neighboring plant, and the IGE could persist after plant senescence to affect ecosystem processes. Utilizing genotypes of *Solidago altissima* and *Solidago gigantea*, we experimentally tested the hypothesis that IGEs on living focal plants would affect litter decomposition rate, as well as nitrogen (N) and phosphorous (P) dynamics after the focal plant senesced. We found that species interactions affected decomposition and N release, genotype interactions affected P immobilization, and "afterlife" effects of IGEs affected decomposition rate and N dynamics. Our results provide insights into above- and belowground linkages by showing that IGEs can drive afterlife effects, tying plant-neighbor interactions to ecosystem processes in novel ways.

INTRODUCTION

Until recently, above- and belowground subsystems had been studied separately, but the processes which occur in each system are tightly linked (Van der Putten *et al.* 2001; Wardle *et al.* 2004; Bardgett & Wardle 2010) with plants serving as a major intermediary. Because plants link these systems, environmental impacts on a plant's phenotype during the growing season have the potential to cross the "living-dead" barrier when, after senescence, plants shed leaves containing important nutrients which enter the belowground system. These "afterlife" effects describe how species- or genotype-based differences in litter quality (e.g., Melillo *et al.* 1982), interactions with herbivores (Choudhury *et al.* 1988; Findlay *et al.* 1996; Wardle *et al.* 2002; Schweitzer *et al.* 2005), ozone (Findlay *et al.* 1996) and UV radiation (Caldwell *et al.* 1995) will feed back to affect ecosystems (Hobbie *et al.* 1992). For example, species in habitats with low nutrient availability generally use their limited resources efficiently and experience limited herbivory, but grow and decompose slowly. This leads to slower rates of nutrient cycling, creating a feedback which further favors plants capable of surviving in nutrient-limited environments (Hobbie *et al.* 1992). In addition to species differences and herbivore-mediated changes to leaf chemistry, litter quality may also be affected by indirect genetic effects (IGEs), which are modifications to the phenotype of one individual due to the expression of genes in another individual (Wolf *et al.* 1998). Indirect genetic effects occur when interactions between plants and their neighbors have a genetic basis, though to our knowledge the possibility that IGEs could initiate “afterlife” effects remains untested.

Decomposition and nutrient dynamics provide an effective way to test how IGEs affect ecosystem processes because litter from neighboring plants frequently decomposes together, causing unique outcomes that may synergistically enhance or slow litter decay or nutrient release. This observation is responsible for an extensive literature on the effects of litter mixing (see reviews by Gartner & Cardon 2004; Hättenschwiler *et al.* 2005; Gessner *et al.* 2010), which presupposes that neighbors interact and examines how decomposition is affected when species with different litter quality (i.e., lignin:N, C:N) decompose together. Many litter mixing studies describe the effects of mixtures as either additive or non-additive, depending upon whether decomposition dynamics in litter mixtures can be predicted using single-species or single-genotype dynamics (e.g., Gartner & Cardon 2004; Schweitzer *et al.* 2005; Madritch *et al.* 2006). “Non-additive” effects result when mixture components interact, either directly through physical and chemical changes to the environment in which the leaves are decomposing (Hansen & Coleman 1998; Salamanca *et al.* 1998), or indirectly by altering decomposer communities (Hansen & Coleman 1998; Wardle 2002). The unpredictable effects of litter mixing on ecosystem processes are common, as 67% and 76% of published studies report non-additive changes to decomposition rate and nutrient release rates, respectively, when species of different litter qualities decompose together (Gartner & Cardon 2004).

In addition to species variation, genotypic variation can also cause differences in decomposition as genotypes can produce tissues that vary in leaf toughness, nutrient concentration, lignin concentration, or susceptibility to leaf-modifying arthropods (Schweitzer *et al.* 2005; Madritch *et al.* 2006; Crutsinger *et al.* 2009; Madritch & Lindroth 2011). When different genotypes decompose together in mixture, studies have demonstrated significant differences in decomposition and nutrient release rates compared with monocultures, although the effects are often weaker than studies comparing species mixtures (Schweitzer *et al.* 2005; Madritch *et al.* 2006; Crutsinger *et al.* 2009). It is important to consider, however, that the chemical properties of leaf litter may be impacted by plant-neighbor interactions during the growing season. Therefore, collecting litter from individual genotypes (or species) which were not grown together and mixing this litter to create experimental treatments (as most previous studies have done) may not provide an accurate picture of how genotype mixtures decompose in natural systems because it does not consider the “afterlife” effects of pre-senescence plant-neighbor interactions. This perspective recognizes the potential role that IGEs could have on ecosystem processes. Specifically, IGEs would be indicated by significant effects of neighbor genotype identity on focal plant traits during the growing season. Many plant traits can be affected by IGEs, including aboveground productivity (Turkington & Harper 1979; Aarssen & Turkington 1985; Fridley *et al.* 2007; Bossdorf *et al.* 2009; Genung *et al.* 2012), fitness (Bossdorf *et al.* 2009), and belowground productivity (Genung *et al.* 2012). IGEs can also have “afterlife” effects on ecosystem processes if the focal plant trait in question is linked with an ecosystem response such as decomposition or nutrient cycling. For example, a neighboring plant could alter a focal plant’s rate of nutrient uptake or pattern of biomass allocation (e.g., Genung *et al.* 2012), which could alter litter inputs from the focal plant. The interpretation of IGEs in community genetics has changed the way genes are “functionally annotated” (Wade 2007), meaning that more information about associated community and ecosystem processes is being attached to particular focal plant genotypes. If pre-senescence plant-neighbor interactions affect plant traits, which then alter decomposition and nutrient dynamics, this would indicate that ecosystem processes (i.e., fluxes of energy and nutrients) are gene-less products of the “afterlife” effects of IGEs.

Solidago altissima and *Solidago gigantea* provide a model system for examining the “afterlife” effects of inter-specific genotype litter mixing because 1) genotypic variation in these species has been shown to affect a wide range of community and ecosystem processes (Maddox & Root 1987; Crutsinger *et al.* 2006, 2009; Genung *et al.* 2010, 2012) and 2) *S. altissima* and *S. gigantea* are among the most commonly co-occurring species pairs in the genus *Solidago* in abandoned agricultural fields. In both species, genotypes display high phenotypic variation. Previous work with interspecific genotype interactions with these species of *Solidago* have found that neighbor genotype identity affected focal plant rhizome, coarse root, and aboveground biomass, showing strong interspecific interactions among neighbors (Genung *et al.* 2012). By affecting plant biomass and resource allocation, these interactions may lead to differences in plant chemistry that drive patterns of nutrient dynamics after plant senescence. The overarching question asked in this study is whether IGEs have “afterlife” effects on ecosystem processes, linking IGEs and ecosystem ecology. We hypothesized that 1) species level differences in litter quality (e.g., litter lignin:N) will lead to species interactions which affect decomposition and nutrient release, and 2) interactions among decomposing genotypes within mixture treatments will cause non-additive patterns of mass loss and nutrient dynamics, due to variation among genotypes in phenotypic traits and the response of decomposers to these traits. Given that neighbor genotype affected focal plant biomass in a previous study (Genung *et al.* 2012), we also predict that 3) decomposition and nutrient dynamics of interacting neighbors will be affected by "afterlife" effects, (i.e., the outcome of IGEs which occurred during the growing season). We found that IGEs during the growing season affected plant biomass and initial litter quality, and also had "afterlife" effects on decomposition rate and N immobilization.

METHODS

In April 2008, a common garden experiment was established at Freels Bend on the reservation of Oak Ridge National Laboratory (Oak Ridge, TN) to examine the community and ecosystem level impacts of IGEs in a *Solidago* sp. system. This common garden included three locally collected genotypes (i.e., clonal lines) each of *S. altissima* and *S. gigantea*. The experimental treatments included genotype monocultures as well as all possible interspecific combinations of *S. altissima* and *S. gigantea* genotypes, planted together in 95 L pots (see Genung *et al.* 2012 for more details). The plants were grown under competitive conditions, and were fertilized once (Miracle Gro, 24:8:16 NPK ratio) at the beginning of the experiment. In October 2008, during leaf senescence, leaf litter was collected from these plants for a litter decomposition experiment (details below).

Study Species and Experimental Design

Solidago altissima is a dominant species in abandoned agricultural fields where it can have large impacts on biodiversity and ecosystem function (Maddox & Root 1987; Crutsinger *et al.* 2006). Genotypes (i.e., intraspecific clonal families) display variation in biomass production, leaf size, green leaf N concentration and leaf litter decomposition and N release (Abrahamson & Weis 1997; Crutsinger *et al.* 2006, 2009). Although *S. altissima* and *S. gigantea* species are ecologically similar perennial plants, they differ in life-history traits (Abrahamson & Weis 1997), allocation of resources to different growth forms, and tolerance for variation in soil moisture (Abrahamson *et al.* 2005). For example, *S. gigantea* allocates relatively more biomass to sexual reproduction, while *S. altissima* is more productive overall (Abrahamson *et al.* 2005).

Litter mixing studies which explicitly examine intraspecific genetic variation face unique issues as genotypes are often morphologically indistinguishable to researchers, but may have chemical traits which make their individual and combined (i.e., genotype by genotype interactions) effects on decomposition unique. The standard design for litter mixing studies involves the incubation of leaf litter in monoculture bags and mixture bags, followed by a comparison of the observed rates of decomposition and nutrient release with expected rates derived from mean monoculture results (e.g., Blair *et al.* 1990; Wardle *et al.* 1997). Generally, the species to be mixed are picked such that they can be visually identified and separated even late into the decomposition process (e.g., Chapman & Newman 2010). Under this standard design for mixture decomposition experiments, experiments that include phenotypically similar species (whose differences cannot be visually identified during the later stages of decomposition) would be unable to determine the mechanisms driving changes in decomposition and nutrient release rates. Therefore, the standard design would be unable to address at least one frequently proposed mechanism – the "priming" effect, through which high nutrient litter creates conditions that allow lower nutrient litter to decompose faster (Briones & Ineson 1996; Salamanca *et al.* 1998).

We collected leaf litter by hand from senescing *S. altissima* and *S. gigantea* plants from the plant neighborhood experiment (described above) in October 2008, when the plants had been growing in the common garden for seven months. Litter from pots containing a genotype monoculture was pooled and mixed, while litter from pots that contained two genotypes was pooled by genotype and mixed. Litter was then pooled for each treatment, which in this case was a focal genotype-neighbor genotype combination. We used a "bag within a bag" design which allowed us to segregate litter by type (*sensu* Wardle *et al.* 2003). This design included smaller bags and larger bags. Smaller bags were used to partition leaf litter by species and genotype identity, and larger bags enclosed two smaller bags to form each experimental replicate. We controlled for position of smaller bags (i.e., top vs. bottom) for equal representation. The larger, exterior bags were 5 cm x 5 cm and were constructed using large diameter mesh (2 mm) on the top to allow access to decomposer organisms, and small diameter mesh (0.25 mm) on the bottom to prevent loss of litter from the bag by fragmentation. The smaller, interior bags were made using large meshed material on both sides (2 mm). This was done to maximize litter interactions between the smaller, interior bags but allow us to keep the material separate throughout decomposition. The interior bags were filled with 1.5 g of leaf litter according to the specific experimental treatments, identified with a labeling tag and placed inside the exterior bags. The design included six monoculture treatments (equivalent to 12 for the purposes of sample size, because two bags were still used in monocultures) in which the focal and neighbor genotypes had the same genotype identity, and 10 genotype mixture treatments in which the focal and neighbor genotypes had different genotype identities. Each of the 22 treatments was replicated three times over three collection dates for a total of 198 small litter bags. The litterbags were placed in the field to decompose on 19 December 2008, and one third of the bags were collected on each of the following dates: 10 February 2009, 25 April 2009, and 22 August 2009, after two, four and eight months in the field, respectively, and after eight months the litter had lost up to 80% of the original mass. We blocked the experimental design by placing the bags at three locations approximately five meters from each other (ten meters maximum for the two blocks which were furthest away from each other) at the same site at Freels Bend where the plants were grown.

After each collection date, the litterbags were removed from the field and all soil and biotic contaminants were removed by hand. The samples were then air-dried in paper sacks, individually weighed and then ground through a 40 mesh screen with a Wiley Mill. Subsamples of the ground leaf material were separately ashed (500°C for 1 h) and oven-dried (70°C for 48 h). All final weights are expressed on an ash-free, oven-dry mass basis (AFODM). Nutrient dynamics (i.e., nutrient immobilization or loss) were assessed for each sample by examining total N and phosphorus (P) concentrations in leaves from each genotype and species individually (i.e., from each of the individual bag samples), initially (time 0) and after each collection date. The remainder of the ground initial litter material was stored at 4°C until lignin analyses could be conducted.

Litter chemical parameters at time 0 were quantified to determine if differences in genotype and the biotic environment in which the plants were grown influenced litter lignin, N and P content. Initial litter lignin was determined using the acid-fiber detergent method using an Ankom 200 fiber analyzer (Ankom Technology, Macedon NY); *Quercus rubrum* leaf litter was used as a standard. Total litter N and P were determined on the initial samples as well as each collection date by modified micro-Kjeldahl digestion (Parkinson & Allen 1975) and analyzed on a Lachat AE Flow Injection Analyzer (Lachat Instruments, Inc., Loveland, CO, USA) using the salicylate and molybdate-ascorbic acid methods, respectively; apple leaves (*Malus* sp. mixture) were used as a standard (SRM 1515, NIST, Gaithersburg, MD, USA).

Statistical Methods

To determine genotype and species-level effects on initial litter chemistry, we used ANOVAs with species identity, and genotype nested within species, as fixed factors. We also used an ANOVA approach to analyze patterns of mass loss and nutrient concentration over time (*sensu* Wieder & Lang 1982) with the factors time, species, and neighbor species in a full factorial design. For mass loss, we did not detect any interaction terms including time, suggesting that a single exponential approach was sufficient to model decomposition. To calculate decomposition rate constants (k), we determined the linear slope of the natural-log-transformed mass loss data. To determine the effects of genotype identity on decomposition and nutrient dynamics, we repeated this analysis with the factors time, species, and genotype nested within species. All analyses were conducted in JMP 9.0 (SAS Institute 2010).

To examine the non-additive effects of genotype mixtures on total N and P immobilization, we compared our observed values to additive expectations. The relative contribution of each genotype to nutrient dynamics changed over time, as *S. altissima* lost mass faster than *S. gigantea*, but our expectations were calculated based on initial conditions when *S. altissima* and *S. gigantea* were present in equal amounts. We calculated expected values for each mixture as the average of the component genotypes in monoculture (Wardle *et al.* 1997). For decomposition rate, we compared observed and expected k-constants. For nutrient dynamics, we used nutrient concentrations, averaged over time and relative to initial values (Madritch *et al.* 2006). If our expected values fell within the 95% confidence intervals of our observed values, we called the effect “additive,” and otherwise we called the effect “non-additive.”

To determine “afterlife” effects, we examined biomass data measured throughout the 2009 growing season (see Genung *et al.* 2012 for details). All biomass data was collected from plants growing in the same common garden from which senescing leaves were collected. From this previous work, we knew that neighbor genotype identity significantly affected three traits –

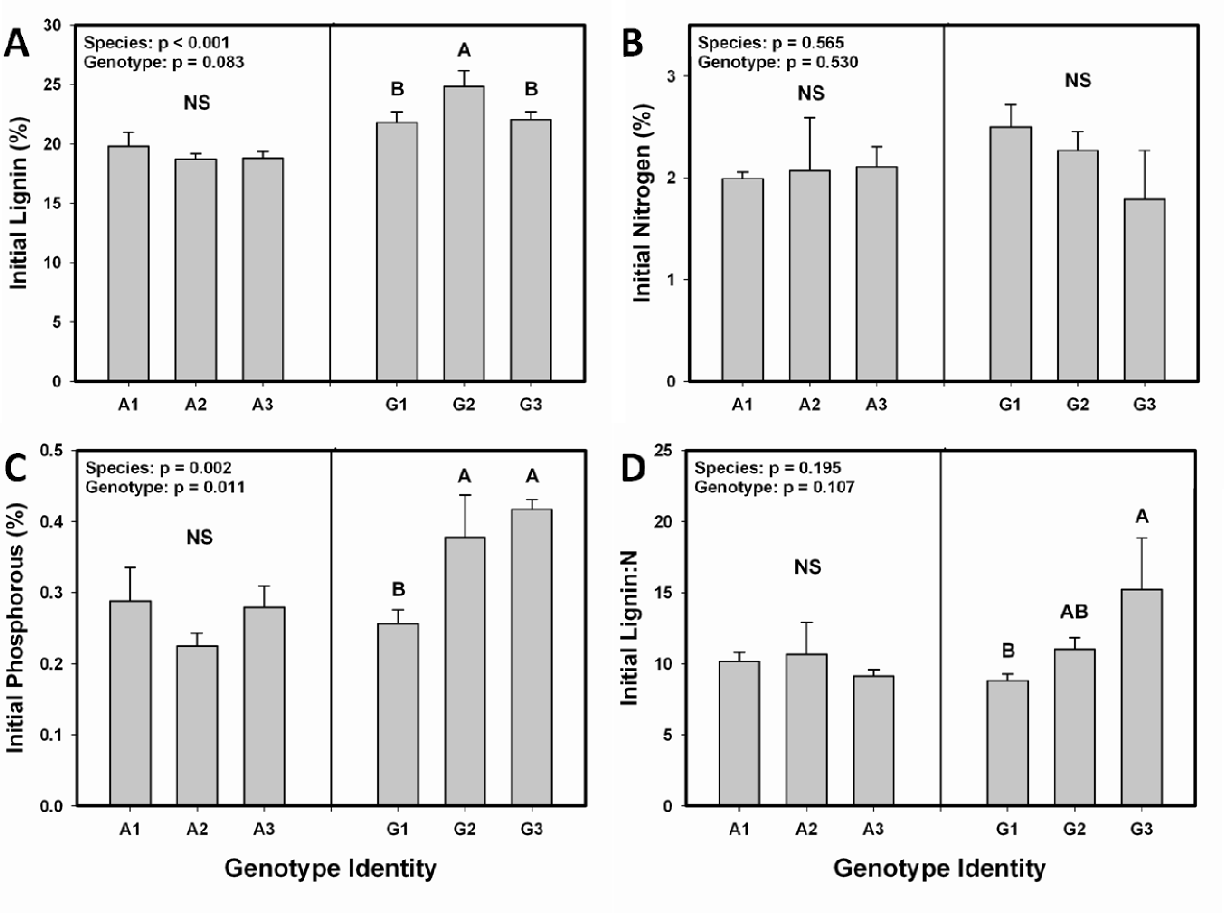
rhizome biomass, coarse root biomass, and aboveground vegetative biomass (Genung *et al.* 2012), so we calculated means for genotype-neighbor genotype pairs (e.g., all measurements from genotype A1 grown with genotype G1) for these traits and compared them to genotype-neighbor genotype means for decomposition rate, average N change (%) and average P change (%) (across all collection dates, relative to initial values for both nutrients). We used mean values for focal genotype-neighbor genotype pairs because litter for decomposition had been pooled, and we could not pair decomposition data points with a matching "growing season" data point. We transformed the focal genotype-neighbor genotype means to meet assumptions of normality, and then used generalized linear models (GLMs) with a normal distribution and identity link function. The factors were rhizome biomass, coarse root biomass, aboveground vegetative biomass, species identity, and genotype nested within species. We used these factors to separately predict litter quality (lignin:N) decomposition rate constant (k), average N change (%), and average P change (%). We included species and neighbor genotype to ensure that plant biomass traits were responsible for changing decomposition rate and nutrient dynamics even after correcting for genotype and species-level differences, and we included litter quality because IGEs affecting litter quality would provide a mechanistic link between plant biomass traits and ecosystem processes.

RESULTS

Initial Litter Chemistry

We found that initial litter chemistry varied between *S. altissima* and *S. gigantea*, and also among genotypes within *S. gigantea* (**Figure 3**). Initial lignin (Fig. 3a) and initial P (Fig. 3c) were 21% and 32% higher, respectively, in *S. gigantea* than *S. altissima*. Initial lignin (Fig. 3a), P (Fig. 3c), and lignin:N (Fig. 3d) also differed across *S. gigantea* genotypes, although we observed no association between P and lignin levels. Because we detected species and genotype level variation for chemical traits are important to decomposition and nutrient dynamics, we would expect to also find species and genotype level effects on mass loss and nutrient immobilization and release. Additionally, because some species and genotype are of higher nutrient quality (e.g., higher P, lower lignin:N), some litter types may "prime" other litter types.

Figure 3. Intraspecific variation affects initial litter chemistry. Initial lignin, foliar nitrogen (N) (B), foliar phosphorous (P) (C), and lignin:N (D) values are presented for three genotypes each of *Solidago altissima* and *Solidago gigantea*. “Genotype” p-values refer to genotype nested within species. In addition to this, post-hoc tests were conducted within each species, corrected for multiple comparisons using reverse Bonferroni corrections (α=0.05), and differences among genotypes within a species are designated by different letters.



