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INVESTIGATING THE ROLE OF GENOMIC MATERIAL COSTS IN ECOLOGICAL, EVOLUTIONARY, AND INVASION DYNAMICS USING THE SOLIDAGO GIGANTEA (GIANT GOLDENROD) POLYPLOID COMPLEX

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By

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A DISSERTATION

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DEDICATION

TO

MARY ("MAE") RANELLA

Dear Nonna,

The last twelve years have been hard without you. I frequently find myself wishing that you were still here to see the woman I grew up to be. Your passion for learning has been inspirational to me, and I often wonder what other amazing things you could have accomplished had you been encouraged, rather than discouraged, from pursuing your education. I want you to know that your passion was inherited. Your daughter has, and your granddaughter will soon have, terminal degrees. I speak for both myself and my mother when I say that we wish you could have watched us earn the title of "Doctor", but rest assured that we both felt your presence with us every step of the way. I love you and miss you beyond words.

Your granddaughter,
Angela

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Author Contribution Statement

Chapter 1: Data collection was done by Angela Walczyk. Both Angela Walczyk and Erika Hersch-Green designed the experiments, analyzed the data, interpreted the data, and wrote and revised the manuscript. This chapter is currently in press in Plant Biology.

Chapter 2: Both Angela Walczyk and Erika Hersch-Green contributed to the conception and design of the experiment; collected, analyzed, and interpreted data; and wrote and revised the manuscript. This chapter is in preparation for submission to New Phytologist.

Chapter 3: Both Angela Walczyk and Erika Hersch-Green contributed to the conception and design of the experiment; collected, analyzed, and interpreted data; and wrote and revised the manuscript. This chapter is in preparation for submission to Oikos.

Chapter 4: Erika Hersch-Green conceived the experiments, and Angela Walczyk, Erika Hersch-Green, and Carsten Külheim analyzed and interpreted the data and wrote and revised the manuscript. Part of Chapter 4 will be incorporated into a future submission(s) to one or two undecided journals.

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Abstract

Polyploidy (whole genome duplication) is large-scale mutation that increases genome size and alters the genomic arrangements and expression patterns, thus influencing morphological and physiological traits. Given that polyploids and progenitor diploids tend to be geographically segregated from each other, and that polyploidy is prevalent in invasive plant taxa, polyploidy might alter responsiveness to the abiotic and/or biotic environment in ways that pre-adapt polyploids to better tolerate variable ecological conditions and/or allow for rapid adaptation in novel environments. Differential responses to soil nitrogen (N) and phosphorus (P) availability are of particular interest because 1) nucleic acids (i.e., DNA and RNA) require large amounts of N and P for their synthesis and structure, potentially disadvantaging polyploids in nutrient-poor environments; and 2) biological invasions usually begin in urbanized areas with locally significant increases in biologically available N and P. The overall goal of this dissertation was to evaluate how the genomic attributes of genome size and polyploidy independently and concurrently influence tolerance to nutrient availability as a means of better understanding the ecological and evolutionary role of genome size and the preponderance of polyploidy in invasive plants. Throughout a series of greenhouse, potted-field, and RNA sequencing studies using diploids, native-tetraploids, invasive-tetraploids, and hexaploids within the autopolyploidy *Solidago gigantea* system we found that: 1) differences in morphology and physiology between tetraploids and diploids might equate to tetraploids being better competitors, regardless of the abiotic environment; 2) polyploids have greater material costs related to genome size than diploids; 3) polyploids exhibit strategies that reduce material costs; 4) polyploids tended to show more

phenotypic plasticity for growth traits than diploids in the most NP enhanced environments, but plasticity did not differ much between native- and invasive-tetraploid populations; and 5) tetraploids down-regulate more genes associated with costly traits (i.e., photosynthesis, defense) relative to diploids in low NP conditions, and differences in gene expression between native- and invasive tetraploids populations was marginal. Together, these studies show that the material costs associated with genome size might limit the ecological success of polyploids in nutrient poor conditions, but mechanisms selectively favored to reduce these costs could lessen the selective pressures favoring small genomes. These studies also highlight the importance that the soil nutrient environment could play in the invasive success of *S. gigantea* and other polyploid invaders, as anthropogenic-caused nutrient enrichment may create environments that release polyploid invaders from nutrient constraints and allow for enhanced investment into fitness and competitive traits.

Introduction

Whole genome duplication (aka polyploidy) is considered to be a major force in plant evolution through the introduction of additional intra- and interspecific variation within plant taxa and its implied role in plant speciation (Van de Peer et al. 2017; Rejlová et al. 2019). Polyploidy results in the inheritance of whole chromosome sets that increases the amount of DNA and chromatin per cell (Robinson et al. 2018; Doyle and Coate 2019) and can subsequently lead to changes in DNA codes and/or the sub- or neofunctionalization of genes (i.e., repetitive gene sequences can result in relaxed functional selection and/or gene sequences can accumulate mutations and evolve new or varied functions; Comai 2005; Chen 2007; Doyle and Coate 2019). These genomic additions can have immediate effects on cell morphology, functioning, and biochemical pathways through alterations in cytology (i.e., cell size, cell number, mitosis rates; Corneillie et al. 2019; Doyle and Coate 2019) and genetic architecture (i.e. changes in gene ordering, gene dosages, gene function, expression patterns, Comai 2005; Liqin et al. 2019; Song and Chen 2015) that can have a downstream effect on key morphological, physiological, and ecological attributes that could lead to evolutionary novelty (Husband and Baldwin 2016; Soltis and Soltis 2016). However, these changes do not ubiquitously result in increased fitness, as polyploidy can have maladaptive consequences on fitness through the disruption of key biochemical pathways and gene expression patterns that manage important biological functions (i.e., gamete formation, mitosis, chromosome pairing; Comai 2005; Cifuentes et al. 2010; Song and Chen 2015). Further support is demonstrated by biological challenges that newly formed polyploids (neopolyploids) face, such as strong reproductive isolation barriers (i.e., triploid block; Marks 1966;

Ramsey and Schemske 1998) and frequency dependent processes (i.e., minority cytotype exclusion; Levin 1975; Fowler and Levin 1984) that would theoretically prevent the establishment of viable polyploid populations. Despite being occasionally considered an evolutionary 'dead-end' (Stebbins 1950; Mayrose et al. 2011; Mayrose et al. 2015), polyploidy *can* be advantageous, but we still do not fully understand which environmental and ecological circumstances positively or negatively influence polyploid success.

Soil nitrogen (N) and phosphorus (P) availability varies across macro- and micro-scales (Ettema and Wardle 2002), which results in competitive and selective pressures that act upon a plant's ability to tolerate nutrient limitations and/or variability (Craine and Dybzinski 2013). In addition to being used in various plant processes, such as growth, photosynthesis, and reproduction (Evans, 1989; Hessen et al., 2010; Hohmann-Marriott & Blankenship, 2011), N and P are also essential in nucleic acid synthesis (i.e., DNA, RNA; Elser et al. 2011). Given that polyploids have larger genomes than diploids, it has been proposed that diploids and polyploids have substantially different N and P requirements and that organisms with larger genomes should be more negatively affected by nutrient limitations (Lewis, 1985; Leitch & Bennett, 2004; Cavalier-Smith, 2005; Faizullah et al., 2021). Hence, the nutrient environment could either help or hinder polyploid establishment and success.

The objective of this dissertation was to investigate how genome size and polyploidy influence plant responses to N and P availability within the contexts of plant ecology and invasive dynamics. In Chapter 1, I tested whether genome size variation

between diploid and tetraploid *Solidago gigantea* plants affected plant morphological and physiological traits based on water and/or nutrient availability. I found that both cytotypes responded similarly to water and nutrient availability, but tetraploids had greater biomasses and photosynthetic rates than diploids regardless of nutrient and water environment. These results suggest that whole genome duplication in *S. gigantea* results in morphological and physiological changes that could give tetraploids a competitive edge over diploids and these differences might have played a role in the invasive success of tetraploids.

In Chapter 2, I tested for direct evidence that material costs increase with genome size by growing diploid, tetraploid, and hexaploid *S. gigantea* in low and high NP conditions. I found that relative to diploids, both polyploids had greater N and P cellular investments and only tetraploids greater growth responses to N-enrichment. Polyploids also exhibited strategies to reduce their genome size-dependent material costs by reducing transcriptome investment relative to their genome size and/or by enhancing N-use efficiencies. Together, these findings provide evidence of increased material costs in plants with larger genome sizes and implies that selection has favored strategies to mitigate the constraining effects of these costs in polyploids.

In Chapter 3, I investigated whether phenotypic plasticity (PP) in *S. gigantea* is positively correlated with increasing ploidy level and if differences in PP between native- and invasive-tetraploid populations result from adaption pre- or post-introduction. In general, I found that PP was complex and varied depending upon the combinations of trait, ploidy level, and change in nutrient environment. Interestingly, higher ploidy levels

exhibited greater PP for physiological traits regardless of nutrient changes and showed greater PP than diploids for growth traits only under the highest nutrient conditions. Native- and invasive tetraploids did not vary much in their plasticity responses, but invasive-tetraploids had higher mean values for all physiological traits than native-tetraploids. These findings highlight that the nutrient environment of invasive habitats might play a role in the success and trajectory of polyploidy invasions, given that polyploids had the most plastic responses when NP was highly enriched. This is especially important as invasions might begin in urbanized habitats exposed to anthropogenic nutrient enrichment.

Finally, in Chapter 4 I examined gene expression patterns between diploids and native-tetraploids, and native- and invasive tetraploids grown under low and high NP availability to determine (1) if tetraploids down-regulate more genes associated with costly traits than diploids when NP is limited, and (2) if selection post-introduction favored altered gene expression patterns for growth and defensive traits in invasive- versus native-tetraploid populations. I found that native-tetraploids tended to down-regulate gene groups associated with photosynthesis and terpene production relative to diploids in low NP conditions. This could imply that tetraploids down-regulate these costly traits as a means of conserving N and P to allocate towards genomic material costs. I also found that both native- and invasive-tetraploids downregulated gene groups associated with defense and responses to biotic stimuli in high versus low NP conditions, and that native-tetraploids tended to express more genes related to N-metabolic processes relative to invasive-tetraploids in low NP conditions. The overall similarity in responsiveness between native- and invasive tetraploids implies that either not enough

evolutionary time has passed for selection to favor alternate expression patterns for growth versus defense in invasive populations and/or the selective environments are similar in native and invasive habitats.

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1 **Chapter 1:** Do water and soil nutrient scarcities differentially impact the performance of diploid and tetraploid *Solidago gigantea* (Giant Goldenrod, Asteraceae)? [1]

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Key Message: Diploid and autotetraploid *Solidago gigantea* responded similarly to water and nutrient limitations, although the larger size and greater photosynthetic capacity of tetraploids might render them more competitive than diploids.

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1.1 Abstract

Rationale: Plants require water and nutrients for survival, although the effects of their availabilities on plant fitness differs amongst species. Genome size variation, within and across species, is suspected to influence plant water and nutrient requirements, but little is known about how variations in these resources concurrently affect plant fitness based on genome size. We examined how genome size variation between autopolyploid cytotypes influences plant morphological and physiological traits, and whether cytotype-specific trait responses differ based on water and/or nutrient availability.

Methods: Diploid and autotetraploid *Solidago gigantea* (Giant Goldenrod) were grown in a greenhouse under four soil water:nitrogen+phosphorus treatments (L:L, L:H, H:L, H:H), and stomata characteristics (size, density), growth (above- and belowground biomass, root/shoot ratio), and physiological (net photosynthetic capacity, transpiration rates, water use efficiency) responses were measured.

Key Results: Resource availabilities and cytotype identity influenced some plant responses but their effects were independent of each other. Plants grown in high-water and nutrient treatments were larger, plants grown in low-water or high-nutrient treatments had higher water use efficiency but lower transpiration rates, and photosynthesis and transpiration rates decreased as plants aged. Autotetraploids also had larger and fewer stomata, greater biomass, and greater photosynthetic capacity than diploids.

Conclusions: Nutrient and water availability could influence intra- and interspecific competitive outcomes. Although *S. gigantea* cytotypes were not differentially affected by resource treatments, genome size may influence cytogeographic range patterning and

population establishment likelihood. For instance, the greater size of autotetraploid *S. gigantea* might render them more competitive for resources and niche space than diploids.

1.2 Introduction

It is becoming increasingly apparent that genome size (nuclear genome DNA content) variation in angiosperms can affect plant developmental, morphological, and physiological traits and ecological interactions (Guignard et al., 2017; Roddy et al., 2020; Faizullah et al., 2021), and plant genome size variation is thought to be an important driver of angiosperm evolutionary trajectories (Doyle and Coate, 2018; Roddy et al., 2020). Genome size variation in angiosperms generally arises from either the accumulation of repeat elements (Wang et al., 2021) or more commonly from polyploidy (McCarthy et al., 2015), the possession of more than two sets of homologous chromosomes within cells following whole genome duplication events (Otto and Whitton, 2000). Polyploidy can arise from either whole genome duplication within a single species' genome, termed autopolyploidy, or by whole genome duplication events coupled with the hybridization of distinct species' genomes, termed allopolyploidy (Otto and Whitton, 2000). While genic (e.g. gene duplications, deletions) and cytological (e.g. altered cell size and density) changes can arise from both forms of polyploidy, most research on the effect of genome duplication on traits and evolutionary dynamics has focused on allopolyploids (Vallejo-Marin et al., 2015; Meeus et al., 2020; Welles and Ellstrand, 2020; Yin et al., 2020). However, a focus on allopolyploids limits our ability to assess how variation in genome size alone, independently of hybridization (and the

additional genetic elements from a separate species), influences plant traits associated with fitness and ecological and evolutionary dynamics. Similarly, a focus on diverse phylogenetic assemblages of plants that vary in genome size also limits our ability to assess how genome size variation influences plant traits associated with fitness and ecological and evolutionary dynamics because the plants represent a diversity of genetic content. Because autopolyploid cytotypes (e.g., diploid and derived autotetraploids) do not vary widely in genic content but do vary in genome sizes, examinations of autopolyploid species systems can add important insights into our understanding of the effect that genome size variation has on traits associated with plant fitness, such as photosynthetic and growth responses.

Photosynthesis is fundamental to plant functioning, growth, and fitness, but a plant's ability to reach and maintain maximum photosynthetic capacity (A_{net}; the net rate in which carbon is fixed) is highly dependent upon their local abiotic environmental conditions (Suzuki et al., 2014; Sharma et al., 2020). For instance, photosynthetic capacity and plant productivity are often higher in tropic and sub-tropic latitudes (Li et al., 2018; Smith et al., 2019), most likely due, in part, to greater water availability in these equatorial environments (Kottek, 2006). Water plays a critical role in photosynthesis because the cleavage of water molecules provides hydrogen ions and electrons that power the reduction of $NADP^+$ to NADPH in Photosystem 1 and helps drive photosynthesis (Rutherford and Boussac, 2004). Research has shown that increasing water availability often translates into increased plant growth and photosynthetic rates (Flexas et al., 2009; Guan et al., 2015; Reich et al., 2018), although too much water can lead to anerobic conditions and reduce photosynthetic capacities

(Pezeshki, 2001). Photosynthetic capacity and primary production have also been found to be positively correlated with nutrient availability, especially that of nitrogen (N: Boussadia et al., 2010; Domingues et al., 2010) and phosphorus (P: Carstensen et al., 2018; Shi et al., 2019), in both terrestrial (Li et al., 2016; Smith et al., 2019) and aquatic environments (Smith and Schindler, 2009). These photosynthetic responses to nutrient enhancement are most likely due to the fact that N and P are major compositional elements in key molecules found within photosynthetic pathways (Elser et al., 2007), such as chlorophyll molecules that absorb light energy, Rubisco that has a variety of roles including catalyzation of various steps in photosynthesis (Evans, 1989; Makino et al., 1992; Evans and Clarke, 2019), and ATP and $NADP^+$ (White and Hammond, 2008) that act as the main energy transfer molecules. Although there is ample evidence that water, N, and P availability can individually and/or jointly affect photosynthesis and growth rates, such impacts do vary across plant species, with some species showing significant responses (Zhu et al., 2014; Maréchaux et al., 2015) and others showing minimal responses (Bänziger et al., 2002; Drenovsky and Richards, 2006). Such an observation begs the question: "does polyploidy influence if and how a plant will respond to changes in water and nutrient availabilities?"

Across a wide range of plant species genome size has been found to be positively correlated with cell size (Doyle and Coate, 2018; Corneillie et al., 2019) and cell size may influence plant water requirements (Cutler et al., 1977; Pereyra-Irujo et al., 2008). In general, larger cell sizes are associated with larger but fewer numbers of stomata per leaf area (Beaulieu et al., 2008; Knight and Beaulieu, 2008) and stomata that have slower closure/response times (Kardiman and Ræbild, 2017; Roddy et al., 2020). These

morphological and physiological changes can affect rates of stomatal conductance (the flux of CO_2 entering, or H_2O vapor exiting via the stomata) and transpiration (*E*; rate of water loss via stomata openings due to evaporation), all of which may translate into plants with larger genomes having different water limitations and photosynthesis rates than plants with smaller genomes. For example, bigger guard cells might lose more water through transpiration and conductance due to longer stomatal closure times (Kardiman and Ræbild, 2017; Roddy et al., 2020) and/or higher rates of gas exchange related to larger pore openings (Meckel et al., 2007; Fanourakis et al., 2015). Conversely, plants with fewer numbers of stomata per unit area (e.g. those with larger cells, Beaulieu et al., 2008; Knight and Beaulieu, 2008), might have fewer overall pore openings, resulting in lower rates of stomatal conductance and transpiration at the whole plant level (Drake et al., 2013; Bertolino et al., 2019). Furthermore, larger xylem conduit cells might increase a plant's total hydraulic conductivity (i.e. the amount of water roots and stems can transport and supply to the leaves; Maherali et al., 2009; Zhang et al., 2017). Depending upon whether pit membrane pores scale with conduit size, a plant could be more (i.e. large conduits and large pit pores) or less (i.e. large conduits and small pit pores) susceptible to cavitation from water deficits (Zhang et al., 2017).

In addition to potentially altering plant tolerances to water limitation, polyploidy or genome size might also influence plant growth and photosynthesis responses to N and P availability. N and P are integral components of nucleic acids (DNA, RNA) and the proteins that make up cell membranes (Sterner and Elser, 2002). It has been theorized that organisms with larger genomes and bigger cells should be more constrained by N and P environmental limitations due to needing more N and P for nucleic acid and cell

synthesis (Lewis Jr, 1985; Leitch and Bennett, 2004; Cavalier-Smith, 2005; Hessen et al., 2010). Given the roles that N and P play in genome and cell building and in photosynthesis and growth, it may be predicted that when nutrients are in limited supply that tradeoffs in N and P investments into the genome over photosynthetic/growth activity will be more pronounced for plants with large genomes. In support of this hypothesis, studies have shown that polyploids, and plants with larger genomes, have greater biomass and fitness gains relative to diploids and plants with smaller genomes with increasing soil nutrient availabilities (Šmarda et al., 2013; Guignard et al., 2016; Bales and Hersch-Green, 2019; Walczyk and Hersch-Green, 2019; Anneberg and Segraves, 2020). These studies are at the forefront of our understanding of the role that cytotype or genome size plays in nutrient mediated responses, but they are not without limitations as they only represent a few plant species and typically do not include other abiotic and/or biotic factors that could influence patterns of nutrient mediated responses.

Several studies with autopolyploid systems have shown that variation within a single abiotic environmental factor can affect plant fitness through changes in photosynthetic and/or growth traits (Kalendar et al., 2000; Maherali et al., 2009; Chao et al., 2013; Bales and Hersch-Green, 2019; Walczyk and Hersch-Green, 2019; Anneberg and Segraves, 2020), but we do not have much information about how variation across multiple abiotic factors concurrently affects plant fitness traits in autopolyploids. Because genome size is known to influence plant morphological and physiological traits related to multiple abiotic tolerances (e.g. water and nutrients; Faizullah et al., 2021), studies focusing on more than one environmental variable are instrumental in our understanding of species boundaries and patterns of cytogeographic range segregation. In this study, we

grew diploid and autotetraploid *Solidago gigantea* (Giant Goldenrod) in a greenhouse under different water and N and P availabilities to test four specific hypotheses. First, we hypothesized that stomatal characteristics (size and density) would be independent of water and nutrient availabilities (Casson and Gray, 2008), but that in general tetraploids would have larger stomata and accordingly fewer stomata per unit leaf area than diploids (Beaulieu et al., 2008). Second, we predicted that growth, photosynthetic activity, and instantaneous water use efficiency (*WUE*) would all increase with increasing water and N+P availability. Third, we hypothesized that the growth and photosynthetic rates of tetraploids would be more impaired by both water and N+P limitations than diploids. We predicted this because data shows that tetraploids tend to have fewer, larger stomata resulting in a reduction of gas exchange rates (Drake et al., 2013; Bertolino et al., 2019), and N and P are important in cell and nucleic acid synthesis. Lastly, because *WUE* is measured as photosynthetic capacity relative to transpiration, we predicted that increases in water would not change *WUE* as both are expected to rise with increasing water availability, but that *WUE* would increase with increasing N+P availability because photosynthesis, but not transpiration, was expected to positively respond to increases in N+P and that this response would be greater in tetraploids.

1.3 Methods

Plant Material — *Solidago gigantea* Aiton (Asteraceae; Giant Goldenrod) is a clonal, perennial aster native to North America, but an aggressive invader in parts of Europe and Asia (Schlaepfer et al., 2008). Within its home range, three ploidy races (cytotypes) exist that are typically spatially segregated (Schlaepfer et al., 2008; Hull-Sanders et al., 2009): diploid (2n = 2x = 18) populations are found along the Atlantic coast, tetraploid

populations (2n = 4x = 36) are predominantly located within the Great Lakes region, and hexaploid populations (2n = 6x = 54) are found in the Great Plains (Schlaepfer et al., 2008; Hull-Sanders et al., 2009, Appendix 1). Polyploids of *S. gigantea* are suspected to be of autopolyploid origin based on a lack of morphological separation between the cytotypes and a lack of distinct clustering among the cytotypes from microsatellite pilot studies (Beck and Semple, personal correspondence and unpublished data).

We collected seeds from a total of 6 wild populations within known diploid and tetraploid ranges in 2017 to be used in the experiments. Due to logistical constraints, we were unable to collect seeds from the known hexaploid ranges of *S. gigantea* to use in this study. Using dried leaves collected from individuals at each sample site (a total of 367 leaves), we verified ploidy level and estimated the nuclear 2C DNA prior to the start of the experiment using a modified protocol of (Baldwin and Husband, 2013; for detailed methods see Appendix 1). Using the Acuri C6 software (BD Biosciences, Franklin Lakes, New Jersey, USA) we removed 241 low quality samples that had a histogram peak coefficient variation (CV) of more than 5% (Dolezel et al., 2007). The average 2c DNA content of diploids was (LS mean ± standard error) 1.96 ± 0.02 pg and tetraploids was 3.77 ± 0.01 pg. 2c DNA content ranged from 1.85 pg to 2.04 pg and 3.56 pg to 4.03 pg in diploids and tetraploids, respectively.

Experimental Design — We germinated 264 seeds from 5 separate diploid (N = 132) and tetraploid (N = 132) maternal lines originating from each of the 6 wild populations. To induce germination, field-collected seeds were washed with a 2.63% bleach solution and rinsed with deionized water to kill fungal spores, cold-stratified in petri dishes lined

with damp filter for 6 weeks at 4°C, and then placed under a light bank for 12 days. Once seedlings were 12 days old, they were transplanted into 2L round pots filled with a 50:50 mixture of vermiculite (Sun Grow Horticulture, Vancouver, British Columbia, Canada) to perlite (PVP Industries, North Bloomfield, Ohio, USA) and randomly assigned to one of four water:N+P treatment groups (low:low, low:high, high:low, high:high; N = 66 per treatment group). These soil media do not have significant nutrient contents and were chosen so that soil N+P concentrations could be more accurately controlled to our intended treatment values (see "Treatments"). The experiment was conducted in a greenhouse at Michigan Technological University (Department of Biological Sciences, Houghton MI) with a light:dark cycle of 16:8h and an ambient air temperature of 21°C from January 2019 to October 2019. Pots were rotated every week to reduce any non-random effects imposed by variable greenhouse conditions. A total of 27 plants, evenly dispersed across treatments, died in the beginning of the experiment (data from these plants are excluded from subsequent analyses), and the experiment concluded after 42 weeks, as plants began to naturally senescence and did not show signs of flowering.

Treatments: Water was administered weekly as 200 mL and 400 mL aliquots for the low- and high- water treatments, respectively. The high-water treatment was chosen by measuring the amount of water needed for a 2L pot of soil medium to become fully saturated, and the low treatment was chosen to be half the volume of water needed to reach full saturation (Poorter et al., 2012). N+P treatments were administered on weeks 6-9 by adding 20 mL of a combined N+P solution of ammonium nitrate and potassium

monophosphate amounting to 7.5 ppm and 75 ppm of N and 0.5 ppm and 5 ppm of P for the low and high treatments, respectively. Phosphorus levels were based on the amount of available total P measured at the field collection sites (Appendix 1), which we expected to represent adequate P availability. Inorganic N was estimated based on these P values using a ratio of 15:1 N:P, which is an optimal N:P ratio for most plants (Güsewell, 2005; Luo et al., 2016). All plants also received 100 ppm of potassium sulfate and 0.590 mL micronutrients (Fertilome chelated liquid iron and other micronutrients; Voluntary Purchasing Groups, Bonham, Texas, USA). Two additional N+P treatments were administered during weeks 21 and 24 to resupply the plants with nutrients that were likely depleted through plant utilization.

Measured Traits — To examine cytotype-specific growth and physiological responses to water and/or N+P levels, we measured traits associated with plant stomata characteristics, growth, and photosynthesis.

Stomata Characteristics: We measured stomata density and stomata size by creating stomata casts on the lower leaf surface of 120 plants (N = 15 per cytotype x treatment combination) during the 20th week of growth, when leaves were large enough to be analyzed and hardy enough to not become damaged from the casts. A thin coat of clear nail polish was painted on the middle-third of a leaf's fully developed underside, extending from the midvein to the edge of the leaf. All leaves were fully expanded and of similar developmental age and position on plants. The nail polish casts dried for two hours, were peeled from the leaf, and then mounted onto a microscope slide where they were examined with an Olympus light microscope (Olympus Corporation, Shinjuku,

Tokyo, Japan). Stomata density was determined by counting the number of stomata present in the field of view (diameter of 550 μm) at 400x total magnification, while stomatal area (μm^2) was calculated by measuring the average of guard cell length and width of four randomly selected stomata at 1000x total magnification.

Growth Traits: At harvest, plants were separated into above- and belowground parts, dried at 60℃, and weighed to the nearest gram. Aboveground biomass was dried for 48hr while belowground biomass was dried for 72hr, as root systems store more water. Root/shoot ratio (R/S) was calculated by dividing belowground biomass by aboveground biomass. This ratio has implications for plant community and competitive dynamics as it is a measure of how a plant invests into belowground (water and nutrients) versus aboveground (sunlight) resource acquisition and present (aboveground biomass) versus future (belowground biomass) reproductive potential (Aerts, 1999; Craine and Dybzinski, 2013). Ploidy level can also influence R/S ratios as cytotypes can have different strategies for adapting to resource limitations (Bales and Hersch-Green, 2019).

Photosynthetic Metrics: From a subset of plants evenly distributed across treatments (N =136), we used a portable infrared CO_2 analyzer system (LI-6400XT; LI-COR Inc., Lincoln, NE, USA) to measure net photosynthetic capacity (A_{net}, μmol CO_2 m^{-2} s^{-1}) and transpiration rate (E, mmol H_2O m^{-2} s^{-1}), which were then used to calculate instantaneous water use efficiency (A_{net}/E; (A_{net}/E; Medrano et al., 2015). These measurements were all taken between 09:00 to 16:00 on sunny or partly sunny days, using the same subset of plants during early- (week 25), mid- (week 29), and late- (week 34) stages of growth.

Measurements were taken on the youngest, fully-developed leaf of a plant and the sampling order was randomized across treatment groups.

The CO_2 analyzer system was equipped with a CO_2 mixer (LI-6400-01) and a $2cm^2$ chamber/red-blue LED light source (LI-6400-40). The mole fraction of CO_2 entering the leaf chamber was maintained at 400 μmol mol^{-1}, and light levels inside the leaf chamber were held constant at a PAR of 1000 μmol m^{-2} s^{-1}. We did not control air temperature inside the chamber as measurements were taken inside a temperature-controlled experimental greenhouse, but equipment was kept shaded to prevent leaf chamber exposure to excessive heat. Measurements were recorded once values for photosynthetic rate and intercellular CO_2 concentrations stabilized (after approx. 3 minutes) and infrared gas analyzers (IRGA) were matched after every 10 sampling measurements to remove any differences between the reference and samples IRGA CO_2 and H_2O readings.

Statistical Analyses — We used analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) models to determine whether cytotype (2x = diploid, 4x = tetraploid), population of origin (by including 'cytotype' nested within 'population of origin'), water availability (low, high), and/or N+P treatment (low, high) influenced plant stomatal characteristics, growth, and/or physiological responses. In all models, factors were treated as fixed effects and model assumptions of normality and homogeneity of variances were tested and transformations were completed if needed. Five separate ANOVA models were used to test whether cytotype, water treatment, N+P treatment, their interactions, and/or population of origin impacted stomata area, stomata density,

biomass accumulation (aboveground, belowground) and/or R/S ratio (square-root transformed). Post hoc analyses were used to determine the nature of significant factors and/or interactions among factors within ANOVA models. Three separate repeated-measure MANOVA models were used to test whether maximum photosynthetic capacity, transpiration rate, and/or water use efficiency were significantly affected by cytotype, water treatment, N+P treatment, their interactions, and/or population of origin and/or if response varied by measurement date (early-, mid-, or late-stage growth). When a 'between subjects' MANOVA model was found to be significant, we performed separate univariate ANOVA models for the response and used post-hoc analyses to test for significant differences among factor-level means independent of the effects of time. All analyses were performed using the statistical software JMP Pro version 14.0 (SAS Institute, Cary, North Carolina, USA) and we report data as untransformed LS means ± 1 SE.

1.4 Results

Do resource treatments and cytotype individually and/or jointly affect stomatal characteristics?

In accordance with our hypothesis, tetraploids had larger and fewer stomata per mm^2 of leaf area than diploids (LS Mean ± 1 SE for 2x: 513.10 ± 12.10 μm^2 and 41.96 ± 1.40; 4x: 667.29 ± 11.76 μm^2 and 31.88 ± 1.36 for stomata area and number respectively; Table S1; Figure 1). Surprisingly, however, stomata density was also affected by N+P treatment, with plants in high-N+P treatments having more stomata than plants in low-

N+P treatments (LS Mean ± 1 SE for low N+P = 32.93 ± 1.42, for high N+P = 40.91 ± 1.36; Table S1). Stomata size was also affected by the N+P treatment, but this effect was dependent upon the water treatment (e.g. the interaction between water x N+P was significant; Table S1; Figure S1). Specifically, plants grown in low-N+P conditions had larger stomata than those grown in high-N+P conditions, but only under the conditions of high-water availability (Figure S1). No other factor or interaction among factors significantly affected stomata size or density (Table S1).

Do resource treatments and cytotype individually and/or jointly affect growth responses?

Water treatment, N+P treatment, and cytotype each had individual effects on above- and belowground biomass accumulation (Table 1; Figure 2). In general, plants grown in high-water and high-N+P treatments had greater above- and belowground biomasses than plants grown in the low treatments (Figure 2). Tetraploids were generally larger than diploids across all treatments (Table 1; Figure 2), but contrary to our expectations, tetraploids did not show evidence of being more impaired, in terms of growth, by the low-water and N+P treatments, than did diploids (Table 1; Figure 2). Belowground biomass for both cytotypes depended upon the interaction between N+P and water availability (Table 1), such that plants grown in both the high-water and high-N+P environments had the greatest belowground biomass relative to the other treatment combinations (Figure 2). Population of origin also had an effect on biomass (Table 1), as plants from a single tetraploid population (Population 7 in Appendix 1) had the greatest

values for above- and belowground biomass compared to the other populations. No other factors nor interactions among factors affected above- or belowground biomass or the R/S ratio (Table 1).

Do resource treatments and cytotype individually and/or jointly affect physiological responses?

Photosynthetic capacity: Overall, cytotype, but not water nor N+P treatment, affected maximum photosynthetic capacity, as measured by A_{net}. Specifically, tetraploids had greater A_{net} than diploids (LS Mean ± 1 SE for 2x = 6.48 ± 0.30 μmol CO_2 m^{-2} s^{-1} and 4x = 7.77 ± 0.30 μmol CO_2 m^{-2} s^{-1}; Table 2; Figure 3A,B). $A_{net,}$ however, did decrease over time dependent upon cytotype and water availability (e.g. the interaction of time x cytotype x water was significant; Table 2). In particular, when water availability was low, A_{net} decreased as both cytotypes aged (Figure 3A,B), whereas when water availability was high, A_{net} responses were more varied between time frames. For instance, during early to mid-stage A_{net} values increased in tetraploids but decreased in diploids, whereas as in mid to late stage A_{net} values decline for both cytotypes (Figure 3A,B). Plants grown in the high-water treatments also had lower A_{net} values during the early stage compared to those in low-water treatments (Figure 3A,B). Values for A_{net} also differed based on population of origin (Table 2), as we found that plants from the tetraploid Population 4 had greater values than the tetraploid Population 3 and the diploid Population 8 (See Appendix 1 for population locations and information). No other factors or interactions among factors were found to significantly affect A_{net} (Table 2).

Transpiration: Overall water and N+P treatments, but not cytotype, affected transpiration rates (*E*) with plants grown in the high-water treatments or low-N+P treatments having greater *E* rates than those grown in the low-water treatments or the high-N+P treatments (Table 2; Figure 3C,D). However, the effects of water on *E* rates changed over time (Table 2). Specifically, during the early to mid-stage only plants grown in the low water treatments experienced a decrease in *E*, whereas from the mid to late stage plants in both the low- and high-water treatment experienced declines in *E* rates (Figure 3C,D). Population of origin also had an effect on *E* (Table 2), as plants from the tetraploid Population 4 and the diploid Population 5 had greater *E* values than the tetraploid Population 3 (See Appendix 1 for population locations and information). No other factors or interactions among factors had a significant effect on *E* rates (Table 2).

Water use efficiency: Overall cytotype, water, and N+P treatments all independently had significant effects on water use efficiency (*WUE*) and none of their effects depended upon each other (Table 2). In general, tetraploids had greater *WUE* values than diploids (LS Mean ± 1 SE for 2x 0.19 ± 0.01μmol mol^{-1} and 4x 0.22 ± 0.01 μmol mol^{-1}), and plants grown in conditions of high-N+P or low-water had greater *WUE* values than those in low-N+P or high-water conditions (Table 2; Figure 3E,F). No other factors or interactions among factors had a significant effect on *WUE* rates (Table 2).