Species and genotype effects on decomposition and nutrient dynamics

As expected based on initial litter quality, we found overall that *S. altissima* decomposed up to 40% faster than *S. gigantea*, although the identity of the neighbor species with which a focal species was decomposed did not affect mass loss (**Figure 4a, Table 4**). Species identity also affected P dynamics, as more P was immobilized in *S. altissima* litter than in *S. gigantea* litter (Fig. 4c, Table 4). A three way interaction between focal species, neighbor species, and time (Fig. 4b, Table 4) affected N dynamics. Nitrogen immobilization peaked for *S. altissima* monocultures at two months and then began declining, while the other mixtures immobilized N throughout the experiment. Averaged across all collection dates, *S. gigantea* monocultures had N concentrations (relative to initial) approximately 15% higher than the three other treatments (Fig. 4b). In the model containing time, species identity, and genotype nested within species, we found that focal genotype predicted N ($p < 0.001$) and P ($p < 0.001$) dynamics, but not decomposition rate, and never interacted with the "time" factor. These results show that the carbon, N, and P dynamics in *Solidago* spp. were driven, in part, by the identity of both the focal species and the neighbor with which it decomposed, and that N and P dynamics were also affected by focal plant genotype.

Non-additivity in genotype mixtures

We did not detect non-additive responses for decomposition rate and N dynamics in any of the five interspecific genotype mixtures, suggesting that there were no "priming effects" in litter mixtures. In contrast, three of five genotype mixtures displayed non-additive responses for total P immobilization (**Figure 5**). One of these responses was 19% lower than expected (Fig. 5d; antagonistic effects), while the other two were 13% and 11% higher than expected (Fig. 5a,c) which is evidence for "priming". These results may be related to initial litter chemistry, as we detected significant genotype and species-level effects on initial P (Fig. 3c) but not initial N (Fig. 3b). We did not detect effects of neighbor species or species interactions on P dynamics (Fig. 4c), suggesting that interspecific genotype interactions are relatively more important drivers of P uptake.

Figure 4. Species-level effects on decomposition and nutrient cycling. At the species level, plant-neighbor interactions drove patterns of decomposition and nutrient release. *Solidago altissima* decomposed faster overall than *Solidago gigantea* (A), a three way interaction between time, species and neighbor species affected nitrogen (N) dynamics (B), and *S. altissima* immobilizes more phosphorous (P) than *S. gigantea* (C). P-values are shown for significant factors (α=0.05) in a fully factorial design which included time, species identity, and neighbor species identity. Non-significant factors are not listed.

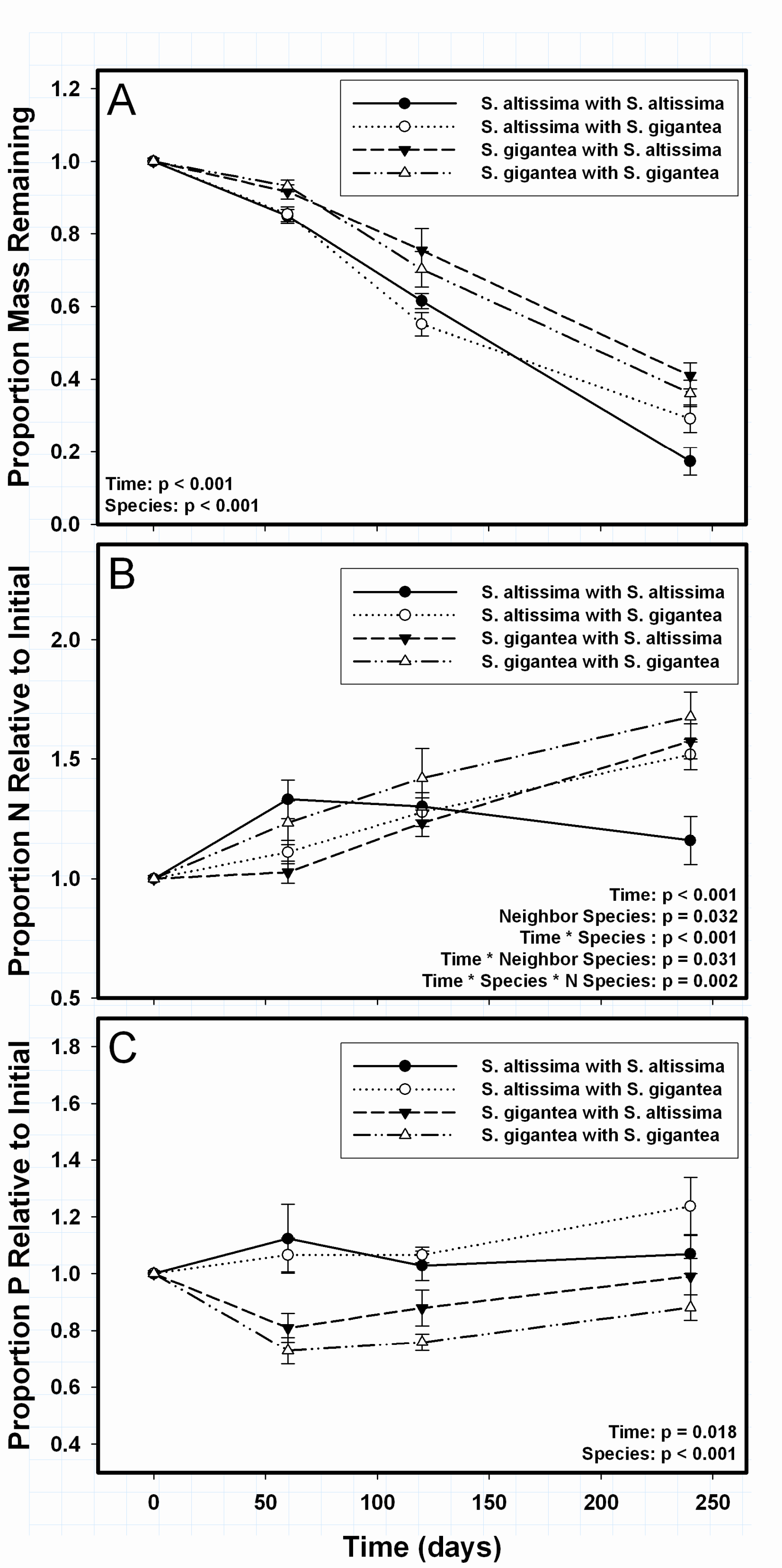
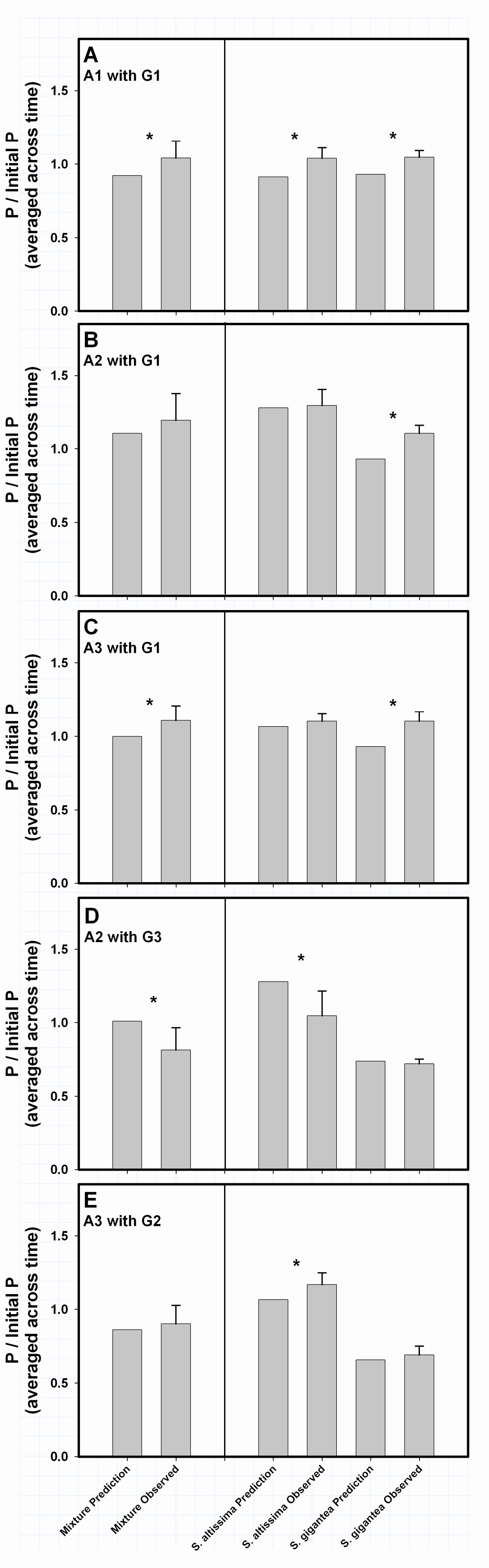
A
B
C
S. altissima with S. altissima
S. altissima with S. gigantea
S. gigantea with S. altissima
S. gigantea with S. gigantea
Proportion Mass Remaining
Proportion N Relative to Initial
Proportion P Relative to Initial
Time (days)
0
50
100
150
200
250
1.2
1.0
0.8
0.6
0.4
0.2
0.0
2.0
1.5
1.0
0.5
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
Time: p < 0.001
Species: p < 0.001
Time: p < 0.001
Neighbor Species: p = 0.032
Time * Species : p < 0.001
Time * Neighbor Species: p = 0.031
Time * Species * N Species: p = 0.002
Time: p = 0.018
Species: p < 0.001

Table 4. Species effects on decomposition and nutrient dynamics. Results of general linear models relating decomposition and nutrient dynamics to species interactions are shown. Mass remaining and phosphorous (P) dynamics were only affected by time and species. Nitrogen (N) dynamics was affected by a three way interaction of time, species, and neighbor species. Italicized, bolded values are significant at α = 0.05.

Factor	**Mass Rem. (%)**	**N Dynamics (%N / Initial %N)**	**P Dynamics (%P / Initial %P)**
	p	p	p
Time	***<0.001***	***<0.001***	***0.018***
Species	***<0.001***	0.121	***<0.001***
Neighbor Species (NS)	0.441	***0.032***	0.533
Time * Species	0.118	***<0.001***	0.207
Time * NS	0.679	***0.031***	0.262
Species * NS	0.550	0.098	0.065
Time * Species * NS	0.092	***0.002***	0.167

Figure 5. Non-additive effects on phosphorous immobilization. Phosphorous (P) immobilization (averaged across collections) was non-additive for three of five interspecific genotype mixtures. Results are presented at both the mixture level (left panels) and individual genotype level (right panels). For mixtures, asterisks indicate that P immobilization for the mixture as a whole was significantly different from additive expectations based on both of the constituent genotype's monoculture P immobilization. For individual genotypes, asterisks indicate that the P immobilization of a focal genotype was different in the presence of a particular interspecific neighbor than in monoculture.


A
A1 with G1
B
A2 with G1
C
A3 with G1
D
A2 with G3
E
A3 with G2
P / Initial P
(averaged across time)
0.0
0.5
1.0
1.5
*
Mixture Prediction
Mixture Observed
S. altissima Prediction
S. altissima Observed
S. gigantea Prediction
S. gigantea Observed

Afterlife effects of pre-senescence indirect genetic effects

The results listed thus far only consider the effects of species- and genotype-level variation, but we also found several significant relationships through which plant biomass traits crossed the "living-dead" barrier and affected decomposition and nutrient dynamics. Low rhizome biomass was associated with faster decomposition rates (**Table 5**). Low coarse root biomass, high rhizome biomass, and high aboveground biomass were associated with more N immobilization (Table 5), and these biomass factors explained a total of 24% of the variation in N immobilization even after including species identity, and genotype nested within species identity. For some of these afterlife effects, the mechanism may involve changes to plant litter quality due to IGEs experienced by a focal genotype during the growing season. For example, low coarse root biomass and high aboveground biomass were correlated with lower lignin:N, an indication of higher litter quality (coarse root biomass: LR $X^2_{(1,16)} = 4.660$, $p = 0.031$; aboveground biomass; LR $X^2_{(1,16)} = 5.129$, $p = 0.024$). However, we did not find any relationship between rhizome biomass and lignin:N (LR $X^2_{(1,16)} = 0.761$, $p = 0.383$). Because all of the genotypes used in the decomposition experiment were grown and decomposed with the same neighbor genotype, and because neighbor genotype is known to affect all of the "biomass" traits listed (Genung *et al.* 2012), the above relationships show the "afterlife" effects of pre-senescence plant-neighbor interactions. For example, *S. altissima* genotype A1 grown in monoculture produced more coarse roots than when it was grown with *S. gigantea* genotype G1 (Genung *et al.* 2012). This led to differences in litter quality which affected decomposition and N immobilization after plant senescence, showing that IGEs can initiate ecological relationships which also influence ecosystem processes (**Figure 6**).

DISCUSSION

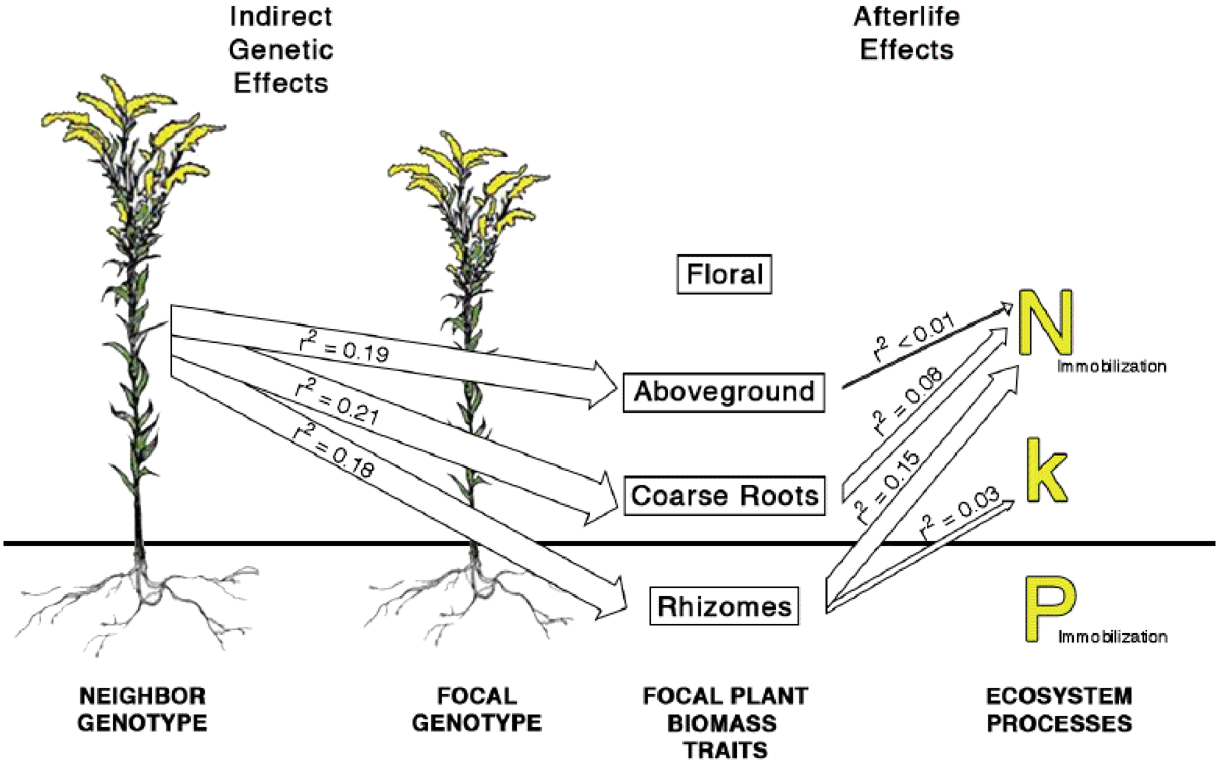
Overall, our results indicate that rates of decomposition and subsequent nutrient release are a legacy of indirect genetic effects (IGEs) that affected plant phenotypes during the growing season. We found that initial litter chemistry varied between *S. altissima* and *S. gigantea*, and also among genotypes within *S. gigantea* (Fig. 3), leading to *S. altissima* decomposing up to 40% faster than *S. gigantea* (Fig. 4a). Nitrogen dynamics were affected by a three way interaction between species, neighbor species, and time (Fig. 4b), but we did not detect a similar interaction

Table 5. Contemporary and afterlife effects on ecosystem processes. Results of generalized linear models (GLMs) relating growing season biomass to decomposition (decomposition rate constants; k) and nitrogen (N) and phosphorus (P) dynamics are shown. Contemporary effects are factors directly tied to leaf litter decomposition, and legacy effects are pre-senescence plant traits which may indirect affect decomposition. Neighbor genotype identity is known to have a significant impact on all listed "biomass" factors, meaning that any effects of a given factor can be interpreted as an extended, ecosystem-level effect of plant-neighbor interactions which occurred during the growing season. All data points are means of a genotype-neighbor genotype pair (e.g., mean of genotype A1 grown with genotype G2). The slope is the parameter estimate which relates the factors to the ecosystem-level responses, and indicates a positive or negative relationship between the factor and response. Slope values are given for untransformed data, and are small because the range of the biomass data greatly exceeds the range of ecosystem-level response data. Genotype was nested within species for all statistical tests. Italicized, bolded values are significant at $\alpha = 0.05$.

Factor	**k-constant**		**N dynamics (%N / Initial %N)**		**P Dynamics (%P / Initial %P)**		**Litter Quality (Lignin:N)**	
	Slope	p	Slope	p	Slope	p	Slope	p
Contemporary								
Species	NA	***0.038***	NA	0.547	NA	***0.003***	NA	0.680
Genotype [Species]	NA	***0.010***	NA	***<0.001***	NA	***<0.001***	NA	0.057
Afterlife								
Coarse Root Biomass (g)	0.001	0.066	***-0.001***	***<0.001***	0.000	0.804	***0.037***	***0.031***
Rhizomes Biomass (g)	***-0.004***	***0.019***	***0.001***	***0.005***	0.000	0.670	0.001	0.383
Aboveground Biomass (g)	0.001	0.837	***0.038***	***<0.001***	0.000	0.660	***-0.004***	***0.024***

NA – not applicable

Figure 6. Indirect genetic effects and afterlife effects on ecosystem processes. Indirect genetic effects alter focal plant biomass traits, and afterlife effects cross the “living-dead” barrier to influence ecosystem processes. Indirect genetic effect values modified from Genung *et al.* (2012) *Ecology Letters*, and show the partial r^2 values of neighbor genotype identity on focal plant biomass traits. Ecosystem processes abbreviations represent nitrogen (N) uptake and release, decomposition rate (k), and phosphorous (P) uptake and release. “Afterlife effects” arrows show the partial r^2 value of plant biomass traits together in a single model which also contained species identity, and genotype nested within species. Arrows are only present for significant relationships. Combined, afterlife effects explained 24% of the variation in N uptake and release even after accounting for species and genotype identity. All r^2 values were determined using the formula (factor sum of squares / full model sum of squares).



for P dynamics (Fig. 4c). However, we detected non-additive effects of genotype mixing on P dynamics in three of the five genotype mixtures (Fig. 5). In one of the three mixtures, P immobilization was decreased, and in the other two more P was immobilized than expected. We also detected "afterlife" effects which linked the above- and belowground systems, as traits expressed by plants during the growing season were correlated with initial litter quality, decomposition and nutrient dynamics (Table 5, Fig. 6). These traits allow us to describe ecosystem processes as the result of changes in plant biomass driven by IGEs which occurred before plant senescence.

Species and genotype interactions influence the dynamics of different nutrients

Species and genotype interactions are ubiquitous in nature and can influence community structure and ecosystem processes such as decomposition and nutrient dynamics. Our study is the first, to our knowledge, to separately examine the components of interspecific genotype mixtures to determine how decomposition and nutrient interactions are affected by species and genotype interactions. Our results show that species interactions drive patterns of N immobilization, as we detected a three-way interaction between time, species, and neighbor species. This effect appears to be driven by *S. altissima* monocultures, in which N immobilization peaked at two months and then declined (Fig. 4b), while each of the other treatments continued to immobilize N throughout the experiment. In contrast with the species-level effects, we detected no effects of interspecific genotype interactions on N dynamics. Previous work has suggested that slowly decomposing litter may decompose faster when mixed with higher quality species, due to a higher N flux and more N availability (e.g., Finzi & Canham 1998). However, we did not detect an increase in *S. gigantea*'s (the lower quality litter) decomposition rate when mixed with *S. altissima* (the higher quality litter). It is possible that we didn't observe priming effects because the magnitude of the difference in lignin:N between *S. altissima* and *S. gigantea* was small (~20%) relative to the difference between high and low quality species in other studies of "priming", which can be over three times that large (e.g., Finzi & Canham 1998). The smaller difference between *S. altissima* and *S. gigantea* may have been insufficient to elicit a strong response from the decomposer communities.