1.5 Discussion

Resource limitation patterns in plants are suspected to be mediated, at least in some part, by genome size and/or ploidy level (Lewis Jr, 1985; Cavalier-Smith, 2005; Hessen et al.,

2010; Guignard et al., 2017), yet we lack an understanding of the role polyploidy has on plant ecological interactions and evolution. Given that we are experiencing unprecedented global changes in precipitation and nutrient deposition patterns (Trenberth, 2011; Goyette et al., 2016), understanding when and how changes in water and nutrient availabilities influence cytotype-specific growth and physiological responses can have wide ramifications for understanding both local plant competitive outcomes and regional biodiversity patterns. Here we examined diploid and tetraploid cytotypes of the autopolyploid *Solidago gigantea* to evaluate whether water and nutrients individually or jointly impact plant growth and physiological responses and whether response patterns were dependent upon the ploidy level of plants. As plants are the primary producers of terrestrial ecosystems and polyploidy is common in plants (Soltis and Soltis, 2016), especially among invasive plant species (Te Beest et al., 2012), findings from our study could inform our understanding of the importance of polyploidy in the fields of community ecology and invasive species biology. In general, we found that resources, such as water, nitrogen, and phosphorus, individually and jointly impacted plant growth and physiological responses. However, contrary to our expectations we found that while cytotypes did differ in some growth and physiological attributes (e.g., biomass accumulation, photosynthetic capacity, water use efficiency), they generally did not respond differently to changes in these resources. We discuss the significance of our findings in terms of plant biodiversity patterns, competitive interactions, and invasive species biology.

Greater resource availability promotes plant growth

Water and nutrient enhancements tend to be positively correlated with plant productivity (Flexas et al., 2009; Boussadia et al., 2010; Kardol et al., 2010; Reich et al., 2014; Harpole et al., 2016) and photosynthetic activity (Guan et al., 2015; Reich et al., 2018; Smith et al., 2019), but the degree to which enhancements translate into individual competitive and/or selective advantages is likely dependent upon stoichiometric and/or physiological trade-off characteristics of individuals (Tilman, 1982; Aerts, 1999; Borer et al., 2014). Here we found that plants grown in the high-resource treatments were generally larger than those grown in low-resource treatments (Figure 2), but we did not find evidence that net photosynthetic activity similarly increased when plants were given more resources (Figure 3). While it is not known why increased biomass was not preceded by increased net photosynthetic capacity, it is possible that observed enhancements in biomass resulted from increased protein synthesis of growth compounds (López-Bucio et al., 2003) and/or increased water and sugar transport (Hesse et al., 2019) triggered by greater water and/or nutrient availability instead of increased net photosynthetic capacity. For instance, under ideal water conditions, fast water transport can make up for the water lost through transpiration, allowing plants to consistently maintain the high-water potentials and turgor pressures needed to sustain the formation and enlargement of cells (Hsiao et al., 1976; Woodruff et al., 2004). Our finding that transpiration rates were greater in high-water conditions provides some additional support for this explanation (Figure 3). Since transpiration rates reflect water transport speed, this finding suggests that plants might relax water conservation strategies, such as *WUE*, in non-limiting conditions to gain the physiological benefits of quicker water transport (Zhang and Cao, 2009; Figure 3).

Cytotypes did not vary in their responses to greater resources

Here we hypothesized that tetraploid *S. gigantea* would be more negatively impacted (in terms of growth, net photosynthetic capacity, and *WUE*) by water and nutrient limitations than diploids. This was predicted because organisms with larger genomes tend to have larger cells which increase rates of water loss through large stomatal pores (Kardiman and Ræbild, 2017; Roddy et al., 2020) and greater nitrogen and phosphorus requirements for nucleic acid and cell synthesis (Lewis Jr, 1985; Cavalier-Smith, 2005; Hessen et al., 2010; Guignard et al., 2017). Contrary to the findings of Bales and Hersch-Green (2019), we did not find evidence of this "diploid advantage" over polyploids in nutrient- and/or water- limited environments as biomass accumulation, photosynthetic capacity, transpiration rates, and water use efficiency of both cytotypes responded similarly to changes in water and nutrients (Figure 2,3). Below we discuss five potential mechanisms underlying this finding.

First, natural selection could have played a role in shaping this observed lack of cytotype-specific responses to available resources if similar strategies for coping with abiotic stressors had been selectively favored at the species, cytotype, or population level. *S. gigantea* is a mesic species (Abrahamson et al., 2005; Weber and Jakobs, 2005) that is known to be more sensitive to water limitations than to other environmental stressors (Shibel and Heard, 2016). Despite existing in separate cyto-geographic ranges within North America (Abrahamson et al., 2005; Weber and Jakobs, 2005; Schlaepfer et al., 2008; Hull-Sanders et al., 2009; Appendix 1), diploid and tetraploid *S. gigantea* tend to occupy similar habitats and ecological niches. Given the essential role of photosynthesis

and water conservation strategies in plant survival, selection pressures on these particular traits could have been especially strong, acting on the species as a whole rather than at the cytotype-level. Thus, highly similar responses to water limitation amongst diploid and tetraploid *S. gigantea* might have been favored. Alternatively, local adaptation at the population and/or cytotype-level might also influence responses to environmental stressors. Despite using seeds collected from multiple, spatially separated populations, we did not find major differences in environmental responses between the populations. Whether our observed lack of response variation is due to convergent local adaptation following whole genome duplication events or strong selective pressures acting on *S. gigantea* as a species is difficult to discern. Studies utilizing neopolyploids and/or species with diploids and polyploids occurring in the same populations would be useful at teasing apart these effects.

Second, selection might have favored different nutrient usage and/or acquisition strategies in tetraploids versus diploids that could have equalized perceived differences in nutrient demands (Gorny and Garczyński, 2008). Other studies have reported that diploids and neo- and/or established polyploids differ in their strategies for coping with resource limitations (Maherali et al., 2009; Hao et al., 2013; Guo et al., 2016; Anneberg and Segraves, 2019; Bales and Hersch-Green, 2019). For instance two studies found that established polyploids can have increased mycorrhizal fungi associations to aid in nutrient uptake (Anneberg and Segraves, 2019) and/or altered R/S ratios, potentially allowing diploids and polyploids the opportunity to occupy separate ecological niches (Bales and Hersch-Green, 2019). While the plants in our study did not appear to use different morphological strategies for adapting to resource limitations (i.e., R/S ratios

were not affected by cytotype nor resource treatments; Table 1), it is possible that cytotypes of *S. gigantea* differ in other nutrient uptake and/or use strategies that we did not investigate here, such as leaf photosynthetic N use efficiency. Future studies investigating nutrient-mediated responses based on cytotype or genome size would benefit from measuring nutrient acquisition and/or conservation strategies ranging from the molecular to the community level.

Third, instead of selecting for strategies to improve nutrient use and/or acquisition, selection could have operated to reduce the nutrient demands of large genomes. This could be achieved, for example, through genome downsizing, which reduces both the nuclear volume and lower limit of cell sizes (Simonin and Roddy, 2018), and/or by selecting for more nitrogen-efficient nucleotide parings (i.e. AT pairings require 7N, while GC parings require 8N; Kelly, 2018). Because RNA is more abundant in cells and also requires large amounts of N and P for nucleic acid synthesis (Sterner and Elser, 2002), plant nutrient demands could depend on transcriptome size rather than genome size, especially if selection has reduced the nutrient costs of the genome. Alternatively, since genome size scales positively with cell size and negatively with cell density (Simonin and Roddy, 2018; Roddy et al., 2020), tradeoffs could exist between cell size and number that result in fewer nuclei, N and P content per unit of tissue mass, potentially reducing DNA synthesis costs in organisms with large genomes and equalizing nutrient demands between cytotypes (Neiman et al., 2009; Raven et al., 2013). While this idea has not yet been widely tested, preliminary data has shown autotetraploid *S. gigantea* to have 36.2% and autotetraploid *Chamerion angustifolium* to have 2.2% less cells than diploids, suggesting that the effect of cell density on plant nutrient demands is

variable and potentially species-specific (Walczyk and Hersch-Green, unpublished data). Therefore, genome size might have little or no effect on plant nutrient demands if investment costs are offset by cell number, which could explain the lack of cytotype-specific responses to N and P availability observed here.

Fourth, morphological and/or physical characteristics in polyploids could have offset the potentially negative effects of large genomes on water and/or nutrient tolerances and resulted in a lack of cytotype-specific responses to resource treatments. For example, we found that despite tetraploids having larger but fewer stomata than diploids, both cytotypes lost water through transpiration at the same rate (Figure 1, 2). Across a wide range of plant taxa a negative correlation exists between stomata size and density (Beaulieu et al., 2008; Knight and Beaulieu, 2008). Because stomatal conductance and transpiration rates increase with increasing stomata size and decreasing density (Meckel et al., 2007; Drake et al., 2013; Fanourakis et al., 2015; Bertolino et al., 2019), any increases in transpiration rate caused by the large stomata pore areas in tetraploids might have been negated by the low density of these openings. Similar results have been reported by Maherali et al. (2009), who also found that both neo- and established polyploids had fewer, but larger stomata relative to diploids, but equal rates of stomatal gas exchange. While alterations to stomatal size and density based on genome size appear consistently across species (Beaulieu et al., 2008; Knight and Beaulieu, 2008; Roddy et al., 2020), whether these alterations are strong enough to induce changes in gas exchange and water loss rates might depend upon other attributes, such as xylem properties.

Finally, our choice of ‘low-’ and ‘high-‘ experimental treatment values might have masked cytotype-specific responses to resource availability. For instance, the similar responses to water treatments we observed between cytotypes could arise because the ‘low-’water treatment was not truly limiting relative to the ‘high’ treatment. We chose our ‘low-’ water treatment to be half the volumetric amount of water needed to fully saturate the soil medium within a plant’s pots. By using this method, we prevented the soil in the ‘low’ treatments from fully drying out. Furthermore, our use of vermiculite as a nutrient-free soil media might have also affected the water treatments, as vermiculite tends to improve water holding capacity in soils. While we still found significant differences in plant phenotypes between the ‘low-‘ and ‘high-‘ water treatments, the vermiculite might have allowed the ‘low-‘ water treatment pots to accumulate more water than initially intended. Therefore, the low treatments may not have been limiting enough (plants never experienced drought) to perceive cytotype-specific differences. Furthermore, our choice of ‘low-’ and ‘high-’ N+P treatments might have been so limiting that both cytotypes were unable to access enough nutrients for ideal functioning and growth. However, it is unlikely that the high-N+P treatment was limiting, as plants generally responded positively in terms of growth when nutrients were added. For instance, post-hoc analyses of growth responses to treatments (calculated as the difference in biomass between high- and low-N+P treatments divided by the averaged biomass of both N+P treatments done separately for plants grown from the same maternal line) showed that plants responded to NP enrichment by increasing their above- and belowground biomass when water was not limited, implying that our high-N+P treatment was not limiting to plant growth (Mean aboveground growth response to N+P enrichment

when H_2O high = 0.19 ± 0.12, when H_2O low = -0.93 ± 0.12; belowground growth response to N+P enrichment when H_2O high = 0.26 ± 0.12, when H2O low = -0.18 ± 0.14). While there is still the possibility that nutrients were limiting and/or in excess, we are unable to fully evaluate that without measuring the elemental ratio of N to P in our experimental plants (Koerselman and Meuleman 1997). Nevertheless, different water and/or nutrient treatment methods, such as a gradual onset of drought and/or the inclusion of 'medium-' treatment levels, might reveal responses to resource availability that differ from our findings here, and are worth investigating in future studies.

Implications for competitive interactions

Competition shapes biodiversity and species distribution patterns in plants (Tilman, 1982; Losapio et al., 2021) and competitive success depends upon a plant's ability to maximize access to shared and/or limited environmental resources (Aerts, 1999; Craine and Dybzinski, 2013). Some attributes that are commonly associated with whole genome duplication, such as increased plant size (due to the possession of large cells; Orr-Weaver, 2015; Segraves and Anneberg, 2016) and wider ecological tolerances (Godfree et al., 2017; Van de Peer et al., 2017), might make both neo- and established polyploids of some species better competitors than diploids (Te Beest et al., 2012; Fowler and Levin, 2016). These potential differences in competitive ability, along with other factors, such as differences in dispersal ability and pollinator clades, could contribute to patterns of cytogeographic segregation found within the ranges of some polyploid species (Schlaepfer et al., 2008; Meimberg et al., 2009; Godfree et al., 2017). Our finding that tetraploid *S. gigantea* were larger, in terms of above- and belowground biomass, had

greater photosynthetic capacity over longer periods of time, and had greater *WUE* than diploids might indicate a greater competitive ability of *S. gigantea* tetraploids over diploids. This is because plants with more aboveground biomass and higher photosynthetic rates are better at competing for light, whereas plants with more belowground biomass are better at competing for water and nutrients in the soil (Aerts, 1999; Craine and Dybzinski, 2013). Additionally, plants with high *WUE* exhibit strategies that reduce water loss while maintaining high photosynthetic and growth rates, making them more competitive in dry environments than plants with low *WUE* (Hatfield and Dold, 2019). While we found tetraploids to have greater *WUE* than diploids, we do not believe that this is representative of a greater drought tolerance or competitive ability than diploids. In order to truly improve drought tolerance through alterations in *WUE,* transpiration rates should decrease while photosynthetic rates simultaneously increase or remain constant (Yoo et al., 2009). Because transpiration rates did not differ between cytotypes (Figure 3), differences in *WUE* between cytotypes was likely driven by the high photosynthetic rates we found in tetraploids.

Polyploidy is highly prevalent in invasive plant species (Te Beest et al., 2012) and given that competitive ability is a vital component to invasion success (Gioria and Osborne, 2014), understanding how polyploidy influences competitive ability could have vast implications in the field of invasive species biology. Our use of *S. gigantea* as a focal species allows us to make inferences surrounding the role of polyploidy in biological invasions, because *S. gigantea* is one of the most prevalent invasive plants in Europe and Asia, but only in its tetraploid form (Schlaepfer et al., 2008; Hull-Sanders et al., 2009; Schlaepfer et al., 2010). This cyto-geographic pattern makes comparisons of *S. gigantea*

cytotypes relevant to our understanding of why only tetraploids have become invasive. Overall, our results suggest that tetraploid *S. gigantea* possess attributes that might be indicative of a superior competitive and hence a potentially greater invasive ability relative to diploids. However, because invasion success is driven by a compilation of multiple traits affecting fitness, an increase in plant size alone may not be strong enough to equate to tetraploids being better invaders than diploids. Studies explicitly testing multiple traits associated with the competitive ability of diploids and polyploids against each other and against other local and non-native plant species under varying resource conditions are needed to more accurately understand how ploidy level translates into competitive and invasive ability.

1.6 Tables

Table 1. Results from fixed-effects ANOVA models for the effects of cytotype, soil water treatment, soil nutrient treatment, their interactions, and population of origin nested within cytotype on aboveground biomass, belowground biomass, and root/shoot ratio (square-root transformed). Overall model for aboveground biomass: $R^2 = 0.25$, $F_{11,225} = 6.98$, $P < 0.0001$, N = 237; for belowground biomass: $R^2 = 0.28$, $F_{11,225} = 7.76$, $P < 0.0001$, N = 237; for root/shoot ratio: $R^2 = 0.06$, $F_{11,225} = 1.41$, $P = 0.1686$, N = 237. Bold values indicate a significant effect at $\alpha = 0.05$.

Source	df	MS	F	Prob > *F*
Aboveground biomass (g)				
Cytotype (C)	1	88.84	28.58	**<0.0001**
Water (W)	1	13.78	4.54	**0.0343**
Nutrients (N)	1	48.99	16.12	**<0.0001**
C x W	1	3.10	1.02	0.3134
C x N	1	2.16	0.71	0.3996
W x N	1	6.43	2.11	0.1473
C x W x N	1	4.44	1.46	0.2278
Population [Cytotype]	4	16.87	5.55	**0.0003**
Model Error	225	3.04		
Belowground biomass (g)				
Cytotype (C)	1	138.76	31.06	**<0.0001**
Water (W)	1	28.70	6.43	**0.0119**
Nutrients (N)	1	89.61	20.06	**<0.0001**
C x W	1	0.55	0.12	0.7258
C x N	1	3.34	0.75	0.3879
W x N	1	21.11	4.73	**0.0308**
C x W x N	1	16.43	3.68	0.0564
Population [Cytotype]	4	86.38	4.83	**0.0009**
Model Error	225	4.47		
Root/shoot ratio				
Cytotype (C)	1	0.03	0.31	0.5768
Water (W)	1	0.00	0.00	0.9899
Nutrients (N)	1	0.11	1.10	0.2943
C x W	1	0.24	2.49	0.1160
C x N	1	0.01	0.12	0.7338
W x N	1	0.47	4.92	0.0275*
C x W x N	1	0.36	3.79	0.0527
Population [Cytotype]	4	0.29	0.77	0.5467
Model Error	225			

* Factor significance negated by the insignificant overall statistical model.

Table 2. Results from repeated measures MANOVA models for the effects of cytotype, soil water treatment, soil nutrient treatment, their interactions, and population of origin nested within cytotype on net photosynthetic capacity, transpiration rate, and water use efficiency (N = 132). Wilk's Λ are given in the footnotes. Bold values indicate a significant effect at α = 0.05.

		Maximum photosynthetic capacity (A_{net})		Transpiration rate (E)		Water use efficiency (WUE)	
Source	df	F	Prob > F	F	Prob > F	F	Prob > F
Between subjects	1						
Cytotype (C)	1	8.46	**0.0043**	0.05	0.8268	10.47	**0.0016**
Water (W)	1	0.01	0.9192	5.52	**0.0204**	9.81	**0.0022**
Nutrients (N)	1	0.69	0.4094	9.81	**0.0022**	8.31	**0.0047**
C x W	1	2.35	0.1280	3.25	0.0738	0.04	0.8359
C x N	1	0.96	0.3280	0.11	0.7363	1.20	0.2761
C x W x N	1	0.06	0.8098	0.03	0.8731	0.10	0.7563
Population [Cytotype]	4	2.71	**0.0334**	2.52	**0.0446**	0.32	0.8642
Whole model	11	2.03	**0.0309**	2.77	**0.0031**	2.97	**0.0016**
Within subjects							
Time (T)	2	82.52	**<0.0001**	197.12	**<0.0001**	32.04	<0.0001*
T x C	2	6.16	**0.0028**	0.89	0.4118	6.19	0.0028*
T x W	2	18.24	**<0.0001**	12.90	**<0.0001**	2.04	0.1348
T x N	2	2.48	0.0882	1.27	0.2856	0.64	0.5267
T x C x W	2	3.52	**0.0328**	0.44	0.6469	1.87	0.1582
T x C x N	2	1.59	0.2091	0.13	0.8790	1.17	0.3141
T x W x N	2	0.12	0.8915	0.69	0.5013	1.23	0.2955
T x C x W x N	2	0.52	0.5937	0.14	0.8699	0.39	0.6796
T x population [Cytotype]	8	0.46	0.8801	1.13	0.3405	0.97	0.4602
Whole model**	22	3.01	**<0.0001**	1.91	**0.0098**	1.57	0.0551

* Factor significance negated by the insignificant overall statistical model.

** A_{net} within interactions Wilk's Λ = 0.61; E within interactions Wilk's Λ = 0.72; WUE within interactions Wilk's Λ = 0.76

Table S1. Results from fixed-effects ANOVA models for the effects of cytotype, soil water treatment, soil nutrient treatment, their interactions, and population of origin nested within cytotype on stomata size (as area in μm^2) and stomata density. Overall model for stomata size: $R^2 = 0.48$, $F_{11,114} = 9.65$, $P < 0.0001$, N = 126; for stomata density: $R^2 = 0.33$, $F_{11,114} = 5.03$, $P < 0.0001$, N = 126. Bold values indicate a significant effect at $\alpha = 0.05$.

Source	df	MS	F	Prob > *F*
Size (μm^2)				
Cytotype (C)	1	729664.40	83.49	**<0.0001**
Water (W)	1	1206.30	0.14	0.7109
Nutrients (N)	1	98553.30	11.28	**0.0011**
C x W	1	3407.30	0.39	0.5336
C x N	1	1789.70	0.20	0.6517
W x N	1	43812.50	5.01	**0.0271**
C x W x N	1	2028.80	0.23	0.6309
Population [Cytotype]	4	15336.90	1.76	0.1428
Model Error	114	8739.20		
Density				
Cytotype (C)	1	3120.99	26.52	**<0.0001**
Water (W)	1	146.41	1.24	0.2670
Nutrients (N)	1	1925.55	16.36	**<0.0001**
C x W	1	250.81	2.13	0.1470
C x N	1	149.49	1.27	0.2620
W x N	1	17.56	0.15	0.7000
C x W x N	1	5.49	0.05	0.8294
Population [Cytotype]	4	236.77	2.01	0.0974
Model Error	114	117.67		

1.7 Figures

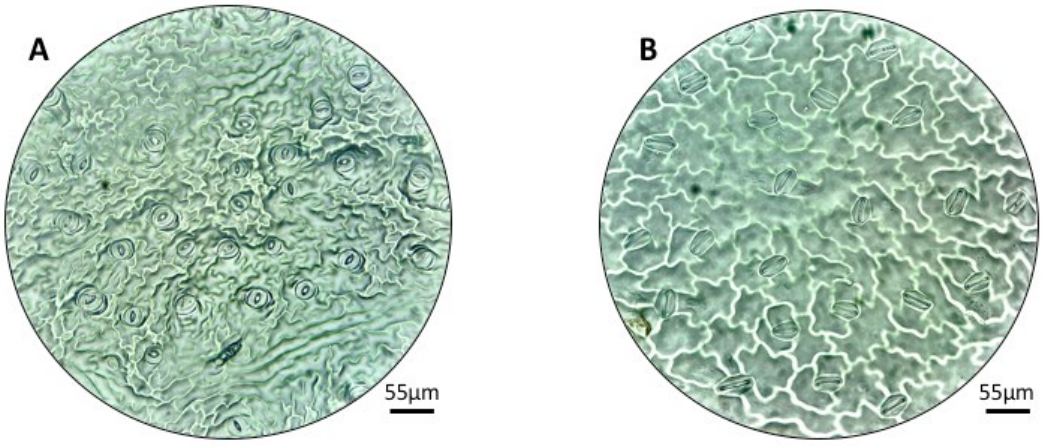

Figure 1. Casts of a diploid (A) and tetraploid (B) leaves at 400x magnification showing differences in stomata size and density. Statistical details in Table S1

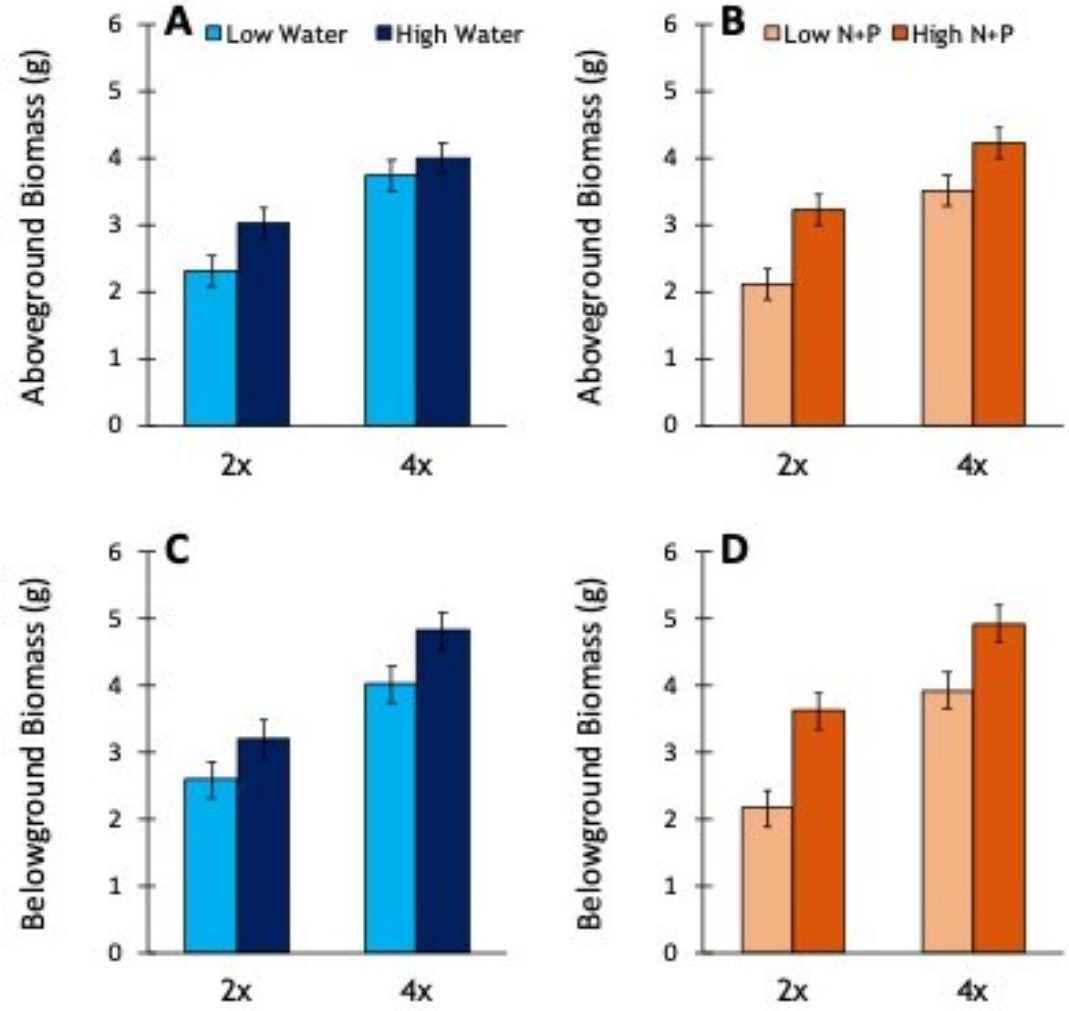

Figure 2. LSMean aboveground (A) and belowground (B) biomass for diploids (2x) and tetraploids (4x) grown in different water (A,C) and N+P (B,D) treatments. Water (aboveground: $F_{1,225} = 4.45$, $P = 0.0343$; belowground: $F_{1,225} = 6.43$, $P = 0.0119$) , N+P (aboveground: $F_{1,225} = 16.12$, $P < 0.0001$; belowground: $F_{1,225} = 20.06$, $P < 0.0001$), and cytotype (aboveground: $F_{1,225} = 28.58$, $P < 0.0001$; belowground: $F_{1,225} = 31.06$, $P < 0.0001$, belowground: $P < 0.0001$) all individually had a significant effect on LS mean above- and belowground biomass, but interactions between these factors did not. Error bars represent ± standard error. Full statistical details in Table 1.

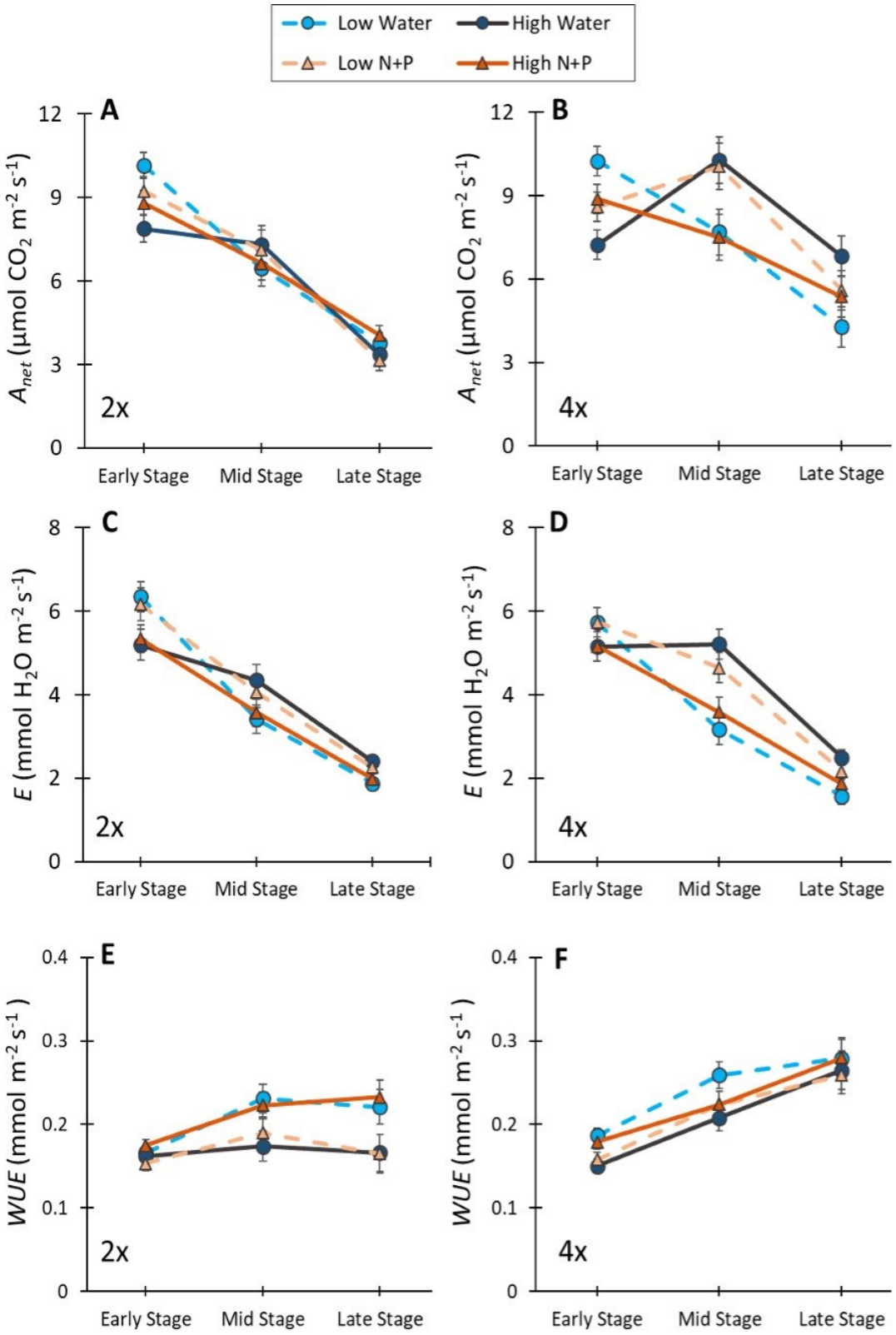

Figure 3: LSMean photosynthetic capacity (A,B), transpiration rates (C,D), and water

use efficiency (E,F) values for diploids (2x) and tetraploids (4x) grown in different water (circles) and N+P (triangles) treatments across early, mid, and late stages of growth. Error bars represent ± standard error. Full statistical details in Table 2.

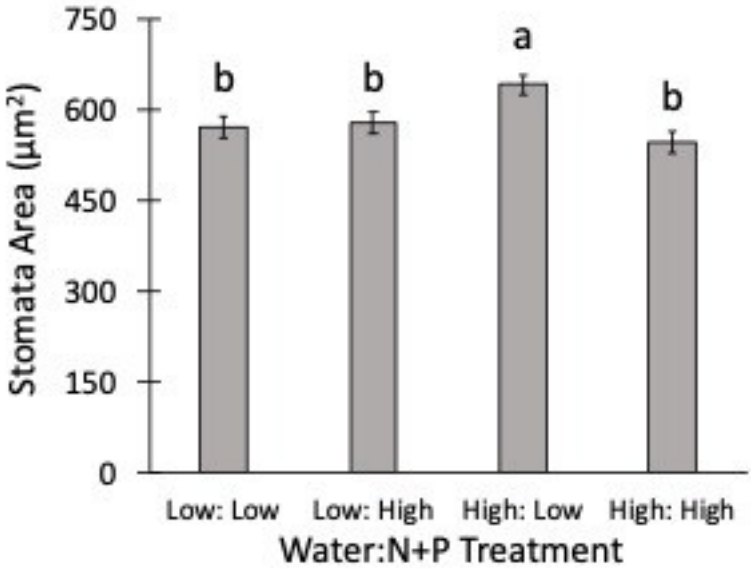

Figure S1. LSMean stomata size within each of the four water:N+P treatments. The results of Tukey's HSD tests between cytotypes when significant ($P < 0.05$) are reported with different letters. Error bars represent ± standard error.

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1.9 Appendix Materials

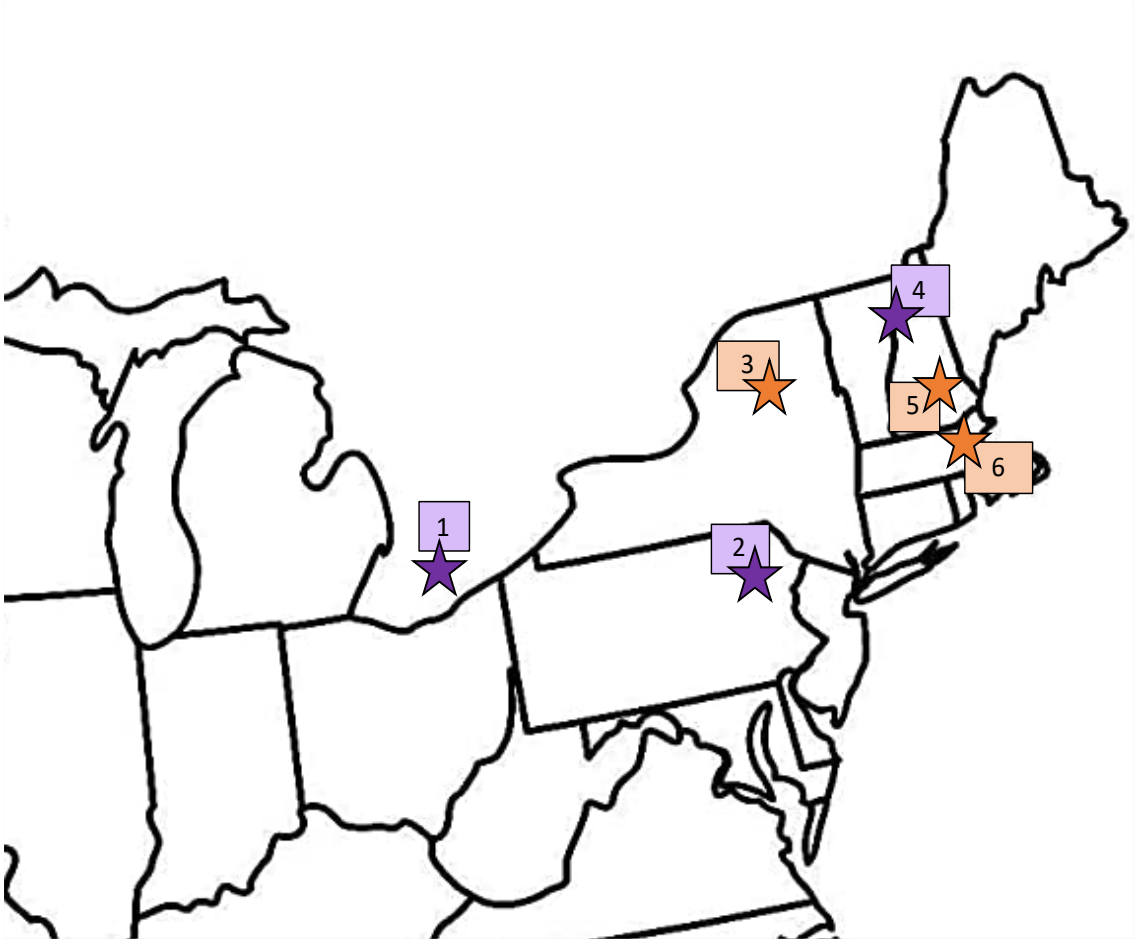

Figure A1. *Solidago gigantea* collection sites. Purple stars depict predominantly tetraploid populations, while orange stars depict predominantly diploid populations. Details on field sites are given below in Table A1.