While at a broad scale it appears that species interactions affect N dynamics, we did not detect an effect of species interactions on P dynamics. Instead, we frequently observed non-additive outcomes for P dynamics in the interspecific genotype mixtures. The non-additive responses were not universal, however, as only certain combinations of genotypes displayed non-additive responses for P dynamics. For example, *S. gigantea* genotype G1 immobilized more P than expected under an additive model in all three mixture treatments in which it was included (Fig. 5a,b,c). However, *S. altissima* genotype A2 only showed an increase in N immobilization when decomposed with *S. gigantea* genotype G3 (Fig. 5b), and not G1 (Fig. 5d). These results show that focal genotype and neighbor genotype may interact to affect rates of P immobilization. This may be due to genotypic differences in resource use efficiency, or neighbor-induced changes to biomass allocation which influence a plant's ability to tightly cycle P, although this experiment was not designed to determine how neighbor genotype could influence P immobilization in mixed litterbags; further experimentation will be required to elucidate these interactions. Recent research suggests that the effect size of inter- and intraspecific variation are similar with respect to ecosystem function (Bailey *et al.* 2009), but the different drivers of N and P immobilization illustrate that the effect size of inter- and intraspecific variation may be dependent upon the ecosystem process in question and whether biotic interactions are considered.

Indirect genetic effects persist after senescence and affect ecosystems

Indirect genetic effects are a fundamental element of the co-evolutionary process (Thompson 2006), through which the genotype of one individual influences the fitness and phenotype of associated interacting individuals (Wolf *et al.* 1998). Indirect genetic effects are fundamental because, among other things, they alter the expected relationship between genotypes and phenotypes (Wolf *et al.* 1998), and because they exist as both an environment and a selective force (Dawkins 1982). Therefore, IGEs of a neighbor species which change the genotype frequencies of a focal species will alter the biotic environment, and the evolutionary conditions, experienced by both species. With respect to the current results, IGEs occurred when the genotypic identity of neighbors influenced the phenotypes of focal plants by altering biomass production (Genung *et al.* 2012). These changes in plant traits then led to "afterlife" effects on decomposition rate and N dynamics, even after genotype identity and species identity were taken into consideration (Fig. 6). Our study does not explicitly test whether coevolution has occurred, because we did not compare randomly collected genotypes to genotype pairs which had grown together. However, our study does illustrate how IGEs, with the potential to drive coevolution, can have “afterlife” effects on ecosystem level processes, which shows a novel link between evolutionary biology and ecosystem ecology.

We identified initial litter quality (lignin:N) as a potential mechanism for how changes in plant biomass traits could have afterlife effects on ecosystem processes. As plants produced more aboveground biomass and less coarse root biomass, litter quality increased (i.e., lower lignin:N; Table 5), and N immobilization also increased. Low coarse root biomass and high aboveground biomass were both also associated with increases in N immobilization. The greater overall N accumulation in high-quality litter could be due to its attractiveness to heterotrophic microbes, resulting in increased microbial biomass and N immobilization (e.g., Blair *et al.* 1992). Although we did not explicitly test how plant-neighbor interactions affected litter quality, increases in litter quality could be due to mechanisms which increase focal plant aboveground biomass, decrease focal plant coarse root biomass, or both. Competitive ability is thought to be related to a plant’s ability to reduce the concentration of limiting nutrients (O’Brien 1974; Tilman *et al.* 1982; others), and because plants allocate resources to maximize the capture of limiting nutrients, neighbors that are weak competitors for N may allow focal plants to allocate less carbon to belowground structures. This could increase focal plant shoot to root ratio as more is carbon available for the production of aboveground biomass. In contrast with coarse root and aboveground biomass, we did not detect a correlation between rhizome biomass and litter quality, suggesting that the effects of rhizome biomass on decomposition rate are due to a different mechanism. One possibility is the translocation of nutrients from mature “mother” ramets to developing “daughter” ramets, which is common in clonal organisms (Caraco & Kelly 1991; others), including *Solidago* (Abrahamson *et al.* 1991). If N translocation occurred, it could explain why plants with rhizome connections to many daughter plants have lower quality litter, and therefore slower decomposition rates, than plants with less rhizome biomass.

These results also provide novel insight into aboveground-belowground interactions by showing that “afterlife” effects can be initiated by IGEs. Previous work had shown that species identity (e.g., Melillo *et al.* 1982), interactions with herbivores (Choudhury *et al.* 1988, Findlay *et al.* 1996), ozone and UV radiation (Caldwell *et al.* 1995; Findlay *et al.* 1996) could all initiate afterlife effects by changing litter quality. To our knowledge, our study is the first to show that IGEs can also initiate afterlife effects, which represents an important advance as it suggests that ecosystem processes can be described as the gene-less products of direct (focal genotype) or indirect (neighbor genotype or IGEs) genetic effects. Our previous work in *Solidago* showed that neighbor genotype identity can affect coarse root, rhizome, and aboveground biomass (Genung *et al.* 2012, Fig. 6), all of which represent types of IGEs. In this study we extended these results by showing that these IGEs can also affect decomposition and nutrient dynamics by affecting plant litter chemistry. This holistic approach significantly advances our understanding of aboveground-belowground interactions as it shows how plants' living interactions influence the quality of their inputs to the organic matter pool, which can influence rates of litter decay, nutrient dynamics and localized nutrient cycles. Future work on ecosystem processes should be undertaken with the understanding that many biotic and abiotic environmental variables, including IGEs, can drive trait expression at multiple stages of a plant's life cycle, and these changes in trait expression can have important impacts on ecosystem processes.

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REFERENCES

Aarssen, L.W. & Turkington, R. (1985). Biotic specialization between neighboring genotypes in *Lolium perenne* and *Trifolium repens* from a permanent pasture. *J. Ecol.,* 73, 605-614.

Abrahamson, W.G., Anderson, S.S. & McKrea, K.D. (1991). Clonal integration – nutrient sharing between sister ramets of *Solidago altissima* (Compositae). *Am. J. Bot.*, 78, 1508-1514.

Abrahamson, W.G. & Weis, A.E. (1997). Evolutionary ecology across three trophic levels: goldenrods, gallmakers, and natural enemies. *Monographs in Population Biology, Volume 29*. Princeton University Press, Princeton, New Jersey, USA.

Abrahamson, W.G., Dobley, K.B, Houseknecht, H.R. & Pecone, C.A. (2005). Ecological divergence among five co-occuring species of old-field goldenrods. *Plant Ecol.*, 177, 43-56.

Bailey, J.K., Schweitzer, J.A., Ubeda, F., Koricheva, J., LeRoy, C.J., Madritch, M.D. *et al.* (2009). From genes to ecosystems: A synthesis of the effects of plant genetic factors across levels of organization. *Philos. T. Roy. Soc. B*, 364, 1607-1616.

Bardgett, R.D. & Wardle, D.A. (2010). *Aboveground-belowground linkages: biotic interactions, ecosystem processes, and global change*. Oxford University Press, Oxford, UK. ISBN 0199546878.

Blair, J.M., Parmelee, R.W. & Beare, M.H. (1990). Decay rates, nitrogen fluxes, and decomposer communities of single- and mixed-species foliar litter. *Ecology*, 71, 1976-1985.

Blair, J.M., Crossley, D.A. & Callaham, L.C (1992). Effects of litter quality and microarthropods on N dynamics and retention of exogenous N-15 in decomposing litter. *Biol. Fert. Soils*, 12, 241-252.

Bossdorf, O., Shuja, Z. & Banta, J.A. (2009). Genotype and maternal environment affect belowground interactions between *Arabidopsis thaliana* and its competitors. *Oikos*, 118, 1541-1551.

Briones, M.J.I. & Ineson, P. (1996). Decomposition of eucalyptus leaves in litter mixtures. *Soil Biol. Biochem.*, 28, 1381-1388.

Caldwell, M., Teramura, A.H., Tevini, M., Bornman, J.F., Bjorn, L.O. & Kulandaivelu, G. (1995). Effects of increased solar ultraviolet radiation on terrestrial plants. *Ambio*, 24, 166-173.

Caraco, T. & Kelly, C.K. (1991). On the adaptive value of physiological integration in clonal plants. *Ecology*, 72, 81-93.

Chapman, S.K. & Newman, G.S. (2009). Biodiversity at the plant-soil interface: microbial abundance and community structure respond to litter mixing. *Oecologia*, 162, 763-769.

Choudhury, D. (1988). Herbivore induced changes in leaf-litter resource quality – a neglected aspect of herbivory in ecosystem nutrient dynamics. *Oikos*, 51, 389-393.

Crutsinger, G.M., Collins, M.D., Fordyce, J.A., Gompert, Z., Nice, C.C. & Sanders, N.J. (2006). Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science*, 313, 966-968.

Crutsinger, G.M., Sanders, N.J. & Classen, A.T. (2009) Comparing intra- and inter-specific effects on litter decomposition in an old-field ecosystem. *Bas. Appl. Ecol.*, 10, 535-543.

Dawkins, R. (1982). *The extended phenotype: the gene as the unit of selection*. Oxford University Press, Oxford, UK.

Findlay, S., Carreiro, M., Krischik, V. & Jones, C.G. (1996). Effects of damage to living plants on leaf litter quality. *Ecol. Appl.*, 6, 269-275.

Finzi, A.C. & Canham, C.D. (1998). Non-additive effects of litter mixtures on net N mineralization in a southern New England forest. *Forest Ecol. Manag.*, 105, 129-136.

Fridley, J.D., Grime, J.P. & Bilton, M. (2007). Genotypic identity of interspecific neighbours mediates plant responses to competition and environmental variation in a species-rich grassland. *J. Ecol.*, 95, 908-915.

Gartner, T.B. & Cardon, Z.G. (2004). Decomposition dynamics in mixed-species leaf litter. *Oikos*, 104, 230-246.

Genung, M.A., Lessard, J.P., Brown, C.B., Bunn, W.A., Cregger, M.A., Reynolds, W.N. *et al.* (2010). Non-additive effects of genotypic diversity increase floral abundance and abundance of floral visitors. *PLoS ONE*, 5, e8711.

Genung, M.A., Bailey, J.K. & Schweitzer, J.A. (2012). Welcome to the neighbourhood: interspecific genotype by genotype interactions in *Solidago* influence above- and belowground biomass and associated communities. *Ecol. Lett.*, 15, 65-73.

Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H. *et al.* (2010). Diversity meets decomposition. *Trends Ecol. Evol.*, 25, 372-380.

Hansen, R.A. & Coleman, D.C. (1998). Litter complexity and composition are determinants of the diversity and species composition of oribatid mites (Acari : Oribatida) in litterbags. *Appl. Soil Ecol.*, 9, 17-23.

Hassall, M., Turner, J.G. & Rands, M.R.W. (1987). Effects of terrestrial isopods on the decomposition of woodland leaf litter. *Oecologia*, 72, 597-604.

H□ttenschwiler, S., Tiunov, A.V. & Scheu, S. (2005). Biodiversity and litter decomposition in terrestrial ecosystems. *Annu. Rev. Ecol. Evol. S.*, 36, 191-218.

Maddox, G.D. & Root, R.B. (1987). Resistance to 16 diverse species of herbivorous insects within a population of goldenrod, *Solidago altissima*: genetic variation and heritability. *Oecologia*, 72, 8-14.

Madritch, M., Donaldson, J.R. & Lindroth, R.L. (2006). Genetic identity of *Populus tremuloides* litter influences decomposition and nutrient release in a mixed forest stand. *Ecosystems*, 9, 528-537.

Madritch, M.D. & Lindroth, R.L. (2011). Soil microbial communities adapt to genetic variation in leaf litter inputs. *Oikos*, 120, 1696-1704.

Melillo, J.M., Aber, J.D. & Muratore, J.F. (1982). Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology*, 63, 621-626.

O'Brien, W.J. (1974). Dynamics of nutrient limitation of phytoplankton algae – model reconsidered. *Ecology*, 55, 135-141.

Parkinson, J.A. & Allen, S.E. (1975). Wet oxidation procedure suitable for determination of nitrogen and mineral nutrients in biological-material. *Commun. Soil Sci. Plan.*, 6, 1-11.

Pregitzer, C.C., Bailey, J.K., Hart, S.C. & Schweitzer, J.A. (2010). Soils as agents of selection: feedbacks between plants and soils alter seedling survival and performance. *Evol. Ecol.*, 24, 1045-1059.

Salamanca, E.F., Kaneko, N. & Katagiri, S. (1998). Effects of leaf litter mixtures on the decomposition of *Quercus serrata* and *Pinus densiflora* using field and laboratory microcosm methods. *Ecol. Eng.*, 10, 53-73.

Schweitzer, J.A., Bailey, J.K., Hart, S.C. & Whitham, T.G. (2005). Non-additive effects of mixing cottonwood genotypes on litter decomposition and nutrient dynamics. *Ecology*, 86, 2834-2840.

Schweitzer, J.A., Madritch, M.D., Felker-Quinn, E. & Bailey, J.K. (2012). From genes to ecosystems: plant genetics as a link between above- and below-ground processes. In: Wall, D.H., Bardgett, R.D., Behan-Pelletier, V., Herrick, J.E., Jones, H., Ritz, K. *et al.* (2012). *Soil Ecology and Ecosystem Services*. Oxford University Press, UK.

Tilman, D., Kilham, S.S. & Kilham, P. (1982). Phytoplankton community ecology – the role of limiting nutrients. *Annu. Rev. Ecol. Syst.*, 13, 349-372.

Turkington, R. & Harper, J.L. (1979). Growth, distribution and neighbor relationships of *Trifolium repens* in a permanent pasture. 4. Fine-scale biotic differentiation. *J. Ecol.*, 67, 245-254.

Wade, M.J. (2007). The co-evolutionary genetics of ecological communities. *Nat. Rev. Genet.*, 8, 185-195.

Wardle, D.A., Bonner, K.I. & Nicholson, K.S. (1997). Biodiversity and plant litter: Experimental evidence which does not support the view that enhanced species richness improves ecosystem function. *Oikos*, 79, 247-258.

Wardle, D.A., Bonner, K.I. & Barker, G.M. (2002). Linkages between plant litter decomposition, litter quality, and vegetation responses to herbivores. *Func. Ecol.*, 16, 585-595.

Wardle, D.A., Nilsson, M.C., Zackrisson, O. & Gallet, C. (2003). Determinants of litter mixing effects in a Swedish boreal forest. *Soil Biol. Biochem.*, 35, 827-835.

Wieder, R.K. & Lang, G.E. (1982). A critique of the analytical methods used in examining decomposition data obtained from litter bags. *Ecology*, 63, 1636-1642.

Wolf, J.B., Brodie, E.D., Cheverud, J.M., Moore, A.J. & Wade, M.J. (1998). Evolutionary consequences of indirect genetic effects. *Trends Ecol. Evol.*, 13, 64-69.

CHAPTER 3.

Belowground interactions shift the relative importance of direct and indirect genetic effects

ABSTRACT

Intraspecific genetic variation can affect decomposition, nutrient cycling, and interactions between plants and their associated belowground communities, such as soil arthropods and microorganisms. However, the effects of genetic variation on ecosystems can also be indirect; in other words, genes in a focal plant may affect ecosystems by altering the phenotype of interacting individuals. We established a common garden experiment which used divided and undivided pots to manipulate the presence or absence of belowground interactions between neighboring *Solidago* plants. We hypothesized that, because the plants in our experiment were nitrogen (N) limited, the most important interactions between focal and neighbor plants would occur belowground. We found that indirect genetic effects (changes in focal plant traits due to the genotype identity of a neighbor) explained over 20 times more variation in belowground biomass than did focal genotype; however, this effect only held in pots which allowed belowground interactions. These results provide a novel insight into IGEs, by showing that, under certain conditions, plant phenotypes can be determined almost exclusively by the expression of genes in its neighbor.

INTRODUCTION

While it is becoming established that intraspecific genetic variation can influence associated communities and ecosystems (e.g., Johnson & Agrawal 2005; Bailey *et al.* 2006; Crutsinger *et al.* 2006; Johnson *et al.* 2006; Whitham *et al.* 2006; Mooney & Agrawal 2008), how genetically based species interactions influence belowground plant traits which are of critical importance to plant competition, nutrient cycling and overall plant fitness is poorly understood. Understanding the aboveground effects of intraspecific genetic variation is important because of its effects on associated communities (Crutsinger *et al.* 2006; Whitham *et al.* 2006; Genung *et al.* 2012), plant fitness (e.g., Johnson *et al.* 2006), species interactions (Bailey *et al.* 2006; Mooney & Agrawal 2008), and many other ecological patterns and processes. However, genetic variation can also drive belowground interactions which affect plant fitness and nutrient cycling (e.g., Madritch *et al.* 2006; Schweitzer *et al.* 2004; Pregitzer *et al.* 2010), as well as the belowground communities associated with plant roots, such as soil arthropods and microorganisms (Schweitzer *et al.* 2008; Crutsinger *et al.* 2009). Research into belowground interactions at the genotype level has received less attention, as most belowground interactions studies have looked at the physiological mechanisms of resource competition or the population and community impacts of species-level competition (see Casper & Jackson 1997 for review). Genotype-level studies of belowground interactions have additional implications, as there are immediate evolutionary consequences because neighbor genotype effects can be interpreted as indirect genetic effects (IGEs). IGEs are environmental influences on the phenotype of a focal species due to the expression of genes in a neighboring species (Wolf *et al.* 1998). This interpretation is contingent on a significant effect of “neighbor genotype identity” on traits in a focal plant. If the IGE is mediated by belowground interactions between a focal plant and its neighbors, and the affected focal plant trait is heritable and has consequences for plant fitness, then belowground interactions may affect genotype frequencies in the next generation by altering the performance and survival of particular genotypes. Understanding the relative roles of direct (genotype) vs. indirect (neighbor genotype) effects on plant phenotypes, and determining whether the importance of these factors varies across plant traits (i.e., aboveground vs. belowground traits) or environments, represents an important step for understanding how IGEs affect belowground interactions.

The relatively few community and ecosystem genetics studies which have looked at belowground plant traits (e.g., Bossdorf *et al.* 2009, Collins *et al.* 2010, Genung *et al.* 2012) have measured total belowground biomass, which can be a poor predictor of a plant’s ability to uptake limiting nutrients (e.g., nitrogen [N] and phosphorous [P]). For example, Caldwell *et al.* (1991) showed that root biomass alone was insufficient to explain the differences in nutrient uptake among three competing species of sagebrush. A better predictor of nutrient uptake is root surface area, which can be substantially different from belowground biomass, especially when fine roots are much more abundant than coarse roots, or vice versa. One factor that can affect the ratio of coarse to fine roots is variation in soil nutrient concentration at very small spatial scales, because plants can respond to this variation by proliferating fine roots in nutrient-rich patches of soil (Drew & Saker 1975; Casper & Jackson 1997). Because neighboring plants can affect the amount of nutrients available to a focal plant (see review by Schwinning & Weiner 1998), focal plants may respond to neighbors by shifting biomass allocation to maximize the capture of limiting resources (e.g., Bloom *et al.* 1985). Changes in root surface area may occur as a result in this change in allocation patterns. Because competition for soil N is believed to be mostly size-symmetric (i.e., resource uptake is roughly correlated with the biomass of the competing individuals; Schwinning & Weiner 1998), patterns of biomass allocation are likely to differ, depending on the intensity of belowground interactions experienced by the focal plant. For example, if a focal plant's neighbor has a relatively large belowground biomass, the focal plant may increase its own allocation to belowground structures to ensure access to limiting soil resources.

The importance of understanding how genotypic variation and IGEs affect the outcome of belowground interactions between neighboring plants is underscored by the observation that plant performance is affected more by belowground competition than by aboveground competition (Wilson 1988). There exists a rich history of belowground competition studies, both at the physiological level and at the population/community level (Casper & Jackson 1997 and references therein). However, to our knowledge, these studies have not taken the perspective of comparing the relative roles of genotypic effects and IGEs to understand more about how evolution may occur in response to belowground interactions. For example, IGEs may have strong effects when they originate in abundant species with major impacts on ecosystem function (i.e., foundation species), and weaker effects when they originate in rare species. Another possibility is that IGEs are strongest for traits related to acquiring limiting nutrients (Genung *et al.* 2012), because interactions involving these traits have presumably been of significant evolutionary importance. Comparing the effect size of genotypic variation with other ecological and evolutionary factors such as belowground interactions and IGEs will help inform a broader effort (e.g., Bailey *et al.* 2009) to understand the relative importance of genotypic variation for associated community structure and ecosystem processes.

Using three genotypes each of *Solidago altissima* and *Solidago gigantea*, we established a common garden experiment which manipulated genotype identity, neighbor genotype identity, and the presence of belowground interactions to examine the effects of interspecific genotype interactions on aboveground plant biomass, belowground plant biomass, and root surface area. The presence of interactions was determined by planting paired plants in custom-made planting boxes that either allowed interactions (no barrier) or prevented belowground interactions (water-tight barrier between individual plants). This experiment allows us to examine how intraspecific genetic variation (i.e., "genotype") and biotic environmental variation (i.e., "neighbor genotype" or IGEs) interact to affect the outcome of plant-neighbor interactions. Given that the plants were nutrient limited, we hypothesized that the effects of neighbor genotype would be largest in pots where belowground interactions were allowed to occur, and that neighbor genotype would have little to no effect on focal plants in the absence of belowground interactions. Specifically, we addressed the following questions: (1) Is plant productivity limited, or is resource allocation shifted, by the experimental exclusion of belowground interactions? (2) Are the effects of genotype and neighbor genotype mediated by the presence of belowground interactions? (3) Does the relative importance of direct and indirect genetic effects for aboveground, belowground, and total plant productivity vary depending on whether belowground interactions are experimentally excluded? When neighbors were allowed to interact belowground, aboveground biomass was mostly determined by genotype identity but belowground biomass was mostly determined by IGEs. When belowground interactions were excluded, focal genotype explained more variation than IGEs for both above- and belowground biomass. These results support the idea that genotypic variation in a plant's neighbors will be most important when neighbor genotypes vary in traits related to acquiring limiting nutrients, and demonstrate that IGEs can play a major role in determining plant traits and allocation strategies.