Flowcytometry methods:

The ploidy level was verified by estimating plant nuclear 2C DNA via flow cytometry as plant nuclear 2C DNA content is positively correlated with chromosome number (Husband and Schemske, 1998). Briefly, approximately 1 cm2 of silica dried *S. gigantea* leaf tissue (a total of 367 leaves were analyzed) was co-chopped with approximately 1 cm^2 live leaf tissue of the internal standards *Zea mays* (2C DNA content = 5.43pg; Dolezel et al., 2007) or *Pisum sativa* (2C DNA content = 9.09pg; Dolezel et al., 2007) in a modified Galbraith nucleiisolation buffer with 50 µg mL-1 RNase and 50 µg mL-1 propidium iodide (see Verloove et al., 2017). Cells were stained for approximately 45

minutes, filtered, and then analyzed on an Accuri C6 flow cytometer and analyzed using CFlow Plus Analysis software (Accuri Inc., Ann Arbor, Michigan, USA). We used a FL2 detector to measure sample fluorescence and generate scatter plots in which we were able to omit residual noise and gate recorded particles within the fluorescence range of *S. gigantea* and either of the internal standards. Mean counts and coefficient variations (CV) were derived from histogram plots within the software. We removed 241 low quality samples that had a histogram peak coefficient variation (CV) of more than 5% (Dolezel et al., 2007). The following formula from Dolezel et al. (2007) was used to calculate sample 2cDNA content (in pg):

$$Sample\ 2cDNA\ value = \ Standard\ 2cDNA\ value * \left(\frac{Sample\ 2C\ mean\ peak\ position}{Standard\ 2c\ mean\ peak\ position}\right)$$

Nutrient soil extraction methods:

A total of 10 soil cores (15 cm deep x 1.9 cm diameter) were taken at each study site, dried at 60°C, and homogenized. Total percent organic nitrogen (N) and carbon (C) were calculated via dry combustion on a Costech elemental analyzer (Costech Analytical Technologies Inc., Valencia, California, USA) at Michigan Technological University. To determine amounts of available P, a colorimetric analysis of extractable P was performed using an ascorbic acid reduction of phosphomolybdate with a Mehlich 3 extraction solution (Mehlich, 1984). The absorbencies of all samples were measured at 880 nm on a Spectrophotometer at Michigan Technological University. The amount of available inorganic N from calculated values of available P using a ratio of 15:1 N:P, which is the optimal N:P ratio for most plants (Güsewell, 2005).

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Table A.1: Location, soil phosphorus, and ploidy level information for each of the 6 field sites depicted in Figure A1.

Number	Study Site	City, State	N 2x	N 4x	Latitude	Longitude	Elevation (m)	Habitat Type	Mean Soil P (ppm)
1.*	Cash-Hetrick Preserve	Geneva, OH	1	22	41.7455	-80.99917	229.61	River Floodplain in Forest	2.377
2.*	Selinsgrove Private Property	Selinsgrove, PA	1	33	40.76664	-76.90432	137.57	Adjacent Agricultural Fields	0.783
3.*	Hoxie Gorge State Forest	Marathon, NY	40	0	42.54805	-76.08305	409.96	Wet Meadow	0.886
4.*	Lyme Loch Lodge	Lyme, NH	0	35	43.82480	-72.14458	140.98	Adjacent Agricultural Fields	4.443
5.*	Nasami Farm New England Wildflower Society	South Deerfield, MA	39	1	42.46162	-72.63932	62.3	Wet Meadow	0.569
6.*	Trout Brook Valley Reserve	Easton, CT	39	0	41.25647	-73.32964	50.44	Adjacent Agricultural Fields	2.071

2 **Chapter 2:** Genome material costs and metabolic-tradeoffs in autopolyploid *Solidago gigantea* (Giant Goldenrod, Asteraceae) series [2]

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[2] This chapter is in preparation to be submitted to the journal New Phytologist. A.M.W conducted the experiment and collected data. Both A.M.W and E.H-G. designed the experiment, analyzed data, interpreted the data, and wrote the manuscript.

2.1 Abstract

- Despite wide variation in genome size (GS) amongst angiosperms, most have small genomes. Selection to reduce genomic "material costs" of nitrogen (N) and phosphorus (P) atoms used in DNA synthesis has been proposed to constrain GS evolution and contribute to genome downsizing, yet direct evidence that material costs increase with GS is limited.
- We grew diploid, tetraploid, and hexaploid *Solidago gigantea* plants under one of four low and high NP treatments and measured traits related to genome and transcriptome sizes, material costs, photosynthesis, and nutrient-use efficiencies.
- Relative to diploids, polyploids had greater N and P cellular investments and growth responses to N-enrichments (tetraploids only), suggesting that material costs increase with GS. Polyploids also exhibited strategies that minimize GS-dependent-material-cost-constraints over both long (reduced monoploid GS) and short [reduced relative transcriptomes (RNA/DNA), enhanced N-use efficiencies (hexaploids only), and reduced photosynthetic activity in nutrient-limited treatments] evolutionary time periods, although patterns were not ubiquitous and varied depending upon cytotype, mechanism, trait, and nutrient conditions.
- Our results that material costs increase with GS lends support to the hypothesis that selection on material costs constrains GS evolution. However, organismal mechanisms that reduce GS-dependent-material-costs could lessen such constraints highlighting that many interacting factors likely contribute to GS-downsizing.

2.2 Introduction

Despite the fact that angiosperms (flowering plants) show the widest variance in genome size (GS) amongst all eukaryotes, ranging about 2,400-fold from the smallest to the largest, most angiosperms have small genomes (Dodsworth *et al.*, 2015; Pellicer *et al.*, 2018). While this preponderancy of small genomes has been attributed to various genome and chromosome diploidization, streamlining, repair, and rearrangement processes (Wendel, 2015; Zenil-Ferguson *et al.*, 2016; Van de Peer *et al.*, 2017; Mandáková & Lysak, 2018; Wang *et al.*, 2021), more recently it has been proposed that natural selection might contribute by operating to minimize nutrient-material-costs of building genomes thereby constraining GS evolution (Lewis, 1985; Leitch & Bennett, 2004; Cavalier-Smith, 2005; Mei *et al.*, 2018; Simonin & Roddy, 2018; Faizullah *et al.*, 2021). Nutrient-material-costs of genomes, specifically nitrogen (N) and phosphorus (P), arise because (1) DNA requires N and P atoms for synthesis (e.g., nucleic acids plus the sugar-phosphate backbone are approximately 12.5% N and 3.4% P; Elser *et al.*, 2011), and (2) organisms with larger genomes have larger cells (Beaulieu *et al.*, 2008; Mueller, 2015; Roddy *et al.*, 2020) requiring more P to synthesize longer phospholipid bilayer cell membranes. Given these huge elemental demands, it has been proposed that plants have substantially different N and P requirements based upon their GS and that organisms with larger genomes should be more negatively affected by nutrient limitations (Lewis, 1985; Leitch & Bennett, 2004; Cavalier-Smith, 2005; Faizullah *et al.*, 2021). Evidence that selection to reduce material costs might constrain GS evolution comes from several studies showing that environmental scarcities in N and/or P favors the abundance,

growth, fitness, and evolution of organisms with smaller genomes (Šmarda *et al.*, 2013; Guignard *et al.*, 2016; Bales & Hersch-Green, 2019; Walczyk & Hersch-Green, 2019; Anneberg & Segraves, 2020). However, a lack of differential GS-dependent growth responses to nutrient amendments has also been observed (Sánchez Vilas & Pannell, 2017; Walczyk & Hersch-Green, in press), implying that multiple factors inherent to organisms and/or the selective context likely contribute to differential growth responses and GS variation.

The transcriptome represents the largest nucleic acid fraction in the cell, is another major N and P sink (Raven, 2013), and transcriptome size differences should also influence overall nutrient-material-costs. While the genome represents a static nutrient requirement for cells that must first be satiated before any nutrients can be allocated to other functions, the transcriptome is phenotypically plastic and gene expression patterns can vary dependent upon nutrient supplies (Jeyasingh & Weider, 2007; Hessen *et al.*, 2010; Raven, 2013) and plant GS (Osborn *et al.*, 2003; Grover *et al.*, 2012; Dodsworth *et al.*, 2015; Wendel *et al.*, 2016). Therefore, organisms might better tolerate nutrient limitations by regulating their gene expression patterns dependent upon GS nutrient-costs (Faizullah *et al.*, 2021; Wang *et al.*, 2021). For example, under nutrient limiting conditions plants with larger genomes might be under stronger pressures to conserve material costs, resulting in lower gene expression and smaller transcriptomes than organisms with smaller genomes (Kelly, 2018; Majda *et al.*, 2021). This molecular tradeoff could offer large GS plants a mechanism to tolerate nutrient poor conditions while also offering flexibility to allocate nutrients to different functions (e.g., growth,

photosynthesis, cell synthesis) depending upon nutrient availability (Faizullah *et al.*, 2021).

N and P investments into macromolecules involved in primary metabolic pathways, such as photosynthesis and associated growth, could also affect GS-dependent nutrient constraints. Proteins, pigments, ATP, and electron transport molecules used in photosynthesis all represent significant N and/or P sinks (e.g., RuBisCo accounts for 20 - 30% of total leaf N in C3 plants; Evans, 1989; Hessen *et al.*, 2010; Hohmann-Marriott & Blankenship, 2011) and these macromolecules might compete with nucleic acids for N and P – potentially leading to resource allocation tradeoffs between nutrient investments into nucleic acids versus primary metabolic pathways (Kelly, 2018; Faizullah *et al.*, 2021). Many plants with larger GS have lower growth (Wyngaard *et al.*, 2005; Beaulieu *et al.*, 2008) and photosynthesis (Herben *et al.*, 2012; Roddy *et al.*, 2020) rates, but these findings are not ubiquitous (Chen *et al.*, 2021; Ulum *et al.*, 2021; Westoby *et al.*, 2021), indicating that GS-dependent metabolic trade-offs might also depend upon nutrient supplies. For example, several studies have found that organisms with smaller genomes have higher growth and/or photosynthesis rates in comparison to organisms with larger genomes only under nutrient limiting conditions (Šmarda *et al.*, 2013; Guignard *et al.*, 2016; Bales & Hersch-Green, 2019; Walczyk & Hersch-Green, 2019; Anneberg & Segraves, 2020).

Differences in organismal traits that influence access to nutrients could also influence GS-dependent nutrient constraints and/or GS-dependent metabolic trade-offs. For instance, organisms with life-history traits rendering them less limited by nutrient

availabilities, such as geophytes, have been found to have larger genomes (Veselý *et al.*, 2013), implying less dependency on environmental nutrient supplies for genome material costs. Furthermore, organisms that display higher nutrient-use efficiencies (i.e., are better able to access and utilize nutrients for important processes) are expected to be more tolerant of nutrient scarcities and faster growing plants have been shown to be more efficient at using N for photosynthesis (Robinson *et al.*, 2001; Hikosaka, 2004). While it is not known whether GS directly influences nutrient-use efficiencies, organisms that are less efficient at using N for photosynthesis (i.e., require more N to fix CO_2) experience stronger selection pressures to reduce N elemental costs in transcripts compared to plants that do not require as much N to fix CO_2 (Kelly, 2018; Majda *et al.*, 2021). Cumulatively, these findings suggest that differences in nutrient-use requirements and efficiencies amongst organisms varying in GS might also influence material cost constraints, patterns of selection on GS, and GS-dependent metabolic function trade-offs.

Studies exploring the relationships between GS, material costs, metabolic processes, and potential mechanisms to reduce material costs are needed to better understand the roles of plant GS in ecological and evolutionary dynamics. Here, we grew diploid, tetraploid, and hexaploid cytotypes of *Solidago gigantea* (Giant Goldenrod) in a greenhouse under varying N and P availabilities to address five hypotheses: (**H1**) Genome downsizing has occurred in this autopolyploid series, potentially indicating selection to reduce material costs. (**H2**) Plants with larger genomes have greater material costs. (**H3**) Plants regulate their transcriptome sizes dependent upon GS and nutrient availability. Specifically, we predicted that plants with smaller genomes would have relatively larger transcriptomes than plants with larger GS, but that discrepancies

amongst cytotypes would be less apparent as nutrients became more plentiful (a relaxation of material cost constraints). (**H4**) Plants adjust their photosynthesis rates to better withstand nutrient poor conditions dependent upon GS. Specifically, we predicted that plant photosynthesis rates would be (a) higher under nutrient enrichments, (b) lower for cytotypes with larger genomes (due to GS-material-cost-photosynthesis tradeoffs), and (c) more dissimilar among cytotypes under nutrient limiting conditions because larger GS organisms would be more constrained by material costs. Furthermore, to maintain optimal photosynthetic functioning, plants must strategically distribute N and/or P into the rate of carboxylation of RuBP by RUBISCO during the Calvin-Bensons cycle (V_{cmax}) and/or the rate of RuBP regeneration via the election transport chain (J_{max}). Therefore, we also predicted that tradeoffs in investments to V_{cmax} versus J_{max} may also occur under low N or P. (**H5**) Plants vary in terms of nutrient-use efficiencies dependent upon GS and cytotypes with larger genomes displaying increased nutrient use efficiencies under nutrient limitations, thereby minimizing increased GS-material costs.

2.3 Methods

Experimental Design — For this research we used *Solidago gigantea* Aiton (Asteraceae; Giant Goldenrod) - a perennial, aster native to North America that has three spatially segregated cytotypes: diploid (2n = 2x = 18) populations grow along the Atlantic coast, tetraploid populations (2n = 4x = 36) grow within the Great Lakes region, and hexaploid populations (2n = 6x = 54) grow within the Great Plains region (Schlaepfer *et al.*, 2008; Hull-Sanders *et al.*, 2009). Polyploidy in *S. gigantea* is thought to have arisen via autopolyploidy (Beck and Semple, unpublished data). During the summers of 2017 and 2019, we collected seeds and leaves from 21 wild populations covering *S. gigantea*'s range and determined the ploidy level (using a modified flow cytometry method of Verloove *et al.*, 2017; Methods S1) of 528 maternal plants to select populations with minimal intrapopulation ploidy variation for subsequent experimentation.

Next, we germinated 240 seeds from different half-sibling maternal lines from three diploid, four tetraploid, and three hexaploid populations in 2L round pots containing a 50:50 mixture of vermiculite to Sun Grow Mix 1 potting soil (Sun Grow Horticulture, Agawam, Massachusetts, USA) in a greenhouse at Michigan Technological University (Department of Biological Sciences, Houghton, Michigan, USA). At 8 weeks growth, we randomly assigned plants to one of four N:P treatments (low:low, low:high, high:low, high:high, N = 60 plants per treatment); low and high treatments were based on the range of soil N and P measured at seed collection sites (see Walczyk & Hersch-Green, in press). The potting soil contained 110 ppm N ($\mu g\ N \cdot g^{-1}$) and 25 ppm P ($\mu g\ P \cdot g^{-1}$)

which we designated respectively as the low N and P treatments, and we added nutrients to the high treatments such that they contained 165 ppm N (μg N · g^{-1}) or 37.5 ppm P (μg P · g^{-1}) for the high N and P treatments, respectively. Treatments were administered as 20 mL solutions of ammonium nitrate (high N), potassium monophosphate (high P), and/or water (low N and low P); all pots received 40 mL total of these solutions plus 20 mL of a potassium sulfate (100 ppm; μg K · g^{-1}) and micronutrient (Fertilome chelated liquid iron and other micronutrients; Voluntary Purchasing Groups, Bonham, Texas, USA) solution. Pots were rotated weekly, and the experiment concluded after 19 weeks of growth. Two plants died during the experiment and were excluded from subsequent analysis.

Measured Traits — We measured traits associated with GS, material costs, photosynthesis, and nutrient-use efficiency to test for evidence of material cost-mediated responses.

Genome Size: We estimated plant holoploid GS (2C DNA content; total amount of DNA in replicated chromosome sets) using flow cytometry methods (Methods S1); the 2C DNA content of eight plants could not be determined due to low quality histogram peaks (N = 3 diploids, N = 3 tetraploids, N = 2 hexaploids; Doležel *et al.*, 2007). Plant monoploid GS (1C DNA content; total amount of DNA in un-replicated chromosome sets; Greilhuber *et al.*, 2005) was estimated by dividing the holoploid GS by plant ploidy level (2, 4, or 6 for diploids, tetraploids, or hexaploids, respectively).

Cell Density: We estimated the average number of cells per mg leaf tissue for each cytotype on a subset of leaves of the same ontogeny as those used in DNA extractions (see below) following methods described by Brown and Rickless (1949). Briefly, a 2cm diameter leaf punch was taken from 52 plants (N = 12 diploids, N = 27 tetraploids, N = 18 hexaploids), weighed, digested in chromic acid, and then the number of cells in a 10uL aliquot was counted on a hemocytometer to estimate cell density per milligram leaf tissue.

Foliar Nutrients: We quantified [N] and [P] foliar contents by first grinding and homogenizing three leaves from two individuals of the same maternal line collected at the 15th week of growth for each cytotype by nutrient combination. To determine [N] per milligram tissue, approximately 5mg of each sample was analyzed on an elemental analyzer (Costech Analytical Technologies Inc., Valencia, California, USA) at the University of Minnesota (N = 37 diploids, N = 39 tetraploids, N = 40 hexaploids). To determining [P] per milligram tissue, approximately 250mg of 65 randomly selected samples (N = 19 diploids, N = 29 tetraploids, N = 17 hexaploids) were digested with nitric acid and hydrogen peroxide (Masson *et al.*, 2010), and analyzed on a Thermo 6500 Duo Inductively Coupled Plasma Spectrometer (Thermo-Fischer Scientific, Waltham, Massachusetts, USA) at Brookside Laboratories Inc. (New Bremen, Ohio, USA).

Biomass: Plants were divided into their above- and belowground parts, dried at 60°C for 48hr (aboveground) or 72hr (belowground) and weighed to the nearest gram.

RNA and DNA: We harvested three leaves from the youngest set of fully mature leaves from 60 individual plants (20 plants per cytotype within the LL and HH treatments) and immediately weighed, froze in liquid nitrogen, and transferred them to a -80°C freezer. Total DNA (from 1 leaf) and total RNA (from 2 leaves to account for the inherent variability of RNA) was then extracted using Qiagen DNeasy Plant Pro and RNeasy Plant Extraction kits (QIAGEN, Hilden, Germany) following manufacturer's instructions. DNA and RNA content in pg per mg leaf tissue was quantified with a Qubit Fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA); in total, nine RNA extractions failed.

Stomata: The density and size of leaf stomata influences gas exchange and photosynthesis rates and generally plants that have more and smaller stomata per leaf area have higher rates of these two processes (Knight & Beaulieu, 2008; Roddy *et al.*, 2020). To assess whether stomata characteristics of *S. gigantea* cytotypes are indicative of photosynthesis rates, we made stomata casts by applying a thin layer of clear nail polish to the lower surface of 122 fully developed leaves (N = 40 diploids, 41 tetraploids, 41 hexaploids). The nail polish casts dried for two hours, were peeled from the leaf, and then mounted onto a microscope slide where they were examined with an Olympus light microscope (Olympus Corporation, Shinjuku, Tokyo, Japan) to calculate average stomata size (μm^2, guard cell length multiplied by width of four randomly selected stomata at 1000x total magnification) and density (number of stomata present in the field of view at 400x total magnification; Hull-Sanders *et al.*, 2009; Walczyk & Hersch-Green, in press).

Photosynthetic Rates: We used a portable infrared CO_2 analyzer system (LI-6800; LI-COR Inc., Lincoln, NE, USA) equipped with a CO_2 mixer and 1x3 cm^2 chamber/red-blue LED light source (LI6800-02) to measure three properties of photosynthesis: net carbon assimilation rate between CO_2 fixation and photorespiration (A_{max}, μmol CO_2 m^{-2} s^{-1}), the maximum rate of RuBP carboxylation ($V_{cmax,}$ $\mu mol \cdot m^{-2} \cdot s^{-1}$), and the maximum rate of electron transport that regenerates RuBP ($J_{max,}$ $\mu mol \cdot m^{-2} \cdot s^{-1}$). Measurements were taken in a random order over three days during the 12th week of growth between the hours of 09:00 to 16:00. Inside the chamber, CO_2 concentration was set 400 ppm, relative humidity to 65%, flow rate to 500 μmol m^{-2} s^{-1}, and light to 1000 μmol m^{-2} s^{-1}. Because measurements were taken inside a temperature-controlled greenhouse, we did not control for temperature within the chamber. Measurements were taken once photosynthetic rate stabilized, and infrared gas analyzers (IRGA) were matched after every 10 sampling measurements.

All measurements were made on leaves from the youngest set of fully mature leaves on a plant at either the 12th (A_{net}) or 13th ($V_{cmax,}J_{max}$) week of growth. A detailed description of methods is in Methods S2. However, briefly A_{net} was measured on 232 plants by taking a snap-shot survey measurement on leaves once they acclimated to chamber conditions. V_{cmax} and J_{max} were estimated from 120 plants (10 plants randomly chosen from each cytotype by nutrient treatment combination) by creating photosynthetic CO_2 response curves (A-Ci curves) and then extracting V_{cmax} and J_{max} values with *tidyverse* and *plantecophys* packages (Duursma, 2015) in R version 4.1.2 (R Core Team,

Vienna, Austria); 18 A-Ci curves were flagged by *plantecophys* for low quality (Duursma, 2015) and were not included in subsequent analyses.

Foliar Terpenes: Terpenes are carbon-based compounds that serve a variety of functions within a plant, including defense against antagonists (Ninkuu et al., 2021). In addition to being a large carbon-sink, terpene biosynthesis also requires N and P for terpene synthase activity and the diphosphates required to synthesize the various carbon skeletons characterizing the different terpene classes (Bohlmann, Meyer-Gauen, and Croteau, 1998; Bustamante et al., 2020).

To measure foliar terpene concentrations, we first harvested leaves of the same ontology (youngest fully developed leaf on a plant) from a total of 65 plants (N = 23 diploid, N = 22 tetraploid, N = 20 hexaploid) of randomized maternal lines originating from 11 separate populations (N = 3 diploid, N = 4 tetraploid, N = 2 hexaploid), and grown in low NP or high NP conditions. The wet mass of collected leaves were weighted to the nearest milligram, flash frozen with liquid nitrogen, and then stored in a -80°C freezer. Terpene extractions followed the methods of Hull-Sanders et al. (2009), in which frozen leaves were ground to a find powder and immediately transferred to a glass vial containing 6 mL of 70% hexane-30% ethyl acetate and 0.26mg of the internal standard nonadecane. The contents of the vials were centrifuged for 10min at 3000 RPM and reduced to a 3 mL volume under a gentle stream of ultra-high purity N_2 has using an Organomation 12-position nitrogen evaporator (Organomation, Berlin, Massachusetts, USA). We then transferred 1.5mL of the concentrated sample to scintillation vials for analysis via gas chromatography-mass spectrometry (GC-MS).

Terpene concentrations of the 65 plants were quantified with a Trace 1310 Gas Chromatograph coupled with an ITQ 1100 Ion Trap MS (ThermoFischer Scientific, Waltham, Massachusetts, USA) at the Great Lakes Research Center at Michigan Technological University using the method of Hull-Sanders et al. (2009). Compounds were separated using a Rtx-5MS (30m x 0.25mm; DF = 0.25um) Low-Bleed GC/MS column (fused silica; Restek. Bellefonte, Pennsylvania, USA) with the injector temperature set to 250°C. Chemical analysis of three technical replicates per concentrated leaf sample was run at an initial oven temperature of 35°C held constant for three minutes before being increased to 280°C at the rate of 10°C per minute. Terpene peaks were grouped by retention time and identified using the NIST2000 software database and Mass Spectrometry Data Center (Chemdata.nist.gov) and then cross-checked against other studies reporting the retention times and identification of terpenes in *Solidago* species (Johnson, Hull-Sanders, and Meyer, 2007; Hull-Sanders et al., 2009; Dobjanschi et al., 2019). We measured total terpene concentrations for mono-, di-, and sesquiterpenes by first averaging the concentration values for each terpene across the three technical replications and then summing the concentration values for each terpene group within each individual sample. A total of 7 samples (N = 1 diploid, N = 2 tetraploid, N = 4 hexaploids) were excluded from the final dataset due to low quality peaks on the GC-MS.

Statistical Analyses — In all analyses, we used analysis of variance (ANOVA) models with a combination of cytotype (2x = diploid, 4x = tetraploid, 6x = hexaploid), soil NP treatment (L = Low N and P, H = High N and P), N-treatment (Low N, High N), P-treatment (Low P, High P), and/or population of origin (nested within ‘cytotype’)

specified as fixed-effect independent variables. Model assumptions of normality and homoscedasticity were examined and, if needed, data transformations were made to meet model assumptions. To compare for significant differences among means: (1) when a single factor was found to be significant, we used post-hoc Tukey's HSD or Student's t tests (for comparisons among ≥3 or 2 means, respectively) and (2) when a significant interaction among factors was found we used controlled contrast tests. All statistical analyses were performed using JMP Pro version 16 (SAS Institute, Cary, North Carolina, USA).

Genome Size: Separate ANOVA models were used to test if the holoploid (N = 231) and monoploid (N = 231) genome sizes significantly differed amongst cytotypes and/or populations of origin.

Material costs: We examined material costs in several ways. First, we examined them as N or P investments per mg tissue, per cell ([N] or [P] per milligram tissue divided by cell density per milligram tissue), and per aboveground biomass ([N] or [P] per milligram tissue multiplied by aboveground biomass) with six separate ANOVA models using cytotypes, N-treatments, P-treatments, their interactions, and a plant's population of origin as factors (N = 116 for [N], N = 65 for [P]). Next, because plants that are more limited by nutrients are thought to respond more strongly to nutrient additions (Boyer, 1982), we also assessed material costs indirectly by examining growth responses to nutrient enrichments. To calculate growth responses to nutrient enrichments, we used the general formula:

$$Growth\ response\ to\ nutrient\ enrichment = \frac{(Combined\ biomass\ in\ high\ treatment - Combined\ biomass\ in\ low\ treatment)}{Average\ combined\ biomass\ across\ low\ and\ high\ treatments}.$$

Growth responses to nutrient enrichments were calculated separately for N and P and for above- and belowground biomass responses (N=10 per cytotype per nutrient for both above- and belowground) and combined biomasses were determined by taking the average of two individuals grown from the same maternal line within a given treatment. Four separate ANOVA models were used to assess whether cytotypes significantly differed in above- and/or belowground N and/or P growth responses.

Relative Transcriptome Size: We measured relative transcriptome size (RNA/DNA) in two ways. First, as "relative transcriptome size per mg leaf tissue" by dividing the concentration of RNA by the concentration of DNA for each RNA extraction replicate (N = 111). Second, as a measure of the "relative transcriptome size per cell" by first determining the concentration of RNA per cell (pg/cell; using the average cell density per milligram of tissue per cytotype) and then dividing this value by cell 2C DNA content (N=111). Next, we examined if relative transcriptome size per mg leaf tissue and/or per cell (both Ln (value)+1 transformed) significantly differed amongst cytotypes, soil N:P treatments, their interaction, and/or a plant's origin with separate ANOVA models (final N = 110 because an outlier was removed).

Photosynthetic Activity: We assessed whether differences in stomata size and density (N = 162) significantly differed between cytotypes and/or plants from different populations using two using ANOVA models. Next, we used three separate 3-way ANOVA models

to examine whether A_{max} (N = 232), Vc_{max} (N = 102), and/or J_{max} (N = 102) significantly differed amongst cytotypes, N-treatments, P-treatments, their interaction, and/or a plant's origin. All 3-way interactions were significant (see Results), and therefore to better tease apart these interactions we used additional ANOVA models to examine whether A_{max}, Vc_{max}, and/or J_{max} significantly differed amongst cytotypes, N-treatments, and/or a plant's origins separately for low and high P-treatments.

Nutrient Use Efficiency: We measured nutrient use efficiencies in two ways. First, we calculated metrics of efficiency that describe how good plants are at converting soil nutrients into biomass (N-use efficiency and P-use efficiency) as the product of nutrient uptake efficiency (processes associated with nutrient uptake and transportation calculated as [N or P] in aboveground biomass / [N or P] nutrient in soil) and nutrient utilization efficiency (processes associated with nutrient assimilation and redistribution calculated as aboveground biomass / [N or P] in the aboveground biomass; Moll *et al.*, 1982; Islam *et al.*, 2021). Second, we calculated metrics of efficiency that describe how well plants use nutrients to fix CO_2 as photosynthetic nitrogen (P_{NUE}) and photosynthetic phosphorus (P_{PUE}) use efficiency by dividing the averaged A_{net} rates of the plants combined for N or P content by [N or P] per mg of leaf tissue. ANOVA models were then used to assess whether cytotypes, N-treatments, P-treatments, their interactions, and/or a plant's origin significantly differed in N-use efficiency (N = 117), P-use efficiency (N = 65), P_{NUE} (N = 117), and P_{PUE} (N = 65). All 3-way interactions were significant (see Results) and therefore to better tease apart these interactions we used additional ANOVA models to

examine whether P_{NUE} and P_{PUE} significantly differed amongst cytotypes, N-treatments, and/or a plant's origins separately for low and high P treatment.

Foliar Terpene Concentrations: Separate ANOVA models were used to test if the total, mono-, sesqui-, and di-terpenes (N = 58; all log-transformed) significantly differed amongst cytotypes, NP treatments (low and high only), their interaction, and/or populations of origin. Two diploid outliers were removed from these analyses.

2.4 Results

Do cytotypes vary in genome sizes? – Holoploid and monoploid GS both significantly differed amongst cytotypes but the patterns were different (Table 1). Specifically, hexaploids have the largest, tetraploids intermediate, and diploids the smallest holoploid GS (LS Means ± 1SE: 2x= 1.99 ± 0.01 pg, 4x= 3.55 ± 0.02 pg, 6x= 5.37 ± 0.01 pg; Table 1), whereas diploids have larger monoploid GS than both tetraploids and hexaploids, which do not significantly differ from each other (LS Means ± 1SE: 2x= 0.99 ± 0.003 pg, 4x= 0.88 ± 0.003 pg, 6x= 0.89 ± 0.004 pg; Table 1).

Do plants with larger genomes have greater material costs? – The significant effects of cytotype, nitrogen-treatment, phosphorus-treatment, and their interactions varied across the levels of [N] and [P] per cell, tissue, and aboveground biomass (Table 2, Fig. **1**). Plants tended to have the most [N] per cell, tissue, and aboveground biomass when soil nitrogen was high and phosphorus was low (Table 2; Fig. **S1**). Plants from different populations significantly differed in [N] and [P] per milligram of tissue and per cell, and cytotypes also significantly differed in [N] per cell and aboveground biomass and [P] per cell (Table 2; Fig. **1c-f**). In general, hexaploids had more, tetraploids intermediate, and diploids had less [N] and [P] per cell (Fig. **1a,b**). Hexaploids also had significantly more [N] per aboveground biomass than diploids, (Fig. **1e,f**), but cytotype differences in [P] per aboveground biomass also depended on N or P treatment (Table 2). Specifically, when N was low or P was high, hexaploids had signifncantly the most [P] per aboveground biomass (controlled contrasts for mean [P] per aboveground biomass in low N: $F_{2,46}$= 6.56, *P*= 0.0034; high P: $F_{2,46}$=6.09, *P*= 0.0045; Fig. S2), but cytotpyes showed

no significant differences when N was high or P was low (controlled contrasts for mean [P] per aboveground biomass in high N: $F_{2,46}$= 0.03, *P*= 0.9705; low P: $F_{2,46}$=0.60, *P*= 0.5541; Table 2; Fig. **S2**).

Cytotypes also showed different aboveground and belowground growth responses to N addition but not to P addition (Table 3), with tetraploids exhibiting significantly greater N-enrichment growth than both diploids and hexaploids, which did not significantly differ from each other (Fig. **2**).

Do plants regulate their transcriptome size dependent upon GS and nutrient availability? – Relative transcriptome sizes per mg leaf tissue and per cell significantly differed amongst cytotypes (Table 4). Specifically, per mg leaf tissue, diploids had significantly larger relative transcriptome sizes than tetraploids, and hexaploids had intermediate sizes that did not significantly differ from the other two cytotypes (untransformed LS Means ± 1SE: 2x= 1.13 ± 0.26, 4x= 0.39 ± 0.26, 6x= 0.42 ± 0.27), while per cell, diploids had significantly larger transcriptome sizes than both tetraploids and hexaploids (untransformed LS Means ± 1SE: 2x= 21.84 ± 3.47, 4x= 11.06 ± 3.52, 6x= 7.72 ± 3.65). Plants grown under high N:P conditions also had significantly larger relative transcriptome sizes per mg tissue than plants grown under low N:P conditions (untransformed LS Means ± 1SE: high N:P= 0.90 ± 0.21, low N:P= 0.39 ± 0.20; Table 4). No other factors nor interactions among factors significantly affected relative transcriptome sizes per mg leaf tissue or per cell.

Do photosynthesis rates vary amongst cytotypes dependent upon nutrient treatments? –Cytotypes significantly differed in average foliar stomatal characteristics with diploids having many small stomata, hexaploids having few large stomata and tetraploids, in comparison, having intermediate sized and density of stomata (LS Means ± 1SE for stomata density: 2x= 66.86 ± 1.56; 4x= 39.70 ± 1.56; 6x= 25.52 ± 1.72; for size: 2x= 440.62 ± 16.52 μm^2; 4x= 586.70 ± 16.58 μm^2; 6x= 723.39 ± 18.27 μm^2; Table S1). Whether cytotype differences in stomatal characteristics were indicative of photosynthesis rates is not clear. For instance, although diploids consistently showed higher rates of maximum photosynthesis (A_{max}), Rubisco carboxylase activity (Vc_{max}), and electron transport during photosynthesis (J_{max}) than the polyploids, such differences were dependent upon N and P (Table S2). To tease apart these interactions, we used additional ANOVA models separately for low and high P-treatments.

Under low P-treatments, cytotype and N-treatment differences in A_{max} depended upon each other and varied amongst population of origin (Table 5). In general, diploids photosynthesized at significantly greater rates than the polyploids when grown under low but not under high N-conditions (A_{max} LS Means ± 1SE under low N: 2x= 10.24 ± 0.77; 4x= 4.83 ± 0.77; 6x= 6.12 ± 0.86; under high N: 2x= 10.58 ± 0.77; 4x= 9.32 ± 0.77; 6x= 8.16 ± 0.87; Fig. **3a**). Furthermore, although plants generally had higher A_{max} under high versus low N-treatments such differences were only significant for tetraploids (Fig. **3a**). Under low P-treatments, plants grown in high N-treatments also had significantly higher Vc_{max} and J_{max} then plants grown in low N-treatments (LS means ± 1SE for Vc_{max}: low N = 31.59 ± 1.92, high N = 39.00 ± 1.93; for J_{max}: low N = 60.71 ± 3.22; high N = 76.38 ±

3.23 μmol·m^{-2}·s^{-1}), although for J_{max} such differences were only significant for hexaploids (Table 5; Fig. **3c,e**). Under high P-treatments, there were significant differences in $A_{max,}$ Vc_{max}, and J_{max} amongst plants from different populations (albeit marginally for Vc_{max}) and cytotypes (Table 5). In general, diploids had greater A_{net} rates than tetraploids and hexaploids had intermediate A_{net} rates that did not significantly differ from the other two cytotypes (Fig. **3b**). Furthermore, both diploids and hexaploids had significantly higher rates of Vc_{max} and J_{max} than tetraploids and they did not differ from each other (Fig. **3b,d,f**). No other factors nor interaction among factors significantly affected A_{max}, Vc_{max}, and J_{max} values.

Do nutrient use efficiencies vary amongst cytotypes dependent upon nutrient treatments? - Nutrient-use efficiencies describe how efficient plants are at converting soil nutrients into biomass. N-use efficiencies differed amongst plants grown in low and high N-treatments dependent upon cytotype or P-treatments (Table 6). Specifically, plants used N significantly more efficiently when grown under low versus high N-conditions only when they were also were grown under high P-conditions (Fig. **4a**). When plants were grown under low but not high N-conditions, hexaploids also used N significantly more efficiently than diploids and tetraploids (Fig. **4b**). P-use efficiencies significantly differed amongst plants grown in low and high P-treatments but such differences were also dependent upon N-conditions and a plant's cytotype (Table 6). Specifically, plants more efficiently converted P into biomass when grown under low versus high P-treatments but only when they were also grown under high N-conditions (Fig. **4c**). Furthermore, when plants were grown under low N-conditions, hexaploids

used P significantly more efficiently than diploids and tetraploids (Fig. **4d**). No other factors nor interaction among factors significantly affected N-use and P-use efficiencies.

Photosynthetic nutrient-use efficiencies describe how efficient plants are at using nutrients to fix CO_2. Because differences in photosynthetic N-use and P-use efficiencies were dependent upon 3-way interactions between cytotype and nutrient treatments (Table S3), we used additional ANOVA models separately for low and high P-treatments to tease apart these interactions. Under low P-treatments, cytotypes significantly differed in their abilities to use N to fix CO_2 and this significance also depended upon N-availability (Table 7, Fig. S3), with tetraploids requiring significantly more N to fix CO_2 (e.g., lower P_{NUE} values) when grown in low versus high N conditions (controlled contrasts for mean P_{NUE} under high versus low N-treatments for 2x: $F_{1,45}$= 3.39, P= 0.0721; for 4x: $F_{1,45}$= 9.18, P= 0.0040; for 6x: $F_{1,45}$= 1.10, P= 0.2991). Under high P-treatments, differences in P_{NUE} depended upon only cytotype and population of origin (Table 7, Fig. S3). Specifically, diploids had significantly greater P_{NUE} values than tetraploids; hexaploids were intermediate and did not significantly differ from the other two cytotypes (Tukey's HSD analysis; LS Means ± SE for P_{NUE} for 2x= 152.68 ± 10.02; 4x= 99.00 ± 9.59; 6x= 120.33 ± 11.70).

Cytotypes differed in their abilities to use P to fix CO_2 (P_{PUE} values) but significance depended upon N-treatment under low P-treatments (Table 7, Fig. S3). While P_{PUE} values of diploids and hexaploids did not vary significantly depending upon soil N, tetraploids had significantly smaller P_{PUE} values (were less efficient) when grown in low versus high N-treatments (controlled contrasts for mean P_{PUE} under high versus

low N-treatments for 2x: $F_{1,18}$= 0.52, *P*= 0.4796; for 4x: $F_{1,18}$= 14.28, *P*= 0.0014; for 6x: $F_{1,18}$= 0.42, *P*= 0.5238; Table 7, Fig. S3). No other factors nor interactions among factors significantly affected P_{NUE} or P_{PUE} values (Table 7).

Do foliar terpene concentrations differ amongst cytotypes dependent upon nutrient availability? - Foliar concentrations of total terpenes, monoterpenes, and sesquiterpenes significantly differed amongst cytotypes (Table 8); diploids had significantly more of these foliar terpene concentrations than tetraploids and hexaploids, which did not differ from each other (LS Means ± SE for total terpenes for 2x= 7.43 ± 0.77; 4x= 3.35 ± 0.76; 6x= 1.56 ± 1.31 mg/mg tissue; for monoterpenes for 2x= 6.89 ± 0.73; 4x= 3.09 ± 0.71; 6x= 1.45 ± 1.23 mg/mg tissue; for sesquiterpenes for 2x=0.48 ± 1.46; 4x= 0.20 ± 0.14; 6x= 0.09 ± 0.25 mg/mg tissue; Table 8). Plants grown under low NP conditions also had significantly more foliar total terpenes and monoterpenes than plants grown in high NP conditions (LS Means ± SE for total terpenes for low NP= 5.12 ± 0.76; high NP= 3.10 ± 0.71 mg/mg tissue; for monoterpenes for low NP= 4.89 ± 0.71; high NP= 2.73 ± 0.67; mg/mg tissue; Table 8). No model factors or interactions among factors significantly affected foliar diterpene concentrations (Table 8).

2.5 Discussion

The preponderance of small genomes in flowering plants despite the fact that GS varies immensely across members of this group (Dodsworth *et al.*, 2015) is puzzling. While traditional explanations of selection for functional genomic attributes (Mei *et al.*, 2018) and genomic rearrangement and deletions to promote stability (Wang *et al.*, 2021) likely explain most instances of genome downsizing, selection to reduce the N and P material costs of building genomes might also contribute (Lewis, 1985; Leitch & Bennett, 2004; Cavalier-Smith, 2005; Mei *et al.*, 2018; Faizullah *et al.*, 2021). Research showing that plants with smaller genomes are selectively favored in nutrient limiting environments (Šmarda *et al.*, 2013; Guignard *et al.*, 2016; Bales & Hersch-Green, 2019; Walczyk & Hersch-Green, 2019; Anneberg & Segraves, 2020), that resource strategies vary dependent upon GS (Bales & Hersch-Green, 2019; Wu *et al.*, 2019; Forrester, Nicole J. *et al.*, 2020), and that nutrient limiting growth environments can impose selection on genomes and transcriptomes towards material cost conserving substitutions (Acquisti *et al.*, 2009a; Acquisti *et al.*, 2009b; Kelly, 2018; Majda *et al.*, 2021), all lend support for this hypothesis. However, it is still not known whether genome material costs at cellular, tissue, and/or whole plant levels increase with GS, thereby imposing stronger selective constraints on organisms with larger genomes. Here, we examined diploid and autopolyploid *S. gigantea* plants grown under varying NP soil conditions and found evidence that N and P cellular and, to a lesser degree, N whole-plant material costs scale positively with GS, but that some traits and resource strategies likely minimize material cost constraints. We discuss these findings in terms of how GS-dependent material costs

might influence evolutionary and ecological dynamics specifically within *S. gigantea* and more generally within flowering plants.

Evidence that material costs increase with GS – Several lines of evidence indicate that material costs likely increase with GS in the *S. gigantea* polyploid complex. First, we found that cellular [N] and [P] increased with increasing GS from diploids to tetraploids to hexaploids (Table 2, Fig. 1), presumably due to the increased elemental costs of synthesizing more DNA (Sterner & Elser, 2002; Elser *et al.*, 2011) and longer phospholipid membranes associated with enlarged cells (Leitch & Bennett, 2004; Cavalier-Smith, 2005; Roddy *et al.*, 2020). Second, although at the tissue level we did not find that [N] and [P] per milligram differed among cytotypes, tetraploids and hexaploids were larger than diploids. When we account for these differences, we found that aboveground [N] significantly and aboveground [P] marginally increased with GS (Table 2, Fig. **1**). Lastly, it is generally thought that plants which are more limited by a resource experience the greatest growth benefits from that resource being added (Boyer, 1982). Therefore, due to our predicted positive relationship between GS and N and P material costs, we also expected to find a similar positive relationship between GS and growth responsiveness to nutrient enrichments (Lewis, 1985; Leitch & Bennett, 2004; Cavalier-Smith, 2005; Faizullah *et al.*, 2021). Although we found that all plants tended to grow more under nutrient enrichments, tetraploids were the most responsive to N-enrichment (Table 3, Fig. **2**); a finding that indirectly suggests that tetraploids are more limited by N then diploids and hexaploids. Several studies report similar findings of diploids, or plants with smaller genomes, showing less responsiveness to nutrient enrichments than

polyploids or plants with larger genomes (Šmarda *et al.*, 2013; Guignard *et al.*, 2017; Bales & Hersch-Green, 2019; Walczyk & Hersch-Green, 2019; Anneberg & Segraves, 2020).

Given our rationale, it was at first surprising that hexaploids, plants with the largest GS, were not as or more responsive to N enrichment as the tetraploids. While the reasoning for this us unknown, the greater ability of hexaploids to more efficiently turn nutrients into biomass (high N and P-use efficiencies) in low N conditions (Table 6; Fig. **4**), may have contributed to their more muted growth responses to N-enrichment as they were able to produce large aboveground biomasses in both low and high N-treatments. Differences amongst cytotypes in nutrient acquisition strategies might have also contributed to cytotype growth responses to nutrients and several studies have found that polyploids had increased microbial (Wu *et al.*, 2019; Forrester, Nicole J *et al.*, 2020) and arbuscular mycorrhizal fungi (Anneberg & Segraves, 2019) associations relative to closely related diploids. Although we did not examine whether rhizospheric changes induced by polyploidization similarly influence *S. gigantea* cytotype nutrient-exchange networks, a post-hoc analysis of N-uptake efficiency (a measure of the N in a plant relative to N in soil) shows that under low N inputs, hexaploids were the most efficient at incorporating N into plant tissues (Table S4). Furthermore, differences in monoploid GS (i.e., chromosome length; Greilhuber *et al.*, 2005) among cytotypes may also have contributed to the muted growth responses of hexaploids to N-enrichment. For example, while both holoploid and monoploid GS have been found to be positively correlated with size-related traits and negatively correlated with cell division and growth-related rates

(Wyngaard *et al.*, 2005; Beaulieu *et al.*, 2008), homoploid GS has been more strongly correlated with the former and monoploid GS with the later (Rhind & Gilbert, 2013; Suda *et al.*, 2015). Thus, while organisms with larger genomes typically grow slower (Wyngaard *et al.*, 2005; Beaulieu *et al.*, 2008), downsizing of the monoploid-genome might counteract the extra time it typically would take to replicate and organize extra chromosome copies during cell division. Therefore, the muted growth responses of hexaploids relative to tetraploids to N-enrichment may have arisen, in part, due to changes in monoploid relative to holoploid GS. For instance, a downsized monoploid genome should be correlated with faster cell division rates and responsiveness to nutrients for both tetraploids and hexaploids, but hexaploids with larger holoploid GS would be more constrained by material costs. In addition, faster cell division rates associated with smaller monoploid GS might have been offset by the additional time it would take to replicate, check, and repair the six-replicates of each hexaploid chromosome during the S and G2 phases of cell division.

Genetic mechanisms might minimize material cost constraints - Greater material costs should theoretically translate into reduced plant growth and competitive success, but various genetic mechanisms that act over long- and short-evolutionary time periods may help offset the higher GS-material costs of polyploids, thereby influencing evo-ecological dynamics. For example, over long-evolutionary time periods, genome downsizing can reduce genomic and cellular material costs (Wang *et al.*, 2021; Table 1) and we found evidence for genome downsizing in *S. gigantea* (i.e., polyploids had smaller monoploid genomes than diploids, Table 1). Interestingly, despite hexaploids

having roughly 1.5 times more holoploid genomic material than tetraploids, monoploid GS did not differ between these polyploids (Table 1). Other studies have reported more extreme genome downsizing in higher ploidy levels (Leitch *et al*. 2004; Wang *et al*. 2021) and we suspect that this more extreme genome downsizing of hexaploids is in response to greater genomic material costs and selective constraints. Over short evolutionary time periods, reductions in transcriptome size (i.e., lower gene expression) could also help minimize realized material costs and reduce selective constraints. For example, the transcriptome is an especially demanding N and P sink in a cell (Raven, 2013), but its material costs vary depending upon the number of transcripts expressed at any given time. Therefore, organisms may reduce/offset their total material costs by reducing their overall gene expression (Faizullah *et al.*, 2021; Wang *et al.*, 2021), and several studies have reported that organisms experiencing resource limitations have smaller transcriptomes (Wu *et al.*, 2004; Forieri *et al.*, 2017; Kelly, 2018; Majda *et al.*, 2021). In support of this hypothesis, we found that 1) relative transcriptome size (RNA/DNA) per mg tissue and per cell were significantly greater in diploids relative to the polyploids (Table 4), 2) that relative transcriptome size per mg tissue was significantly lower for plants grown under NP limiting conditions, and 3) diploids had greater total RNA per cell than hexaploids, with tetraploid total cellular RNA being intermediate ($F_{2,109} = 3.92$, P = 0.0227; LSMean ± SE for 2x= 3.47 ± 0.11, 4x = 3.88 ± 0.11; 6x= 3.30 ± 0.12 pg). However, contrary to our expectations we did not find that polyploids showed greater transcriptome size reductions under more nutrient limiting conditions (Table 4).

Resource investment trade-offs might minimize material cost constraints – Metabolic processes that a are major N and P sinks for a plant, such as photosynthesis (Evans, 1989; Hessen *et al.*, 2010; Hohmann-Marriott & Blankenship, 2011) and the synthesis of defensive compounds (i.e., N and P are used in upstream terpene biosynthesis pathways; Bohlmann, Meyer-Gauen, and Croteau, 1998; Bustamante et al., 2020) might compete with nucleic acids for allocation of available N and P. Therefore, plants with larger GS and greater material costs might invest less into these costly processes to reduce material expenditures (Kelly, 2018; Faizullah *et al.*, 2021). We found support for this hypothesis as diploids tended to have greater photosynthetic activity than tetraploids (Table 5; Fig. **3**) and greater foliar total and monoterpene concentrations than both tetraploids and hexaploids (Table 8). Contrary to our expectations, hexaploids had similar A_{net}, V_{Cmax}, and J_{max} rates to diploids (Fig. **3**) despite having the largest genome size. This pattern might result from differing investment patterns being present among cytotypes wherein tetraploids allocate more resources into growth than photosynthesis, as evidenced by their greater growth responses to N-enrichment (Fig. **2**). Further evidence of such GS-mediated tradeoffs come from studies showing plants with larger GS to have lower growth (Wyngaard *et al.*, 2005; Beaulieu *et al.*, 2008) and photosynthesis (Herben *et al.*, 2012; Roddy *et al.*, 2020) rates, but these findings are not ubiquitous (Chen *et al.*, 2021; Ulum *et al.*, 2021; Westoby *et al.*, 2021), indicating that GS-dependent metabolic trade-offs might only be apparent under nutrient limiting conditions (Kelly, 2018; Faizullah *et al.*, 2021). In support of this, we also found that polyploids showed reduced photosynthetic activity relative to diploids when *both* N and P were limiting (e.g., grey bars in Fig. **3 a, c, e**), and greater gains in photosynthetic activity when N and P were no

longer limiting (e.g., striped bars in Fig. **3 b, d, f**). Together this suggests that polyploids may rely on mechanisms that show plasticity in the partitioning of resources between the genome and other costly factions over short periods of evolutionary time.

Conclusion – The preponderancy of small genomes in angiosperms has led to speculations of selective forces disproportionately favoring small over large genomes ((Lewis, 1985; Leitch & Bennett, 2004; Cavalier-Smith, 2005; Mei *et al.*, 2018; Simonin & Roddy, 2018; Faizullah *et al.*, 2021). Our results support this by showing that material costs increase with GS and selection has favored mechanisms to reduce these GS-dependent-material-costs across a range of evolutionary timescales. Furthermore, our results provide insight into the ecological dynamics of the *S. gigantea* cytotype complex which could be used to better understand why only the tetraploids have become noxious invaders in non-native ranges (Schlaepfer *et al*. 2008). For instance, hexaploids were more N-use efficient and photosynthetically active than tetraploids, but the greater growth responsiveness of tetraploids following nutrient enrichment might be a more adaptive trait in certain non-native habitats. Finally, our findings have implications for understanding the future ecological and evolutionary dynamics of polyploids and plant species with large genomes. Global N and P availability is increasing across terrestrial and aquatic ecosystems as a result anthropogenic activities (e.g., fertilizing practices; landscape changes; Penuelas *et al.*, 2013; Fowler *et al.*, 2015; Goyette *et al.*, 2016; Asabere *et al.*, 2018) and could result in a large-scale release from GS-mediated nutrient constraints in many plant species affecting the individual fitness and competitive

performances of species with large genomes in ways that can shift community assemblages (Šmarda *et al.*, 2013), decrease biodiversity (Borer *et al.*, 2014), and influence patterns of biological invasions (Te Beest *et al.*, 2012; Suda *et al.*, 2015; Luo *et al.*, 2019). Studies investigating the relationship between GS, material costs, and the nutrient environment have enhanced our understanding of GS evolution in angiosperms, but perhaps most importantly, they have provided a framework upon which to better predict how GS might dictate plant responses to rapidly changing global environments.

2.6 Tables

Table 1. Results from fixed-effects ANOVA models for the effects of cytotype (diploid = 2x, tetraploids = 4x, hexaploid = 6x) and population nested within cytotype on holoploid GS (2C DNA content) and monoploid GS (1C DNA content). Overall model results are reported in the footnotes, bold values indicate a significant effect at $\alpha = 0.05$, and if a factor was found to be significant, Tukey's HSD tests ($\alpha = 0.05$) were used to determine significant differences between means and patterns are indicated.

Source	df	MS	F	Prob > *F*	Tukey's HSD
Holoploid GS					
Cytotype	2	165.10	18346.60	**<0.0001**	6x>4x>2x
Population [Cytotype]	8	0.04	4.10	**0.0001**	
Model Error	231	44.81			
Monoploid GS					
Cytotype	2	0.26	441.10	**<0.0001**	2x>4x=6x
Population [Cytotype]	7	0.00	3.20	**0.0030**	
Model Error	230	0.06			

Overall model for holoploid GS: $R^2 = 1.00$, $F_{10,231} = 4970.46$, $P < 0.0001$, N = 232
Overall model for monoploid GS: $R^2 = 0.81$, $F_{9,230} = 106.78$, $P < 0.0001$, N = 232

Table 2. Results from fixed-effects ANOVA models for the effects of cytotype (diploid = 2x, tetraploids = 4x, hexaploid = 6x), nitrogen (low, high), phosphorus (low, high), their interactions, and/or population nested within cytotype on traits associated with material costs: [N] / mg tissue, [P] / mg tissue, [N] / cell, [P] / cell, [N] / aboveground biomass, and [P] / aboveground biomass. Overall model results are reported in the footnotes, bold values indicate a significant effect at $\alpha = 0.05$, and if a factor was found to be significant, Tukey's HSD tests ($\alpha = 0.05$) were used to determine significant differences between means and patterns are indicated.

Source	df	MS	F	Prob > *F*	Tukey's HSD
[N] / cell					
Cytotype	2	2.81×10^{-9}	173.49	**<0.0001**	6x > 4x > 2x
Nitrogen (N)	1	1.53×10^{-10}	7.12	**0.0089**	H > L
Phosphorus (P)	1	7.49×10^{-11}	4.63	**0.0340**	L > H
C x N	2	1.01×10^{-11}	0.62	0.5383	
C x P	2	6.08×10^{-13}	0.04	0.9632	
N x P	1	4.81×10^{-10}	29.71	**<0.0001**	HL > HH=LH=LL
C x N x P	2	1.72×10^{-11}	1.06	0.3498	
Population [Cytotype]	7	9.59×10^{-11}	5.93	**<0.0001**	
Model Error	97	1.62×10^{-11}			
[P] / cell					
Cytotype	2	4.72×10^{-11}	57.41	**<0.0001**	6x > 4x > 2x
Nitrogen (N)	1	5.45×10^{-14}	0.07	0.7997	
Phosphorus (P)	1	1.01×10^{-14}	0.01	0.9121	
C x N	2	1.06×10^{-11}	1.29	0.2837	
C x P	2	1.30×10^{-13}	0.16	0.8545	
N x P	1	1.02×10^{-12}	1.24	0.2718	
C x N x P	2	6.42×10^{-13}	0.78	0.4636	
Population [Cytotype]	7	2.51×10^{-12}	3.05	**0.0101**	
Model Error	46	8.21×10^{-13}			
[N] / mg tissue					
Cytotype	2	1.20×10^{-6}	0.74	0.4799	
Nitrogen (N)	1	1.53×10^{-5}	9.26	**0.0030**	H > L
Phosphorus (P)	1	1.23×10^{-6}	7.45	**0.0075**	L > H
C x N	2	8.93×10^{-7}	0.54	0.5844	
C x P	2	1.10×10^{-6}	0.66	0.5199	
N x P	1	6.31×10^{-6}	38.20	**<0.0001**	HL > HH=LH >LL
C x N x P	2	3.50×10^{-7}	0.21	0.8094	

Population [Cytotype]	7	1.45×10^{-6}	8.75	**<0.0001**	
Model Error	97	1.65×10^{-6}			
[P] / mg tissue					
Cytotype	2	8.04×10^{-8}	0.82	0.4463	
Nitrogen (N)	1	3.57×10^{-8}	0.64	0.5489	
Phosphorus (P)	1	1.73×10^{-9}	0.02	0.8949	
C x N	2	1.08×10^{-7}	1.10	0.3407	
C x P	2	1.82×10^{-8}	0.19	0.8307	
N x P	1	1.82×10^{-7}	1.57	0.1796	
C x N x P	2	1.46×10^{-7}	1.50	0.2348	
Population [Cytotype]	7	3.62×10^{-7}	3.70	**0.0030**	
Model Error	46	9.79×10^{-8}			
[N] / aboveground biomass					
Cytotype	2	1.31×10^{-5}	3.17	**0.0465**	6x (=4x) > 2x (=4x)
Nitrogen (N)	1	2.69×10^{-3}	65.30	**<0.0001**	H > L
Phosphorus (P)	1	5.62×10^{-5}	1.16	0.2461	
C x N	2	1.02×10^{-5}	2.46	0.0907	
C x P	2	3.87×10^{-5}	0.94	0.3952	
N x P	1	1.02×10^{-3}	24.80	**<0.0001**	
C x N x P	2	4.97×10^{-5}	1.21	0.3040	
Population [Cytotype]	7	7.64×10^{-4}	2.64	**0.0152**	
Model Error	97	1.09×10^{-5}			
[P] / aboveground biomass					
Cytotype	2	3.80×10^{-7}	3.07	0.0562	
Nitrogen (N)	1	4.05×10^{-6}	32.91	**<0.0001**	H > L
Phosphorus (P)	1	2.20×10^{-7}	1.79	0.1880	
C x N	2	4.10×10^{-7}	3.31	**0.0455**	
C x P	2	4.30×10^{-7}	3.53	**0.0375**	
N x P	1	1.80×10^{-7}	1.50	0.2276	
C x N x P	2	3.50×10^{-7}	2.82	0.0701	
Population [Cytotype]	7	3.90×10^{-7}	3.13	**0.0086**	
Model Error	46	1.23×10^{-6}	4.76	**<0.0001**	

Overall model for [N] / cell: $R^2 = 0.83$, $F_{18,115} = 26.59$, $P < 0.0001$, N = 116
Overall model for [P] / cell: $R^2 = 0.81$, $F_{18,64} = 10.71$, $P < 0.0001$, N = 65
Overall model for [N] / mg tissue: $R^2 = 0.54$, $F_{18,115} = 6.31$, $P < 0.0001$, N = 116
Overall model for [P] / mg tissue: $R^2 = 0.44$, $F_{18,64} = 1.99$, $P = 0.0304$, N = 65
Overall model for [N] / aboveground biomass: $R^2 = 0.57$, $F_{18,115} = 7.26$, $P < 0.0001$, N = 116
Overall model for [P] / aboveground biomass: $R^2 = 0.65$, $F_{18,64} = 4.76$, $P < 0.0001$, N = 65

Table 3. Results from fixed-effects ANOVA models for the effects of cytotype (diploid = 2x, tetraploids = 4x, hexaploid = 6x) on traits associated with material costs: aboveground growth response to N, aboveground growth response to P, belowground growth response to N, and belowground growth responses to P. Overall model results are reported in the footnotes, bold values indicate a significant effect at $\alpha = 0.05$, and if a factor was found to be significant, Tukey's HSD tests ($\alpha = 0.05$) were used to determine significant differences between means and patterns are indicated.

Source	df	MS	F	Prob > *F*	Tukey's HSD
Aboveground growth response to N					
Cytotype	2	1.00	4.82	**0.0165**	4x(=2x) > 6x(=2x)
Model Error	20	0.20			
Aboveground growth response to P					
Cytotype	2	0.13	0.52	0.6031	
Model Error	20	0.25			
Belowground growth response to N					
Cytotype	2	0.49	4.38	**0.0230**	4x(=2x) > 6x(=2x)
Model Error	20	0.11			
Belowground growth response to P					
Cytotype	2	0.02	0.20	0.8176	
Model Error	20	0.10			

Overall model for aboveground growth response to N: $R^2 = 0.27$, $F_{2,28} = 4.82$, $P = 0.0165$, N = 29
Overall model for aboveground growth response to P: $R^2 = 0.04$, $F_{2,28} = 0.52$, $P = 0.6031$, N = 29
Overall model for belowground growth response to N: $R^2 = 0.25$, $F_{2,28} = 4.38$, $P = 0.0230$, N = 29
Overall model for belowground growth response to P: $R^2 = 0.02$, $F_{2,28} = 0.20$, $P = 0.8176$, N = 29

Table 4. Results from fixed-effects ANOVA models for the effects of cytotype (diploid = 2x, tetraploids = 4x, hexaploid = 6x), soil N:P treatments (L= low N and P and H= high N and P, only), their interaction, and population of origin nested within cytotype on relative transcriptome size per milligram tissue and relative transcriptome size per cell (both Ln (value)+1 transformed). Overall model results are reported in the footnotes, bold values indicate a significant effect at $\alpha = 0.05$, and if a single factor was found to be significant, Tukey's HSD tests ($\alpha = 0.05$) were used to determine significant differences between means.

Source	df	MS	F	Prob > *F*	Tukey's HSD
Relative transcriptome size per milligram tissue					
Cytotype (C)	2	3.84	4.65	**0.0118**	2x>4x=6x
Treatment (T)	1	3.96	4.80	**0.0309**	H>L
C x T	2	0.40	0.48	0.6209	
Population [Cytotype]	7	0.79	0.96	0.4681	
Model Error	97	0.83			
Relative transcriptome size per cell					
Cytotype (C)	2	6.01	12.23	**<0.0001**	2x>4x=6x
Treatment (T)	1	0.02	0.04	0.8342	
C x T	2	0.15	0.30	0.7446	
Population [Cytotype]	7	0.18	0.37	0.9177	
Model Error	97	0.49			

Overall model for relative transcriptome size per milligram tissue: $R^2 = 0.20$, $F_{12,109} = 1.96$, $P = 0.0359$, N = 110

Overall model for relative transcriptome size per cell: $R^2 = 0.24$, $F_{12,109} = 2.51$, $P = 0.0065$, N = 110

Table 5. Results from fixed-effects ANOVA models for the effects of cytotype (diploid = 2x, tetraploids = 4x, hexaploid = 6x), soil N treatment (L = low, H = high), their interactions, and population nested within cytotype on maximum photosynthetic rate (A_{max}), the maximum rate of Rubisco carboxylase activity (Vc_{max}), and the maximum rate of photosynthetic electron transport (J_{max}) for plants grown in low or high P conditions. Statistics for the accompanying three-way ANOVAs can be found in Table S2. Overall model results are reported in the footnotes, and bold values indicate a significant effect at α = 0.05.

	Low P Treatment				High P Treatment			
Source	df	MS	F	Prob > *F*	df	MS	F	Prob > *F*
A_{max}								
Cytotype (C)	2	114.20	10.40	**<0.0001**	2	44.92	4.06	**0.0201**
Nitrogen (N)	1	173.39	15.80	**0.0001**	1	29.24	2.64	0.1070
C x N	2	105.67	4.81	**0.0101**	2	14.15	1.28	0.2826
Population [Cytotype]	7	175.84	2.29	**0.0331**	8	11.06	5.60	**<0.0001**
Model Error	102	10.98			103			
Vc_{max}								
Cytotype (C)	2	58.06	0.68	0.5105	2	1309.11	9.19	**0.0006**
Nitrogen (N)	1	622.23	7.33	**0.0100**	1	101.47	0.71	0.4042
C x N	2	192.61	2.27	0.1169	2	214.24	1.50	0.2356
Population [Cytotype]	7	108.22	1.28	0.2878	7	314.98	2.21	0.0557
Model Error	39	84.87			37	142.50		
J_{max}								
Cytotype (C)	2	272.21	1.14	0.3300	2	3534.23	10.20	**0.0003**
Nitrogen (N)	1	2779.83	11.65	**0.0015**	1	381.64	1.10	0.3008
C x N	2	885.00	3.71	**0.0335**	2	752.73	2.17	0.1283
Population [Cytotype]	7	366.54	1.54	0.1839	7	1049.71	3.03	**0.0128**
Model Error	39	238.59			37	346.61		

Overall model for $A_{max:}$ in low P: $R^2 = 0.41$, $F_{12,114} = 5.92$, $P < 0.0001$, N = 115; in high P: $R^2 = 0.44$, $F_{12,114} = 6.12$, $P < 0.0001$, N = 117
Overall model for Vc_{max} in low P: $R^2 = 0.39$, $F_{12,51} = 2.10$, $P = 0.0407$, N = 52; in high P: $R^2 = 0.50$, $F_{12,49} = 3.08$, $P = 0.0043$, N = 50
Overall model for J_{max} in low P: $R^2 = 0.47$, $F_{12,51} = 2.85$, $P = 0.0066$, N = 52; in high P: $R^2 = 0.55$, $F_{12,49} = 3.84$, $P = 0.0008$, N = 50

Table 6: Results from fixed-effects ANOVA models for the effects of cytotype (2x, 4x, 6x), soil N treatment (low, high), soil P treatment (low, high), their interactions, and population nested within cytotype for N-use efficiency and P-use efficiency. Overall model results are reported in the footnotes, and bold values indicate a significant effect at $\alpha = 0.05$.

Source	df	MS	F	Prob > *F*
N-use efficiency				
Cytotype (C)	2	1.30×10^{-3}	4.75	**0.0108**
Nitrogen (N)	1	5.01×10^{-4}	1.84	0.1785
Phosphorus (P)	1	7.45×10^{-4}	2.73	0.1017
C x N	2	1.79×10^{-3}	6.56	**0.0021**
C x P	2	5.64×10^{-4}	2.07	0.1318
N x P	1	3.18×10^{-3}	11.64	**0.0009**
C x N x P	2	6.40×10^{-4}	2.35	0.1010
Population (Cytotype)	7	8.32×10^{-4}	3.05	**0.0060**
Model Error	97			
P-use efficiency				
Cytotype (C)	2	0.01	1.82	0.1736
Nitrogen (N)	1	0.21	34.77	**<0.0001**
Phosphorus (P)	1	0.16	26.68	**<0.0001**
C x N	2	0.02	4.02	**0.0246**
C x P	2	0.01	1.24	0.2990
N x P	1	0.03	5.79	**0.0202**
C x N x P	2	0.01	1.17	0.3196
Population (Cytotype)	7	0.01	2.09	0.0640
Model Error	46	0.01		

Overall model for N-use efficiency: $R^2 = 0.47$, $F_{18,115} = 4.82$, $P < 0.0001$, N = 116
Overall model for P-use efficiency: $R^2 = 0.70$, $F_{18,64} = 5.92$, $P < 0.0001$, N = 65

Table 7. Results from fixed-effects ANOVA models for the effects of cytotype (diploid = 2x, tetraploids = 4x, hexaploid = 6x), soil N treatment (L = low, H = high), their interactions, and population nested within cytotype on photosynthetic N- and P-use efficiency for plants grown under low or high P conditions. Statistics for the accompanying three-way ANOVAs can be found in Table S4. Overall model results are reported in the footnotes, and bold values indicate a significant effect at $\alpha = 0.05$.

	Low P Treatments				High P Treatments			
Source	df	MS	F	Prob > *F*	df	MS	F	Prob > *F*
Photosynthetic N-Use Efficiency								
Cytotype (C)	2	7.96×10^3	5.53	**0.0071**	2	1.28×10^4	7.54	**0.0015**
Nitrogen (N)	1	2.26×10^3	1.57	0.2170	1	5.41×10^3	3.20	0.0806
C x N	2	8.57×10^3	5.95	**0.0051**	2	1.96×10^3	1.16	0.3240
Population [Cytotype]	7	2.08×10^3	1.44	0.2117	7	5.12×10^3	3.02	**0.0109**
Model Error	45	1.44×10^3			45	1.69×10^3		
Photosynthetic P-Use Efficiency								
Cytotype (C)	2	18.84	0.25	0.7800	2	210.78	2.03	0.1567
Nitrogen (N)	1	218.67	2.92	0.1044	1	67.42	0.65	0.4298
C x N	2	321.66	4.30	**0.0297**	2	83.07	0.80	0.4631
Population [Cytotype]	7	123.38	1.65	0.0848	7	114.22	1.10	0.3997
Model Error	18	74.77			21	104.00		

Overall model for photosynthetic N-use efficiency in low P: $R^2 = 0.47$, $F_{12,57} = 3.33$, $P = 0.0016$, N = 58; in high P: $R^2 = 0.51$, $F_{12,57} = 3.86$, $P = 0.0005$, N = 58

Overall model for photosynthetic P-use efficiency in low P: $R^2 = 0.63$, $F_{12,30} = 2.50$, $P = 0.0384$, N = 31; in high P: $R^2 = 0.40$, $F_{12,33} = 1.17$, $P = 0.3636$, N = 34

Table 8. Results from fixed-effects ANOVA models for the effects of cytotype (diploid = 2x, tetraploids = 4x, hexaploid = 6x), soil N:P treatments (L= low N and P and H= high N and P, only), their interaction, and population of origin nested within cytotype on the concentrations of foliar total terpenes, monoterpenes, sesquiterpenes, and diterpenes (all log-transformed). Overall model results are reported in the footnotes, bold values indicate a significant effect at $\alpha = 0.05$, and if a single factor was found to be significant, Tukey's HSD tests ($\alpha = 0.05$) were used to determine significant differences between means.