METHODS

Study Species

Solidago altissima is a dominant species in abandoned agricultural fields where it can have large impacts on biodiversity and ecosystem function (Maddox & Root 1987; Crutsinger *et al.* 2006). Intraspecific genetic variation in *S. altissima* has been shown to affect many community and ecosystem level responses (e.g., Maddox & Root 1987; Crutsinger *et al.* 2006; Genung *et al.* 2012). *Solidago gigantea* and *S. altissima* frequently co-occur in old fields (Abrahamson *et al.* 2005) but differ in a range of life-history traits (Abrahamson & Weis 1997; Abrahamson *et al.* 2005; Genung *et al.* 2012). *Solidago altissima* is highly clonal and produces more rhizome biomass than *S. gigantea*, while *S. gigantea* allocates a greater percentage of its biomass to inflorescences (Abrahamson *et al.* 2005). *Solidago altissima* and *S. gigantea* are both known to produce shorter rhizomes, and overall less rhizome biomass, in fertilized soil relative to unfertilized soil (Schmid & Bazzaz 1992), suggesting that belowground biomass in these species is plastic with regard to soil nutrient availability. Previous work with the genotypes used in this experiment has shown that the *S. altissima* genotypes used in this study vary in rhizome biomass, while the *S. gigantea* genotypes differ in coarse root biomass, aboveground vegetative biomass, and floral biomass (Genung *et al.* 2012).

Garden Design

In March 2010, a common garden experiment was established at the East Tennessee Research and Education Center in Knoxville, TN, USA. This common garden included three locally collected genotypes (i.e., clonal families) of both *S. altissima* and *S. gigantea*. The *S. altissima* and *S. gigantea* clones we utilized were originally propagated by G.M. Crutsinger, and clones were maintained at Freels Bend Ecological Research Park on the reservation of Oak Ridge National Laboratory. The genotypes were collected from random locations around the study site at Freels Bend; sampled individuals from both species were carefully collected from unique connected genets that were at least 50–150 m apart (Crutsinger *et al.* 2006; Supplementary Material). Rhizomes were collected from connected ramets to ensure they were from the same genet. The three *S. altissima* genotypes were originally collected and determined as unique genotypes using AFLP (Crutsinger *et al.* 2006, Supplementary Material); however, molecular data is unavailable for the *S. gigantea* genotypes.

The experimental treatments included genotype monocultures as well as all possible interspecific combinations of *S. altissima* and *S. gigantea* genotypes, planted together in custom-built, open-top cubic containers (each side = 0.33 m). Half of the containers were centrally divided using a waterproof, airproof, polypropylene sheet to create two equal halves, a design which aimed to prevent belowground interactions from occurring in these containers. Although this treatment could potentially reduce the amount of area a plant in the divided treatment could explore relative to a strong competitor in the non-divided pots, we rarely observed root-bound plants when belowground biomass was collected, and we found no differences in total plant biomass in divided pots vs. open pots. Treatments consisted of interspecific genotype-neighbor genotype pairs (i.e., *S. altissima* genotype A1 grown with *S. gigantea* genotype G1) either in divided pots or non-divided pots. There were 6 genotypes monoculture (one for each genotype), 9 genotypes mixtures (all factorial combinations of 3 *S. altissima* genotypes x 3 *S. gigantea* genotypes), and presence/absence of belowground interactions (excluded or permitted) for a total of 30 treatments. We replicated each treatment seven times for a total of 210 pots, or 420 plants. Of the

210 pots, 208 are used in data analyses, due to at least one plant dying in two of the pots early in the experiment.

All plants were propagated from cloned stocks of genotypes. A 3-cm rhizome of each species and genotype were grown, in greenhouse flats, outdoors in shaded conditions and watered as needed. When the plants were *c.* 15 cm in height they were transplanted into the pots at the field site. After transplanting, the initial aboveground biomass of individuals was estimated using allometric equations (see Genung *et al.* 2012 Supplementary Information for equation), and this estimate of initial biomass was used as a covariate in our statistical models. Each pot initially included two individuals, but variation in plant density occurred due to clonal production of new ramets beginning during the growing season (2010). In monocultures, both individuals were clones of the same genotype. In genotype mixtures, each pot initially contained one individual of each genotype (total of two plants/pot). The pots were randomly placed in a grid formation within an old field with *c.* 1 m separating each pot from its neighbors. The surrounding field was mown frequently during the experiment, and supplementary water was added to each pot in equal amounts when conditions required. Water was allowed to drain through small holes drilled into the bottom of the pots. The bottom quarter of the pots was filled with gravel (to aid draining). Inside the pots, the gravel was covered with shade cloth and Sunshine Growing Mix #4 (Sun Gro Horticulture, Vancouver, BC, Canada). Invading plants were removed throughout the experiment. Approximately 10 g of fertilizer (24/8/16, Miracle-Gro, Marysville, OH, USA) was applied once to each pot in April 2010.

Trait Measurements

We measured belowground plant biomass at the conclusion of the growing season by destructively sampling all plants. We removed entire blocks of soil from the pots, and water-filtered soil through a 1 mm sieve (USA Standard Testing #18) to remove coarse and fine roots from the soil. Roots were then oven-dried (70° C for 48 h) before weighing to determine belowground biomass. After weighing, we re-hydrated each root sample with deionized water and determined root surface area using the program WinRhizo (Regent Instruments, Nepean, ON, Canada). We measured ANPP near the height of the growing season (September 28) using non-destructive, allometric techniques (see Genung *et al.* 2012 Supplementary Material for allometric equation).

Statistical Methods

Effects of belowground interactions

We used generalized linear models (GLMs) with normal distributions and identity link functions to determine how plant biomass and allocation strategies were affected by excluding belowground interactions. The predictor variable was presence/absence of belowground interactions (i.e., pots with or without divider), and the response variables were root surface area, belowground biomass, aboveground biomass, total biomass, and shoot to root ratio. We square root transformed aboveground and belowground biomass, cubic root transformed total biomass, and quartic root transformed root to shoot ratio, to meet assumptions of normality. These same transformations are used throughout the following analyses.

Genetic controls on aboveground biomass and root surface area

Because plants can reduce the ability of their neighbors to acquire limiting nutrients, and because root surface area provides a good estimate of a plant's ability to uptake nutrients, we tested whether genotype, neighbor genotype, and belowground interactions affected root surface area. We separated our data by species identity and used GLMs with normal distributions and identity link functions. Factors included were genotype, neighbor genotype, initial biomass (at time of planting in the common garden), and presence/absence of belowground interactions. We allowed presence/absence of belowground interactions to interact with all other model terms to determine whether the other factors were affected by experimentally manipulating belowground interactions. For this analysis, as well as those listed below, we only used pots containing individuals of two different species (i.e., monocultures were excluded). We did this because the factors genotype and neighbor genotype were identical in monocultures, and because comparing interspecific competition to competition among genetically identical individuals is beyond the scope of this study.

After testing which factors affected root surface area, we examined whether root surface area was linked with aboveground biomass production. We separated our data by species identity and used GLMs with normal distributions and identity link functions. Factors included genotype, neighbor genotype, initial biomass (at time of planting in the common garden), root surface area, and presence/ absence of belowground interactions (i.e., pot type – with or without dividers). We allowed pot type to interact with all other model terms to determine whether the other factors were affected by experimentally manipulating belowground interactions. The response variable for this analysis was aboveground biomass.

Relative importance of genotype and neighbor genotype

We calculated the proportion of variation explained by genotype and neighbor genotype for each plant trait in the presence/absence of belowground interactions. We used general linear models and determined the proportion of variance explained by each trait as factor sum of squares divided by the full model sum of squares (*sensu* Johnson *et al.* 2008). Because there were significant species level differences for the relative effects of genotype and neighbor genotype in a previous study (Genung *et al.* 2012), we did this analysis for each focal species separately and repeated it for each plant trait (i.e., aboveground biomass, belowground biomass, total biomass). We predicted that neighbor genotype effects for plant biomass traits would be relatively more important than genotype in pots which allowed neighboring plants to compete belowground, since belowground competition is frequently more intense than aboveground competition, especially when light is plentiful (Wilson 1988, Wilson & Tilman 1993).

RESULTS

Effects of belowground interactions

Excluding belowground interactions had no effect on belowground biomass (LR $X^2_{(1,99)} = 1.941$, $p = 0.164$), aboveground biomass (LR $X^2_{(1,99)} = 0.077$, $p = 0.781$), or total biomass (LR $X^2_{(1,99)} = 2.995$, $p = 0.084$); however, we did find an effect on shoot to root ratio (LR $X^2_{(1,99)} = 7.543$, $p = 0.006$). Plants produced more aboveground biomass, relative to belowground biomass, when belowground interactions were experimentally excluded, resulting in 55% greater shoot to root ratio. These results indicate that total plant productivity is not limited by the experimental treatments; that is, productivity does not appear to be limited by having a smaller volume of soil in which to forage in divided pots. However, the presence of belowground interactions did cause plants to shift their allocation strategies, leading to more investment in belowground structures and less investment in vegetative growth.

Genetic controls on aboveground biomass and root surface area

We did not find any main effects of genotype, neighbor genotype, or presence/absence of belowground interactions on root surface area. We did, however, detect a significant interaction between neighbor genotype and presence/absence of belowground interactions which predicted root surface area for *S. altissima* focal plants (**Table 6**). This interaction arose because *S. gigantea* neighbors only exerted neighbor genotype effects on *S. altissima* focal plants' root surface area when belowground interactions were allowed. Because we observed no main effect of "presence/absence of belowground interactions", belowground interactions did not necessarily decrease root surface area of *S. altissima* focal plants. However, this result does show that belowground interactions are crucial to the transmission of IGEs from a neighboring genotype to a focal plant phenotype.

Root surface area, as a measure of nutrient uptake ability, may be related to aboveground biomass, but this relationship might be dependent upon neighbor genotype effects and the presence/absence of belowground interactions. We found that genotype identity affected aboveground biomass in both *S. altissima* and *S. gigantea* (**Table 7**). However, for *S. altissima*, focal plants, the main effect of genotype interacted with the presence of belowground interactions (Table 7). Specifically, *S. altissima* focal plants only expressed genotypic variation for aboveground biomass when belowground interactions were experimentally excluded. This result could be due to other factors, such as belowground interactions or neighbor genotype, being more important drivers of aboveground productivity when belowground interactions were allowed.

Belowground interactions shift the relative influences of genotype and neighbor genotype

On average, focal genotype explained approximately twice as much variation in plant biomass traits (24%) as did neighbor genotype (13%). However, the relative importance of these two factors varied considerably by species identity, and depending upon whether belowground interactions were allowed or experimentally excluded (**Figure 7**). For example, for *S. altissima* focal plants there was no significant difference in the percent of variance explained by genotype (23%) and neighbor genotype (18%), while for *S. gigantea* focal plants, focal genotype explained more variation (25%) than did neighbor genotype (8%). Belowground interactions also affected

Table 6. Indirect genetic effects on root surface area depend on belowground interactions. The results of GLMs linking genotype, neighbor genotype, and belowground interactions to root surface area are presented below. The significant interaction term is due to neighbor genotype effects (i.e., indirect genetic effects) being significant only in pots in which belowground interactions were allowed. Significant values ($\alpha = 0.05$) are in bold italics.

Response: Root Surface Area	Focal *S. alt*, Neighbor *S. gig*	Focal *S. gig*¸ Neighbor *S. alt*
	p-value	p-value
Genotype	0.1607	0.5076
Neighbor Genotype	0.3765	0.2791
Belowground Interactions (Yes/No)	0.2864	0.8804
Genotype * B. Interactions	0.8434	0.4492
Neighbor Genotype * B. Interactions	***0.0088***	0.8525

“Belowground interactions” is abbreviated as “B. Interactions” and indicates whether a divider experimentally prevented plants from interacting with their neighbor belowground.

Solidago altissima is abbreviated as *S. alt*, and *Solidago gigantea* is abbreviated as *S. gig*.

“Focal” indicates the species identity of plants for which traits were measured, and “Neighbor” indicates the species identity of neighboring plants.

Table 7. Belowground interactions determine the expression of genotypic variation. The results of GLMs linking genotype, neighbor genotype, belowground interactions, and root surface area to aboveground biomass. The significant interaction term is due to genotypic variation being expressed only in pots in which belowground interactions were experimentally excluded. Significant values (α = 0.05) are in bold italics.

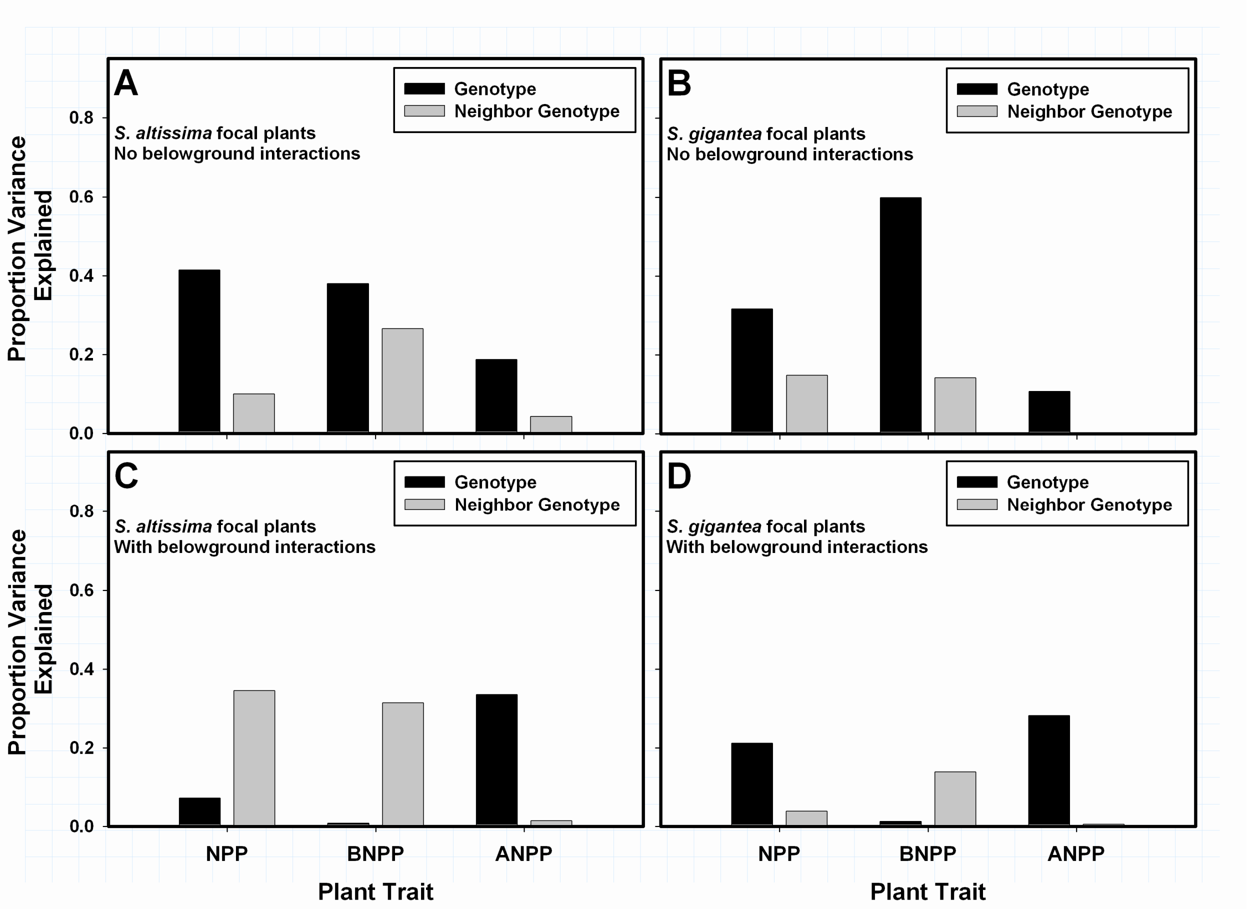
Response: Aboveground Biomass	Focal *S. alt*, Neighbor *S. gig*	Focal *S. gig*, Neighbor *S. alt*
	p-value	p-value
Genotype	***<0.001***	***0.016***
Neighbor Genotype	0.111	0.187
Root Surface Area	*0.063*	0.413
Belowground Interactions (Y/N)	0.493	0.837
Genotype * B. Interactions	***0.002***	0.204
Neighbor Genotype * B. Interactions	0.386	0.441
Root Surface Area * B. Interactions	0.333	0.157

"Belowground interactions" is abbreviated as "B. Interactions" and indicates whether a divider experimentally prevented plants from interacting with their neighbor belowground.

Solidago altissima is abbreviated as *S. alt*, and *Solidago gigantea* is abbreviated as *S. gig*.

"Focal" indicates the species identity of plants for which traits were measured, and "Neighbor" indicates the species identity of neighboring plants.

Figure 7. Belowground interactions shift the importance of genotype and neighbor genotype. The proportion variance explained by focal genotype and neighbor genotype varies depending on whether belowground interactions are excluded (a,b) or allowed (c,d), and whether the focal plant is an individual of *Solidago altissima* (a,c) or *Solidago gigantea* (b,d). Proportion variation in plant and community traits (along *x*-axis), as explained by focal plant genotype identity (black bars) and neighbor genotype identity (grey bars), is shown. NPP, BNPP, ANPP refer to total, belowground, and aboveground plant productivity, respectively, over the course of one growing season.



the relative influences of genotype and neighbor genotype. When plants were separated belowground, focal genotype explained much more variation (33%) than neighbor genotype (12%). However, when belowground interactions were allowed, focal genotype explained roughly the same amount of variation (15%) as did neighbor genotype (14%). This pattern becomes even more striking when comparing genotype and neighbor genotype effects on belowground biomass in pots which allowed belowground interactions, as neighbor genotype explained over 20 times more variation than did focal genotype (23% and 1%, respectively). This result shows that belowground interactions shift the relative importance of direct (focal genotype) and indirect (neighbor genotype) genetic effects as drivers of plant phenotypes.

DISCUSSION

Overall, we found that the presence of belowground interactions allowed for IGEs from neighboring plants to have significant effects on belowground traits in focal plants, and that the relative importance of genotype and neighbor genotype varied considerably across plant traits and environmental conditions. The experimental exclusion of belowground interactions resulted in plants shifting their allocation away from belowground growth and towards aboveground biomass. Neighbor genotype effects (i.e., IGEs) affected root surface area in *S. altissima* focal plants, but only when *S. gigantea* neighbors were allowed to interact with the focal plants belowground (Table 6). Belowground interactions also regulated the expression of genotypic variation for aboveground biomass in *S. altissima*, as genotypic differences were only apparent when belowground interactions (with *S. gigantea* neighbors) was excluded (Table 7). There were striking patterns related to the relative importance of genotypic variation and IGEs (Fig. 7), especially regarding the relative explanatory power of genotype and neighbor genotype for belowground biomass in pots which allowed belowground interactions. These results help inform how the relative importance of direct (focal genotype) and indirect (neighbor genotype) genetic effects varies, depending on the trait in question and how the neighboring plants are interacting.

It is well known that an individual's phenotype is the result of interacting genetic and environmental influences, and in this study we found that genotypic variation and IGEs were contingent on an experimental manipulation of the "environment" – specifically, whether belowground interactions were allowed or excluded. For example, we found significant "genotype" effects on aboveground biomass, but only when *S. altissima* focal plants were grown in divided (i.e., no belowground interactions) pots with *S. gigantea* neighbors (Table 7), suggesting that the expression of genotypic variation is controlled by belowground interactions. One explanation for this pattern is that genotype effects were overwhelmed by belowground interactions in non-divided pots. Additionally, root surface area in *S. altissima* focal plants was affected by IGEs from their *S. gigantea* neighbors, but only when belowground interactions were allowed (Table 6). In other words, post-hoc tests for significant neighbor genotype effects only showed significant differences within the treatment that allowed belowground interactions. While root surface area provides more insight into belowground competition than belowground biomass (Caldwell *et al.* 1991, Casper and Jackson 1997), care should be taken when viewing root surface area as a direct measurement of competitive ability as there are scenarios under which the relationship between root surface area and competition break down. Plants can temporally or spatially partition the way they acquire nutrients such that nutrient depletion zones do not overlap (Mooney *et al.* 1986; Fernandez & Caldwell 1975), the location within the soil where roots are deployed (i.e., areas of high nutrient density or low nutrient density) can override the effects of root surface area, or root competition can occur between the roots of the same plant (Casper & Jackson 1997). However, we did not detect any evidence of plants partitioning the way they acquire resources, because total biomass was not increased when belowground interactions were experimentally excluded. Because all plants were grown in the same potting mix, it is also unlikely that nutrient availability was heterogeneous within the pots. Therefore, in this study, root surface area is likely a good estimator of a plant's ability to uptake nutrients belowground with important implications for plant productivity and fitness. The context-dependent effects of genotype and neighbor genotype on aboveground biomass and root surface area, respectively, both indicate that neighbor genotype effects become more important when plants are allowed to interact belowground.

Similar to the results of previous studies (Genung *et al.* 2012), we found that IGEs (i.e., neighbor genotype effects) played a major role in determining belowground biomass. When plants were allowed to interact belowground, IGEs explained 20 times more variation in belowground biomass than did genotype (Fig. 7). The effect-size difference between genotype and neighbor genotype was more pronounced for *S. altissima*, but was also present in *S. gigantea*. This suggests at least some generality to the pattern, which is likely driven by intense belowground competition in a non-light limited environment (Wilson 1988, Wilson and Tilman 1993). This observation extends the results of our previous work (Genung *et al.* 2012) by explicitly testing, and supporting, the hypothesis that neighbor genotype effects would be stronger when plants were allowed to interact belowground. Additionally, because IGEs explained much more variation in belowground biomass than did genotypic variation, this result also suggests that the focal plant genotypic variation for belowground traits in *Solidago* is more related to exerting IGEs on neighbors than to biomass production in the focal plant. One possible explanation for this pattern involves allelopathy, through which plants exude chemicals which can positively or negatively affect interacting organisms (see Schenk 2006 for review). *Solidago* is known to produce allelopathic chemicals, specifically polyacetylenes and diterpenes (Hegnauer 1977). This allows *Solidago* to negatively affect neighboring species, especially those without a shared co-evolutionary history, e.g., when invading European ecosystems (Abhilasha *et al.* 2008). Although we did not test for the potential effects of allelopathy, the strong effects of IGEs on belowground biomass production of focal plants warrant further investigation.

Our results provide a novel perspective on the importance of direct vs. indirect genetic effects in plant-neighbor interactions by showing that, in *Solidago*, a focal plant's belowground biomass phenotype can be almost exclusively determined by IGEs from its neighbor. This observation has important implications for evolutionary processes acting on the interacting plants (Dawkins 1982, Wolf *et al.* 1998), and suggests that plant competition studies at the genotype level should measure both above- and belowground biomass, especially if they are interested in correctly understanding the influence of neighbors in nutrient limited environments (Genung *et al.* 2012). For example, neighboring plants may have large effects on each other's belowground biomass, which may not be apparent from patterns of aboveground biomass (Fig. 7) but nonetheless can affect the fitness of the interacting plants. While it is well known that IGEs have important ecological and evolutionary consequences due to alterations of the expected relationship between genotype and phenotype (Dawkins 1982), our results provide a new case study which shows that belowground interactions can be a mechanism for IGEs. Interesting possibilities for future work involve determining whether, for belowground biomass traits, evolutionary causes have driven IGEs to be so strong relative to focal genotype effects, and developing better mechanistic understandings of how these belowground IGEs occur.

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REFERENCES

Abhilasha, D., Quintana, N., Vivanco, J. & Joshi, J. (2008). Do allelopathic compounds in invasive *Solidago canadensis* s.l. restrain the native European flora? *J. Ecol.*, 96, 993-1001.

Abrahamson, W.G., Dobley, K.B, Houseknecht, H.R. & Pecone, C.A. (2005). Ecological divergence among five co-occuring species of old-field goldenrods. *Plant Ecol.*, 177, 43-56.

Bailey, J.K., Wooley, S.C., Lindroth, R.L. & Whitham, T.G. (2006). Importance of species interactions to community heritability: a genetic basis to trophic-level interactions. *Ecol. Lett.*, 9, 78-85.

Bailey, J.K., Schweitzer, J.A., Ubeda, F., Koricheva, J., LeRoy, C.J., Madritch, M.D. *et al.* (2009). From genes to ecosystems: A synthesis of the effects of plant genetic factors across levels of organization. *Philos. T. Roy. Soc. B*, 364, 1607-1616.

Bloom, A.J., Chapin, F.S. & Mooney, H.A. (1985). Resource limitation in plants – an economic analogy. *Ann. Rev. Ecol. Systemat.*, 16, 363-392.

Bossdorf, O., Shuja, Z. & Banta, J.A. (2009). Genotype and maternal environment affect belowground interactions between *Arabidopsis thaliana* and its competitors. *Oikos*, 118, 1541-1551.

Caldwell, M.M., Manwaring, J.H. & Jackson, R.B. (1991). Exploitation of phosphate from fertile soil microbes by 3 Great Basin perennials when in competition. *Func. Ecol.*, 5, 757-764.

Casper, B.B & Jackson, R.B. (1997). Plant competition underground. *Ann. Rev. Ecol. Systemat.*, 28, 545-570.

Collins, A., Hart, E.M., & Molovsky, J. (2010). Differential response to frequency-dependent interactions: an experimental test using genotypes of an invasive grass. *Oecologia*, 164, 965-969.

Crutsinger, G.M., Collins, M.D., Fordyce, J.A., Gompert, Z., Nice, C.C. & Sanders, N.J. (2006). Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science*, 313, 966-968.

Crutsinger, G.M., Sanders, N.J. & Classen, A.T. (2009). Comparing intra- and inter-specific effects on litter decomposition in an old-field ecosystem. *Bas. Appl. Ecol.*, 10, 535-543.

Dawkins, R. (1982). *The extended phenotype: the gene as the unit of selection*. Oxford University Press, Oxford, UK.

Drew, M.C. and Saker, L.R. (1975). Nutrient supply and growth of seminal root system in barley. 2. Localized, compensatory increases in lateral root growth and rates of nitrate uptake when nitrate supply is restricted to only part of root system. *J. Exp. Bot.*, 26, 79-90.

Fernandez, O.A. & Caldwell, M.M. (1975). Phenology and dynamics of root growth of three cool semi-desert shrubs under field conditions. *J. Ecol.*, 63, 703-714.

Genung, M.A., Bailey, J.K. & Schweitzer, J.A. (2012). Welcome to the neighbourhood: interspecific genotype by genotype interactions in *Solidago* influence above- and belowground biomass and associated communities. *Ecol. Lett.*, 15, 65-73.

Hegnauer, R. (1977). *The chemistry of Compositae*. In *The biology and Chemistry of the Compositae* (eds. Heywood, V.H., Harborne, J.B. & Turner, B.L.), 1, 283-335. Academic Press, London.

Johnson, M.T.J. & Agrawal, A.A. (2005). Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). *Ecology*, 86, 874-885.

Johnson, M.T.J., Lajeunesse, M.J. & Agrawal, A.A. (2006). Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecol. Lett.*, 9, 24-34.

Johnson, M.T.J. (2008). Bottom up effects of plant genotype on aphids, ants, and predators. *Ecology*, 89, 145-154.

Maddox, G.D. & Root, R.B. (1987). Resistance to 16 diverse species of herbivorous insects within a population of goldenrod, *Solidago altissima*: genetic variation and heritability. *Oecologia*, 72. 8-14.

Madritch, M., Donaldson, J.R. & Lindroth, R.L. (2006). Genetic identity of *Populus tremuloides* litter influences decomposition and nutrient release in a mixed forest stand. *Ecosystems*, 9, 528-537.

Mooney, K.A. & Agrawal, A.A. (2008). Plant genotype shapes ant-aphid interactions: Implications for community structure and indirect plant defense. *Am. Nat.*, 171, E195-E205.

Mooney, H.A., Hobbs, R.J., Gorham, J. & Williams, K. (1986). Biomass accumulation and resource utilization in cooccurring grassland annuals. *Oecologia*, 70, 555-558.

Pregitzer, C.C., Bailey, J.K., Hart, S.C. & Schweitzer, J.A. (2010) Soils as agents of selection: feedbacks between plants and soils alter seedling survival and performance. *Evol. Ecol.*, 24, 1045-1059.

Schenk, H.J. (2006). Root competition: beyond resource depletion. *J. Ecol.*, 94, 725-739.

Schmid, B. & Bazzaz, F.A. (1992). Growth responses of rhizomatous plants to fertilizer application and interference. *Oikos,* 65, 13-24.

Schweitzer, J.A., Bailey, J.K., Rehill, B.A., Martinsen, G.D., Hart, S.C., Lindroth, R.L. *et al.* (2004). Genetically based trait in a dominant tree affects ecosystem processes. *Ecol. Lett.*, 7, 127-134.

Schwinning, S. & Weiner, J. (1998). Mechanisms determining the degree of size asymmetry in competition among plants. *Oecologia*, 113, 447-455.

Whitham, T.G., Bailey, J.K., Schweitzer, J.A., Shuster, S.M., Bangert, R.K., LeRoy, C.J. *et al.* (2006) A framework for community and ecosystem genetics: From genes to ecosystems. *Nat. Rev. Genet.,* 7, 510-523.

Wilson, J.B. (1988). Shoot competition and root competition. *J. Appl. Ecol.*, 25, 279-296.

Wilson, S.D. & Tilman, D. (1993). Plant competition and resource availability in response to disturbance and fertilization. *Ecology*, 74, 599-611.

Wolf, J.B., Brodie, E.D., Cheverud, J.M., Moore, A.J. & Wade, M.J. (1998). Evolutionary consequences of indirect genetic effects. *Trends Ecol. Evol.*, 13, 64-69.

CHAPTER 4.

Genetic variation and community change: selection, evolution, and feedbacks

ABSTRACT

There is relatively little information on how evolutionary processes that alter genetic variation in a focal species may affect interactions with other species, impact the structure or function of the community and ecosystem, and affect evolutionary feedbacks among interacting species through time. Because evolution can occur at ecological time-scales, it is important to understand how major selective events, such as climatic changes, can impact the community of interacting species and ecosystem processes by changing intraspecific genetic variation. The evidence linking genetic variation and evolution to community change and feedbacks has arisen from several different approaches whose results have not been synthesized into one conceptual framework, and whose commonalities may not be fully understood. This review synthesizes several different experimental approaches on how evolution may impact communities and ecosystems and focuses on five main issues: (i) the genetic basis to communities and ecosystems; (ii) the community and ecosystem consequences of among-population genetic differentiation; (iii) the role of local adaptation and co-evolution; (iv) the effects of trans-generational feedbacks and the eco-evo dynamic and; (v) the integration of community and ecosystem genetics and multi-level selection. Our review found extensive evidence for the idea that evolution can alter intraspecific genetic variation to affect indirect genetic effects and feedbacks. Future studies should investigate how communities and ecosystems are affected when evolution causes the strength of feedbacks to change.

REVIEW AND SYNTHESIS

Background

An important current issue in community and ecosystem genetics research is determining the relevance of intraspecific genetic variation (i.e., polymorphism) and *genetic differentiation* (i.e., divergence) (**Table 8**) to ecological and evolutionary processes at the community and ecosystem level. Intraspecific genetic variation in a focal species not only affects the composition of associated communities, but can cause community members to evolve in response to genetic differentiation across the focal species' range, which provides a strong argument for the necessity of considering intraspecific genetic variation in ecological studies. The genetic analysis of ecological and evolutionary interactions involving multiple species may seem complicated,

Table 8. Lexicon of common ecological genetics terms.

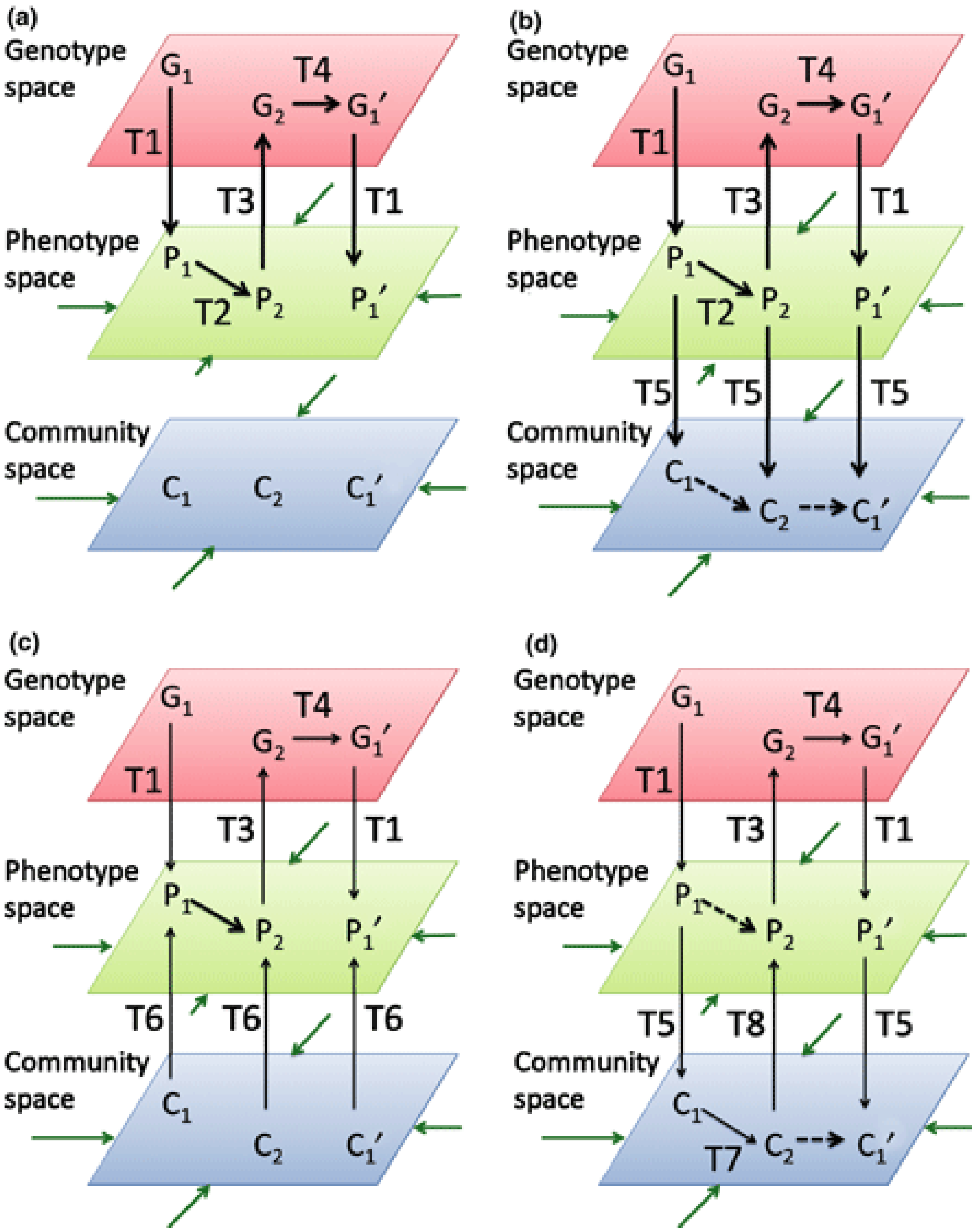
Term	**Definition**
All home and away	An experimental design in which common gardens are established at every location from which individuals were collected
Co-evolution	Evolutionary changes in traits of one population followed by an evolutionary change in traits of a second population to the first
Common garden	An experimental design in which environmental differences are minimized by planting all individuals in a common site
Community heritability	The degree to which genetically related individuals support similar communities and drive similar ecosystem processes
Community and ecosystem phenotypes	The effects of genes on levels of organization above the population; occur due to species interactions in which the genes of one species affect the phenotype of the other species
Diffuse co-evolution	Selection imposed reciprocally by one species on another is dependent on the presence or absence of other species
Eco-evo feedback	Reciprocal interactions between ecology and evolution in which populations alter their environment and those changes subsequently affect the evolution of the population
Feedback	Change in fitness of an organism due to its alteration of its biotic and abiotic environments
Genetic cline	A gradual change in allele frequencies across a portion of a species' geographic range
Genetic differentiation or divergence	Genetic differences between populations across the geographic range of a species
Indirect genetic effect	Genotype of one individual affects the phenotype and fitness of an associated conspecific individual
Interspecific indirect genetic effect	Environmental influences on the phenotype of one species are due to expression of genes in another species
Local adaptation	Higher fitness, within a given habitat, of individuals born in that habitat relative to individuals from other habitats

because *community and ecosystem phenotypes* represent complex traits related to genetically based interactions among species, and because abiotic and biotic environmental variation have a large effect on their expression. However, in both controlled *common garden* environments and across broad biogeographic regions, genetic variation in one species can have predictable and heritable effects on associated communities and ecosystems (Whitham *et al.* 2003, 2006; Johnson & Stinchcombe 2007; Bangert *et al.* 2008; Bailey *et al.* 2009; Johnson, Vellend & Stinchcombe 2009). It is crucial that we further our understanding of these processes, because when genetic variation in one species drives the expression of community and ecosystem phenotypes, selection can alter that genetic variation, resulting in evolution in one species which will further affect associated communities and ecosystems (Bailey *et al.* 2011).

The attempt to understand how evolution acts on genetic variation within a species to affect associated communities is not a new endeavor – for decades, both ecologists and evolutionary biologists have been interested in understanding how evolution in one species affects other species in the community (e.g., Pimentel 1961, 1968; Janzen 1980; Reznick & Endler 1982; Antonovics 1992; Thompson 1998; Grant & Grant 2002; Whitham *et al.* 2006). For example, Pimentel (1968) proposed that evolution in one species could regulate the population of another through a 'genetic feedback mechanism' through which the selective pressure on host plants increases with herbivore densities, causing changes in the genetic makeup of the host populations such that they become more resistant. This change, in turn, imposes selection on herbivores to overcome plant resistance. Moreover, it has been clearly shown that evolution can occur at ecologically relevant time-scales (Thompson 1998; Hendry & Kinnison 1999; Bohannan & Lenski 2000; Kinnison & Hendry 2001; Grant & Grant 2002; Yoshida *et al.* 2003; Hairston *et al.* 2005) and this observation is a unifying element to every approach linking ecology and evolution, including the geographic mosaic theory of *co-evolution* (Thompson 1999), *diffuse co-evolution* (Janzen 1980; Fox 1981; Herrera 1982; Strauss, Sahli & Connor 2005; Wade 2007), *community heritability* (Whitham *et al.* 2003, 2006; Shuster *et al.* 2006), and *eco-evo feedbacks* (Post & Palkovacs 2009). This has important implications because it suggests that major selective events such as climate change, increased habitat fragmentation, and species invasions can have extended ecological consequences by changing intraspecific genetic variation. While rapid evolution has already been incorporated into invasion biology through the 'evolution of increased competitive ability' hypothesis (see review by Bossdorf *et al.* 2005) and the ‘hybridization increases invasiveness’ hypothesis (see review by Ellstrand & Schierenbeck 2000) the demonstration that rapid evolution can alter species interactions and ecosystem properties has broad implications that are not commonly addressed in many other subfields of ecology including conservation biology, plant-pollinator interactions, and restoration ecology.

Although decades of research clearly shows that intraspecific genetic variation has community-level consequences, the extent to which evolutionary forces change genetic variation in one species and affect associated communities, and whether these changes feed back to affect the original species, is less clear. One conceptual model for understanding the extended effects of evolution acting on intraspecific genetic variation involves expanding Lewontin’s (1974) representation of population genetic transformations occurring in genotype and phenotype space to include another level of ‘community space’ (**Figure 8**). In this diagram, ‘spaces’ represent a set of possible values for genotypes, phenotypes, and communities, respectively, and arrows represent the ‘transformations’ between different spaces. For example, the transformation beginning in genotype space and ending in phenotype space represents the results of different genotypes developing in various environments (Lewontin 1974). In the expanded version the transformation beginning in phenotype space and ending in community space represents the community phenotype of genes being expressed by the species of interest. In this model, we assume that all community-level effects of genotype are mediated through the expressed phenotypes. In some systems, intraspecific genetic variation may have little or no effect on associated communities (Fig. 8a), suggesting that other ecological factors determine community composition. For example, Tack *et al.* (2010) reported that insect communities associated with *Quercus robur* were determined more by an environmental factor, spatial connectivity, than by host-plant genotype. When intraspecific genetic variation does affect associated communities, then evolution can lead to changes in the associated community (Fig. 8b), represented by transformations between the phenotype and community spaces. Transformations can also begin in community space and end in phenotype space, if the community represents an environmental factor driving differences in an organism’s phenotype independent of genetics (Fig. 8c). In some cases, evolution in a community context will feed back to affect the fitness and performance of the focal species (Fig. 8d). In this case, the transformation in phenotype space (dashed arrow) is primarily driven by the transformation in community space, which represents natural selection acting to change the composition of associated communities.

Figure 8. Extended phenotypes and eco-evo feedbacks in genotype, phenotype, and community space. Genetically based feedbacks through which one species alters its associated community, and community changes exert a reciprocal effect on the fitness of the original species, may occur when a series of conditions are met. In this diagram, ‘spaces’ represent a set of possible values for genotypes, phenotypes, and communities. Transformations are represented by arrows and refer to changes either within or between spaces; for example, a transformation beginning in phenotype space and ending in community space represents the community phenotype of genes in the species of interest. Dashed arrows represent transformations which are driven, at least primarily, by dynamics in another space. G_1 and G_1' represent the beginning of new generations, while G_2 or P_2 represents the second value for a genotype or phenotype within a generation. The transformations involving genotype and phenotype space are (modified from Lewontin 1974): [T1] laws that give the distribution of phenotypes that result from the development of various genotypes in various environments; [T2] laws of mating, migration, and natural selection which transform the distribution of phenotypes within one generation; [T3] relations which allow inference of a distribution of genotypes corresponding with a distribution of phenotypes; [T4] genetic rules which allow the prediction of genotypes in the next generation, given the array of parental genotypes. The transformations involving phenotype and community space describe: [T5] the community phenotypes associated with a species based on the distribution of phenotypes (i.e., community heritability); [T6] environmental factors driving differences in the phenotype of the plant independent of plant genetics; [T7] the differential fitness values of community members associated with a species which transform the distribution of communities within one generation; [T8] how changes in community composition feed back to affect the distribution of phenotypes in the species of interest. (a) represents a system in which plant genetic variation does not affect associated communities; (b) represents a system in which plant genetic variation affects associated communities but there are no feedbacks; (c) represents a system in which the herbivore community drives differences in a plant’s phenotype; and (d) represents a system in which plant genetic variation affects herbivore communities and these changes feed back to affect the plant species. Arrows pointing inwards to the boundaries of phenotype and community space are placeholders for extrinsic effects of the biotic and abiotic environment on expression of phenotypes and community structure.