Source	df	MS	F	Prob > *F*	Tukey's HSD
Total Terpenes					
Cytotype (C)	2	13.41	9.57	**0.0003**	2x > 4x = 6x
Treatment (T)	1	3.95	5.65	**0.0213**	L > H
C x T	2	0.54	0.38	0.6830	
Population [Cytotype]	7	2.76	3.94	**0.0017**	
Model Error	50	0.70			
Total Monoterpenes					
Cytotype (C)	2	12.70	7.12	**0.0012**	2x > 4x = 6x
Treatment (T)	1	3.92	4.75	**0.0340**	L > H
C x T	2	0.38	0.46	0.6353	
Population [Cytotype]	7	3.07	3.73	**0.0025**	
Model Error	50	0.82			
Total Sesquiterpenes					
Cytotype (C)	2	5.80	4.88	**0.0116**	2x > 4x = 6x
Treatment (T)	1	3.14	2.64	0.1103	
C x T	2	0.19	0.16	0.8539	
Population [Cytotype]	7	1.43	1.20	0.3206	
Model Error	50	1.19			
Total Diterpenes					
Cytotype (C)	2	6.24	4.43	*0.0169	
Treatment (T)	1	0.15	0.11	0.7442	
C x T	2	0.30	0.21	0.8095	
Population [Cytotype]	7	1.38	0.98	0.4571	
Model Error	50	1.41			

Overall model for total terpenes: $R^2 = 0.58$, $F_{12,62} = 5.77$, $P < 0.0001$, N = 63
Overall model for total monoterpenes: $R^2 = 0.55$, $F_{12,62} = 5.05$, $P < 0.0001$, N = 63
Overall model for total sesquiterpenes: $R^2 = 0.38$, $F_{12,62} = 2.54$, $P = 0.0106$, N = 63
Overall model for total diterpenes: $R^2 = 0.29$, $F_{12,62} = 1.66$, $P = 0.1047$, N = 63
*Significant model factor negated by insignificant overall ANOVA model

Table S1. Results from fixed-effects ANOVA models for the effects of cytotype (2x, 4x, 6x) and population nested within cytotype on stomata area (μm^2) and stomata density. Overall model results are reported in the footnotes, bold values indicate a significant effect at $\alpha = 0.05$, and Tukey's HSD test ($\alpha = 0.05$) were used to determine significant differences between cytotype means and these results are reported.

Source	df	MS	F	Prob > *F*	Tukey's HSD
Stomata Area (μm^2)					
Cytotype	2	650322.90	66.29	**<0.0001**	6x>4x>2x
Population [Cytotype]	7	15354.10	1.57	0.1531	
Model Error	112	206518.00			
Stomata Density					
Cytotype	2	14658.85	168.45	**<0.0001**	2x>4x>6x
Population [Cytotype]	7	251.36	2.89	**0.0082**	
Model Error	112	3717.13			

Overall model for stomata area: $R^2 = 0.63$, $F_{9,121} = 21.05$, $P < 0.0001$, N = 122
Overall model for stomata density: $R^2 = 0.77$, $F_{9,121} = 42.72$, $P < 0.0001$, N = 122.

Table S2. Results from fixed-effects ANOVA models for the effects of cytotype (2x, 4x, 6x), soil N treatment (low, high), soil P treatment (low, high), their interactions, and population nested within cytotype on maximum photosynthetic rate (A_{max}), the maximum rate of Rubisco carboxylase activity (Vc_{max}), and the maximum rate of photosynthetic electron transport (J_{max}). Overall model results are reported in the footnotes, and bold values indicate a significant effect at $\alpha = 0.05$.

Source	df	MS	F	Prob > *F*
A_{max}				
Cytotype (C)	2	90.41	7.58	**0.0007**
Nitrogen (N)	1	171.05	14.34	**0.0002**
Phosphorus (P)	1	8.28	0.69	0.4058
C x N	2	16.80	1.41	0.2469
C x P	2	7.07	0.59	0.5537
N x P	1	30.49	2.56	0.1114
C x N x P	2	51.98	4.36	**0.0140**
Population (Cytotype)	8	50.13	4.21	**0.0001**
Model Error	212			
Vc_{max}				
Cytotype (C)	2	640.71	5.27	**0.0070**
Nitrogen (N)	1	675.60	5.56	**0.0207**
Phosphorus (P)	1	22.02	0.18	0.6714
C x N	2	28.07	0.23	0.7942
C x P	2	658.43	5.42	**0.0061**
N x P	1	258.38	2.13	0.1485
C x N x P	2	412.62	3.40	**0.0382**
Population (Cytotype)	7	208.91	1.72	0.1155
Model Error	83	121.48		
J_{max}				
Cytotype (C)	2	1607.01	4.94	**0.0094**
Nitrogen (N)	1	2695.11	8.28	**0.0051**
Phosphorus (P)	1	0.04	0.00	0.9912
C x N	2	177.21	0.54	0.5823
C x P	2	1954.43	6.00	**0.0037**
N x P	1	1013.96	3.11	0.0813
C x N x P	2	1591.14	4.89	**0.0098**
Population (Cytotype)	7	717.44	2.20	**0.0420**
Model Error	83	325.55		

Overall model for A_{max}: $R^2 = 0.36$, $F_{19,231} = 6.17$, $P < 0.0001$, N = 232
Overall model for Vc_{max} : $R^2 = 0.37$, $F_{18,101} = 2.71$, $P = 0.0012$, N = 102
Overall model for J_{max}: $R^2 = 0.42$, $F_{18,101} = 3.29$, $P = 0.0001$, N = 102

Table S3. Results from fixed-effects ANOVA models for the effects of cytotype (2x, 4x, 6x), soil N treatment (low, high), soil P treatment (low, high), their interactions, and population nested within cytotype on photosynthetic N-use efficiency and photosynthetic P-use efficiency. Overall model results are reported in the footnotes and bold values indicate a significant effect at $\alpha = 0.05$.

Source	df	MS	F	Prob > *F*
Photosynthetic N-use efficiency				
Cytotype (C)	2	20156.47	12.35	**<0.0001**
Nitrogen (N)	1	7295.00	4.47	**0.0371**
Phosphorus (P)	1	4716.51	2.89	0.0924
C x N	2	1077.95	0.66	0.5190
C x P	2	253.60	0.16	0.8563
N x P	1	250.43	0.15	0.6962
C x N x P	2	9541.38	5.84	**0.0040**
Population (Cytotype)	7	4720.76	2.89	**0.0087**
Model Error	97	1632.66		
Photosynthetic P-use efficiency				
Cytotype (C)	2	135.15	1.48	0.2376
Nitrogen (N)	1	291.50	3.20	0.0803
Phosphorus (P)	1	33.34	0.37	0.5482
C x N	2	86.18	0.95	0.3958
C x P	2	42.90	0.47	0.6275
N x P	1	49.11	0.54	0.4666
C x N x P	2	346.31	3.80	**0.0297**
Population (Cytotype)	7	143.04	1.57	0.1685
Model Error	46	91.13		

Overall model for photosynthetic N-use efficiency: $R^2 = 0.44$, $F_{18,115} = 4.22$, $P < 0.0001$, N = 116
Overall model for photosynthetic P-use efficiency: $R^2 = 0.42$, $F_{18,64} = 1.87$, $P = 0.0453$, N = 65

Table S4. Results from fixed-effects ANOVA models for the effects of cytotype (2x, 4x, 6x), soil N treatment (low, high), soil P treatment (low, high), their interactions, and population nested within cytotype on N-uptake efficiency. Overall model results are reported in the footnotes, bold values indicate a significant effect at $\alpha = 0.05$, and Tukey's HSD test ($\alpha = 0.05$) were used to determine significant differences between cytotype means and these results are reported.

Source	df	MS	F	Prob > *F*	Tukey's HSD
N-Uptake efficiency					
Cytotype (C)	2	2.87×10^{-7}	4.98	**0.0087**	6x > 4x = 2x
Nitrogen (N)	1	6.85×10^{-9}	0.24	0.6269	
Phosphorus (P)	1	6.36×10^{-9}	0.22	0.6394	
C x N	2	1.22×10^{-7}	4.23	**0.0174**	
C x P	2	4.70×10^{-8}	1.63	0.2006	
N x P	1	6.41×10^{-7}	22.27	**<0.0001**	
C x N x P	2	5.66×10^{-8}	1.96	0.1458	
Population [Cytotype]	7	7.05×10^{-7}	2.45	**0.0235**	
Model Error	97	2.88×10^{-8}			

Overall model for N-uptake efficiency: $R^2 = 0.43$, $F_{18,115} = 4.14$, $P < 0.0001$, N = 116

2.7 Figures

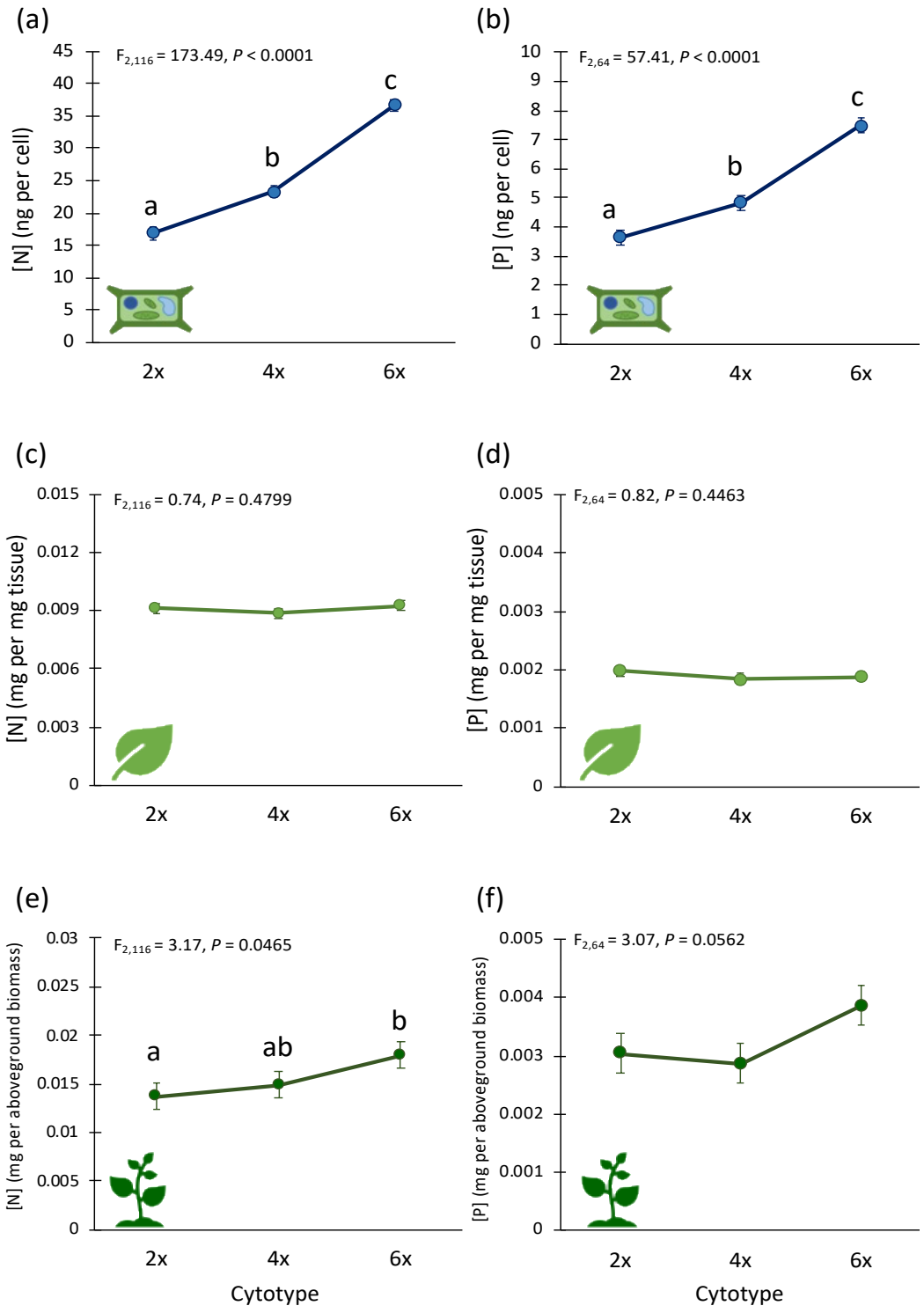
(a)
$F_{2,116}$ = 173.49, P < 0.0001
[N] (ng per cell)
45
40
35
30
25
20
15
10
5
0
a
b
c
2x
4x
6x
(b)
$F_{2,64}$ = 57.41, P < 0.0001
[P] (ng per cell)
10
9
8
7
6
5
4
3
2
1
0
a
b
c
2x
4x
6x
(c)
$F_{2,116}$ = 0.74, P = 0.4799
[N] (mg per mg tissue)
0.015
0.012
0.009
0.006
0.003
0
2x
4x
6x
(d)
$F_{2,64}$ = 0.82, P = 0.4463
[P] (mg per mg tissue)
0.005
0.004
0.003
0.002
0.001
0
2x
4x
6x
(e)
$F_{2,116}$ = 3.17, P = 0.0465
[N] (mg per aboveground biomass)
0.03
0.025
0.02
0.015
0.01
0.005
0
a
ab
b
2x
4x
6x
Cytotype
(f)
$F_{2,64}$ = 3.07, P = 0.0562
[P] (mg per aboveground biomass)
0.005
0.004
0.003
0.002
0.001
0
2x
4x
6x
Cytotype

Fig. **1**: LSMean values [N] and [P] mg per cell (a,b), per mg tissue (c,d), and per aboveground biomass for diploids (2x), tetraploids (4x), and hexaploids (6x). A plant's cytotype had a significant effect on [N] and [P] ng per cell (a,b) and [N] per aboveground biomass (e). The results of Tukey's HSD tests between cytotypes are reported with different letters when significant. Error bars represent ± standard error. Full statistical details in Table 2.

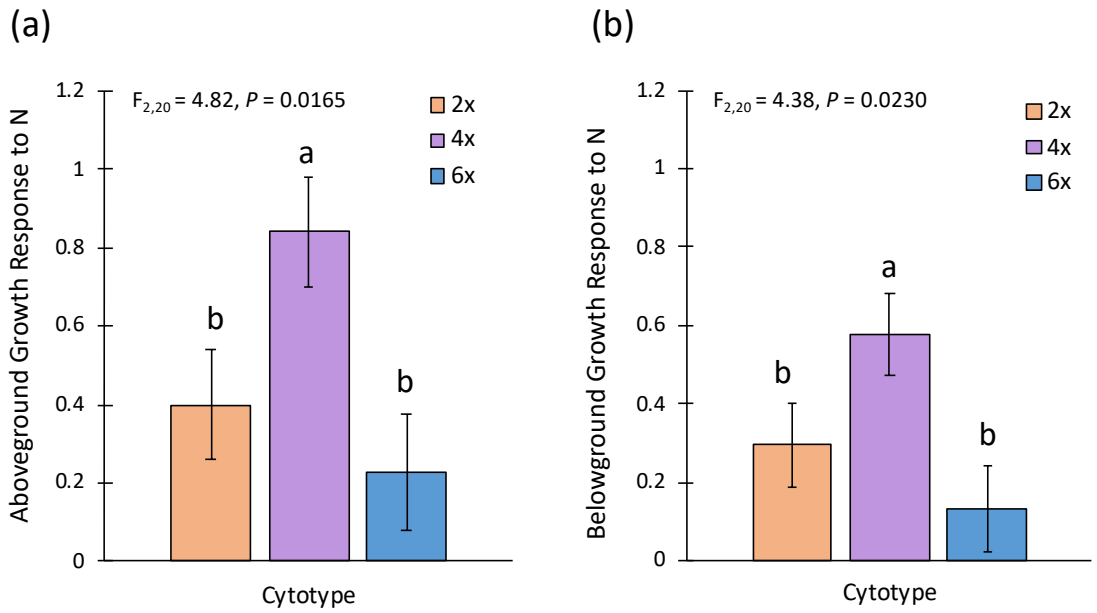

Fig. **2**: LSMean values for (a) aboveground and (b) belowground response to nitrogen (N) additions for diploids (2x), tetraploids (4x), and hexaploids (6x). A plant's cytotype had a significant effect on both above- and belowground responses to N additions. The results of Tukey's HSD tests between cytotypes are reported with different letters when significant ($\alpha = 0.05$). Error bars represent ± standard error. Full statistical details in Table 3.

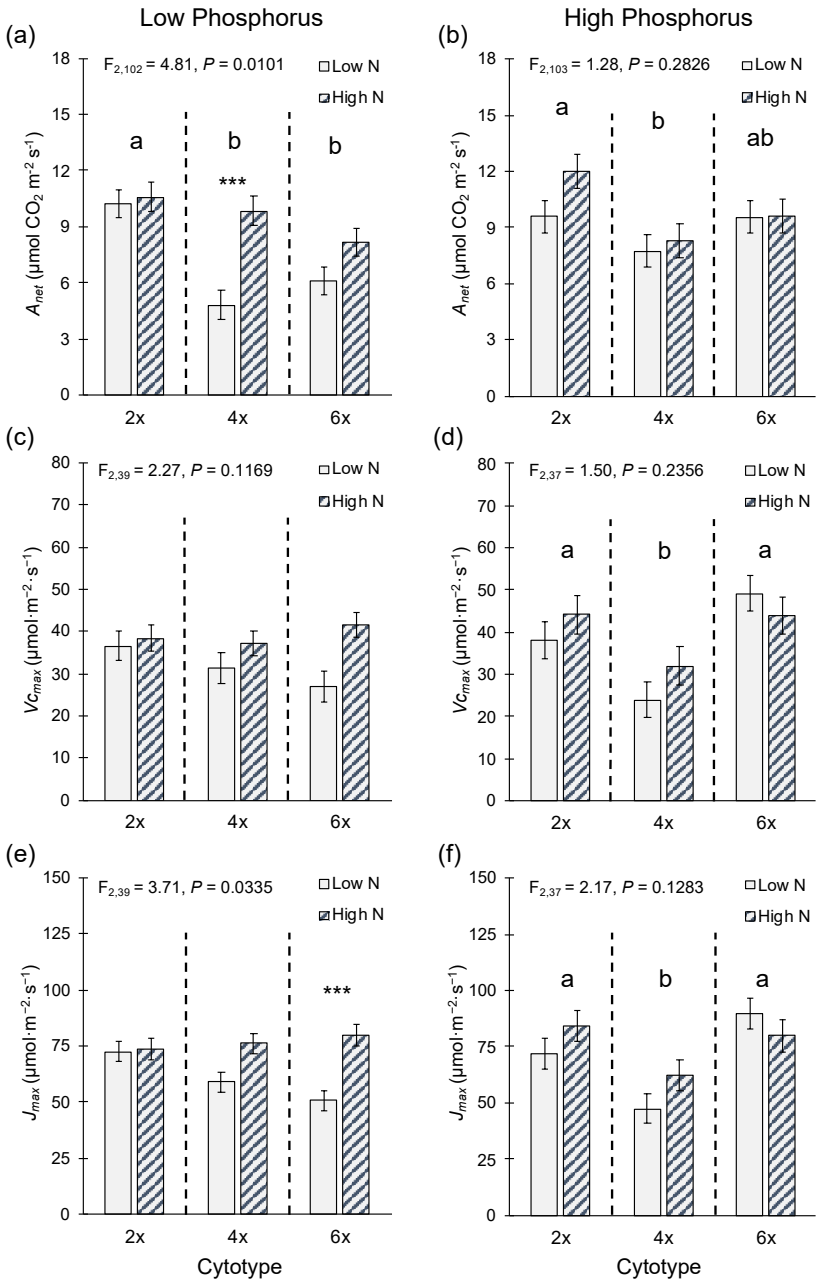

Fig. **3**: LSMean values for net photosynthetic rate (A_{net}; a,b), the maximum rate of Rubisco carboxylase activity (Vc_{max}; c,d), and the maximum rate of photosynthetic electron transport (J_{max}; e,f) showing the significant effects of the interaction between cytotype (2x = diploid, 4x = tetraploid, 6x = hexaploid) and nitrogen availability (a,c,e) and the effect of cytotype alone (b,d,f) under low and high phosphorus availabilities, respectfully. Significantly different mean values were determined with controlled

contrasts when interactions were significant and are noted with stars. These contrast tests revealed that under low P (1) diploids had significantly greater A_{net} rates than tetraploids and hexaploids (a; mean A_{net} of diploids vs. mean of tetraploids and hexaploids in low N-treatments: $F_{1,102}$= 24.72, P < 0.0001; in high N-treatments: $F_{1,102}$= 2.71, P = 0.1030), (2) diploids photosynthesized at significantly greater rates than the polyploids when grown under low but not high N-condition (a; mean A_{net} of diploids vs. tetraploid. vs. hexaploids in low N-treatments: $F_{1,102}$= 13.43, P < 0.0001; in high N-treatments: $F_{1,102}$= 2.23, P = 0.1124), (3) only tetraploids significantly increased A_{net} values in high versus low N-treatments (a; mean A_{net} under high versus low N-treatments for 2x: $F_{1,102}$= 0.10, P = 0.7560; for 4x: $F_{1,102}$= 21.64, P < 0.0001; for 6x: $F_{1,102}$= 3.69, P = 0.0575), and (4) only hexaploids significantly increased J_{max} values in high versus low N-treatments (e; mean J_{max} under high versus low N-treatments for 2x: $F_{1,39}$= 0.02, P = 0.8889; for 4x: $F_{1,39}$= 3.46, P = 0.0706; for 6x: $F_{1,39}$= 16.41, P = 0.0002). Tukey's HSD tests were used to determine significant differences among cytotype means when grown under high or low P are also reported with different letters when significant. Error bars represent ± standard error. Full statistical details in Table 5.

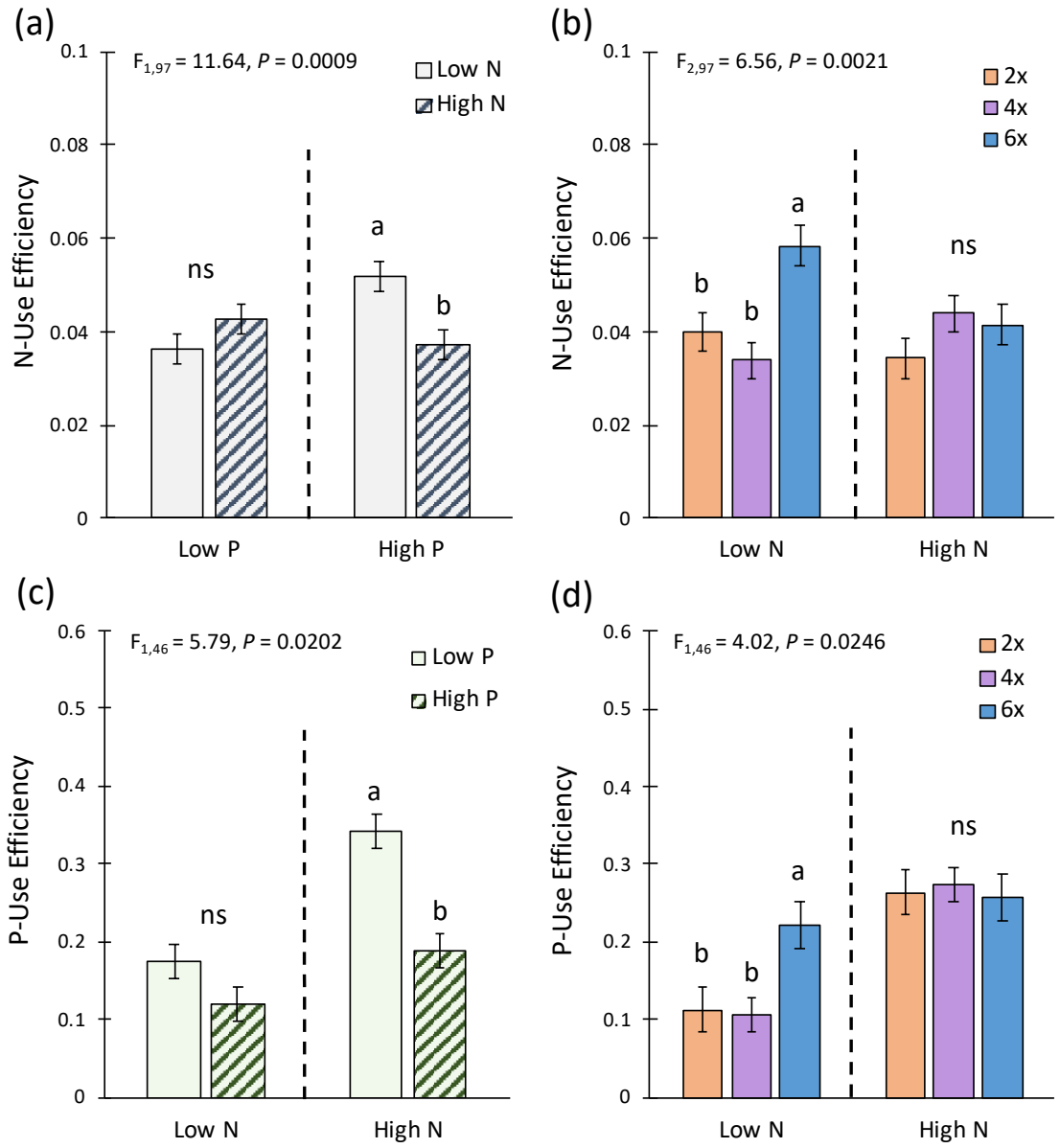

Fig. **4**: LSMean values of nitrogen-use efficiency (a,b) and phosphorus-use efficiency (c,d) for the significant interactions between nitrogen and phosphorus availability (a,c) and between plant cytotype (2x = diploid, 4x = tetraploid, 6x = hexaploid) and nitrogen availability (b,d). Significantly different mean values were determined with controlled contrasts and are noted with different letters. These controlled contrasts revealed that (1) when P was not limiting, plants were significantly more N-use efficient when grown in low versus high N-conditions (a; mean N-use efficiency for plants grown in low vs. high N-conditions under low P: $F_{1,97}$= 2.12, P = 0.1482; under high P: $F_{1,97}$= 11.31, P =

0.0011), (2) plants more efficiently converted P into biomass when grown under low versus high P-treatments only when they were also grown under high N-conditions (c; mean P-use efficiency for plants grown in low vs. high P-conditions under low N: $F_{1,46}$= 3.80, P = 0.0572:under high N: $F_{1,46}$= 26.94, P < 0.0001), and (3) hexaploids were significantly more N-use efficient and P-use efficient than diploids and tetraploids when N was limited (b,d; mean N-use efficiency of hexaploids vs. mean of diploids and tetraploids in low N-treatments: $F_{1,97}$= 18.43, P < 0.0001; in high N-treatments: $F_{1,97}$= 0.21, P = 0.6496; mean P-use efficiency of hexaploids vs. mean of diploids and tetraploids in low N-treatments: $F_{1,46}$= 10.78, P = 0.0020; in high N-treatments: $F_{1,46}$= 0.10, P = 0.7548). Error bars represent ± standard error. Full statistical details in Table 6.

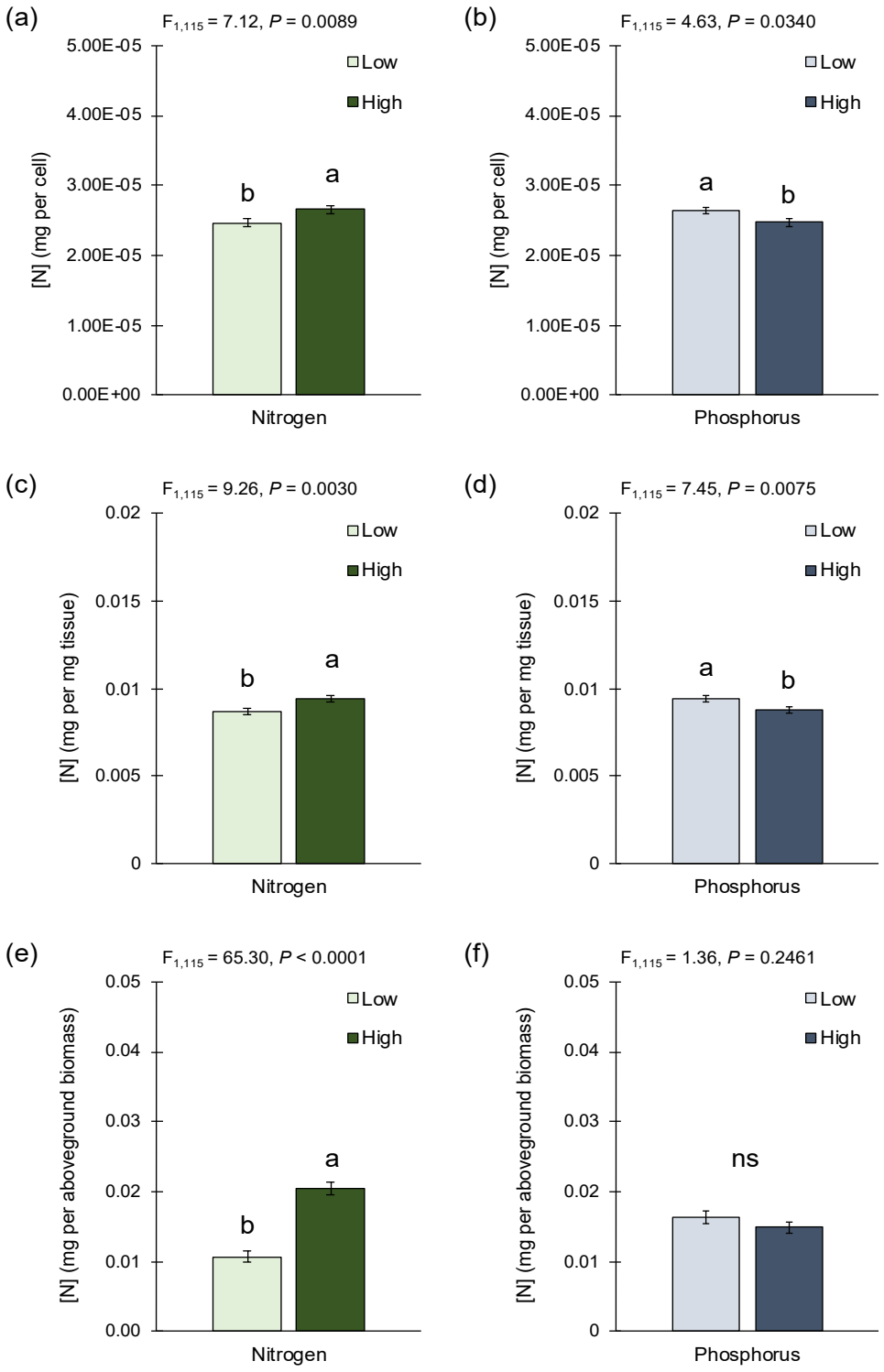

Fig. **S1:** LSMean values [N] mg per cell (a,b), per mg tissue (c,d), and per aboveground biomass in low and high N or P treatments. The availability of both N and P had a significant effect on [N] per cell (a,b), per mg tissue (c,d), and per aboveground biomass

(e; N-availability only). The results of Tukey's HSD tests between cytotypes are reported with different letters when significant. Error bars represent ± standard error. Full statistical details in Table 2.

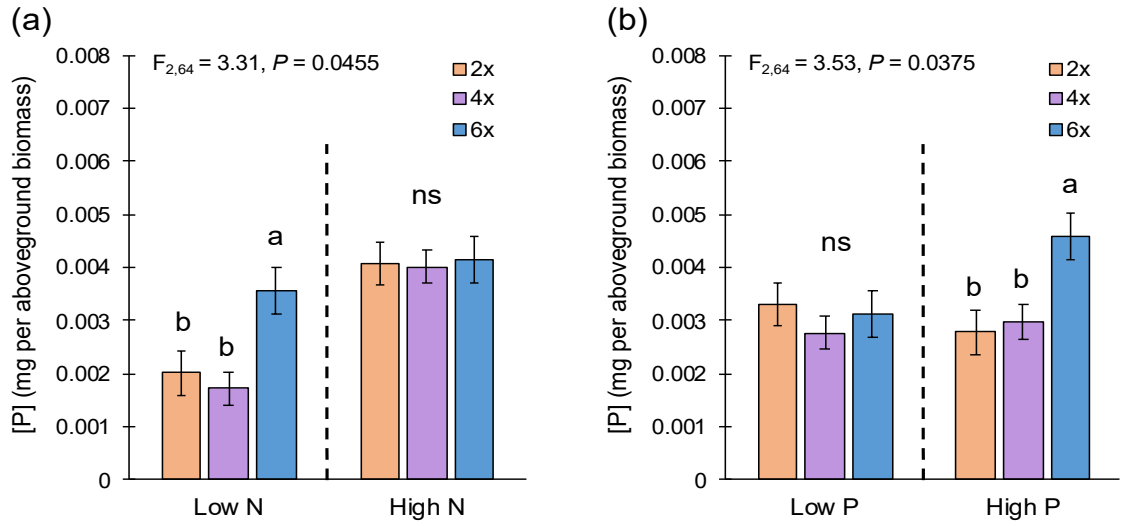

Fig. **S2**: LSMean values for [P] per aboveground biomass for diploids (2x), tetraploids (4x), and hexaploids (6x). Hexaploids had significantly more [P] per aboveground biomass than the other cytotypes in low N and high P treatments. The results of Tukey's HSD tests between cytotypes are reported with different letters when significant. Error bars represent ± standard error. Full statistical details in Table 2.

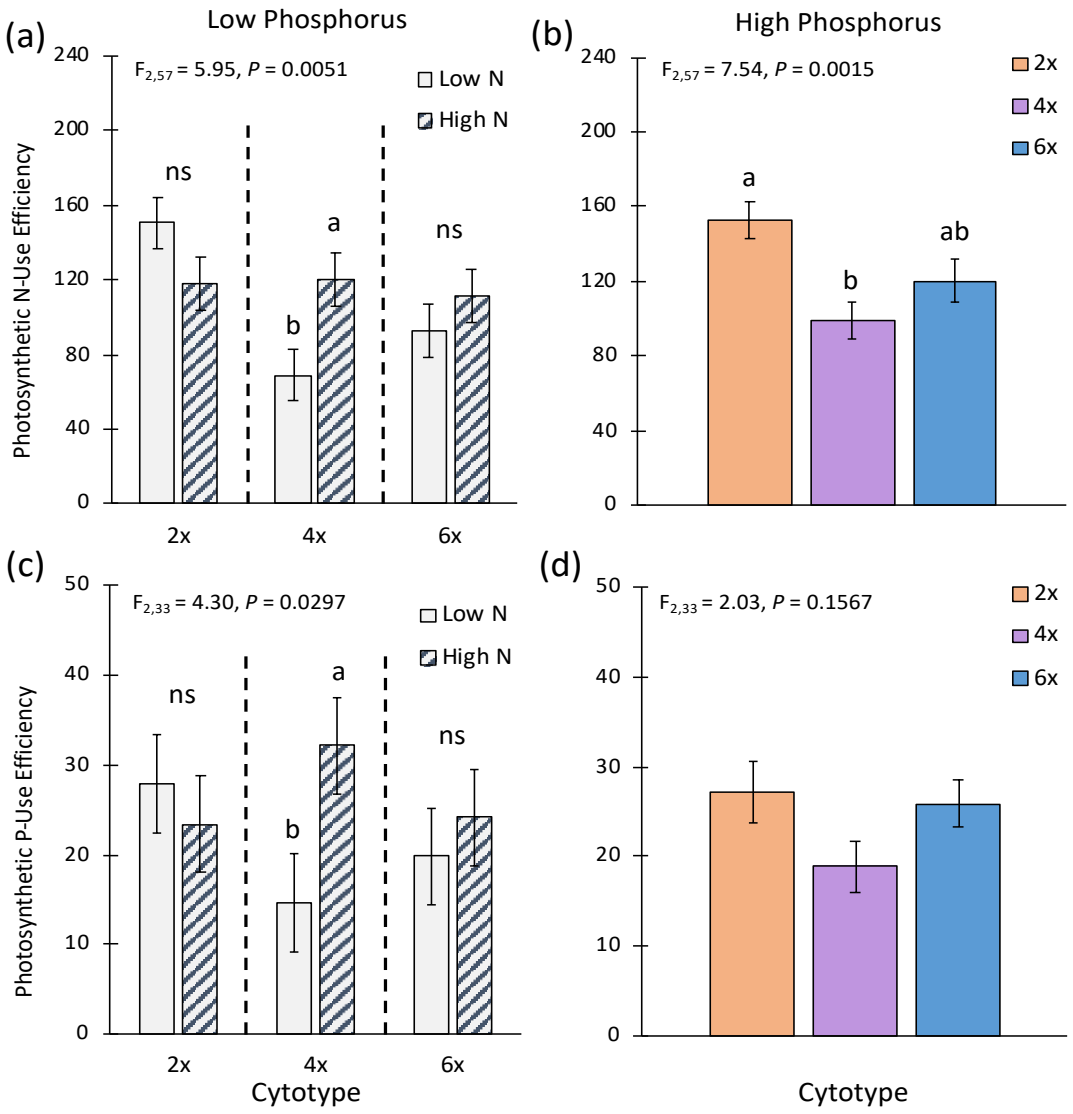

Fig. **S3**: LSMean values for photosynthetic nitrogen-use efficiency (a,b) and phosphorus-use efficiency (c,d) for the significant interactions between plant cytotype (2x = diploid, 4x = tetraploid, 6x = hexaploid) and nitrogen availability (a,c) and the independent effect of cytotype (b,d) under low and high phosphorus, respectively. Significantly different mean values were determined with controlled contrasts when interactions were significant and are noted with different letters. These contrast tests revealed that under low P, tetraploids were significantly more efficient at using N and P for photosynthesis when grown in high versus low N-treatments (a,c; mean P_{NUE} under high versus low N-treatments for 2x: $F_{1,45}$= 3.39, P = 0.0721; for 4x: $F_{1,45}$= 9.18, P = 0.0040; for 6x:

$F_{1,45}$= 1.10, P = 0.2991; mean P_{PUE} under high versus low N-treatments for 2x: $F_{1,18}$= 0.52, P = 0.4796; for 4x: $F_{1,18}$= 14.28, P = 0.0014; for 6x: $F_{1,18}$= 0.42, P = 0.5238). Tukey's HSD tests were used to determine significant differences among cytotype means when grown under high P are also reported with different letters when significant (b,d). Error bars represent ± standard error. Full statistical details in Table 7.

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2.9 Appendix Materials

Supplemental Methods 1

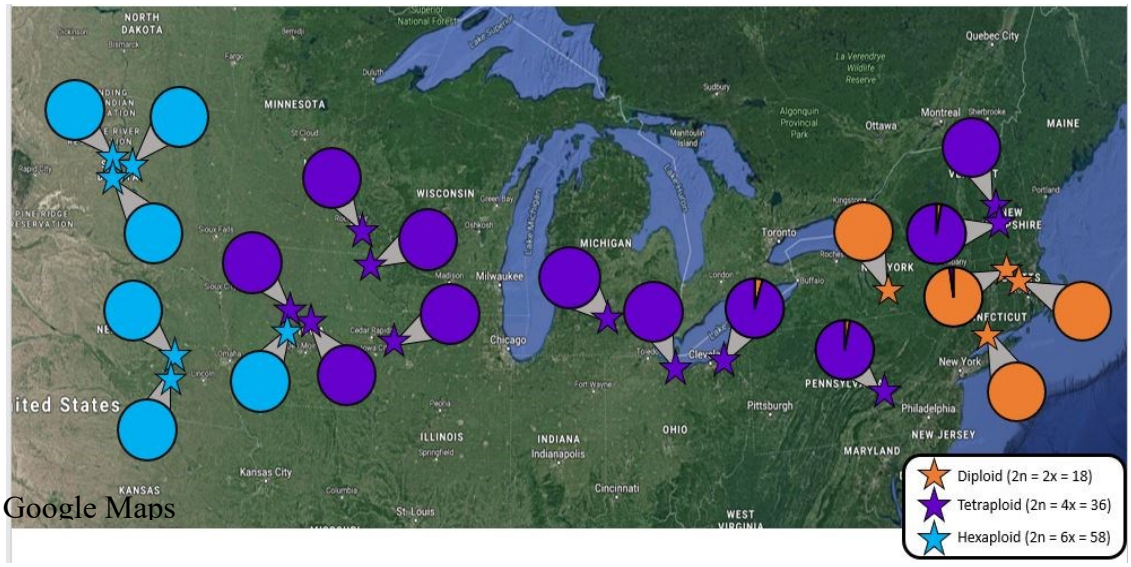
Flow cytometry methods:

The ploidy level of 20 plants per population was verified by estimating plant nuclear 2C DNA via flow cytometry as plant nuclear 2C DNA content is positively correlated with chromosome number (Husband and Schemske, 1998). Briefly, approximately 1 cm2 of silica dried (for field screening; N = 528) or fresh *S. gigantea* leaf tissue (for holoploid genome estimation on experimental plants; N = 238) was co-chopped with approximately 1 cm^2 fresh leaf tissue of the internal standards *Zea mays* (2C DNA content = 5.43pg; Dolezel et al., 2007) or *Pisum sativa* (2C DNA content = 9.09pg; Dolezel et al., 2007) in a modified Galbraith nucleiisolation buffer with 50 µg mL-1 RNase and 50 µg mL-1 propidium iodide (see Verloove et al., 2017). Cells were stained for approximately 45 minutes, filtered, and then analyzed on an Accuri C6+ flow cytometer and analyzed using CFlow Plus Analysis software (BD Biosciences, Franklin Lakes, New Jersey, USA). We used a FL2 detector to measure sample fluorescence and generate scatter plots in which we were able to omit residual noise and gate recorded particles within the fluorescence range of *S. gigantea* and either of the internal standards. Mean counts and coefficient variations (CV) were derived from histogram plots within the software. We removed low quality samples that had a histogram peak coefficient variation (CV) of more than 5% (Dolezel et al., 2007). The following formula from Dolezel et al. (2007) was used to calculate sample 2cDNA content (in pg):

$$Sample\ 2cDNA\ value = Standard\ 2cDNA\ value * \left(\frac{Sample\ 2C\ mean\ peak\ position}{Standard\ 2c\ mean\ peak\ position}\right)$$

The average 2C DNA content of diploids was (LS mean ± standard error) 1.96 ± 0.01 pg, tetraploids was 3.76 ± 0.01 pg, and hexaploids was 5.51 ± 0.02 pg. 2C DNA content ranged from 1.85 - 2.04 pg, 3.56 - 4.03 pg, and 5.41 - 5.63 pg in diploids, tetraploids, and

hexaploids, respectively. Mixed ploidy populations were rare, as only four of the 21 populations were mixed (see below). These mixed populations were made up diploids and tetraploids, with the minority cytotype only representing one individual within these four mixed populations.



Solidago gigantea collection sites. Orange stars depict predominately diploid populations, while purple stars depict predominantly tetraploid populations, and blue stars depict predominantly hexaploids populations. Pie graphs represent the proportion of each cytotype present in each of the 21 surveyed populations.

Google Maps, 2022. *Solidago gigantea population and ploidy levels*. Google Maps [online].

Supplemental Methods 2

We used a portable infrared CO_2 analyzer system (LI-6800; LI-COR Inc., Lincoln, NE, USA) equipped with a CO_2 mixer and 1x3 cm^2 chamber/red-blue LED light source (LI6800-02) to measure net carbon assimilation rate between CO_2 fixation and photorespiration (A_{net}, μmol CO_2 m^{-2} s^{-1}) on 232 plants. Measurements were taken in a random order over three days during the 12th week of growth between the hours of 09:00 to 16:00. Inside the chamber, CO_2 concentration was set 400 ppm, relative humidity to 65%, flow rate to 500 μmol m^{-2} s^{-1}, and light to 1000 μmol m^{-2} s^{-1}. Because measurements were taken inside a temperature-controlled greenhouse, we did not control for temperature within the chamber. Measurements were taken once photosynthetic rate stabilized, and infrared gas analyzers (IRGA) were matched after every 10 sampling measurements.

To maintain optimal photosynthetic functioning, plants must strategically distribute N and/or P into the carboxylation of RuBP by RUBISCO during the Calvin-Bensons cycle and/or the regeneration of RuBP via the electron transport chain. Investment into one process over the other depends upon limitations to photosynthesis imposed by the current environment (e.g., light, water, CO_2, N, and/or P; Quebbeman and Ramirez 2016). To measure the maximum rates of RuBP carboxylation (V_{cmax}) and of electron transport that regenerates RuBP (J_{max}), we performed photosynthetic CO_2 response curves (A-Ci curves) using the LI-6800 portable infrared CO_2 analyzer system set at multiple CO_2 concentrations. A-Ci curves were measured over 5 days during the 13th week growth on a subset of 120 plants between the hours of 09:00 to 16:00. Using an undamaged mature leaf, V_{cmax} and J_{max} were extracted from each curve constructed from 400, 300, 200, 100, 50, 0, 400, 400, 600, 800, 1000, 1200 ppm CO_2 concentrations with relative humidity set to 65%, flow rate to 500 μmol m^{-2} s^{-1}, light set to 1500 μmol m^{-2} s^{-1}, and temperature to 25°C. Using the *tidyverse* and *plantecophys* packages (Duursma 2015) in R version 4.1.2, V_{cmax} and J_{max} were extracted from net assimilation responses (*Anet*) to internal CO_2 concentrations (*Ci*), and a total of 18 A-Ci curves were flagged by *plantecophys* for low quality and were removed.

3 **Chapter 3:** Investigating the effects of whole genome duplication on phenotypic plasticity: implications for the invasion success of Giant Goldenrod (*Solidago gigantea*)[3]

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[3] This chapter is in preparation to be submitted to the journal Oikos. A.M.W conducted the experiment and collected data. Both A.M.W and E.H-G. designed the experiment, analyzed data, interpreted the data, and wrote the manuscript.

3.1 Abstract

Polyploidy commonly occurs in invasive species and phenotypic plasticity (PP), defined as the ability to alter one's phenotype in different environmental contexts, is predicted to be greater in polyploids and to contribute to their invasive success. However, empirical support that increased PP is associated with polyploidy and/or confers invasive success is limited. Here, we grew diploid, tetraploid (collected from both their native and invasive ranges), and hexaploid *Solidago gigantea* in pots placed outside under low, medium, and high soil nitrogen and phosphorus (NP) conditions, and measured growth, asexual reproductive, physiological, and resistance traits to examine relationships between PP, polyploidy, and species invasiveness. PP patterns were complex and dependent upon ploidy-level, plant origin, trait of interest, and nutrient changes. For example, in comparison to diploids, tetraploid and hexaploid cytotypes generally exhibited greater PP for physiological traits, but only greater PP for biomass traits when nutrients shifted from medium to high levels. Furthermore, native-tetraploids exhibited PP patterns more similar to diploids, their putative ancestor, than hexaploids but greater PP and mean values for biomass traits in comparison to invasive-tetraploids. These results indicate that PP for biomass in invasive-tetraploids might have been reduced by genetic drift and/or by selection for specific trait values better adapted to invasive habitats. Interestingly invasive-tetraploids had higher mean values for all physiological traits than native-tetraploids, although they did not differ in PP for these traits. Cumulatively, our findings highlight that PP is complex and can vary depending upon changes in the environment, ploidy-level, and selective and neutral processes. Furthermore, our results also imply that the nutrient environment of invasive habitats might play a role in the success and

trajectory of polyploidy invasions. This is especially important as invasions typically began in urbanized habitats exposed to anthropogenic nutrient enrichment.

3.2 Introduction

Phenotypic plasticity (PP) is the ability of a single genotype to change its phenotype in response to environmental change (Pigliucci et al. 2006, Fusco and Minelli 2010, Gianoli and Valladares 2012). PP is thought to be a contributor of invasive species success, with more phenotypically plastic species being more likely to be successful invaders for two main reasons (Callaway et al. 2003, Pigliucci, et al. 2006, Richards et al. 2006, Gratani 2014, Colautti et al. 2017, Fox et al. 2019). First, invasive species are often exposed to novel environmental conditions which they are not adapted to upon introduction (e.g., new climatic conditions, enemies, and/or competitors), and PP might allow invaders to tolerate these novel and/or changing environments and to maintain fitness before adaptation can occur (Agrawal et al. 2008, Chevin et al. 2010, Gratani 2014, Colautti, et al. 2017, Fox, et al. 2019). Second, new populations of invasive species often suffer from low genetic diversity following genetic drift (e.g., bottleneck effects, inbreeding depression; Charlesworth and Willis 2009, Rosche et al. 2016), and high degrees of PP might enhance a population's phenotypic variation when genetic variation cannot easily be increased (Pérez et al. 2006, Ardura et al. 2017). Despite the strong arguments for the importance of PP in successful biological invasions, empirical evidence of its role is both limited and mixed, as studies have found invasive populations to be more (Porté et al. 2011, Knop and Reusser 2012, Matesanz et al. 2012, Luo et al. 2019, Bufford and Hulme 2021), less (Lamarque et al. 2013, Wang et al. 2018, Plantamp et al. 2019, Albarrán-Mélzer et al. 2020), or equally (Peperkorn et al. 2005, Palacio-López and Gianoli 2011, Matzek 2012, Ryan and Gunderson 2021) plastic relative to non-invasive populations. As a result, we do not know whether invasive species are generally more

phenotypically plastic than non-invasive species and/or if PP is an innate ability (i.e., 'pre-adapted') verses an evolved ability selected for post-introduction.

Such discrepancies in our understanding of PP in invasive systems could be, in part, due to differences in experimental methodology amongst studies (e.g., PP measured in a species' invasive or native range but not both, Pichancourt et al. 2012, VanWallendael et al. 2018, Amat-Trigo et al. 2019, Granata et al. 2020) and/or due to species-, population-, and/or trait-specific plasticity responses to specific environmental manipulation. Polyploidy (the state of containing three or more chromosome sets per cell) is one species-specific trait commonly attributed to many successful invasive plant species (e.g., *Centaurea stoebe, Fallopia japonica, Rosa multiflor*a; Pandit et al. 2006, Pandit et al. 2011, Te Beest et al. 2012), as polyploidization can rapidly change a plant's morphology, physiology, and ecological interactions in ways that promote invasive success in certain ecological contexts (e.g., stages of invasion, climates; Te Beest, et al. 2012, Van de Peer et al. 2017, Wani et al. 2018, Van de Peer et al. 2020). Until recently, data on the ploidy levels of native and invasive flora was sparce (Pyšek and Richardson 2008; but see Pyšek and Richardson 2010, Pandit, et al. 2011, Te Beest, et al. 2012, Suda et al. 2015) and ploidy level variation tended to be overlooked in studies aiming to identify factors aiding in the likelihood of a species becoming invasive (Edwards et al. 1998, Jakobs et al. 2004, Zou et al. 2007, Hillstrom and Cipollini 2011; but see Schlaepfer et al. 2010, Callaway et al. 2011, Hahn et al. 2012, Te Beest, et al. 2012). Studies investigating polyploidy as a potential invasive trait have revealed that in some species (1) polyploids are the majority cytotype present in invasive ranges (Pandit, et al. 2006, Pandit, et al. 2011, Te Beest, et al. 2012), (2) phenotypic traits and ecological

tolerances vary between ploidy levels (McIntyre 2012, Te Beest, et al. 2012, Chao et al. 2013, Ramsey and Ramsey 2014), and (3) PP responses might be influenced by polyploidy (Schlichting 1986, Hahn, et al. 2012, Sánchez Vilas and Pannell 2017, Wei et al. 2019).

The relationship between polyploidy and PP is suspected to arise via alterations in gene number, function, expression, and/or arrangement patterns following whole genome duplication events (Jackson and Chen 2010, Vogt 2017, Ding and Chen 2018, Liqin et al. 2019). For example, duplicate chromosome copies could make polyploids more plastic than diploids for a particular trait(s) through 1) increased heterozygosity and genetic variability (Comai 2005, Chen 2010, Soltis et al. 2015), 2) the sub- or neo-functionalization of genes (i.e., repetitive gene sequences accumulate mutations to evolve new or varied functions; Vogt 2017, Ding and Chen 2018, Doyle and Coate 2019), and/or 3) gene dosage effects that broaden the range of quantitative trait variation (Bastiaanse et al. 2019). PP could contribute to a polyploid's invasive success as either a pre-adaptation or as a trait selectively favored in the novel invasive environment, and research is needed to distinguish amongst these alternatives. For instance, if polyploid populations of a species are more phenotypically plastic than diploid populations in their native ranges, this would suggest that polyploidy serves as a *pre-adaption* to invasive success. On the other hand, if polyploid populations of a species tend to be more phenotypically plastic than diploid populations only in the invasive range and/or if genotypes from the invasive range are, on average, more plastic than genotypes from the native range, this would suggest that the non-native selective environment *favored* genotypes that were the most plastic (e.g., *post-introduction selection*). Despite this strong theoretical framework

connecting PP, polyploidy, and invasive ecology, the hypothesis that increased PP is associated with polyploidy lacks a large body of empirical testing (but see Blanc-Mathieu et al. 2017, Gallego-Tévar et al. 2018, Kornstad et al. 2022), and even fewer studies have explicitly tested the influence of polyploidy on PP in invasive model systems (but see Hahn, et al. 2012, Sánchez Vilas and Pannell 2017, Wei, et al. 2019, Harms et al. 2021).

Here, we asked whether polyploidy and/or post-introduction selection might influence trait PP responses to changes in soil nutrients. We were particularly interested in PP responses to changes in soil nutrients because plants require sufficient amounts of soil nutrients to reach and maintain optimal growth and fitness and thus, soil nutrient availability likely exerts strong selective pressure on patterns of PP. For instance, strong plasticity responses in particularly nutrient-demanding traits (e.g., photosynthesis; Evans 1989) could allow plants to tolerate nutrient poor conditions by investing less resources into traits when environmental nutrient availability is low and more into these traits when environmental nutrient availability is high. Additionally, biological invasions usually begin in urbanized areas where long-term anthropogenic activities have resulted in locally significant increases in biologically available N and P (Penuelas et al. 2013, Fowler et al. 2015, Goyette et al. 2016, Asabere et al. 2018). Given that N and P availability is also increasing at the global scale in many terrestrial and aquatic ecosystems (Penuelas, et al. 2013, Fowler, et al. 2015, Goyette, et al. 2016, Asabere, et al. 2018), examining whether alterations in N and P bioavailability differentially effects the PP responses of polyploids versus diploids will also increase our understanding of the potential threats imposed by polyploid invasive species in ecosystems that are at the most risk for continuous and severe eutrophication (Luo, et al. 2019).

To meet our overall objective of understanding the relationship between PP, polyploidy, and invasion ecology we grew four *Solidago gigantea* geo-cytotypes (diploid, invasive-tetraploid, native-tetraploid, hexaploid) under low, medium, or high soil nitrogen and phosphorus conditions (NP) in pots that were placed outside and addressed the following questions and predictions: **(1)** Are polyploids more plastic than diploids? We predicted that PP would scale positively with ploidy level stemming from genomic alterations and novelty following whole genome duplication events. **(2)** Does nutrient availability differentially influence PP dependent upon ploidy level? Given that DNA require large amounts of N and P for synthesis and structure, polyploids are thought to have greater N and P demands than diploids to satisfy the material costs associated with maintaining larger genomes (Lewis 1985, Leitch and Bennett 2004, Cavalier-Smith 2005, Hessen et al. 2010, Guignard et al. 2017, Bales and Hersch-Green 2019, Faizullah et al. 2021; Walczyk and Hersch-Green 2020, 2022). As a result, polyploids might exhibit higher levels of PP than diploids in response to variations in soil nutrient conditions to diminish negative fitness consequences associated with nutrient limitations, and this might be especially apparent in nutrient-demanding traits. Recent empirical data supports this hypothesis, in that polyploids appear to have significantly higher fitness and growth responses when grown under high versus low nutrient conditions whereas the fitness and growth responses of diploids often do not changes as much when grown under high versus low nutrient conditions (Šmarda et al. 2013, Guignard et al. 2016, Bales and Hersch-Green 2019, Walczyk and Hersch-Green 2019). **(3)** Does PP for growth, photosynthetic, and resistance traits differ between native and invasive genotypes? We predict that invasive genotypes (i.e., post-selection genotypes) will be more plastic than

native genotypes for some growth and photosynthetic traits response to the nutrient environment for two reasons. First, PP is thought to increase tolerance to novel and/or variable environmental stimuli (Pigliucci, et al. 2006, Fusco and Minelli 2010, Gianoli and Valladares 2012), potentially resulting in a selectively favored competitive advantage in genotypes displaying the most PP in the non-native habitat. Second, if the non-native habitat lacks herbivores and/or other antagonists, the selective pressures formerly favoring defensive traits in the native range might relax to a point where reduced investment into defense becomes favored in invasive populations (Blossey and Notzold 1995, Keane and Crawley 2002, Bossdorf et al. 2005, Joshi and Vrieling 2005). Such reductions in defensive investment might allow non-native populations to reallocate resources that would have otherwise been invested into defense into increasing the expression and/or plasticity of other competitive traits in relation to native genotypes (Blossey and Notzold 1995, Keane and Crawley 2002, Bossdorf, et al. 2005, Joshi and Vrieling 2005).

3.3 Methods

Plant Material – To address our hypotheses, we examined *Solidago gigantea* Aiton (Asteraceae, Giant Goldenrod), which is an insect-pollinated perennial aster native to North America where it occurs as three spatially distinct cytotypes: diploid (2n = 2x = 18) populations are found along the Atlantic coast, tetraploid populations (2n = 4x = 36) are found within the Great Lakes region, and hexaploid populations (2n = 6x = 58) are found within the Great Plains region (Schlaepfer, et al. 2008, Hull-Sanders et al. 2009, Schlaepfer, et al. 2010). In contrast, *S. gigantea* is an exotic and highly invasive species in Europe and Asia (Schlaepfer, et al. 2008, Schlaepfer, et al. 2010) where most accounts indicate that it is a tetraploid (Schlaepfer, et al. 2008, Schlaepfer, et al. 2010). During the summers of 2017 to 2019, we collected seeds from 21 populations across *S. gigantea*'s native range and from 15 invasive populations near Zurich, Switzerland; the ploidy level of all plants were verified following flow cytometry methods (Walczyk and Hersch-Green 2022).

Experimental Design – Two biological replicates from four half-sibling maternal lines collected from three populations per geo-cytotype (diploid = 2x, native tetraploid = $4x^N$, invasive tetraploid = $4x^I$, hexaploid = 6x; N = 288 total plants) were germinated in seed trays in a greenhouse at Michigan Technological University. Once large enough to tolerate outside conditions (~two weeks), plants were transplanted to 7.6L round pots containing a 50:50 mixture of vermiculite to Sun Grow Mix 1 potting soil (Sun Grow Horticulture, Agawam, Massachusetts, USA) were randomly arranged in an open field in

Houghton, Michigan on land belonging to Michigan Technological University and divided into one of three N:P treatments (low, medium, high; N = 24 per geo-cytotype per treatment). The potting soil already contained 110 ppm N (μg N ⋅ g^{-1}) and 25 ppm P (μg P ⋅ g^{-1}), which we designated as the low treatment, and we added nutrients to the medium and high treatment so they totaled 165 ppm N (μg N ⋅ g^{-1}) and 37.5 ppm P (μg P ⋅ g^{-1}), and 220 ppm N (μg N ⋅ g^{-1}) and 50 ppm P (μg P ⋅ g^{-1}), respectively. NP treatment values were based on the range of soil N and P measured at a subset of seed collection sites (see Walczyk and Hersch-Green in press; Chapter 1). All plants also received 100 ppm of potassium sulfate (μg K ⋅ g^{-1}) and 3.22 mL micronutrients (Fertilome chelated liquid iron and other micronutrients; Voluntary Purchasing Groups, Bonham, Texas, USA), which were mixed in with the NP treatments, and all treatments were administered as 50mL shots three times throughout the course of the experiment on weeks 2, 6, and 10 of experimental growth. Plants were exposed to natural precipitation and temperatures and were given additional water as needed. The experiment ran from early June to early October 2020 and concluded after 21 weeks.

Measured Traits – We measured traits associated with growth and photosynthetic activity to test whether PP and/or mean trait values differed between native cytotypes and/or between the invasive vs. native tetraploids.

Growth and Resource Allocation Traits: Above- and belowground biomasses can influence plant competitive ability for light, space, water, and soil nutrients, with larger biomasses typically displaying the most competitiveness (Aerts 1999, Craine and

Dybzinski 2013). At harvest, plants were severed at the soil line and separated into their above- and belowground parts, dried in a drying oven (48hr for aboveground, 72hr for belowground), and then weighed to the nearest gram. The number of clonal ramets (i.e., genetically identical vegetative growths attached to a "parent plant" that have the potential to flower; Dong et al. 2014) produced by a single plant were also counted at harvest. They are a metric of asexual fitness as they have the potential to allow a single individual to dominate a large portion of a habitat and the resources within it (Dong, et al. 2014, Yu et al. 2016).

We also calculated the root:shoot ratio (R:S ratio) by dividing dry aboveground biomass by dry belowground biomass. This ratio is a measure of both investment into current vs. future reproductive potential and investment into below vs. aboveground resource acquisition (both indicated by low root:shoot ratios; Gioria and Osborne 2014, Goldberg et al. 2017). Ploidy level can also influence R:S ratios as cytotypes could have different strategies for adapting to resource limitations and/or may differ in their sensitivity to nutrient availability (Bales and Hersch-Green 2019).

Photosynthetic Traits: We used a portable infrared CO_2 analyzer system (LI-6800; LI-COR Inc., Lincoln, NE, USA) equipped with a CO_2 mixer and 1x3 cm^2 chamber/red-blue LED light source (LI6800-02) to measure net carbon assimilation rate between CO_2 fixation and photorespiration (A_{max}, µmol CO_2 m^{-2} s^{-1}) and transpiration rate (E, mmol H2O m-2 s-1), which were used to calculate instantaneous water use efficiency (*WUE;* A_{max}/E; Medrano et al. 2015) on a subset of 144 plants (N = 12 per geo-cytotype per treatment). Inside the chamber, CO_2 concentration was set 400 ppm, relative humidity to

65%, flow rate to 500 μmol m^{-2} s^{-1}, and light to 1000 μmol m^{-2} s^{-1} and remained constant throughout sampling. Measurements were taken once photosynthetic rate stabilized (approx. 2 min), and infrared gas analyzers (IRGA) were matched after every 10 sampling measurements. Survey snap-shot measurements were taken on the youngest, fully developed leaf on a plant in a random order spread over two sampling days between the hours of 09:00 to 16:00 during the 10^{th} and 14^{th} week of growth.

Resistance Traits: Because cytotypes (Thompson et al. 1997, Nuismer and Thompson 2001, Segraves and Anneberg 2016) and native versus invasive populations could differ in their ability to resist antagonists (Bossdorf, et al. 2005, Huang et al. 2010), we chose to expose geo-cytotypes to naturally occurring enemies in *S. gigantea*'s native range via an outdoor experimental design to evaluate plant resistance to insect and fungal pathogens. At the end of the experiment (week 20), we quantified leaf damage on all 288 plants by counting the number of leaves on each plant showing by fungal pathogens and insect herbivores and dividing this number by the total number of leaves. Next, we calculated insect and fungal resistance by subtracting the percent of fungal or insect damaged leaves from a value of "1" (Rausher and Simms 1989; Fornoni, et al. 2004).

Phenotypic Plasticity Calculations - Degrees of PP were calculated between successive nutrient level changes (i.e., low to medium, medium to high, low to high) to provide a more detailed and accurate description of plasticity responses to nutrient changes (Arnold et al. 2019). For example, should the greatest degree of PP occur between the low to medium treatments, a comparison of only the low to high treatments could be misleading

and inaccurate to the true response to environmental change (Arnold, et al. 2019; Figure S1).

To calculate PP, we first created reaction norm plots (Pigliucci, et al. 2006, Agrawal, et al. 2008, Gianoli and Valladares 2012) by averaging values of the 7 measured growth, photosynthetic, and resistance traits of each maternal line within each of the three nutrient levels. These averaged values from each maternal line were then plotted and the absolute value of the slope of the line between two nutrient treatment pairs (i.e., low to medium, medium to high, low to high) were calculated. A zero-slope value indicates that no PP is present, and larger slope values correspond to more PP.

Statistics – All statistical models consisted of a combination of the following fixed-effect independent factors: geo-cytotype (2x = diploid, $4x^N$ = native-tetraploid, $4x^I$ = invasive-tetraploid, 6x = hexaploid), NP-treatment (L = low, M = medium, H = high), nutrient-level change (L to H = low to high, L to M = low to medium, M to H = medium to high), and/or maternal line (nested within 'geo-cytotype'). Data transformations were made as needed to meet model assumptions of normality and homoscedasticity and are noted below. We used the following post-hoc analyses to test for differences among means: 1) Tukey's HSD analysis (comparisons between ≥3 levels) or Student's t-test (comparisons between two levels) for significant single model factors, and 2) controlled contrasts for significant interactions among model factors. When 'geo-cytotype' and/or the interaction between 'geo-cytotype x nutrient-level change (or NP- treatment)' was significant within statistical models, we used controlled contrasts to test whether 1) native diploids, tetraploids, and hexaploids, and 2) invasive- and native-tetraploids significantly differed

from each other independently or within changes to the nutrient environment. All statistical analyses were performed using JMP Pro version 16 (SAS Institute, Cary, North Carolina, USA).

Phenotypic Plasticity Differences-We first examined for significant differences in growth trait plasticity using four analysis of variance (ANOVA) models that tested whether the |slope values| for aboveground biomass, belowground biomass, the number of clonal ramets, R:S ratio (log10 transformed), insect resistance, and fungal resistance significantly differed based upon geo-cytotype, nutrient-level change, their interaction, and/or maternal line. Next, we used three separate repeated-measures multivariate analysis of variance models (MANOVA) to test whether the PP values of net photosynthetic capacity (A_{max}), transpiration rate (E), and/or water use efficiency (WUE) responses differed over time (10^{th} or 14^{th} week of growth) and/or were significantly affected by geo-cytotype, nutrient-level change, their interaction, and/or maternal line. When a "between subjects" MANOVA model was significant, we preformed separate univariate ANOVA models for the significant response and then used post-hoc analyses to test for significance differences among factor-level means independent of the effects of time.

Trait Level Differences- Using four ANOVA models, we examined whether significant differences in aboveground biomass, belowground biomass, the number of clonal ramets, and R:S ratio (log10 transformed) significantly differed based upon geo-cytotype, nutrient-level change, their interaction, and/or maternal line. We then used three separate repeated-measures multivariate analysis of variance models (MANOVA) to test whether

A_{max}, E, and/or WUE changed over time (10^{th} or 14^{th} week of growth) and/or were significantly affected by geo-cytotype, nutrient-level change, their interaction, and/or maternal line. When a "between subjects" MANOVA model was significant, we then preformed separate univariate ANOVA models for the significant response and then used post-hoc analyses to test for significance differences among factor-level means independent of the effects of time.

3.4 Results

Does growth trait plasticity differ between native cytotypes, native vs. invasive tetraploids, and/or nutrient level changes?

PP for both above- and belowground biomass significantly differed based upon a plant's maternal line, geo-cytotype, and changes in the nutrient environment, with the latter two model factors being dependent on each other (i.e., the interaction of geo-cytotype x nutrient level change was significant; Table 1, Figure 1,2). In general, plants tended to show the most PP for both above- and belowground biomass as nutrient levels increased from low to medium, regardless of ploidy level (Tukey's HSD Analysis; Table 1, Figure 1).

Native-tetraploids tended have more aboveground plasticity than hexaploids, with diploids not significantly differing from the other two cytotypes (Table 1, Figure 1a), and diploids tended to be more plastic for belowground biomass than native-tetraploids and hexaploids, which did not differ from each other (Table 1, Figure 1b). However, these differences in plasticity were only significant under certain changes in the nutrient environment. Native cytotypes did not significantly differ from each other in above- and belowground biomass PP when nutrient availability increased from low to high (controlled contrast for the |plasticity slopes| between diploids, native-tetraploids, and hexaploids for aboveground biomass: $F_{2,88}= 2.41$, $P = 0.0960$; for belowground biomass: $F_{2,88}= 1.24$, $P = 0.2948$; Figure 1ab). Rather, significant differences between native cytotypes in both above- and belowground biomass PP only existed when nutrient availability increased from either low to medium or medium to high conditions (Table 1;

Figure 1). For aboveground biomass plasticity, diploids and native-tetraploids had similar plasticity values that were both significantly greater than hexaploids when the nutrientt environment changed from low to medium NP conditions (controlled contrast for the |plasticity slopes| between diploids, native-tetraploids, and hexaploids: $F_{2,88}$= 27.42, P<0.0001; Figure 1a). However, when the nutrient levels increased from medium to high NP hexaploids were significantly the most plastic for aboveground biomass, native-tetraploids intermediate, and diploids the least (controlled contrast for the |plasticity slopes| between diploids, native-tetraploids, and hexaploids: $F_{2,88}$= 14.24, P<0.0001; Figure 1a). For belowground biomass plasticity, diploids were significantly the most plastic, native-tetraploids intermediate, and hexaploids the least plastic when nutrients increased from low to medium availability (controlled contrast for the |plasticity slopes| between diploids, native-tetraploids, and hexaploids: $F_{2,88}$= 17.24, P<0.0001; Figure 1b). But when nutrients increased from medium to high availability, hexaploids were significantly more plastic for belowground biomass than native-tetraploids and diploids, which did not significantly differ from each other (controlled contrast for the |plasticity slopes| between diploids, native-tetraploids, and hexaploids: $F_{2,88}$= 4.70, P=0.0115; Figure 1b).

Invasive-tetraploids tended to be less plastic for both above- and belowground biomass than native-tetraploids (Table 1, Figure 2ab), but these differences were only significant under some nutrient level changes. For aboveground biomass, native-tetraploids were only significantly more plastic than invasive-tetraploids when nutrient levels changed from low to high and from low to medium (controlled contrast for aboveground biomass | plasticity slopes| between native- and invasive- tetraploids from

low to high: $F_{2,88} = 5.20$, $P = 0.0250$; low to medium: $F_{2,88} = 21.14$, $P < 0.0001$, medium to high: $F_{2,88} = 0.00$, $P = 0.9574$; Figure 2a). Native-tetraploids exhibited significantly more belowground biomass plasticity than invasive-tetraploids only when nutrient availability shifted from low to medium (controlled contrast for aboveground biomass | plasticity slopes| between native- and invasive- tetraploids from low to high: $F_{2,88} = 0.93$, $P = 0.3365$; low to medium: $F_{2,88} = 9.93$, $P = 0.0022$, medium to high: $F_{2,88} = 0.00$, $P = 0.9501$; Figure 2b).