(a)
Genotype space
Phenotype space
Community space
G1
G2
G1′
P1
P2
P1′
C1
C2
C1′
T1
T2
T3
T4
T1
(b)
Genotype space
Phenotype space
Community space
G1
G2
G1′
P1
P2
P1′
C1
C2
C1′
T1
T2
T3
T4
T1
T5
T5
T5
(c)
Genotype space
Phenotype space
Community space
G1
G2
G1′
P1
P2
P1′
C1
C2
C1′
T1
T3
T4
T1
T6
T6
T6
(d)
Genotype space
Phenotype space
Community space
G1
G2
G1′
P1
P2
P1′
C1
C2
C1′
T1
T3
T4
T1
T5
T8
T5
T7

The perspective outlined in Figure 8 is valuable because understanding how this range of *indirect genetic effects* and *feedbacks* varies spatially and temporally (e.g., Thompson 2005) in response to abiotic and biotic environmental variation represents an important frontier. Each of the possibilities listed above have been detected in different experimental designs, each with its own strengths and weaknesses, and each one providing different information about how evolution in one species may affect community and ecosystem properties in associated species (**Table 9**). For example, designs that provide valuable information about co-evolutionary processes and feedbacks are considerably more expensive and labor-intensive than a single-location common garden study, so investigators should consider the experimental scale at which their question can be best addressed.

Of all the above approaches, studies examining how feedbacks affect co-evolution and eco-evo interactions have received the least attention (but see Lankau & Strauss 2007; Palkovacs & Post 2008; Palkovacs *et al.* 2009; Post & Palkovacs 2009; Pregitzer *et al.* 2010); however, these studies are crucial to linking ecological and evolutionary processes. Although ecological feedbacks in natural systems are just beginning to be formally studied at the genetic level (e.g., Lankau & Strauss 2007; Pregitzer *et al.* 2010), studies of this nature have broad implications to many scientific disciplines, some of which currently do not incorporate genetic variation as a part of their research. Many types of feedbacks exist, but a common feature is that feedbacks occur when an organism modifies its abiotic (e.g., increased leaf area causes decreased ground temperature) or biotic (e.g., changes in prey community structure due to predation pressure) environment, and that modification in turn affects the fitness of the organism or other community members (Van der Putten, Vandijk & Peters 1993; Bever 1994; Jones, Lawton & Shachak 1997; Post & Palkovacs 2009). Feedbacks are fundamental to the co-evolutionary process (Thompson 2005) and local adaptation (Clausen, Keck & Hiesey 1940; Gandon & Nuismer 2009) because in each of these cases the interacting species can exert reciprocal fitness consequences. Additionally, feedbacks are an important part of the maintenance of biodiversity (Bascompte & Jordano 2007; Lankau & Strauss 2007; Duffy & Forde 2009; Laine 2009). For example, in a recent review, Laine (2009) reported that variation in the strength of feedbacks led to population-level divergence in the traits of interacting species. Finally, there has been virtually no consideration of the extended consequences of particular feedbacks in community or ecosystem ecology; for example, if a feedback alters the fitness and performance of one species,

Table 9. Experimental approaches for eco-evo feedback studies. Experimental approaches which address how evolution in one species changes associated communities and how these changes can feed back to affect the original species. Types of questions which can be answered by each type of study are also included.

Type of Study	Description	Genetic basis of communities and ecosystems	Evolution	Co-evolution	Eco-evo feedbacks	Eco-evo feedback dynamics
Local common garden	Collect individuals locally and plant in local garden(s)	Yes	Yes*	No	No	No
Genetic differentiation	Collect individuals from across the range of a species and plant them in one location	Yes	Yes	No	No	No
Local adaptation	Collect and grow individuals and associated species in all home and away environments	Yes	Yes	Yes	Yes	No
Trans-generational feedback	Examine systems in which genetic differentiation has occurred in multiple environments and infer the effects on trans-generational feedbacks	Yes	Yes	Yes	Yes	Yes

*Assumptions of evolution in common garden studies may be too strong, due to the effects of gene flow between genetic individuals being stronger than selection.

how does that alter interactions with other species and the ecosystem services they mediate? Although recent studies have begun to address the dynamic nature of ecological and evolutionary feedbacks (e.g., Lankau & Strauss 2007; Palkovacs & Post 2008; Pregitzer *et al.* 2010), a more complete understanding of these feedbacks remains a major challenge.

As the field of community and ecosystem genetics is currently focused on documenting the extended effects of genetic variation in a focal species, this review provides a broad conceptual framework for understanding how evolution may impact communities and ecosystems. We discuss five main elements relating to ‘genes to ecosystems’ research, including: (i) intraspecific genetic variation affects communities and ecosystems, although these effects can be dependent on abiotic and biotic environmental context; (ii) evolution in one species across its native range leads to genetic differentiation which affects associated communities; (iii) genetically differentiated populations of species can co-evolve interactions with other species which maximize the focal species’ fitness in a given environment; (iv) genetically differentiated populations can affect the success of subsequent generations of their offspring and the offspring of other community members through trans-generational feedbacks; and lastly, (v) feedbacks may vary in space and time and may be a consequence of the community of interacting species or even the ecosystem processes that those communities mediate (Lankau & Strauss 2007; Bailey *et al.* 2009). This suggests that the relative fitness of any individual may vary depending upon characteristics of the specific ‘group’ (i.e., population, community, and ecosystem) to which it belongs and suggests that concepts associated with multi-level selection may be useful for understanding the potential impacts of selection on populations as a consequence of the particular ‘groups’ with which they are associated. In this last section, we use the Price equation to define mathematically how multi-level selection may act within the context of the community and ecosystem. In this review, we attempted to bring together these five components whose synthesis suggests that if the evolutionary dynamic in a system changes, then the ecological dynamic also likely changes (and vice versa) through indirect genetic effects and feedbacks, with consequences that can extend from the phenotype of an individual within a population to affect associated communities and ecosystem processes. Our examples largely focus upon plant-herbivore interactions, but the ideas and conceptual framework proposed are general and may apply to any system.

Genetic basis to communities and ecosystems

Linking genetic variation within species to patterns and processes that occur above the population level is critical to understanding how genetic changes in one species may affect the ecology of associated species (Bailey *et al.* 2010). One approach to this problem involves quantifying the predictability of community and ecosystem responses with respect to genetic factors in a given species. A common experimental approach to this question has been to collect plants from a specific population and then to plant all collected specimens in a 'common garden' at one location. Within the common garden, trait differences across genotypes can be assessed in the absence of high environmental variation. Common garden studies have been instrumental in building our understanding of the extended effects of genotypic variation on traits at the community (Whitham *et al.* 2003, 2006; Johnson & Agrawal 2005; Bailey *et al.* 2006; Crutsinger *et al.* 2006; Johnson, Lajeunesse & Agrawal 2006; Tetard-Jones *et al.* 2007; see also Fig. 1a), and ecosystem (Whitham *et al.* 2003, 2006; Crutsinger *et al.* 2006; Madritch, Donaldson & Lindroth 2006; Schweitzer *et al.* 2008; Pregitzer *et al.* 2010) level. However, the effects of genetic variation on associated communities are often context dependent, varying across different environments (genetic (G) × environment (E) interactions, e.g., Johnson & Agrawal 2005; Bangert *et al.* 2008; Bossdorf, Shuja & Banta 2009) or due to the presence of genetic variation in other species in addition to non-genetic biotic and abiotic environmental effects (G × G × E interactions, e.g., Tetard-Jones *et al.* 2007). For example, Tetard-Jones *et al.* (2007) manipulated aphid and barley genotypes, and showed that the presence of rhizobacteria significantly altered the genotype by genotype interactions between aphids and barley. Genetic by environment interactions are important because the context dependency of genetic effects sets up the possibility of community-level responses to genetic differentiation and *local adaptation* if a species' range includes many different environments. These interactions also suggest that studies of *genetic clines* along elevational or latitudinal gradients could improve our knowledge of how strong the effects of genetic variation and differentiation are in the context of varying environmental conditions (Bailey *et al.* 2010).

The higher-level ecological effects of genetic variation were first termed 'extended phenotypes' by Dawkins (1982) because they are caused by the expression of genes in an individual. Extended phenotypes have been further characterized as community and ecosystem phenotypes, respectively, which describe the tendency for genetically related individuals to support similar communities and affect ecosystem processes in similar ways (Whitham *et al.* 2003, 2006). A common garden experimental framework allows the estimation of variation in the community or ecosystem phenotype and the extent to which it is explained by variation in underlying genetic factors. The heritability of community phenotypes involves using traditional diversity indices (i.e., Simpson's or Shannon–Weiner) or an ordination technique to convert multivariate community data (associated with individuals of a focal species) into a univariate response which can be analyzed with standard statistical tools, allowing for the estimation of the broad sense heritability of community traits (see Johnson & Agrawal 2005; Shuster *et al.* 2006; Whitham *et al.* 2006 for details). For example, in *Populus* spp., the heritability of communities associated with particular genotypes has been estimated as 0.56–0.63 for arthropod communities and 0.70 for soil microbial communities or microbial pools of nutrients (Shuster *et al.* 2006; Schweitzer *et al.* 2008).

Many investigators argue that community and ecosystem phenotypes represent complex traits related to variation in the fitness consequences of indirect genetic effects (IGEs) among species (Thompson 2005; Shuster *et al.* 2006; Whitham *et al.* 2006; Tetard-Jones*et al.* 2007). In their most basic form, IGEs are environmental influences of genes in one individual on the phenotype and fitness of an associated conspecific individual (Moore, Brodie & Wolf 1997; Agrawal, Brodie & Wade 2001). When IGEs occur between different species, they are referred to as *interspecific IGEs* (IIGEs, Shuster *et al.* 2006; Whitham *et al.* 2006). Based on theory outlined by Shuster *et al.* (2006), genetically based variation in community phenotypes provides evidence that: (i) fitness effects arise due to IIGEs, and (ii) through differential fitness consequences of IIGEs, selection can occur in a community context. While it has been suggested that a finding of significant community heritability provides evidence of evolution in a community context, in practice, the relationship between community heritability and co-evolution by natural selection is less clear.

For associated species to evolve in response to underlying plant genetic variation there are four important requirements. First, plant genotypes have to act as an agent of selection affecting the fitness and phenotype of the associated species (described in the preceding paragraphs). Second, the effects of plant genetic variation on arthropods must be mediated by genetic variation for arthropod traits (Lewontin 1970). Third, the variation in arthropod traits must also covary with arthropod fitness (Lewontin 1970). Fourth, some spatial isolation or other mechanism is required to prevent gene flow between populations that are evolving in response to different selective environments (i.e., associations with different host-plant genotypes). For example, Heschel & Paige (1995) showed that introducing pollen from a larger population of scarlet gilia (*Ipomopsis aggregata*) to a smaller population was enough to ease extinction risk caused by small population size, illustrating the well-known principle that even very low levels of gene flow can have relatively large effects on the genetic structure of populations (Wright 1931; Hartl & Clark 1997). Although divergent adaptation in the face of gene flow is possible, and often observed in phytophagous insects (see reviews by Dres & Mallet 2002; Coyne & Orr 2004; Bolnick & Fitzpatrick 2007), the conditions are restrictive (Lenormand 2002) and the strength of selection needed to overcome gene flow might be rare among genotypes of a single host plant population (but see Van Zandt & Mopper 1998). Immigration can affect the rate at which arthropod populations became locally adapted to host plant genotypes. For example, Tack & Roslin (2010) reported that arthropod abundance was higher on local genotypes of *Quercus robur*, compared to genotypes transplanted from different parts of the species' range, when immigration rates were low; however, the opposite pattern was observed (higher arthropod abundance on transplanted genotypes than local genotypes) when immigration rates were high. The effects of immigration on community heritability are important because most estimates of community heritability are from within a single common garden site where gene flow among the pool of possible herbivore populations is not limited. A better understanding of how genetic variation in one species affects associated community members requires that experiments are carried out at larger spatial scales (i.e., along environmental gradients or among races) such that realistic levels of gene flow can be compared with the effects of selection.

Community and ecosystem consequences of among population genetic differentiation

Identifying and understanding genetic differentiation along specific environmental gradients (Endler 1977; Storfer 1999; Foster *et al.* 2007) provides an important tool for addressing the hypothesis that evolution in one species can lead to change at the community and ecosystem level. One promising approach to examining how evolution in one species leads to change in associated communities and ecosystem processes involves investigating whether a species is genetically differentiated across its geographic range and then determining whether that genetic differentiation has affected associated communities and ecosystem processes. For example, in a 15-year-old plantation forest trial, Barbour *et al.* (2009a) found that (i) genetically based variation in *Eucalyptus globulus* expressed at the race and family level was correlated with differences in the associated arthropod community; (ii) community divergence among races was correlated with leaf physical and chemical traits; and (iii) community dissimilarity was correlated with neutral marker and quantitative genetic dissimilarity. Because variation among populations as well as between families was detected, this indicates that *E. globulus* had genetically differentiated across its range, and that this differentiation had led to changes in the associated community. This type of experiment is represented in Fig. 8b, in which the transformations in genotype and phenotype space represent the process of genetic differentiation, and the subsequent transformations in community space represent the unique community composition of each family of *E. globulus*. Using the same plantation forest, differences among races in litter arthropod communities (Barbour *et al.* 2009b) and soil nitrate (NO_3^-) availability (Bailey *et al.* 2011) were also detected, demonstrating broad community and ecosystem phenotypes of *E. globulus*. Genetic differentiation along latitudinal gradients can also affect plant palatability to herbivores (B. T. Moles, S. P. Bonser, I. R. Wallis & W. J. Foley, unpublished data). For example, Pennings, Siska & Bertness (2001) allowed herbivorous insects to choose between conspecific plants collected from northern and southern environments within the United States and reported that herbivores preferred the northern plants, a pattern which held across herbivore species. These studies indicate that genetic differentiation across broad geographic ranges can result in variation to foliar and litter arthropod communities and soil nitrogen availability (Bailey *et al.* 2010). Based on theory established in Shuster *et al.* (2006), one may expect that community differences resulting from divergence in one species (e.g., *E. globulus*) suggest that all interacting species are differentiating in a co-evolutionary manner. However, the frequency and intensity of co-evolution will vary; co-evolution is most likely to be observed when the strength of feedbacks is large relative to gene flow. Because genetic variation interacts with differences in the regional species pool, strong inferences about the co-evolutionary dynamics are difficult to make without placing the differentiated population (e.g., *E. globulus*) within all potential communities to test for feedbacks (see local adaptation below).

Another way that evolution can affect change in associated communities is reticulate evolution, which can occur in hybridizing species complexes. In *Populus (P. angustifolia* X *P. fremontii*), Bailey *et al.* (2010) showed that introgression at a single molecular marker had important effects on associated arthropod communities; for example, total arthropod richness increased by 23% and gall-forming arthropod abundance increased by 72% on trees carrying the *P. fremontii* allele at the molecular marker RFLP-755. Introgression also caused a 56% increase in ramet production and a 51% decrease in the production of salicortin, an anti-herbivore defensive compound (Palo 1984; Rehill *et al.* 2006; Bailey *et al.* 2007). A multiple regression model indicated ramet production, but not salicortin, predicted arthropod abundance, suggesting that the increase in arthropod abundance was due to increased vegetative branching (Price 1991). Reticulate evolution can also lead to the evolution of invasiveness by introduced species (see review by Ellstrand & Schierenbeck 2000), which is important because of the large effects these invaders can have on associated communities and ecosystems. For example, invasion by a hybrid cordgrass, *Spartina alterniflora* × *foliosa*, affected sediment properties and associated communities, increasing sediment organic matter and decreasing benthic macrofaunal density by 75% (Neira, Levin & Grosholz 2005). These results indicate that community phenotypes can be sensitive to even small genetic changes, suggesting that evolutionary changes in associated communities may occur more often, and through a simpler mechanism, than previously thought.

Genetic variation may be expressed and evolutionary processes may act in a variety of ways to affect patterns of community structure and ecosystem function. Moreover, there is emerging evidence from a variety of taxa, including vertebrates (e.g., Palkovacs & Post 2008), which clearly indicates that evolutionary dynamics can operate on ecological time-scales (Thompson 1998; Hendry & Kinnison 1999; Bohannan & Lenski 2000; Kinnison & Hendry 2001; Grant & Grant 2002; Yoshida *et al.* 2003; Hairston *et al.* 2005). While genetic variation in one species can have community and ecosystem consequences, little is understood about whether there are feedbacks from the community or ecosystem on the fitness and performance of a focal population, and how these feedbacks may affect ecological and evolutionary dynamics.

Local adaptation, the co-evolutionary process, and feedbacks

Genetic differentiation that is a result of natural selection commonly results in strong patterns of local adaptation. Local adaptation occurs when individuals born in a given habitat have higher fitness within that habitat (i.e., 'home' environment), than individuals from other habitats (i.e., 'away' environments). Such local adaptation may be associated with factors such as differences in temperature or precipitation along elevational or latitudinal gradients. For example, Clausen, Keck & Hiesey (1940) showed that clones of sticky cinquefoil (*Potentilla glandulosa*) performed better when grown near their native elevation than at other elevations, suggesting that the populations were adapted to local abiotic environmental conditions.

Local adaptation can also occur in response to biotic environmental variation, and this type of local adaptation can result in the co-evolutionary process. Local adaptation associated with biotic environmental variation is largely associated with species interactions which vary across a species' range (Pennings, Siska & Bertness 2001; Thompson 2005; Johnson *et al.* 2010; Bailey *et al.* 2011). For example, Johnson *et al.* (2010) collected ecotypes of *Andropogon gerardii* from across its range and planted them in *all home and away* conditions with respect to both soils and arbuscular mycorrhizal (AM) fungi inoculates, and showed that ecotypes were adapted to local soil environments and AM fungi communities to maximize transfer of the most limiting nutrients between plants and fungi. Because this study manipulated ecotype identity in 'all possible home and away' environments from which individuals were collected, it confirms that subpopulations of *A. gerardii* had both genetically differentiated and co-evolved interactions with particular AM fungi strains in different environments across its range. However, the 'all possible home and away' approach can be financially and logistically intractable depending upon the number of families collected from each population, the number of populations sampled across the range of the species and the number of different abiotic and biotic environments that are tested (Barbour *et al.* 2009a).

When local adaptation and co-evolution are detected in genetically differentiated populations (e.g., Johnson *et al.* 2010), genetically based feedbacks can be inferred because certain populations display greater fitness and performance in their 'home' environment (biotic and abiotic) vs. an 'away' environment. When a population performs better in its home environment relative to away conditions it experiences a *positive feedback*. When a population performs better under away conditions, it experiences a *negative feedback*. Similar to the design of local adaptation studies, experiments to test for feedbacks would be conducted by establishing common gardens at every location from which a subpopulation was collected; however, in contrast to local adaptation studies, feedback studies examine the dynamic nature of organisms' modifying their environment and the fitness consequences of those modifications.

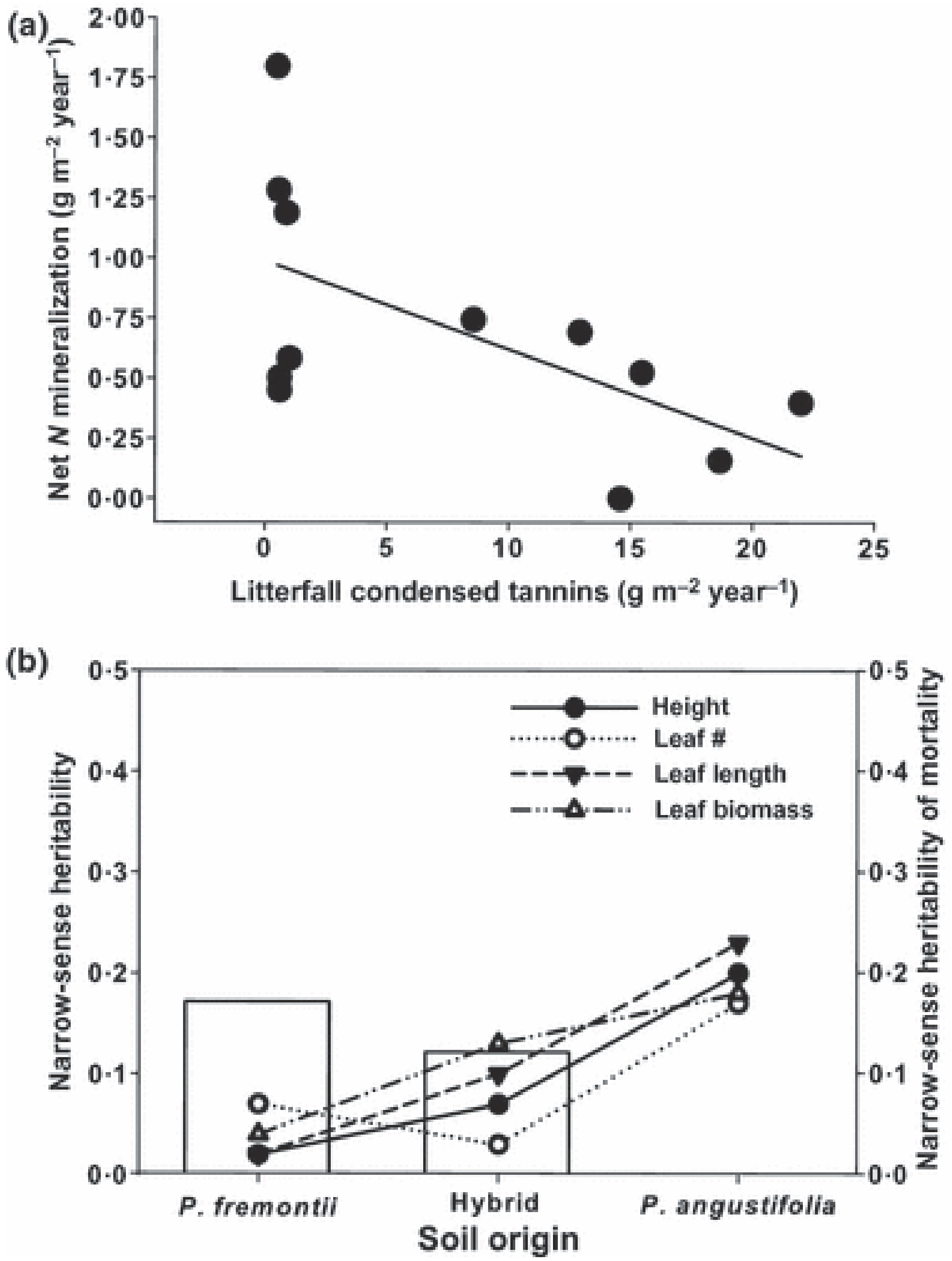
Feedbacks could be observed in a variety of species interactions, including those between plants and herbivores, plants and their soil communities, plants and neighboring plants, animals and their prey, and many others. It is likely that species experience multiple positive and/or negative feedbacks from many different interactions simultaneously, and understanding how multiple feedbacks may interact is largely uninvestigated. For example, a plant may experience a positive feedback in genotype monocultures because it is able to cultivate a specific microbial community below-ground (Johnson *et al.* 2010); conversely, a plant may experience a negative feedback in a genotype mixture due to temporal and spatial partitioning of resource acquisition (Crutsinger *et al.* 2006). Theoretically, feedbacks with fitness consequences are implicit in the measurement of the heritability of community composition because different communities are assumed to have different fitness consequences for any given species. However, quantification of such feedbacks requires the experimental manipulation of the associated community and the subsequent measurement of the original species' fitness and performance. Furthermore, even if plant fitness and performance change with the removal of the associated community, understanding the strength of the feedback through time is an additional challenge.

Trans-generational feedbacks and the eco-evo dynamic

Feedbacks involving reciprocal interactions of ecology and evolution over relatively short time-scales have been termed eco-evo feedbacks, which are defined as occurring when a population alters its environment (abiotic or biotic) and those environmental changes influence the fitness of the original population (Post & Palkovacs 2009) or subsequent generations (i.e., trans-generational feedback, Pregitzer *et al.* 2010). While community and ecosystem phenotypes clearly indicate the potential of feedbacks to occur between associated species, simply detecting a community or ecosystem phenotype does not specifically examine such interactions or speak to the dynamic nature of eco-evo feedbacks. Intraspecific genetic variation provides a means for testing how eco-evo feedbacks affect species and their environments through time, although it is not technically a prerequisite condition for eco-evo feedbacks to occur (Post & Palkovacs 2009). Examples of systems with a high potential for eco-evo feedbacks include those in which intraspecific genetic variation is high (Yoshida *et al.* 2003) or species interactions are continuous throughout the year, rather than intermittent (Palkovacs & Post 2008). One example of eco-evo feedbacks comes from Lankau & Strauss (2007), who showed that genetic variation for sinigrin content in *Brassica nigra* is responsible for a feedback which maintains diversity in plant communities, due to an intransitive (i.e., rock-paper-scissors) competitive relationship in which high-sinigrin *B. nigra* individuals can invade patches of heterospecific neighbors, low-sinigrin *B. nigra* individuals can invade patches of high sinigrin *B. nigra*, and heterospecific neighbors can invade patches of low-sinigrin *B. nigra*. This result indicates that genetic variation for sinigrin content is responsible for feedbacks which maintain genetic diversity in *B. nigra*, and species diversity in neighboring plants. This type of experiment is represented in Fig. 8d, in which genetic variation in *B. nigra* for sinigrin content drives changes in the associated plant community, which in turn affects which genotypes of *B. nigra* will perform well in the next generation. While we recognize that *B. nigra* may reduce the species diversity by outcompeting its neighbors in some cases, we discuss this study to illustrate the potential for genetic differentiation to cause community changes which feedback to affect traits in the original species.

Another mechanism through which transgenerational feedbacks can operate involves offspring performing better in their parent's soil than in an 'away' soil environment. For example, Pregitzer *et al.* (2010) showed that *P. angustifolia* seedlings performed better when grown in soil conditioned by their parent trees compared with soil conditioned by other *Populus* species, even though the 'home' soils were the most N-limited (i.e., highest C : N ratio and highest microbial N pools, **Figure 9**). Because parent species affect soil microbial communities and nutrient cycling (Schweitzer *et al.* 2004, 2008; Whitham *et al.* 2006), these results indicate that soils, which are a common abiotic and biotic environment for both parents and their offspring, can act as agents of selection (Pregitzer *et al.* 2010). In the same sense that we must determine the relative importance of intraspecific genetic variation, future studies should investigate the importance of feedbacks as agents of selection, relative to 'traditional' (i.e., unidirectional)

Figure 9. Soils as agents of selection. Genetic variation in *Populus* can affect soils, and these changes can feed back to affect fitness and performance of the next generation of *Populus* trees. In field and laboratory incubations, condensed tannin in litterfall was best related to soil net N mineralization. Condensed tannin in litterfall explained 65% of the variation in annual rates of net N mineralization in soils from 12 stands of gallery cottonwood forests (a, Schweitzer *et al.* 2004). Trees with low condensed tannins were from the *P. fremontii* zone, trees with high tannins were from the *P. angustifolia* zone, with hybrids intermediate, on average. Soils were collected from all three zones, and *P. angustifolia* seedlings were planted in each soil type. *P. angustifolia* survived best in its own soil type, even though these soils were the most nutrient limited; additionally, *P. angustifolia* also experienced the most mortality in soils collected from beneath *P. fremontii*, even though these soils were the least nutrient limited (b, Pregitzer *et al.* 2010). Bars represent the narrow-sense heritability of seedling mortality. Symbols represent the narrow-sense heritability of performance traits. Originally published in Schweitzer *et al.* (2004), *Ecology Letters* (a) and Pregitzer *et al.* (2010), *Evolutionary Ecology* (b).


(a)
Net *N* mineralization (g m⁻² year⁻¹)
2·00
1·75
1·50
1·25
1·00
0·75
0·50
0·25
0·00
0
5
10
15
20
25
Litterfall condensed tannins (g m⁻² year⁻¹)
(b)
Narrow-sense heritability
Narrow-sense heritability of mortality
0·5
0·4
0·3
0·2
0·1
0·0
Height
Leaf #
Leaf length
Leaf biomass
P. fremontii
Hybrid
P. angustifolia
Soil origin

ecological and evolutionary factors. Because genetic variation in one species can have community and ecosystem phenotypes that affect genetic variation in subsequent generations, it becomes difficult to determine at which level of organization (e.g., population, community, or ecosystem) the relative fitness of an individual depends.

Multi-level selection…whose phenotype is it and how is selection acting?

While no experimental approach for multi-level selection is widely accepted, multi-level selection provides an important theoretical framework for understanding how higher level selection may influence individual level evolutionary processes. Multi-level selection is generally defined as variation in an individual's relative fitness that can be partitioned into within-group and between-group components, with 'level' defined by the question to be addressed (Keller 1999; Collins & Gardner 2009). Partitioning variation in fitness is an important approach if we are to model the change in the average value of a specific character across all individuals, in all species, in all communities in an ecosystem (i.e., a phenotype that is common to all organisms in an ecosystem such as biomass, plant and soil nutrient content (i.e., carbon, nitrogen, phosphorous), body size, or offspring). We recognize that ecosystems are more than a set of communities, and that some ecosystem properties would go unmeasured even if every parameter at the community level was assessed. Our approach is only one method of using the flexible Price equation to partition across levels of organization. This approach provides an all-encompassing view of natural selection by recognizing that organisms are embedded not only in a complex abiotic environment, but also a matrix of interacting species in which the phenotype of one organism is the environment of another (i.e., the relative fitness of an individual depends on the group to which it belongs). One common theoretical framework for understanding multi-level selection is the Price equation (Price 1972; Frank 1998; Gardner 2008; Collins & Gardner 2009). The Price equation mathematically describes change via natural selection in a completely general way and has recently been applied successfully in the field of community ecology (Loreau & Hector 2001; Fox 2006; Collins & Gardner 2009) and its use for understanding how evolutionary processes may alter communities and ecosystem function may be fundamental.

Consider an ecosystem formed by a set of communities C, in which each community is assigned a unique index $i \in C$. Each community contains a set of species Si and we assign each species a unique index $j \in Si$. Each species contains a set of different individual phenotypes Pij, to which we assign each phenotype a unique index $k \in Pij$. Notice that for our purposes communities are mutually exclusive groups of species and phenotypes within an ecosystem, and species are mutually exclusive groups of individuals within a community. Communities can be defined in different ways depending on the character considered and the interest of the researcher as long as they retain their hierarchical and mutually exclusive nature.

Let $wijk$ be the fitness of an individual with phenotype k in species j in community i. The average (arithmetic mean) fitness of all individuals in species j in community i is $w_{ij} = E_{P_{ij}}[w_{ijk}]$ (where EA denotes the statistical expectation taken over the set A). Set A is a generic set which can refer to phenotypes (P), communities (C), or ecosystems (E). The average fitness of all species in community i is $w_i = E_{S_i}[w_{ij}]$. The average fitness of all phenotypes in the ecosystem is $\bar{w} = E_c\left[E_{S_i}\left[E_{P_{ij}}[w_{ijk}]\right]\right]$.

Let $zijk$ be the value of the character of interest corresponding to phenotype k in species j in community i. The average value of character z in species j in community i is $z_{ij} = E_{P_{ij}}[z_{ijk}]$. The average value of character z in community i is $z_i = E_{S_i}[z_{ij}]$. The average value of character z in the ecosystem is $z = E_C[z_i] = E_C\left[E_{S_i}\left[E_{P_{ij}}[z_{ijk}]\right]\right] = \bar{z}$.

Consider the value of the character of interest z and z' in times t_1 and t_2 respectively. Let $\Delta z = z' - z$ be the change of the character value from one generation to the next. A species changes when the frequency of the different phenotypes within the species changes over time. A community changes when its constituent species change and/or when the frequency of species changes over time. Finally, an ecosystem changes when the frequency of communities changes over time.

We applied the Price equation (Price 1972; Frank 1998; Gardner 2008) to model the change in the average value of a specific character across a whole ecosystem. In particular, the total effect of selection can be partitioned into among-community, among-species within communities, and among-phenotypes within species of a community (Frank 1998; Gardner 2008):

$$\Delta\bar{z} = E_C\left[E_{S_i}\left[C_{P_{ij}}\left[\frac{w_{ijk}}{\bar{w}}, z_{ijk}\right]\right]\right]$$
$$+E_C\left[C_{S_i}\left[\frac{w_{ij}}{\bar{w}}, z_{ij}\right]\right] + C_C\left[\frac{w_i}{\bar{w}}, z_i\right]$$

where *CA* denotes the statistical covariance taken over the set *A*. Here, we assumed that there is no environmental induction or bias in the transmission of a particular phenotype between generations (i.e., $E_{P_{ij}}\left[w_{ijk}\Delta z_{ijk}\right] = 0$) but an additional partition could be added to account for such changes (Collins & Gardner 2009).

This equation shows that the change, owing to selection, in the average value of a character across the whole ecosystem during the time considered is given by the sum of three terms. The first term is the covariance between a phenotypic character value (*zijk*) and its fitness relative to the average fitness of all phenotypes in the ecosystem considered ($\frac{w_{ijk}}{\bar{w}}$) averaged over all species and all communities in the ecosystem. This term captures whether phenotypes with higher character value increase or decrease within their species and community and describes the average change in phenotypic composition within species. It represents the change in the total or average phenotype of the ecosystem that can be explained by variation in fitness within species.

The second term is the covariance between the average character in each species (*zij*) and the average fitness of that species relative to the overall average fitness in the ecosystem ($\frac{w_{ij}}{\bar{w}}$) averaged over all communities in the ecosystem. This term captures whether species with higher character value increase or decrease within their community and describes the change in species composition of a community. The third term is the covariance between the average character in each community (*zi*) and the fitness of that community relative to the average fitness in the ecosystem ($\frac{w_i}{\bar{w}}$). This term captures whether communities with higher character value increase or decrease within the ecosystem and describes the change in community composition of an ecosystem. Note that covariance between a trait and fitness need not imply that the trait is responsible for variation in fitness (Lande & Arnold 1983). Particularly for more inclusive partitions (i.e., species), strong covariances among traits make correlated responses to selection highly likely. For example, average biomass might change owing to differential extinction of prokaryotes and eukaryotes driven by a chemical with physiological effects functionally unrelated to size. Because phenotypic covariances are often different at different levels, partitioning the Price equation can help expose effects of correlated selection (Collins & Gardner 2009).

Note also that the average within-species selection might often be very near zero if different species are changing in opposite directions. Thus, the degree of inclusiveness, detail, and manner of partitioning must all be chosen carefully to most effectively address any specific question. The Price equation is a ‘statistical tautology’ that follows from the definitions of the phenotype, fitness, and grouping variables (Gardner 2008; Collins & Gardner 2009). As such, it is both general and exact, but does not specify mechanisms or functional forms of selection. The levels-of-selection partition of the Price equation shows how the contribution of natural selection to changes at the phenotype, species, and community levels can be distinguished and combined additively to explain the total change of a character value over time. The equation provides a foundation for creating a theoretical framework whereby changes in the phenotypic composition of a population (evolutionary processes) can affect the species and community composition of the ecosystem (ecological processes) and *vice versa* through ecological and evolutionary dynamic feedbacks. Specifically, this partition allows us to assess eco-evo feedbacks because it separates the contributions of ecological and evolutionary components to the total change. This allows us to determine how a particular set of evolutionary conditions affects the ecological properties of a community or ecosystem, and vice versa. Such processes may favor one species or community over another (*sensu* Swenson, Wilson & Elias 2000) resulting in changing patterns on the landscape. The total change in a system will be affected most by whichever covariance is greatest.

CONCLUSIONS

In this review, we have synthesized evidence from different sub-fields of research to provide a broad conceptual framework in which to consider how evolution may impact communities and ecosystems. We focused on five main issues: (i) Within local populations, intraspecific genetic variation affects community and ecosystem properties, although these effects can be dependent on abiotic and biotic environmental context; (ii) across a species’ range, genetically differentiated populations have different effects on associated communities, showing that evolution in one species can drive community change; (iii) genetically differentiated populations can also co-evolve interactions with other species, resulting in feedbacks and strong patterns of local adaptation; (iv) the effects of feedbacks can alter the environment in a way which affects future generations; and (v) multi-level selection, which provides a framework for considering how the fitness of an individual depends on the group (i.e., population, community, or ecosystem) of which it is a part.

The evidence linking genetic variation and evolution to community change and feedbacks comes from several different approaches whose results have not been synthesized into one conceptual framework, and whose commonalities may not be fully understood. Together, this synthesis suggests that the evolutionary and ecological dynamics of systems are connected through species interactions, specifically indirect genetic effects and feedbacks, and the consequences of this ecological-evolutionary linkage begin with the phenotype of an individual within a population and extend to the associated community and ecosystem. Potential research questions for future studies in this field include: (i) how does the strength of indirect genetic effects and feedbacks change over time; (ii) if a feedback alters the fitness and performance of one species, how does that alter interactions with other species and the ecosystem functions they mediate; and (iii) what are the consequences of multiple feedbacks (i.e., feedbacks from insect herbivores, neighboring plants, and soil microbial communities) acting on a population simultaneously? Understanding the linkages between evolutionary processes and ecological outcomes is particularly important given the continuing global loss of genetic variation (Butchart *et al.* 2010), as well as the potential for community and ecosystem-level consequences when large selective events change the range of genetic variation present in a species.

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REFERENCES

Agrawal, A.F., Brodie, E.D. & Wade, M.J. (2001). On indirect genetic effects in structured populations. *Am. Nat.*, 158, 308-323.

Antonovics, J. (1992). Toward community genetics. In *Plant Resistance to herbivores and pathogens: ecology, evolution, and genetics*. University of Chicago Press, Chicago, IL, USA.

Bailey, J.K., Wooley, S.C., Lindroth, R.L. & Whitham, T.G. (2006). Importance of species interactions to community heritability: a genetic basis to trophic-level interactions. *Ecol. Lett.*, 9, 78-85.

Bailey, J.K., Schweitzer, J.A., Rehill, B.J., Irschick, D.J., Whitham T.G. & Lindroth, R.L. (2007). Selective herbivory by elk results in rapid shifts in the chemical composition of aspen forests. *Biol. Invas.*, 9, 715-722.

Bailey, J.K., Schweitzer, J.A., Úbeda, F., Koricheva, J., LeRoy, C.J., Madritch, M.D. *et al.* (2009). From genes to ecosystems: a synthesis of the effect of plant genetic factors across levels of organization. *Philos. T. R. Soc. B,* 364, 1607-1616.

Bailey, J.K., Schweitzer, J.A., Úbeda, F., Zinkgraf, M., Fitzpatrick, B.M., O'Reilly-Wapstra, J. *et al.* (2010). From Genes to Ecosystems: Emerging Concepts Bridging Ecological and Evolutionary Dynamics. *Ecological Reviews*. Cambridge University Press, Cambridge, UK.

Bangert, R.K., Lonsdorf, E.V., Wimp, G.M., Shuster, S.M., Fischer, D., Schweitzer, J.A. *et al.* (2008). Genetic structure of a foundation species: scaling community phenotypes from the individual to the region. *Heredity*, 100, 121-131.

Barbour, R.C., O'Reilly-Wapstra, J.M., De Little, D.W., Jordan, G.J., Steane, D.A., Humphreys, J.R. *et al.* (2009a). A geographic mosaic of genetic variation within a foundation tree species and its community-level consequences. *Ecology,* 90, 1762-1772.

Barbour, R.C., Forster, L.G., Baker, S.C., Steane, D.A. & Potts, B.M. (2009b). Biodiversity consequences of genetic variation in bark characteristics within a foundation tree species. *Conserv. Biol.*, 23, 1146-1155.

Bascompte, J. & Jordano, P. (2007). Plant-animal mutualistic networks: The architecture of biodiversity. *Ann. Rev. Ecol. Systemat.*, 38, 567-593.

Bever, J.D. (1994). Feedback between plants and their soil communities in an old-field community. *Ecology*, 75, 1965-1977.

Bohannan, B.J.M. & Lenski, R.E. (2000). Linking genetic change to community evolution: insights from studies of bacteria and bacteriophage. *Ecol. Lett.*, 3, 362-377.

Bolnick, D.I. & Fitzpatrick, B.M. (2007). Sympatric speciation: Models and empirical evidence. *Ann. Rev. Ecol. Evol. S.*, 38, 459-487.

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W.E., Siemann, E. & Prati, D. (2005). Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia*, 144, 1-11.

Bossdorf, O., Shuja, Z. & Banta, J.A. (2009). Genotype and maternal environment affect belowground interactions between Arabidopsis thaliana and its competitors. *Oikos*, 118, 1541-1551.

Butchart, S.H.M., Walpole, M., Collen, B., van Strien, A., Scharlemann, J.P.W., Almond, R.E.A *et al.* (2010). Global Biodiversity: Indicators of Recent Declines. *Science*, 328, 1164-1168.

Clausen, J., Keck, D.D. & Hiesey, W.M. (1940). *Experimental studies on the nature of species. 1. Effects of varied environments on western North American plants.* Carnegie Institute of Washington, Washington DC, USA.

Collins, S. & Gardner, A. (2009). Integrating physiological, ecological and evolutionary change: a Price equation approach. *Ecol. Lett.*, 12, 744-757.

Coyne, J.A., & Orr, H.A. (2004). *Speciation*. Sinauer Associates, Sunderland, MA, USA.