Plasticity in the number of clonal ramets produced by a plant significantly differed between maternal lines and nutrient level changes, under which geo-cytotype variation was only found to have a significantly effect (i.e., the interaction of geo-cytotype x nutrient level change was significant; Table 1). While plants generally were the most plastic when nutrients increased from low to medium availability (Tukey's HSD; LSMean ± SE for | plasticity slopes| for clonal ramets between low to high= 3.75 ± 0.39; low to medium= 5.66 ± 0.39; medium to high= 4.41 ± 0.39; Table 1, Figure S1), geo-cytotypes were only found to significantly differ from each other under certain nutrient level changes. Native cytotypes significantly differed from each other only when nutrients increased from medium to high availability (controlled contrast for clonal ramet |plasticity slopes| between diploids, native-tetraploids, and hexaploids from low to high: $F_{2,88} = 0.65$, $P= 0.5232$; low to medium: $F_{2,88} = 0.56$, $P= 0.5751$; medium to high: $F_{2,88} = 5.57$, $P= 0.0053$; Table 1, Figure S1). Within this nutrient level change, hexaploids were significantly more plastic for clonal shoot production than diploids, with native-tetraploids not significantly differing from the other two cytotypes (LSMean ± SE for clonal ramet | plasticity slopes| under medium to high nutrient levels for diploids: 2.71 ±

0.78; for tetraploids: 4.25 ± 0.78, for hexaploids: 6.36 ± 0.78; Figure S1). Invasive- and native-tetraploids only significantly differed from each other when nutrient availability shifted from medium to high (controlled contrast for clonal ramet |plasticity slopes| between invasive- and native-tetraploids from low to high: $F_{2,88} = 0.50$, $P = 0.4811$; low to medium: $F_{2,88} = 8.01$, $P = 0.058$; medium to high: $F_{2,88} = 00$, $P = 0.9598$; Table 1), in which native-tetraploids were significantly more plastic for clonal ramet production than invasive-tetraploids (LS Means ± SE of |slope value| for: invasive-tetraploid = 3.90 ± 0.45; for native-tetraploid = 5.17 ± 0.45).

We found that neither geo-cytotype, nutrient level change, nor their interaction had a significant effect on the R:S plasticity (Table 1). However, R:S plasticity did significantly differ between maternal lines.

Does physiological trait plasticity differ between native cytotypes, native vs. invasive tetraploids, and/or nutrient level changes?

The plasticity of maximum carbon assimilation rates (A_{max}) varied between nutrient level changes and over time. Plants exhibited significantly less plasticity responses for $A_{max}$from low to high nutrient level shifts relative to shifts from low to medium and medium to high, which did not significantly differ from each other (Tukey's HSD analysis; LS Means ± SE for |plasticity slope| of $A_{max}$from low to high= 1.67 ± 0.22; for low to medium= 3.48 ± 0.22; for medium to high= 3.17 ±0.22; Table 2; Figure 3a-c). Plasticity for A_{max}also significantly decreased over time (Student's t-test; LS Means ± SE for A_{max}|plasticity slopes| during early season= 3.20 ± 0.23; late season= 2.34 ± 0.18; Table 2). Maternal line also had a significant effect on the plasticity of A_{max}, both

dependent and independent of the effect of time (Table 2). No other model factors or interactions among model factors had a significant effect on the plasticity values of A_{max} in *S. gigantea* plants (Table 2).

Both geo-cytotype and nutrient level change had significant effects on the PP of transpiration rates (*E*) that acted independently of each other (Table 2). Native-tetraploids and hexaploids exhibited similar values for *E* plasticity, and both were significantly more plastic than diploids (controlled contrast for the |plasticity slopes| between diploids, native-tetraploids, and hexaploids: $F_{2,88}$= 4.27, *P*=0.0170; Table 2; Figure 3d-f). Invasive- and native-tetraploids did not significantly differ from each other in *E* plasticity (controlled contrast for the |plasticity slopes| between invasive- and native-tetraploids: $F_{2,88}$= 2.85, *P*=0.0950; LS Means ± SE for the |plasticity slope| of *E* in invasive-tetraploids= 1.65 ± 0.12; native-tetraploids= 1.35± 0.12; Table 2). Additionally, *E* plasticity was significantly lower when nutrient levels shifted from low to high relative to shifts from low to medium and medium to high, which did not significantly differ from each other (Tukey's HSD analysis; LS Means ± SE for |plasticity slope| of A_{net} from low to high= 0.74 ± 0.11; for low to medium= 1.56 ± 0.11; for medium to high= 1.79 ±0.11; Table 2; Figure 3d-f). Time also had a significant effect on *E* plasticity, as plasticity was greater during the early season than late season of growth (Student's t-test; LS Means ± SE for *E* |plasticity slopes| during early season = 1.60 ± 0.12; late season= 1.13 ± 0.09; Table 2). We also found that a plant's maternal line also significantly affected *E* plasticity both independent of and dependent upon the effect of time (Table 2). No other factors or interactions among model factors had a significant effect on the plasticity of *E* (Table 2).

Plant water-use efficiency (*WUE*) plasticity depended upon the independent effects of geo-cytotype and nutrient level changes (Table 2; Figure 3g-i). Diploids were significantly the least, native-tetraploids intermediate, and hexaploids the most plastic for *WUE* (controlled contrast for the |plasticity slopes| between diploids, native-tetraploids, and hexaploids: $F_{2,88}$= 15.08, P<0.0001; Figure 3g-i), while invasive- and native-tetraploids did not significantly differ from each other (controlled contrast for the |plasticity slopes| between invasive- and native-tetraploids: $F_{1,88}$= 1.27, P=0.2622; LS Means ± SE for the |plasticity slope| of *WUE* in invasive-tetraploids= 0.12 ± 0.01; native-tetraploids= 0.14 ± 0.01; Table 2). PP for *WUE* between the medium to high nutrient level changes was significantly greater than low to high changes, while nutrient level changes from low to medium did not significantly differ from the two (Tukey's HSD analysis, LS Means ± SE for |plasticity slope| of *WUE* from low to high= 0.07 ± 0.01; for low to medium= 0.10 ± 0.01; for medium to high= 0.13 ± 0.01).

WUE plasticity also significantly changed over time dependent upon a plant's geo-cytotype (i.e., the interaction between time and geo-cytotype was significant; Table 2). In general, both plasticity values for *WUE* (Student's t-test; LS Means ± SE for *WUE* |plasticity slopes| during early season= 0.08 ± 0.01; late season= 0.12 ± 0.01) and the differences in PP between the three native cytotypes increased over time (Table 2). *WUE* plasticity between native cytotypes did not differ in the earlier in the growing season (controlled contrast for WUE |plasticity slopes| between diploids, native-tetraploids, and hexaploids: $F_{2,88}$= 0.91, P=0.4061; LS Means ± SE for diploids= 0.05 ± 0.01; for native-tetraploids= 0.07 ± 0.01; for hexaploids= 0.07 ± 0.01), but as time progressed, native-tetraploids became significantly the most, hexaploids intermediate, and diploids the least

plastic for *WUE* (controlled contrast for WUE |plasticity slopes| between diploids, native-tetraploids, and hexaploids: $F_{2,88}$= 16.78, P<0.0001; LS Means ± SE for diploids= 0.05 ± 0.01; for native-tetraploids= 0.20 ± 0.01; for hexaploids= 0.13 ± 0.01). During the early season, invasive-tetraploids were significantly more plastic than native-tetraploids (controlled contrast for the |plasticity slopes| between invasive- and native-tetraploids: $F_{1,88}$= 4.12, P=0.0455; LS Means ± SE for the |plasticity slope| of invasive-tetraploids= 0.11 ± 0.01; native-tetraploids= 0.07 ± 0.01). But this pattern was reversed in the late season, with native-tetraploids becoming significantly more plastic for WUE than invasive-tetraploids (controlled contrast for the |plasticity slopes| between invasive- and native-tetraploids: $F_{1,88}$= 7.65, P=0.0069; LS Means ± SE for the |plasticity slope| of invasive-tetraploids= 0.13 ± 0.02; native-tetraploids= 0.20 ± 0.02). Plasticity values for *WUE* also differed based upon maternal line, both dependent and independent of the effect of time (Table 2). No other factors or interactions among model factors significantly affected the plasticity of *WUE* (Table 2).

Do growth traits differ between native cytotypes, native vs. invasive tetraploids, and/or nutrient level changes?

Geo-cytotype had a significant effect on aboveground biomass, while nutrient treatment, maternal line, and the interaction between nutrient treatment and geo-cytotype significantly affected both above- and belowground biomass values (Table 3, Figure 4ab). In general, plant above- and belowground biomass significantly increased with increasing NP availability (Tukey's HSD analysis; Table 3, Figure 4ab), but means both varied within each NP treatment dependent upon geo-cytotype.

Diploids and native-tetraploids tended to have larger aboveground biomasses than hexaploids (Table 3, Figure 4a), but differences in above- and belowground biomass between native cytotypes were only significant within certain nutrient treatments. For instance, native cytotype above- and belowground biomass values did not significantly differ from each other when grown under low NP (controlled contrast between diploids, native-tetraploids, and hexaploids for aboveground biomass: $F_{2,231}$= 1.60, P=0.2046; for belowground biomass: $F_{2,231}$= 2.71, P=0.0683; Figure 4a), but under medium NP conditions both diploids and native-tetraploids had significantly more above- and belowground biomass than hexaploids (controlled contrast between diploids, native-tetraploids, and hexaploids for aboveground biomass: $F_{2,231}$= 17.18, P<0.0001; for belowground biomass: $F_{2,231}$= 14.31, P<0.0001; Figure 4ab). Under high NP conditions, native-tetraploids had significantly larger aboveground biomass than both diploids and hexaploids (controlled contrast between diploids, native-tetraploids, and hexaploids: $F_{2,231}$= 5.50, P=0.0046; Figure 4a), while diploids and hexaploids had significantly larger belowground biomasses than native-tetraploids (controlled contrast between diploids, native-tetraploids, and hexaploids: $F_{2,231}$= 3.89, P=0.0218; Figure 4b).

Invasive-tetraploids tended to have smaller aboveground biomasses than native-tetraploids (Table 3), but this difference in biomass was only significant under both medium and high NP treatments (controlled contrast between invasive- and native-tetraploids under low NP: $F_{1,231}$= 1.23, P=0.2685; medium NP: $F_{1,231}$= 4.15, P=0.0427; High NP: $F_{1,231}$= 11.14, P=0.0010; Figure 5). Invasive- and native-tetraploids did not significantly differ in their belowground biomass (Table 3), nor did their belowground biomass values differ from each other dependent upon NP treatment (controlled contrast

between invasive- and native-tetraploids under low NP: $F_{1,231}$= 1.28, *P*=0.2586; medium NP: $F_{1,231}$= 3.68, *P*=0.0564; High NP: $F_{1,231}$= 0.01, *P*=0.9393). No other model factors had a significant effect on above- or belowground biomass values (Table 3).

Cytotype, NP treatment, and maternal line had significant but independent effects on clonal ramet production (Table 3). Hexaploids produced significantly fewer clonal ramets than diploids and native-tetraploids, which did not significantly differ from each other (controlled contrast between diploids, native-tetraploids, and hexaploids: $F_{2,231}$= 14.09, *P*<0.0001; LS Means ± SE for diploids= 11.83 ± 0.46; for native-tetraploids= 12.07 ± 0.49; for hexaploids= 8.76 ± 0.51), and invasive- and native-tetraploids did not significantly differ in clonal ramet production (controlled contrast between invasive- and native-tetraploids: $F_{1,231}$= 2.25, *P*=0.1347; LS Means ± SE for invasive-tetraploids= 11.06 ± 0.49; for native-tetraploids= 12.07 ± 0.49). Plants grown in medium NP conditions produced significantly the most, plants in high NP conditions produced significantly intermediate, and plants in low NP produced significantly the least clonal ramets (Tukey's HSD analysis; LS Means ± SE for low NP= 6.78 ± 0.41; medium NP= 11.72 ± 0.41; high NP= 14.28 ± 0.41; Table 3). No other model factor or interaction among model factors significantly affected clonal ramet production (Table 3).

Plant investment into root growth vs shoot growth (R:S ratio) significantly differed based upon geo-cytotype, NP treatment, and their interaction (Table 3). Hexaploids and plants grown in low NP treatments tended to have the largest R:S ratios (Table 3; Figure 4c), but differences between geo-cytotypes were dependent upon NP availability. When NP was low, both hexaploids and native-tetraploids had significantly

greater R:S ratios than diploids (controlled contrast between diploids, tetraploids, and hexaploids: $F_{2,231}$= 7.91, *P*=0.0005; Figure 4c). As NP availability increased, these differences between native cytotypes changed. Specifically, hexaploids alone had the greatest R:S ratio under medium NP conditions (controlled contrast between diploids, native-tetraploids, and hexaploids: $F_{2,231}$= 4.04, *P*=0.0188; Figure 4c), while tetraploids had the lowest R:S ratio when NP was high (controlled contrast between diploids, native-tetraploids, and hexaploids: $F_{2,231}$= 7.17, *P*=0.0010; Figure 4c). Invasive- and native tetraploids did not significantly differ from each other, regardless of NP treatment (controlled contrast between invasive- and native tetraploids under low NP: $F_{1,231}$= 0.20, *P*=0.6573; medium NP: $F_{1,231}$= 0.32, *P*=0.5715; High NP: $F_{1,231}$= 3.55, *P*=0.0608).

Do physiological traits differ between native cytotypes, native vs. invasive tetraploids, and/or nutrient level changes?

Geo-cytotype, NP treatment, and maternal line all had a significant, independent effect on A_{max} values (Table 4, Figure 6ab, 7a). Hexaploids had significantly greater A_{net} values than diploids and native-tetraploids, which did not differ from each other (controlled contrast between diploids, native-tetraploids, and hexaploids: $F_{2,231}$= 36.48, *P*<0.0001; Figure 6a), while invasive-tetraploids had significantly greater A_{max} values than native-tetraploids (controlled contrast between invasive- and native-tetraploids: $F_{1,231}$= 12.27, *P*=0.0006; Figure 7a). Additionally, plants grown in medium NP conditions had significantly the greatest A_{max} values, while plants grown in high NP conditions had intermediate A_{max} values, and those grown in low NP conditions had the lowest (Tukey's HSD analysis; Figure 6b).

A_{max} values tended to decrease over time (Student's t-test; Figure 6ab) but were dependent upon geo-cytotype or NP treatment (Table 4; Figure 6ab). In particular, during the early period of growth, A_{max} values for diploids and native-tetraploids did not differ from each other and were both significantly lower than the A_{max} values of hexaploids (controlled contrast between diploids, native-tetraploids, and hexaploids: $F_{2,231}$= 16.02, P<0.0001; Figure 4a), whereas during the latter growing period diploids had significantly the lowest, native-tetraploids intermediate, and hexaploids the greatest A_{max} values (controlled contrast between diploids, native-tetraploids, and hexaploids: $F_{2,231}$= 35.73, P<0.0001; Figure 4a). Invasive-tetraploids had significantly greater A_{net} values than native-tetraploids during the early season (controlled contrast between invasive- and native-tetraploids during the early season: $F_{1,231}$= 10.67, P=0.0012; LS Means ± SE during the early season for invasive-tetraploids = 12.20 ± 0.44, for native-tetraploids= 10.17 ± 0.44 mmol CO_2 m^{-2} s^{-1}). As time progressed these differences disappeared, as invasive- and native-tetraploids did not significantly differ from each other later in the growing season (controlled contrast between invasive- and native-tetraploids during the late season: $F_{1,231}$= 10.67, P=0.0012; LS Means ± SE during the late season for invasive-tetraploids = 11.49 ± 0.38, for native-tetraploids= 10.61 ± 0.38 mmol CO_2 m^{-2} s^{-1}).

Plants grown in different NP conditions also varied in A_{max} over time. Earlier in the growing season, plants in medium and high NP conditions had significantly greater A_{max} values than plants grown in low NP conditions (Tukey's HSD analysis, Figure 6b). Later in the growing season, plants grown in medium NP conditions had significantly greater A_{max} values than plants grow in both low and high NP treatments, which did not significantly differ from each other (Tukey's HSD analysis, Figure 6b). Additionally, a

plant's maternal line also had a significant effect on A_{max} rates both independent and dependent of time, and no other model factors or interaction among factors affected A_{max} values (Table 4).

E rates significantly differed based upon the independent effects of geo-cytotype, NP treatment, and maternal line (Table 4, Figure 6cd, 7b), and the latter two model factors also significantly affected *E* rates dependent upon time (Table 4, Figure 6cd). Both hexaploids and diploids had similar *E* rates that were significantly greater than native-tetraploids (controlled contrast between diploids, native-tetraploids, and hexaploids: $F_{2,231}$= 5.89, P=0.0032; Figure 6c), and invasive-tetraploids had significantly greater *E* rates than native-tetraploids (controlled contrast between invasive- and native-tetraploids: $F_{1,231}$= 27.10, P<0.0001; Figure 7b). Plants grown in medium NP conditions had the greatest *E* rates relative to the other NP treatments (Tukey's HSD analysis; Figure 6d). *E* rates decreased over time (Student's t-test; Table 4; Figure 6cd), and *E* rates between plants grown in different NP conditions differed within the early and late growing season (Table 4; Figure 6d). During early periods of growth, plants grown in medium NP conditions had significantly greater *E* rates than those grown in low NP conditions, while plants in high conditions did not differ from the two (Tukey's HSD analysis; Figure 6d). However, during latter season growth, plants grown in low and medium NP conditions had *E* rates that did not differ from each other while also being significantly greater than those of plants grown in high NP conditions (Tukey's HSD analysis; Figure 6d). *E* rates were not significantly affected by any other factors or interaction among factors (Table 4).

WUE significantly differed amongst plants depending upon the individual effects of geo-cytotype, nutrient treatment, and maternal line (Table 4). Hexaploids and native-tetraploids had similar *WUE* values that were both significantly greater than diploids (controlled contrast between diploids, native-tetraploids, and hexaploids: $F_{2,231}$= 19.60, P<0.0001; LS Means ± SE for diploids= 0.27 ± 0.01; for native-tetraploids= 0.39 ± 0.01; for hexaploids= 0.38 ± 0.02 mmol H_2Om^{-2} s^{-1}), while native-tetraploids were significantly more water use efficient than invasive-tetraploids (controlled contrast between invasive- and native-tetraploids: $F_{1,231}$= 13.66, P=0.0003; Figure 7c). *WUE* significantly decreased as NP availability decreased, with plants in high NP treatments being the most, medium NP treatments intermediate, and low NP treatments the least water use efficient (Tukey's HSD analysis; LS Means ± SE for low NP= 0.29 ± 0.01; for medium NP= 0.33 ± 0.01; for high NP= 0.39 ± 0.02 mmol H_2Om^{-2} s^{-1}). No other model factor or interaction among model factors had a significant effect on *WUE* values (Table 4).

Does insect and fungal resistance plasticity differ between native cytotypes, native vs. invasive tetraploids, and/or nutrient level changes?

Plant insect resistance plasticity was not significantly affected by geo-cytotpye, changes in the NP environment, their interaction, nor population of origin (Table 5). However, fungal resistance did display significant patterns of plasticity in response to changes in the NP environment (Table 5). Specifically, plants displayed significantly more resistance plasticity as NP availability shifted from low to medium and from medium to high conditions than shifts from low to high (LS Means ± SE for fungal resistance |plasticity slopes| between low to high = 2.66 ± 0.75; low to medium = 4.21 ±

0.75; and medium to high = 5.32 ± 0.75). This plasticity pattern was driven by the finding that plants were significantly more resistant in medium NP conditions than in high NP conditions, with plants grown in the high conditions not significantly differing from those grown in low or medium conditions (LS Means ± SE for fungal resistance for low NP = -59.44 ± 1.94, medium NP = -52.72 ± 1.94, high NP = -58.00 ± 1.94).

3.5 Discussion

Biological invasions are a global ecological and economic threat (Ehrenfeld 2010, Diagne et al. 2021). Changes in local environments from anthropogenic activity (e.g., nutrient enrichment, moisture availability, predation) have the potential to alter the selective environment in ways that may favor the invasive success of some species and/or populations within a species over others (Hulme 2017). Throughout the last several decades, research efforts have attempted to identify traits that pre-dispose a species to be a successful invader and/or allow for rapid adaptation to novel, non-native habitats (Van Kleunen et al. 2010, Matzek 2012). Both PP and polyploidy are suspected to contribute to the invasive success of some plant species, but studies that examine their independent and joint roles have been lacking until recently (but see Hahn, et al. 2012, Sánchez Vilas and Pannell 2017, Wei, et al. 2019, Harms, et al. 2021). Here, we aimed to better understand the relationship between PP, polyploidy, and invasion ecology by investigating what attributes might have led to the invasive success of tetraploid *S. gigantea*. To do so, we asked whether PP and mean values for traits related to successful invasions differ between 1) three ploidy levels of *S. gigantea*, 2) native and invasive populations of tetraploid *S. gigantea*, and/or 3) different levels of N and P enrichment. In general, we found that PP is complex and can vary depending upon changes in the nutrient environment and/or ploidy-level. Below we synthesize our findings and discuss them within the context of invasion and cytotype dynamics in both native and non-native environments.

Pre-introduction advantage: polyploids may be more pre-adapted to successfully invade in nutrient enriched environments

The success of a biological invasion may be due, in part, to traits and/or strategies already present in the invading species (van Kleunen et al. 2011, Oh et al. 2021, Kaushik et al. 2022). Enhanced PP in traits typically associated with competition and fitness (e.g., size, root:shoot ratio; WUE, photosynthetic rate; flower phenology; Van Kleunen et al. 2010) is one strategy thought to play a role in promoting the invasive success of some plant species (Richards et al. 2006; Pigliucci et al. 2006; Gratani et al. 2014; Colautti et al. 2017). Furthermore, greater PP in polyploids relative to diploids has been suggested as an explanation to the prevalence of polyploidy in invasive plant taxa (Pandit, et al. 2006, Pandit, et al. 2011, Te Beest, et al. 2012), as the genomic alterations following polyploidization events has the potential to change expressed phenotypes and patterns of gene expression (Comai 2005, Chen 2010, Soltis, et al. 2015). Here, we tested the hypothesis that PP differs between cytotypes in response to nutrient enrichment, with the prediction that the degree of plasticity generally increasing with ploidy level and NP availability. We also predicted that greater PP in polyploids over diploids might have aided in the invasive success of tetraploid *S. gigantea* in parts of Europe and Asia (Schlaepfer, et al. 2008, Schlaepfer, et al. 2010). We found that PP tended to increase with NP availability for all traits except for R:S ratio, and plasticity responses for most traits varied dependent upon cytotype. We did not detect any differences in plasticity for both net photosynthetic rates and R:S ratios (Figure 3; Table 1,2) between cytotypes; although photosynthetic rate plasticity did increase with NP availability and decreased over time (Figure 3; Table 1,2). A lack of plasticity differences between cytotypes have also been reported in other plant species such as *Dactylis glomerata, Arrhenatherum elatius,* and *Mercurialis annua* for morphological and fitness traits (Petit and Thompson

1997, Bretagnolle and Thompson 2001, Sánchez Vilas and Pannell 2017, Wei, et al. 2019). The lack of plasticity between cytotypes of *S. gigantea* for these two traits might have been due to selective pressures at the species level favoring consistently high photosynthetic rates to promote plant growth and productivity when nutrients become more favorable (Van Kleunen, et al. 2010, Kingsolver et al. 2012) and/or high plasticity in root vs. shoot growth to allow all cytotypes to readily adapt to changes in resource availability in their local environments (Van Kleunen, et al. 2010, Kingsolver, et al. 2012).

Nevertheless, we still found support for our hypothesis as polyploids tended to be more plastic for some traits than diploids, either regardless of changes in the NP environment (Figure 3, S2) or only under certain NP level changes (Figure 1). Both native-tetraploids and hexaploids were more plastic than diploids for their *E* rates and *WUE*, implying that polyploid *S. gigantea* may have more precise control over water retention than diploids. While polyploids have been found to display greater trait plasticity over diploids in other studies (Hahn, et al. 2012, Gallego-Tévar, et al. 2018, Kornstad, et al. 2022), only one other study has tested the plasticity of traits pertaining to water retention and drought resistance in a polyploid system and found no plasticity differences between cytotypes (Mráz et al. 2014). The ability to better control water loss could be especially useful in novel habitats that are drier and/or more variable than that which the invading species has been adapted to (Mráz, et al. 2014), and other studies have found drought resistance to be superior in polyploids relative to related diploids (Maherali et al. 2009, Rao et al. 2020, Li et al. 2021, Osipova et al. 2022) and invading species relative to native-counterparts and/or local species (Antunes et al. 2018, Abbas et

al. 2019, Orbán et al. 2021). Given that tetraploid and hexaploid *S. gigantea* occupy the central and western-most portions of *S. gigantea*'s range in North America (Schlaepfer, et al. 2008, Schlaepfer, et al. 2010), selection might have favored traits allowing polyploid populations to be better adapted to the more variable and/or limiting water availability. Walczyk and Hersch-Green (in press) investigated differences in drought-tolerance in diploid and tetraploid *S. gigantea* and found that both cytotypes were equally impaired by water limitation and showed no differences in traits related to water retention. But due to a lack of hexaploids in the aforementioned study, it remains unknown if hexaploids differ from diploids and tetraploids in their drought tolerance.

Interestingly, for some growth traits polyploids only showed greater plasticity than diploids when the nutrient environment changed from medium to high availability (e.g., aboveground biomass, belowground biomass, Figure 1). This response might be due to the additional genomic material costs assumed by the larger genome sizes of tetraploids and hexaploids relative to diploids (Lewis 1985, Leitch and Bennett 2004, Cavalier-Smith 2005, Hessen, et al. 2010, Guignard, et al. 2017, Faizullah, et al. 2021). Larger genome sizes could impose constraints on polyploid, but not diploid, plants grown in environments where nutrient availability was insufficient to maintain investment into the genome and other costly plant traits, such as growth (Cavalier-Smith 2005, Faizullah, et al. 2021, Walczyk and Hersch-Green in press). Other studies have found this to be true and have reported instances where polyploids (or organisms with large genomes) experience significant gains in growth and/or fitness relative to diploids (or organisms with small genomes; Šmarda, et al. 2013, Guignard, et al. 2016, Bales and Hersch-Green 2019, Walczyk and Hersch-Green 2019). We found similar findings here, in that as we

increased NP availability from medium to high tetraploids became more plastic than diploids for aboveground biomass and hexaploids became more plastic for both above- and belowground biomass relative to the two other cytotypes (Figure 1). In comparison, when NP availability shifted from low to medium, hexaploids were the least plastic for both above- and belowground biomass, with tetraploids being equally and less plastic than diploids for above- and belowground biomass, respectively (Figure 1). The strong response of hexaploids and the sometimes-intermediate response of tetraploids supports the idea that polyploid *S. gigantea* are released from nutrient constraints in NP enrichment environments, and that this release can result in elevated plasticity responses. Given that biological invasions often begin in environments heavily altered by humans and likely to experience N and P enrichment from development, landscaping, and farming practices (Penuelas, et al. 2013, Fowler, et al. 2015, Goyette, et al. 2016, Asabere, et al. 2018, Luo, et al. 2019), strong plasticity responses to highly available N and P could have played a strong role in the invasive success of tetraploid *S. gigantea* and other polyploid invaders. However, additional studies testing the plasticity responsiveness to nutrient enrichment in other polyploids invasive systems are needed to know the generality of our findings.

While both tetraploids and hexaploids tended to display more plasticity than diploids, the majority of *S. gigantea* in its invasive range are tetraploid (Schlaepfer, et al. 2008, Schlaepfer, et al. 2010). Thus, implying that there may be other biological factors, in addition to PP, that aided in the invasive spread of tetraploids. Differences in phenotypic traits between cytotypes could also play a role in the invasive success of one cytotype over others (Te Beest, et al. 2012), either independently or in conjunction with

differences in PP. Therefore, we also investigated whether trait values differed between cytotypes and/or the nutrient environment. We predicted that tetraploids and hexaploids would show greater enhancements in trait values than diploids as NP increased, due to their larger genome sizes, and that tetraploids would have the highest mean values for these invasive traits, given their invasive status. We found that plants experienced gains in their growth and physiological traits as NP availability increased, but we did not find much support for our prediction that polyploids would respond to NP enrichment by having larger mean values than diploids. While cytotype above- and belowground biomass did not differ from each other in low NP conditions (i.e., no "diploid advantage" was present, Bales and Hersch-Green 2019), aboveground biomass was the only trait in which a polyploid had a significantly larger mean value than diploids in NP enriched conditions (i.e., in high NP conditions tetraploids had greater aboveground biomasses than diploids and hexaploids; Figure 4). Other studies have reported similar findings where in polyploids display greater biomasses than diploids in N and/or P enriched conditions (Šmarda, et al. 2013, Guignard, et al. 2016, Bales and Hersch-Green 2019, Walczyk and Hersch-Green 2019). For example, Walczyk and Hersch-Green (2019) found that tetraploid *Chamerion angustifolium* experienced significant gains in biomass upon N-enrichment while diploid plant biomasses were not affected by nutrient additions. However, here we found that across the three NP environments, tetraploids and diploids tended to have similar trait values for growth traits and that one or both cytotypes also tended to have larger trait values than hexaploids (e.g., aboveground biomass in medium or high conditions, belowground biomass in medium conditions clonal ramet production; Figure 4). There was a similar lack of interaction between ploidy level and the NP

environment on the values of physiological traits, as hexaploids tended to have greater A_{net}, *E,* and *WUE* values than diploids and/or tetraploids, regardless of NP availability (Figure 6; Table 4). Similar trait responses to changing nutrient levels among cytotypes might occur if, for example, selection has favored different nutrient use and/or acquisition strategies in polyploids that allow them to overcome the potential limitations and growth constraints of having to supplement a large genome in nutrient poor conditions (Maherali, et al. 2009, Guo et al. 2016, Anneberg and Segraves 2019, Bales and Hersch-Green 2019). We found evidence of differing strategies in response to the NP environment here, as hexaploids consistently invested more into root growth over shoot growth relative to diploids and/or tetraploids across the three NP level treatments (Figure 4) Furthermore, under low NP environments tetraploids had significantly greater root versus shoot investment than diploids, but under high NP conditions diploids had greater R:S ratios than tetraploids (Figure 4). These findings suggest that the three cytotypes of *S. gigantea* have different investment strategies for root versus shoot investment to maximize access to either above or belowground resources. Specifically, hexaploids might invest primarily into their root systems as a means of accessing more nutrients to supplement their large genomes regardless of NP availability, while tetraploids adjust their investment strategy dependent upon NP availability. This finding is similar to that of Bales and Hersch-Green (2019) who found that resource allcoation strategies differed in diploid and tetraploid *C. angustifolium* in N-enriched environments in that diploids exhibited strategies favoring future reproduction while tetraploids exhibited strategies favoring current season reproduction.

These cytotype-specific differences in invasive traits values, coupled with our findings regarding differences in plasticity responses might offer some insight as to why tetraploids became the invasive cytotype over diploids and hexaploids. While tetraploids did not have the greatest values for all growth and physiological traits, they did tend to have greater aboveground biomass values, produce more clonal ramets, and invested more into aboveground biomass versus belowground biomass in medium and high NP conditions over hexaploids (Figure 4). Relative to diploids, tetraploids tended to be more plastic, had greater aboveground biomass in high NP conditions, were more water use efficient, and had greater net photosynthetic rates later in the growth season (Figure 4). It is difficult to tell if the invasion success of tetraploids might simply be due to the chance event of only tetraploids being introduced in Europe and Asia or if these cytotype differences equate to a competitive advantage in *S. gigantea's* non-native habitat. Comparisons of the three cytotypes grown in their non-native habitat would be beneficial in testing whether cytotype differences in plasticity and/or trait values persist or change in the presence of novel biotic and abiotic pressures. Such studies would help us to more fully understanding the invasion biology of polyploid systems.

Post-introduction adaptation: invasive populations may have adapted via enhanced physiological traits rather than PP

PP has been associated with successful biological invasions in plant species (Porté, et al. 2011, Knop and Reusser 2012, Matesanz, et al. 2012, Luo, et al. 2019, Bufford and Hulme 2021), but whether high levels of PP is a trait that evolved within invasive populations or if it is a pre-adaptive trait already found in invading populations

is not well known and likely varies across invasive taxa. By comparing native and invasive tetraploid populations, we aimed to better understand if and how *S. gigantea* utilized PP in its invasive success. We hypothesized that native and invasive tetraploid populations would exhibit differences in PP for growth, physiological, and resistance traits that could be associated with biological invasions. Specifically, our overarching prediction was that invasive populations would be more plastic than native populations if high degrees of plasticity for a given trait was selectively favored as an evolved adaptation in the non-native environment. In general, we did not find support for this prediction in that invasive populations tended to be less plastic for growth traits (Figure 2) and equally plastic for most physiological and resistance traits relative to native tetraploid populations. It is possible that the potential for evolving increased PP was reduced in invasive populations via genetic drift, as a lack of genetic variation and/or continuous genetic admixture can limit the emergence of PP (Schlichting and Pigliucci 1998, Murren, et al. 2015). While some studies also report a lack of plasticity difference between native and invasive populations (Peperkorn, et al. 2005, Palacio-López and Gianoli 2011, Griffith et al. 2014, Ryan and Gunderson 2021) or greater plasticity in native populations (Lamarque, et al. 2013, Wang, et al. 2018, Plantamp, et al. 2019, Albarrán-Mélzer, et al. 2020), others have found invasive populations to be more plastic than their native counterparts (Porté, et al. 2011, Knop and Reusser 2012, Matesanz, et al. 2012, Luo, et al. 2019, Bufford and Hulme 2021). This implies that differences in PP between native and invasive populations are likely to be highly species-specific, dependent upon different abiotic and/or biotic variables, requires significant amounts of evolutionary time (i.e., not enough evolutionary time has passed), and/or may vary across

different stages of a biological invasion (e.g., plasticity differences might be most visible early in an invasion before adaptation can occur; Palacio-López and Gianoli 2011). For instance, high plasticity for growth traits may be more advantageous in native populations of *S. gigantea* in that a greater phenotypic range for aboveground and belowground biomasses and the number of clonal ramet stems produced by a plant might render native populations more successful at competing for access to limited space, sunlight, water, and nutrients against other species endemic and locally adapted to mesic grassland ecosystems (Henn et al. 2018). In the non-native range, invading *S. gigantea* populations might have possessed traits (e.g., large plant size, fast growth rates; allelopathy; Van Kleunen, et al. 2010) and/or been introduced to ideal environments (e.g., high nutrient availability, wet soil, lack of enemies; Keane and Crawley 2002, Te Beest, et al. 2012, Luo, et al. 2019) that gave them a competitive edge over the endemic flora without needing to rely on PP to succeed. Given that PP is costly for an organism to maintain and execute (Wolfe and Mazer 2005, Auld, et al. 2010, Murren, et al. 2015), selection for PP in invasive populations may have relaxed over time in favor of investing resources into fixed trait values associated with high fitness. In this case, invasion success in *S. gigantea* might be more associated with the ability to adapt to specific ecological niches than with PP.

Our finding that native and invasive tetraploids differed from each other in their aboveground biomass, net photosynthetic rates, transpiration rates, and water use efficiency (Figure 5,7), suggests that the invasive population might have adapted to some aspects of the non-native habitat. High photosynthetic rates in plants tend to be positively correlated with biomass accumulation and growth (Peng et al. 1991, Arntz et al. 1998, Li

et al. 2016) and with reproduction (Arntz, et al. 1998, Zhang et al. 2005, Choi et al. 2016). But here the greater photosynthetic rates of invasive tetraploids did not equate to larger biomass (Figure 5,7), as native tetraploids tended to have greater aboveground biomasses than invasive tetraploids when NP was not limiting (e.g., medium, high NP conditions; Figure 5) and belowground biomass did not differ between the two tetraploid populations (Figure 5). One possibility for this finding is that the increased photosynthetic activity in invasive populations translates into enhanced reproductive traits, such as flower production, seed production, and seedling viability, and/or increased investment into rhizospheric symbiotes relative to native populations (Feng et al. 2007, Feng and Fu 2008, Li et al. 2016, Lin et al. 2019). But because plants did not flower throughout the duration of this study (possibly due to *S. gigantea* being a perennial species grown directly from seed; Albani and Coupland 2010), we were unable to determine if reproductive traits differed between native and invasive populations.