Crutsinger, G.M., Collins, M.D., Fordyce, J.A., Gompert, Z., Nice, C.C. & Sanders, N.J. (2006). Genotypic diversity predicts community structure and governs an ecosystem process. *Science*, 313, 966-968.

Dawkins, R. (1982). *The extended phenotype: the gene as the unit of selection*. Oxford University Press, Oxford, UK.

Dres, M., & Mallet, J. (2002). Host races in plant-feeding insects and their importance in sympatric speciation. *Philos. T. R. Soc. B*, 357, 471-492.

Duffy, M.A. & Forde, S.E. (2009). Ecological feedbacks and the evolution of resistance. *J. Anim. Ecol.*, 78, 1106-1112.

Ellstrand, N.C. & Schierenbeck, K.A. (2000). Hybridization as a stimulus for the evolution of invasiveness in plants? *Proc. Natl. Acad. Sci. USA*, 97, 7043-7050.

Endler, J.A. (1977). *Geographic variation, speciation and clines*. Princeton University Press, Princeton, NJ, USA.

Foster, S.A., McKinnon, G.E., Steane, D.A., Potts, B.M. & Vaillancourt, R.E. (2007). Parallel evolution of dwarf ecotypes in the forest tree Eucalyptus globulus. *New Phytol.*, 175, 370-380.

Fox, J.W. (2006). Using Price equation to partition the effects of biodiversity loss on ecosystem function. *Ecology*, 87, 2687-2696.

Fox, L.R. (1981). Defense and dynamics in plant-herbivore systems. *Am. Zool.*, 21, 853-864.

Frank, S.A. (1998). *Foundations of social evolution.* Princeton University Press, Princeton, NJ, USA.

Gardner, A. (2008). The Price equation. *Current Biol.*, 18, R198-R202.

Gandon, S. & Nuismer, S.L. (2009). Interactions between genetic drift, gene flow, and selection mosaics drive parasite local adaptation. *Am. Nat.*, 173, 212-224.

Grant, P.R. & Grant, B.R. (2002). Unpredictable evolution in a 30-year study of Darwin's finches. *Science*, 296, 707-711.

Hairston, N.G., Ellner, S.P., Geber, M.A., Yoshida, T. & Fox, J.A. (2005). Rapid evolution and the convergence of ecological and evolutionary time. *Ecol. Lett.*, 8, 1114-1127.

Hartl, D.L. & Clark, A.G. (1997). *Principles of population genetics*, 3rd edn. Sinauer Associates, Sunderland, MA, USA.

Hendry, A.P. & Kinnison, M.T. (1999). Perspective: The pace of modern life: measuring rates of contemporary microevolution. *Evolution,* 53, 1637-1653.

Herrera, C.M. (1982). Seasonal variation in the quality of fruits and diffuse coevolution between plants and avian dispersers. *Ecology*, 63, 773-785.

Heschel, M.S. & Paige, K.N. (1995). Inbreeding depression, environmental stress, and population size variation in scarlet gilia (Ipomopsis-Aggregata). *Conserv. Biol.*, 9, 126-133.

Janzen, D.H. (1980). When is it coevolution? *Evolution*, 34, 611-612.

Johnson, M.T.J. & Agrawal, A.A. (2005). Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (Oenothera biennis). *Ecology*, 86, 874-885.

Johnson, M.T.J., Lajeunesse, M.J. & Agrawal, A.A. (2006). Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecol. Lett.*, 9, 24-34.

Johnson, M.T.J. & Stinchcombe J.R. (2007). An emerging synthesis between community ecology and evolutionary biology. *Trends Ecol. Evol.*, 22, 250-257.

Johnson, M.T.J., Vellend, M. & Stinchcombe, J.R. (2009). Evolution in plant populations as a driver of ecological changes in arthropod communities. *Philos. T. R. Soc. B*, 364, 1593-1605.

Johnson, N.C., Wilson, G.W.T., Bowker, M.A., Wilson, J.A. & Miller, R.M. (2010). Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proc. Natl. Acad. Sci. USA*, 107, 2093-2098.

Jones, C.G., Lawton, J.H. & Shachak, M. (1997). Positive and negative effects of organisms as ecosystem engineers. *Ecology*, 78, 1946-1957.

Keller, L. (1999). *Levels of Selection in Evolution.* Princeton University Press, Princeton, NJ, USA.

Kinnison, M.T. & Hendry, A.P. (2001). The pace of modern life II: from rates of contemporary microevolution to pattern and process. *Genetica*, 112, 145-164.

Laine, A.L. (2009). Role of coevolution in generating biological diversity: spatially divergent selection trajectories. *J. Exp. Bot.*, 60, 2957-2970.

Lande, R. & Arnold S.J. (1983). The measurement of selection on correlated characters. *Evolution*, 37, 1210-1226.

Lankau, R.A. & Strauss, S.Y. (2007). Mutual feedbacks maintain both genetic and species diversity in a plant community. *Science*, 317, 1561-1563.

Lenormand, T. (2002). Gene flow and the limits to natural selection. *Trends Ecol. Evol.*, 17, 183-189.

Lewontin, R.C. (1970). The units of selection. *Ann. Rev. Ecol. Systemat.*, 1, 1-18.

Lewontin, R.C. (1974). *The genetic basis to evolutionary change*. Columbia University Press, New York, NY, USA.

Loreau, M. & Hector, A. (2001). Partitioning selection and complementarity in biodiversity experiments. *Nature*, 412, 72-76.

Madritch, M., Donaldson, J.R. & Lindroth, R.L. (2006). Genetic identity of Populus tremuloides litter influences decomposition and nutrient release in a mixed forest stand. *Ecosystems*, 9, 528-537.

Moore, A.J., Brodie, E.D. & Wolf, J.B. (1997). Interacting phenotypes and the evolutionary process. 1. Direct and indirect genetic effects of social interactions. *Evolution*, 51, 1352-1362.

Neira, C., Levin, L.A. & Grosholz, E.D. (2005). Benthic macrofaunal communities of three sites in San Francisco Bay invaded by hybrid Spartina, with comparison to uninvaded habitats. *Mar. Ecol.-Prog. Ser.*, 292, 111-126.

Palkovacs, E.P. & Post, D.M. (2008). Eco-evolutionary interactions between predators and prey: can predator-induced changes to prey communities feed back to shape predator foraging traits? *Evol. Ecol. Res.*, 10, 699-720.

Palkovacs, E.P., Marshall, M.C., Lamphere, B.A., Lynch, B.R., Weese, D.J., Fraser, D.F. *et al.* (2009). Experimental evaluation of evolution and coevolution as agents of ecosystem change in Trinidadian streams. *Philos. T. R. Soc. B*, 364, 1617-1628.

Palo, R.T. (1984). Distribution of birch (Betula spp), willow (Salix spp), and poplar (Populus spp) secondary metabolites and their potential role as chemical defense against herbivores. *J. Chem. Ecol.*, 10, 499–520.

Pennings, S.C., Siska, E.L. & Bertness, M.D. (2001). Latitudinal differences in plant palatability in Atlantic coast salt marshes. *Ecology*, 82, 1344-1359.

Pimentel, D. (1961). Animal population regulation by genetic feedback mechanism. *Am. Nat.*, 95, 65-79.

Pimentel, D. (1968). Population regulation and genetic feedback. *Science*, 159, 1432-1437.

Post, D.M. & Palkovacs, E.P. (2009). Eco-evolutionary feedbacks in community and ecosystem ecology: interactions between the ecological theatre and the evolutionary play. *Philos. T. R. Soc. B*, 364, 1629-1640.

Pregitzer, C.C., Bailey, J.K., Hart, S.C. & Schweitzer, J.A. (2010). Soils as agents of selection: feedbacks between plants and soils alter seedling survival and performance. *Evol. Ecol.*, 24, 1045-1059.

Price, G.R. (1972). Fisher's "fundamental theorem" made clear. *Ann. Hum. Genet.*, 36, 129-140.

Price, P.W. (1991). The plant vigor hypothesis and herbivore attack. *Oikos*, 62, 244-251.

Rehill, B.J., Whitham, T.G., Martinsen, G.D., Schweitzer, J.A., Bailey, J.K. & Lindroth, R.L. (2006). Developmental trajectories in cottonwood phytochemisty. *J. Chem. Ecol.*, 32, 2269-2285.

Reznick, D. & Endler, J.A. (1982). The impact of predation on life-history evolution in Trinidadian guppies (Poecilia reticulata). *Evolution*, 36, 160-177.

Schweitzer, J.A., Bailey, J.K., Rehill, B.J., Martinsen, G.D., Hart, S.C., Lindroth, R.L. *et al.* (2004). Genetically based trait in a dominant tree affects ecosystem processes. *Ecol. Lett.*, 7, 127-134.

Schweitzer, J.A., Bailey, J.K., Fischer, D.G., Leroy, C.J., Lonsdorf, E.V., Whitham, T.G. *et al.* (2008). Plant-soil-microorganism interactions: Heritable relationship between plant genotype and associated soil microorganisms. *Ecology*, 89, 773-781.

Shuster, S.M., Lonsdorf, E.V., Wimp, G.M., Bailey, J.K. & Whitham, T.G. (2006). Community heritability measures the evolutionary consequences of indirect genetic effects on community structure. *Evolution*, 60, 991-1003.

Strauss, S.Y., Sahli, H., & Connor, J.K. (2005). Toward a more trait-centered approach to diffuse (co)evolution. *New Phytol.*, 165, 81-89.

Storfer, A. (1999). Gene flow and local adaptation in a sunfish-salamander system. *Behav. Ecol. Sociobiol.*, 46, 273-279.

Swenson, W., Wilson, D.S. & Elias, R. (2000). Artificial ecosystem selection. *Proc. Natl. Acad. Sci. USA*, 97, 9110–9114.

Tack, A.J.M., Ovaskainen, O., Pulkkinen, P. & Roslin, T. (2010). Spatial location dominates over host plant genotype in structuring an herbivore community. *Ecology*, 91, 2660-2672.

Tack, A.J.M. & Roslin, T. (2010). Overrun by the neighbors: Landscape context affects strength and sign of local adaptation. *Ecology*, 91, 2253-2260.

Tetard-Jones, C., Kertesz, M.A., Gallois, P. & Preziosi, R.F. (2007). Genotype-by-genotype interactions modified by a third species in a plant-insect system. *Am. Nat.*, 170, 492-499.

Thompson, J.N. (1998). Rapid evolution as an ecological process. *Trends Ecol. Evol.*, 13, 329-332.

Thompson, J.N. (1999). Specific hypotheses on the geographic mosaic of coevolution. *American Naturalist*, 153, S1-S14.

Thompson, J.N. (2005). Coevolution: the geographic mosaic of coevolutionary arms races. *Current Biol.*, 15, R992-R994.

Van der Putten, W.H., Vandijk, C. & Peters, B.A.M. (1993). Plant-specific soil-borne diseases contribute to succession in foredune vegetation. *Nature*, 362, 53-56.

Van Zandt, P.A. & Mopper, S. (1998). A meta analysis of adaptive deme formation in phytophagous insect populations. *Am. Nat.*, 152, 595-604.

Wade M.J. (2007). The co-evolutionary genetics of ecological communities. *Nat. Rev. Genet.*, 8, 185-195.

Whitham, T.G., Young, W.P., Martinsen, G.D., Gehring, C.A., Schweitzer, J.A., Shuster, S.M. *et al.* (2003). Community and ecosystem genetics: A consequence of the extended phenotype. *Ecology*, 84, 559-573.

Whitham, T.G., Bailey, J.K., Schweitzer, J.A., Shuster, S.M., Bangert, R.K., LeRoy, C.J. *et al.* (2006). A framework for community and ecosystem genetics: From genes to ecosystems. *Nat. Rev. Genet.,* 7, 510-523.

Wright, S. (1931). Evolution in Mendelian populations. *Genetics*, 16, 97-159.

Yoshida, T., Jones, L.E., Ellner, S.P., Fussmann, G.F. & Hairston, N.G. (2003). Rapid evolution drives ecological dynamics in a predator-prey system. *Nature*, 424, 303-306.

CONCLUSIONS.

In a broad sense, my dissertation has followed the tradition of previous studies in community and ecosystem genetics (Whitham *et al.* 2003, 2006; Bailey *et al.* 2006; Crutsinger *et al.* 2006, 2008; Johnson *et al.* 2006; Mooney & Agrawal 2008; Genung *et al.* 2010, 2012; others) by examining the effects of genes beyond the population level (i.e., communities and ecosystems). More specifically, my dissertation has examined how direct and indirect genetic effects (i.e., effects on a focal plant's phenotype due to the expression of genes in a neighboring plant; modified from Wolf *et al.* 1998) drive the outcomes of focal plant-neighbor plant interactions. To address this question, I used two common garden experiments in which we manipulated the genotypic identity of both focal and neighbor plants, a decomposition experiment, and a literature review. From my field work, I found indirect genetic effects could affect belowground plant biomass, which altered plant litter quality and therefore influenced rates of decomposition and nutrient cycling. This result shows a novel link between evolutionary biology and ecosystem ecology which could change the way ecologists think about ecosystem processes; that is, instead of being considered a product of environmental conditions and species identity, ecosystem processes can also be thought of as the gene-less products of genetic interactions.

In Chapter 1, I established a common garden near ORNL and showed that IGEs can be equivalent to the direct effects of genes as influences on belowground plant biomass. However, the direct effects of genes still explained more variation in aboveground biomass, floral biomass, and pollinator visitation. Additionally, we detected a G x G interaction for pollinator visitation, suggesting that neighboring plants can influence the sexual reproductive success of a focal plant. In Chapter 2, I collected leaf litter plants which had grown together in the ORNL common garden and conducted a decomposition experiment to see how genotype effects, IGEs, and G x G interactions affected ecosystem processes. Combined, the results from the first common garden and decomposition experiment showed that IGEs changed belowground plant traits, and these changes also affected litter quality at the time of plant senescence. These shifts in litter quality extended to affect ecosystem processes, specifically decomposition rate and nitrogen (N) immobilization. This result shows that IGEs can initiate "afterlife effects", linking aboveground-belowground interactions with evolutionary processes.

Because IGEs strongly affected belowground plant traits in the ORNL common garden, I established a second common garden which manipulated the presence of belowground interactions between neighboring plants. The goal of this experiment was to determine whether IGEs occur due to belowground interactions. In Chapter 3, I found that, in pots which permitted belowground interactions, IGEs explained over 20 times more variation in belowground biomass than did the direct effects of focal plant genotype. This result shows that considering belowground processes is crucial to understanding how direct and indirect genetic effects, and therefore evolutionary processes, occur in natural systems.

Beyond the field work described above, I wanted to end the dissertation by reviewing relevant current literature and searching for commonalities among the different subfields of ecology that have interest in how genetic variation affects communities and ecosystems. In Chapter 4, I conducted a literature review which found that many subfields of ecology and evolutionary biology have examined how natural selection drives community changes by operating on genetic variation in a focal species. These fields are united by the observation that evolution takes place rapidly, at a pace which is relevant to ecological processes. Along with my collaborators, I presented a heuristic model for understanding extended phenotypes, IGEs, and eco-evo feedbacks in a well-known population genetics framework (Lewontin 1974).

The driving idea behind my dissertation research has been: In natural systems, the ecological impacts of intraspecific genetic variation are more common and farther reaching than currently recognized. I have shown that G x G interactions can drive pollinator visitation, with implications for plant sexual reproductive success and fitness, showing that G x G interactions between plant can extend to affect associated communities (Genung *et al.* 2012). I have also shown that IGEs can have large effects on plant productivity, especially belowground, and that these effects can persist after plant senescence to affect ecosystem processes such as decomposition and N dynamics. Future work should examine whether these effects are consistent outside of *Solidago*, and whether the importance of IGEs is maintained in natural settings (i.e., not common gardens) when many other ecological factors may influence plants and their associated communities.

REFERENCES

Crutsinger, G.M., Collins, M.D., Fordyce, J.A., Gompert, Z., Nice, C.C. & Sanders, N.J. (2006). Genotypic diversity predicts community structure and governs an ecosystem process. *Science*, 313, 966-968.

Crutsinger, G.M., Collins, M.D., Fordyce, J.A. & Sanders, N.J. (2008). Temporal dynamics in non-additive responses of arthropods to host-plant genotypic diversity. *Oikos*, 117, 255-264.

Genung, M.A., Lessard, J.P., Brown, C.B., Bunn, W.A., Cregger, M.A., Reynolds, W.N. *et al.* (2010). Non-additive effects of genotypic diversity increase floral abundance and abundance of floral visitors. *PLoS ONE*, 5, e8711.

Genung, M.A., Bailey, J.K. & Schweitzer, J.A. (2012). Welcome to the neighbourhood: interspecific genotype by genotype interactions in *Solidago* influence above- and belowground biomass and associated communities. *Ecol. Lett.*, 15, 65-73.

Johnson, M.T.J., Lajeunesse, M.J. & Agrawal, A.A. (2006). Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecol. Lett.*, 9, 24-34.

Lewontin, R.C. (1974). *The genetic basis to evolutionary change*. Columbia University Press, New York, NY, USA.

Mooney, K.A. & Agrawal, A.A. (2008). Plant genotype shapes ant-aphid interactions: Implications for community structure and indirect plant defense. *Am. Nat.*, 171, E195-E205.

Whitham, T.G., Young, W.P., Martinsen, G.D., Gehring, C.A., Schweitzer, J.A., Shuster, S.M. *et al.* (2003). Community and ecosystem genetics: A consequence of the extended phenotype. *Ecology*, 84, 559-573.

Whitham, T.G., Bailey, J.K., Schweitzer, J.A., Shuster, S.M., Bangert, R.K., LeRoy, C.J. *et al.* (2006). A framework for community and ecosystem genetics: From genes to ecosystems. *Nat. Rev. Genet.,* 7, 510-523.

Wolf, J.B., Brodie, E.D., Cheverud, J.M., Moore, A.J. & Wade, M.J. (1998). Evolutionary consequences of indirect genetic effects. *Trends Ecol. Evol.*, 13, 64-69.

APPENDICES.

Appendix 1. Allometric equation used to determine aboveground biomass estimates

We sampled natural populations of *S. altissima* and *S. gigantea* from natural populations at ORNL, recorded the height of the stems and major braches (>10 cm), and oven dried each individual (24 hr at 70 °C). We weighed each sample (n=15 per species) and determined an allometric equation which estimates biomass (Weight (g) = (-0.071 + 0.0346 * height (cm) ^ 2). We found no significant differences between the allometric equations for *S. altissima* and *S. gigantea*; therefore, we pooled the data and used only one equation. This equation explained 83 % of the variation in biomass (dry weight) and both the linear ($p < 0.001$) and quadratic terms ($p = 0.007$) were statistically significant.

Appendix 2. Taxonomic groups for pollinator identification

Pollinators were grouped into taxa based on differences the surveyor (MAG) could consistently identify, from a range that did not influence pollinators (3m from the pot). *Atteva aurea* and *Chaliognathus pennsylvanicus* were very common, and distinct enough to be identified to the species level. *Apis* spp. and *Bombus* spp. visitors were identified at the genus level. We also identified the following Lepidoptera and Hymenoptera families Halictidae, Hesperiidae, Papilionoidea, Spechidae, Syrphidae, Tiphiidae and superfamily Vespoidea that commonly visited flowers in the treatment pots. Especially common pollinators and the taxa in which they were grouped included *Apis mellifera* (*Apis*), *Bombus impatiens* (*Bombus*), *Agapostemon* sp. (Halictidae), *Junonia coenia* (Papilionoidea), *Toxomerus* sp. (Syrphidae), *Scolia dubia* (Vespoidea), *Polistes metricus* (Vespoidea), and *Polistes fuscatus* (Vespoidea). The most common pollinator taxa on *S. gigantea* were halictid bees, especially *Agapostemon* species, and the most common pollinator taxa on *S. altissima* were *Apis* species and *Bombus* species. Following the visual survey, flowering panicles were shaken onto a sheet of white paper to assess insect pollinators which were present but had not moved between plants during the survey time; by far the most common pollinator recorded in this way was *Chauliognathus pennsylvanicus*.

Appendix 3. Species Diversity Effects

As expected due to low diversity (i.e., only two genotypes per pot), we did not detect an effect of species diversity (monocultures vs. interspecific genotype mixtures) on most traits (but see per-flower visitation) (Coarse root biomass: $p = 0.953$; Rhizome biomass: $p = 0.402$; Aboveground vegetative biomass: $p = 0.609$; Floral biomass: $p = 0.536$; Pollinator visitation: $p = 0.189$; Per-flower visitation: $p = 0.003$).

VITA

Mark Genung was born in Knoxville, TN to Donna and Richard Genung. He has one younger sister, Linda Genung. He attended elementary and high school in Farragut, TN. After graduating, he enrolled in the University of Tennessee where he developed an interest in ecology while working in Dr. Nathan Sander's community ecology lab. He obtained a Bachelor's of Science from the University of Tennessee in May 2007. He accepted a graduate teaching assistantship at the University of Tennessee in the Department of Ecology and Evolutionary Biology, where he worked in the community and ecosystem genetics laboratory with Dr. Jennifer Schweitzer and Dr. Joseph Bailey. He graduated with a Doctor of Philosophy degree in Ecology and Evolutionary Biology in May 2012.