Enhanced photosynthetic activity in invasive populations might also be indictive of an adaptive trade-off involving the reduction of costly defensive traits in favor of investing into photosynthesis and growth and/or reproduction (Blossey and Notzold 1995). An "escape" from specialist antagonistic species is well-reported across invasive plant taxa (Vila et al. 2005, Liu et al. 2007, Correia et al. 2016, Hartshorn et al. 2022), and in some species the release from this selection pressure allowed for invasive populations to exhibit greater competitive, growth, and/or fitness traits (Blossey and Notzold 1995, Keane and Crawley 2002, Joshi and Vrieling 2005, Hull-Sanders et al. 2007) relative to their native counterparts. For example, a recent study of the invasive plant *Rumex crispus* by Costan et al. (2022) showed that invasive populations were less

affected by above- and belowground herbivores and had greater aboveground biomass relative to native populations, indicating that a release from herbivore pressure might have allowed invasive populations to invest more into growth. While we compared differences in resistance plasticity towards naturally occurring insect and fungal enemies between the native- and invasive tetraploids, we did not detect any differences in damage patterns of plasticity or resistance. This may be due to the damage being inflicted by generalist enemies, which both populations may experience to some extent in their respective habitats (Joshi and Vrieling 2005), owing to a need for studies that incorporate both above- and belowground generalist and specialist enemies when quantifying investment into defensive traits to explore local adaption in the context of enemy release.

Finally, it is also possible that the native and invasive populations have adapted to different climates and/or growing seasons, as evidenced by their differing photosynthetic rates early in the season, the greater transpiration and photosynthetic rates in invasive populations, and the greater *WUE* in native populations (Figure 7). Our invasive populations originate from Zürich, Switzerland, which has a wet climate with a long growing season (Begert et al. 2005), while our native populations originate from three states within the United States (Ohio, Iowa, New Hampshire) that encompass a range of summer precipitation totals and growing seasons. Selection might have favored physiological traits that increase carbon uptake at the expense of increasing water loss (e.g., higher transpiration rates, stomata staying open in drier conditions; low water use efficiency; Hatfield and Dold 2019) in invasive populations given the wetter climate. However, because we were only able to include a small number of populations representative of *S. gigantea*'s invasive range relative to the populations we included

form the native range (Appendix 1), our speculations of which traits have been selectively favored in non-native habitats may not apply to all *S. gigantea* populations within its invasive range. Future studies in this invasive system would greatly benefit from incorporating more geographic diversity in both the native and invasive populations.

Conclusion

Plasticity responses are highly complex and can depend upon a multitude of factors including species or cytotype-specific traits and responses, environmental attributes, and/or selective and neutral processes. Here, we highlight the need to consider ploidy level in comparisons between native and non-native populations of a species, as both phenotypic traits and plasticity can vary greatly dependent upon cytotype and the abiotic environment. Our findings also emphasize the importance of examining PP across more than two environmental treatment levels, as discussed in Arnold, et al. (2019). The addition of a third nutrient treatment level resulted in much greater resolution of the underlying shape of our reaction norms. By doing so, we found that polyploids tended to have greater plasticity responses between our medium to high nutrient level changes relative to diploids. The inclusion of only the low and high NP treatments would have masked this interesting finding, especially as geo-cytotype plasticity seldom differed between the low to high NP level changes.

3.6 Tables

Table 1. Results from fixed-effects ANOVA models for the effects of geo-cytotype (diploid = 2x, native-tetraploids = $4x^N$, invasive-tetraploids = $4x^I$, hexaploid = 6x), soil nutrient level changes (LtoH= low to high, LtoM= low to medium, MtoH= medium to high), their interaction, and maternal line nested within geo-cytotype on the |phenotypic plasticity slope values| of aboveground biomass, belowground biomass, clonal ramet production, and R:S ratio (log10 transformed). Overall model results are reported in the footnotes, bold values indicate a significant effect at $\alpha = 0.05$. If the model factors 'geo-cytotype' and/or "geo-cytotype x NP treatment' were significant, controlled contrasts were used to determine significant differences between means of native cytotypes (2x, $4x^N$, 6x) and/or tetraploid origin ($4x^N$, $4x^E$) independently or between nutrient level changes. Tukey's HSD tests ($\alpha = 0.05$) were used to determine significant differences between means when nutrient level change was significant.

Source	df	MS	F	Prob > *F*	Controlled Contrasts and/or Tukey's HSD
Aboveground biomass					
Geo-cytotype (C)	3	14.64	6.03	**0.0009**	$4x^N$ (= 2x) > 6x (= 2x) $4x^N > 4x^I$
Nutrient level change (NL)	2	8.34	3.43	**0.0367**	LtoM (=LtoH) > MtoH (= LtoH)
C x NL	6	34.67	14.27	**<0.0001**	
Maternal line [Geo-cytotype]	44	5.86	2.41	**0.0002**	
Model Error	143	2.43			
Belowground biomass					
Geo-cytotype (C)	3	115.65	6.70	**0.0004**	2x > $4x^N$ = 6x $4x^N > 4x^I$
Nutrient level change (NL)	2	95.22	5.52	**0.0055**	LtoM > LtoH = MtoH
C x NL	6	119.96	6.95	**<0.0001**	
Maternal line [Geo-cytotype]	44	40.57	2.35	**0.0003**	
Model Error	143	17.26			

Clonal ramets					
Geo-cytotype (C)	3	13.83	1.91	0.1342	
Nutrient level change (NL)	2	45.39	6.26	**0.0029**	LtoM (=MtoH) > LtoH (= MtoH)
C x NL	6	19.13	2.64	**0.0212**	
Maternal line [Geo-cytotype]	44	17.44	2.41	**0.0002**	
Model Error	143	7.25			
R:S ratio					
Geo-cytotype (C)	3	0.16	0.67	0.5702	
Nutrient level change (NL)	2	0.22	0.89	0.4133	
C x NL	6	0.17	0.71	0.6388	
Maternal line [Geo-cytotype]	44	0.45	1.84	**0.0080**	
Model Error	143	0.24			

Overall model for aboveground biomass: $R^2 = 0.71$, $F_{55,143} = 3.94$, $P < 0.0001$, N = 144
Overall model for belowground biomass: $R^2 = 0.67$, $F_{55,143} = 3.21$, $P < 0.0001$, N = 144
Overall model for clonal ramets: $R^2 = 0.61$, $F_{55,143} = 2.54$, $P < 0.0001$, N = 144
Overall model for R:S Ratio: $R^2 = 0.50$, $F_{55,143} = 1.62$, $P = 0.0221$, N = 144

Table 2. Results from repeated measures MANOVA models for the effects of geo-cytotype (diploid = 2x, native-tetraploids = $4x^N$, invasive-tetraploids = $4x^I$, hexaploid = 6x), nutrient level change (LtoH= low to high, LtoM= low to medium, MtoH= medium to high), their interactions, and maternal line nested within geo-cytotype on the |phenotypic plasticity slope values| maximum photosynthetic capacity (A_{max}), transpiration rate (E), and water use efficiency (WUE; N = 144). Wilk's Λ are given in the footnotes. Bold values indicate a significant effect at α = 0.05. If the model factors 'geo-cytotype' and/or "geo-cytotype x NP treatment' were significant, controlled contrasts were used to determine significant differences between means of native cytotypes (2x, $4x^N$, 6x) and/or tetraploid origin ($4x^N$, $4x^E$) independently or between nutrient level changes. Tukey's HSD tests (α = 0.05) were used to determine significant differences between means when nutrient level change was significant.

		Maxiumum photosynthetic rate (A_{max})		Transpiration rate (E)		Water use efficiency (WUE)	
Source	df	F	Prob > F	F	Prob > F	F	Prob > F
Between subjects							
Geo-Cytotype (C)	3,88	2.08	0.1087	5.18	**0.0024**	11.11	**<0.0001**
Nutrient level change (NL)	2,88	18.44	**<0.0001**	26.20	**<0.0001**	9.86	**0.0001**
C x NL	6,88	1.99	0.0756	1.21	0.3097	1.21	0.3113
Maternal line [Geo-cytotype]	44,88	1.92	**0.0049**	2.48	**0.0001**	1.94	**0.0043**
Whole model	55,88	2.53	**<0.0001**	3.35	**<0.0001**	2.65	**<0.0001**
Within subjects							
Time (T)	1,88	14.20	**0.0003**	17.30	**<0.0001**	18.48	**<0.0001**
T x C	3,88	0.27	0.8500	1.71	0.1711	6.49	**0.0005**
T x NL	2,88	2.77	0.0683	1.26	0.2881	1.95	0.1489
T x C x NL	6,88	1.09	0.3755	0.83	0.5510	0.91	0.4929
T x Maternal line [Geo-cytotype]	44,88	1.66	**0.0222**	2.58	**<0.0001**	2.78	**<0.0001**
Whole model**	55,88	1.56	**0.03016**	2.30	**0.0002**	2.75	**<0.0001**

** A_{net} within interactions Wilk's Λ = 0.98; E within interactions Wilk's Λ = 1.44; WUE within interactions Wilk's Λ = 1.72.

Table 3: Results from fixed-effects ANOVA models for the effects of geo-cytotype (diploid = 2x, native-tetraploids = $4x^N$, invasive-tetraploids = $4x^I$, hexaploid = 6x), soil NP treatments (L= low, M=medium, H=high), their interaction, and geo-cytotype nested within maternal line on the mean values of aboveground biomass, belowground biomass, clonal ramet production, and R:S ratio (log10 transformed). Overall model results are reported in the footnotes, bold values indicate a significant effect at $\alpha = 0.05$. If the model factors 'geo-cytotype' and/or "geo-cytotype x NP treatment' were significant, controlled contrasts were used to determine significant differences between means of native cytotypes (2x, $4x^N$, 6x) and/or tetraploid origin ($4x^N$, $4x^E$) independently or between treatment levels. Tukey's HSD tests ($\alpha = 0.05$) were used to determine significant differences between means when NP treatment was significant.

Source	df	MS	F	Prob > *F*	Controlled Contrasts and/or Tukey's HSD
Aboveground biomass					
Geo-cytotype (C)	3	24.85	5.02	**0.0022**	$2x = 4x^N > 6x$ $4x^N > 4x^I$
NP Treatment (NP)	2	1322.69	267.01	**<0.0001**	H > M > L
C x NP	6	36.74	7.42	**<0.0001**	
Maternal line [Cytotype]	44	18.16	6.67	**<0.0001**	
Model Error	231	4.95			
Belowground biomass					
Geo-cytotype (C)	3	61.01	1.48	0.2211	
NP Treatment (NP)	2	7072.45	171.40	**<0.0001**	H > M > L
C x NP	6	195.81	4.75	**0.0001**	
Maternal line [Geo-cytotype]	44	109.90	2.66	**<0.0001**	
Model Error	231	41.26			
Clonal ramets					
Geo-cytotype (C)	3	146.19	9.40	**<0.0001**	$2x = 4x^N > 6x$ $4x^N = 4x^I$

NP Treatment (NP)	2	1394.96	89.69	**<0.0001**	H > M > L
C x NP	6	22.27	1.43	0.2033	
Maternal line [Geo-cytotype]	44	45.88	2.95	**<0.0001**	
Model Error	231				
R:S ratio					
Geo-cytotype (C)	3	0.19	6.41	**0.0003**	$6x > 2x = 4x^N$ $4x^N = 4x^I$
NP Treatment (NP)	2	0.16	5.33	**0.0055**	L (= M) > H (= M)
C x NP	6	0.11	3.56	**0.0021**	
Maternal line [Geo-cytotype]	44	0.04	1.28	0.1244	
Model Error	231	0.03			

Overall model for aboveground biomass: $R^2 = 0.77$, $F_{55,286} = 13.72$, $P < 0.0001$, N = 287
Overall model for belowground biomass: $R^2 = 0.68$, $F_{55,286} = 8.97$, $P < 0.0001$, N = 287
Overall model for clonal ramets: $R^2 = 0.60$, $F_{55,286} = 6.35$, $P < 0.0001$, N = 287
Overall model for R:S Ratio: $R^2 = 0.32$, $F_{55,286} = 1.97$, $P = 0.0003$, N = 287

Table 4. Results from repeated measures MANOVA models for the effects of geo-cytotype (diploid = 2x, native-tetraploids = $4x^N$, invasive-tetraploids = $4x^I$, hexaploid = 6x), soil NP treatments (L= low, M=medium, H=high), their interaction, and geo-cytotype nested within maternal line on the mean values of maximum photosynthetic capacity (A_{max}), transpiration rate (E), and water use efficiency (WUE; N = 237). Wilk's Λ are given in the footnotes. Bold values indicate a significant effect at α = 0.05. If the model factors 'geo-cytotype' and/or "geo-cytotype x NP treatment' were significant, controlled contrasts were used to determine significant differences between means of native cytotypes (2x, $4x^N$, 6x) and/or tetraploid origin ($4x^N$, $4x^E$) independently or between treatment levels. Tukey's HSD tests (α = 0.05) were used to determine significant differences between means when NP treatment was significant.

		Maximum photosynthetic rate (A_{max})		Transpiration rate (E)		Water use efficiency (WUE)	
Source	df	F	Prob > F	F	Prob > F	F	Prob > F
Between subjects							
Geo-cytotype (C)	3,231	26.03	**<0.0001**	9.17	**<0.0001**	14.33	**<0.0001**
NP Treatment (NP)	2,231	28.68	**<0.0001**	11.06	**<0.0001**	16.93	**<0.0001**
C x NP	6,231	1.12	0.3527	2.12	0.0515	1.79	0.1020
Maternal line [Geo-cytotype]	44,231	2.22	**<0.0001**	2.48	**<0.0001**	1.69	**0.0073**
Whole model	55,231	4.28	**<0.0001**	3.14	**<0.0001**	2.97	**<0.0001**
Within subjects							
Time (T)	1,231	5.62	**0.0186**	155.62	**<0.0001**	75.45	*<0.0001
T x C	3,231	4.19	**0.0066**	2.02	0.1119	5.38	*0.0013
T x NP	2,231	21.18	**<0.0001**	7.29	**0.0009**	3.34	*0.0371
T x C x NP	6,231	0.91	0.4908	1.31	0.2538	1.37	0.2267
T x Maternal line [Geo-cytotype]	44,231	1.44	**0.0466**	1.86	**0.0018**	1.05	0.4026
Whole model**	55,231	2.23	**<0.0001**	2.02	**0.0002**	1.36	0.0634

* Factor significance negated by the insignificant overall statistical model.
** A_{net} within interactions Wilk's $\Lambda = 0.53$; E within interactions Wilk's $\Lambda = 0.48$; WUE within interactions Wilk's $\Lambda = 0.32$

Table 5: Results from fixed-effects ANOVA models for the effects of geo-cytotype (diploid = 2x, native-tetraploids = $4x^N$, invasive-tetraploids = $4x^I$, hexaploid = 6x), soil nutrient level changes (LtoH= low to high, LtoM= low to medium, MtoH= medium to high), their interaction, and maternal line nested within geo-cytotype on the |phenotypic plasticity slope values| of insect resistance and fungal resistance. Overall model results are reported in the footnotes, bold values indicate a significant effect at $\alpha = 0.05$..If the model factors 'geo-cytotype' and/or "geo-cytotype x NP treatment' were significant, controlled contrasts were used to determine significant differences between means of native cytotypes (2x, $4x^N$, 6x) and/or tetraploid origin ($4x^N$, $4x^E$) independently or between treatment levels. Tukey's HSD tests ($\alpha = 0.05$) were used to determine significant differences between means when NP treatment was significant.

Source	df	MS	F	Prob > *F*	Controlled Contrasts and/or Tukey's HSD
Insect Resistance					
Geo-cytotype (C)	3	21.20	0.83	0.4820	
NP Treatment (NP)	2	85.28	3.17	*0.0453	
C x NP	6	19.73	0.73	0.6231	
Maternal line [Geo-cytotype]	8	56.11	2.09	*0.0418	
Model Error	124	26.88			
Fungal Resistance					
Geo-cytotype (C)	3	39.38	0.33	0.8069	
NP Treatment (NP)	2	1819.20	15.04	**<0.0001**	MtoH=LtoM >LtoH
C x NP	6	140.07	1.16	0.3331	
Maternal line [Geo-cytotype]	8	236.97	1.96	0.0570	
Model Error	124	120.95			

Overall model for insect resistance: $R^2 = 0.19$, $F_{19,143} = 1.57$, $P = 0.0739$, N = 144
Overall model for fungal resistance: $R^2 = 0.30$, $F_{19,143} = 2.81$, $P = 0.0003$, N = 144
*Insignificant overall model negates significant model factor

3.7 Figures

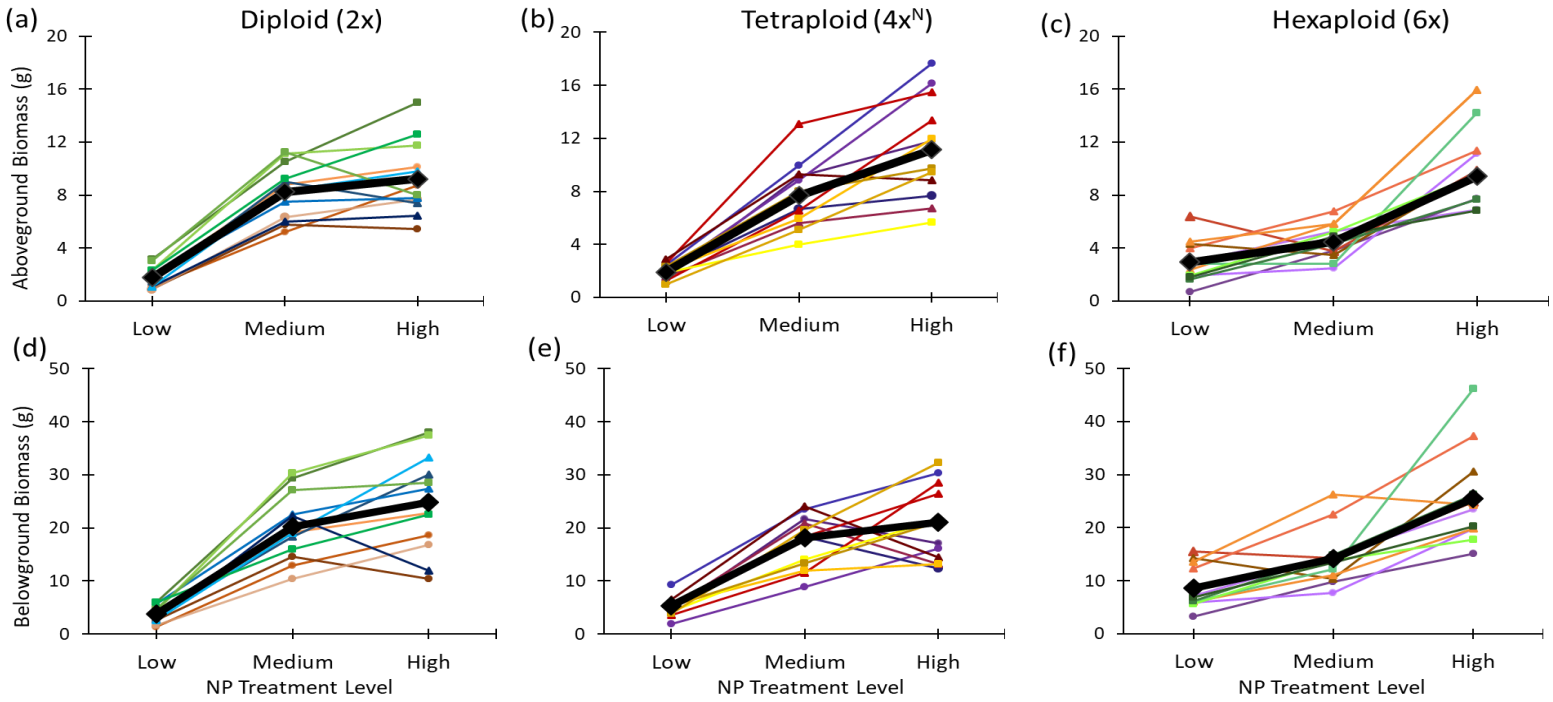

Figure 1: Reaction norm plots depicting phenotypic plasticity for aboveground biomass (a-c) and belowground biomass (d-f) in diploids (a,d), native-tetraploids (b,e), and hexaploids (c,f). The colored symbols represent the averaged means of two individuals of the same maternal line at a given nutrient treatment, and the different symbol shapes (circle, triangle, square) are representative of the three different populations within a geo-cytotype. The colored lines connecting the NP treatments are the phenotypic plasticity reaction norm slopes, wherein a larger slope indicates more plasticity between treatment levels. The black symbols and lines represent the mean value and mean phenotypic plasticity reaction norm slopes, respectively, for a geo-cytotype. Full statistical details surrounding differences in plasticity between geo-cytotypes, nutrient level changes, their interaction, and maternal line can be found in Table 1.

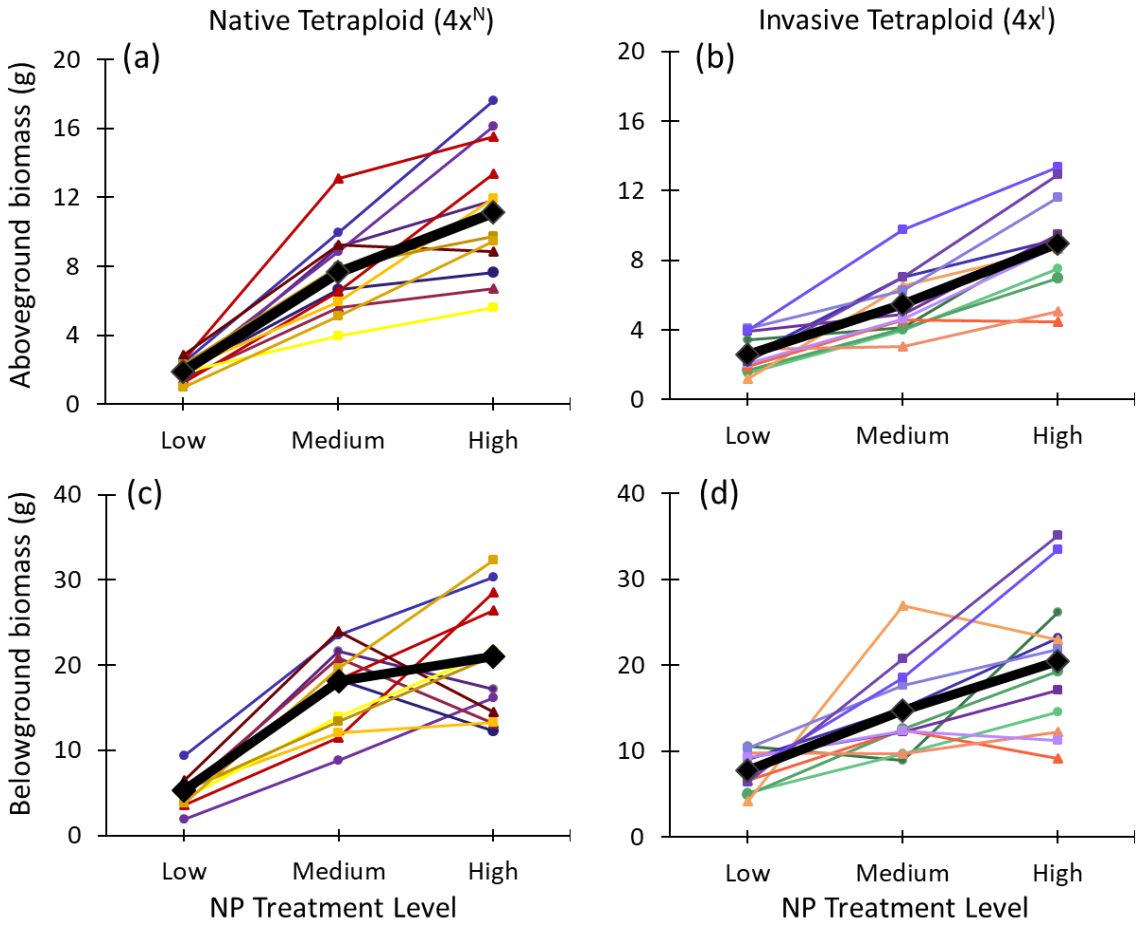

Figure 2: Reaction norm plots depicting phenotypic plasticity for aboveground biomass (a,b) and belowground biomass (c,d) in native-tetraploids (a,c), and invasive-tetraploids (b,d). The colored symbols represent the averaged means of two individuals of the same maternal line at a given nutrient treatment, and the different symbol shapes (circle, triangle, square) are representative of the three different populations within a geo-cytotype. The colored lines connecting the NP treatments are the phenotypic plasticity reaction norm slopes, wherein a larger slope indicates more plasticity between treatment levels. The black symbols and lines represent the mean value and mean phenotypic plasticity reaction norm slopes, respectively, for a geo-cytotype. Full statistical details surrounding differences in plasticity between geo-cytotypes, nutrient level changes, their interaction, and maternal line can be found in Table 1.

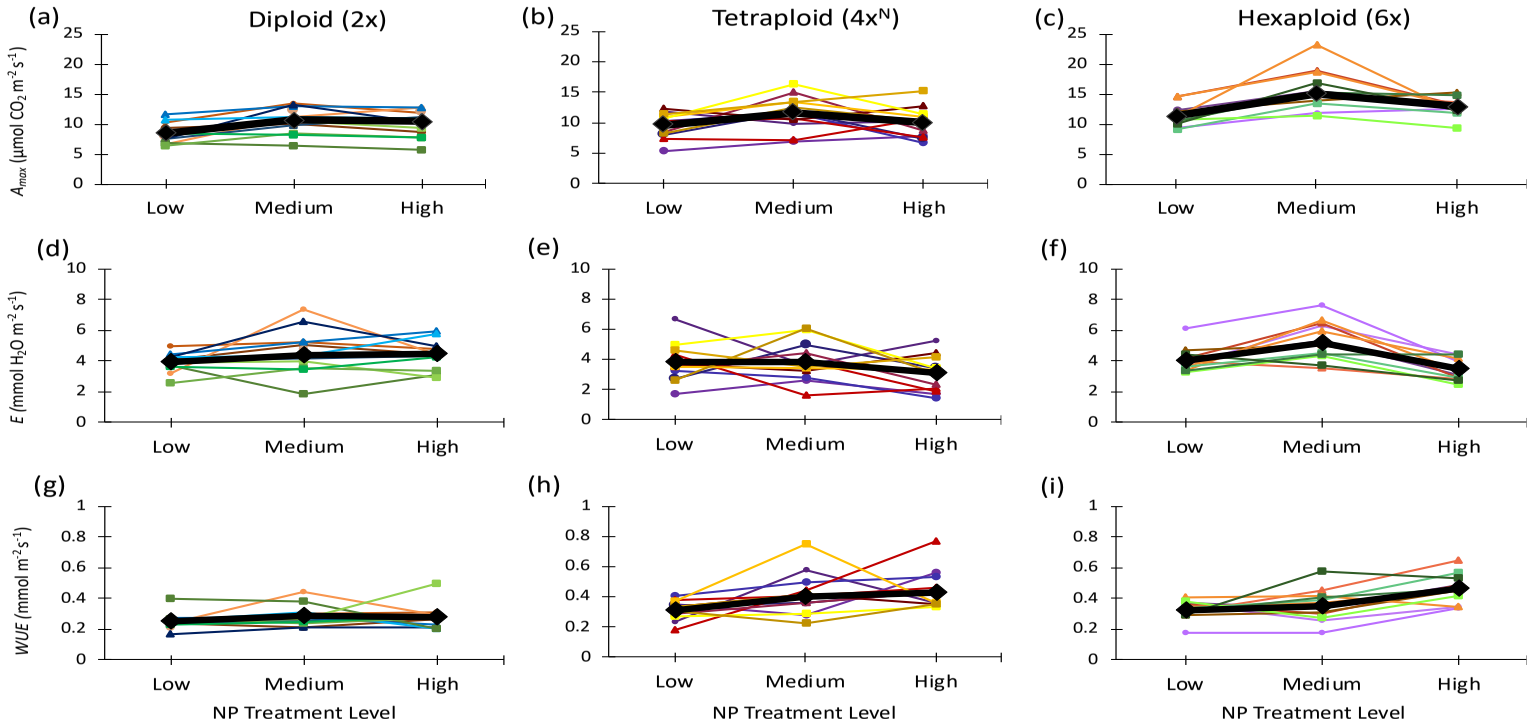

Figure 3: Reaction norm plots depicting phenotypic plasticity for maximum photosynthetic activity (A_{max}, a-c), transpiration rates (E, d-f), water use efficiency (WUE, g-i) in diploids (a,d,g) in native-tetraploids (b,e,h), and hexaploids (c,f,i). The colored symbols represent the averaged means of two individuals of the same maternal line at a given nutrient treatment, and the different symbol shapes (circle, triangle, square) are representative of the three different populations within a geo-cytotype. The colored lines connecting the NP treatments are the phenotypic plasticity reaction norm slopes, wherein a larger slope indicates more plasticity between treatment levels. The black symbols and lines represent the mean value and mean phenotypic plasticity reaction norm slopes, respectively, for a geo-cytotype. Full statistical details surrounding differences in plasticity between geo-cytotypes, nutrient level changes, their interaction, and maternal line can be found in Table 2.

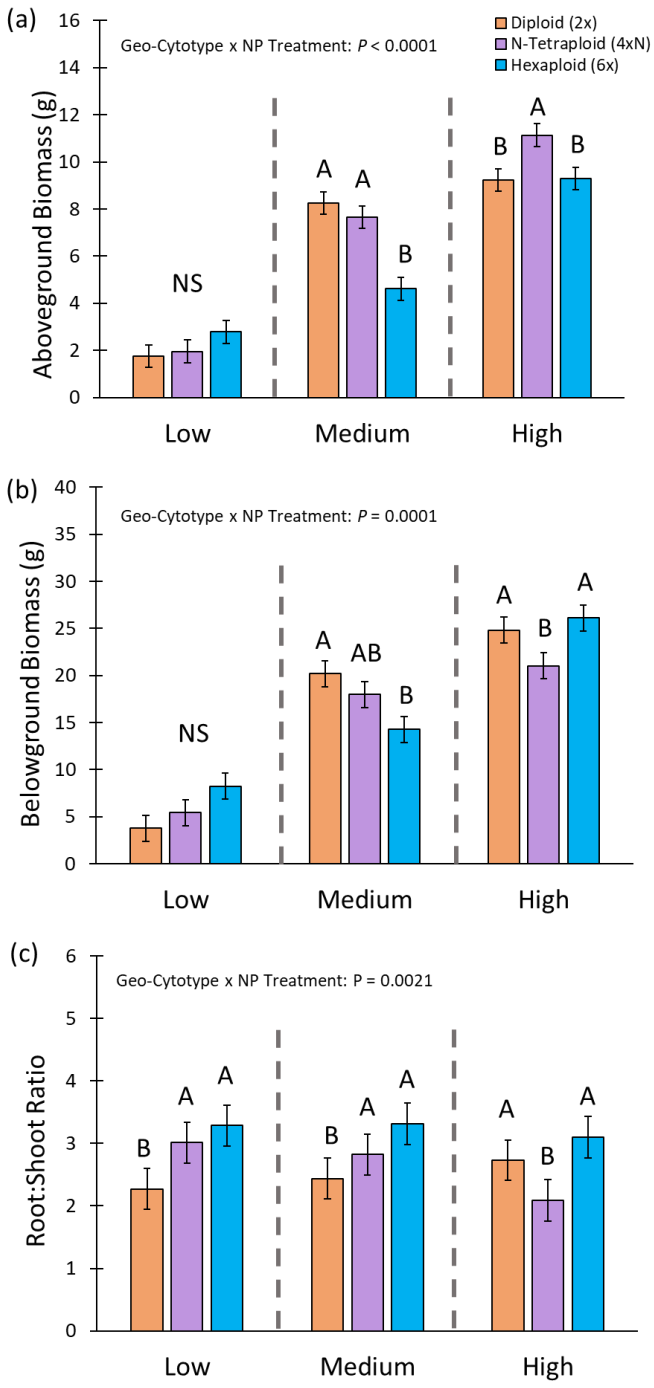

Figure 4: LSMean values for aboveground biomass (a), belowground biomass (b), and root:shoot ratio (c) for the controlled contrasts between native-cytotypes grown under each nutrient level within the significant main interaction between NP treatment and plant geo-cytotype. Significantly different mean values determined by the controlled contrasts are noted with different letters, and error bars represent ± standard error. Full statistical details can be found in Table 3 and the main text.

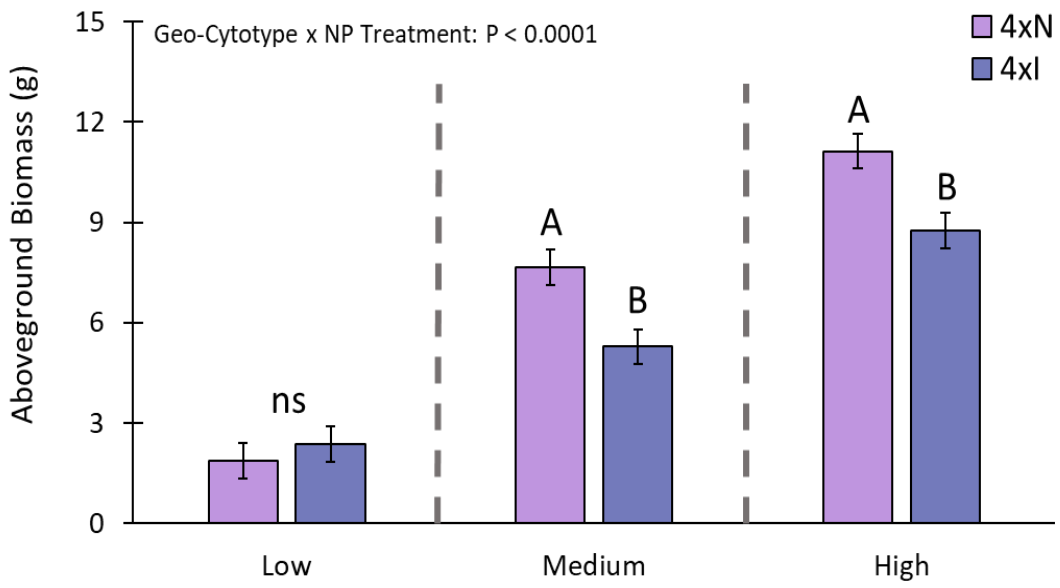

Figure 5: LSMean values for aboveground biomass for the controlled contrasts between invasive- (4xI) and native-tetraploids (4xI) grown under each nutrient level within the significant main interaction between NP treatment and plant geo-cytotype. Significantly different mean values determined by the controlled contrasts are noted with different letters, and error bars represent ± standard error. Full statistical details can be found in Table 3 and the main text.

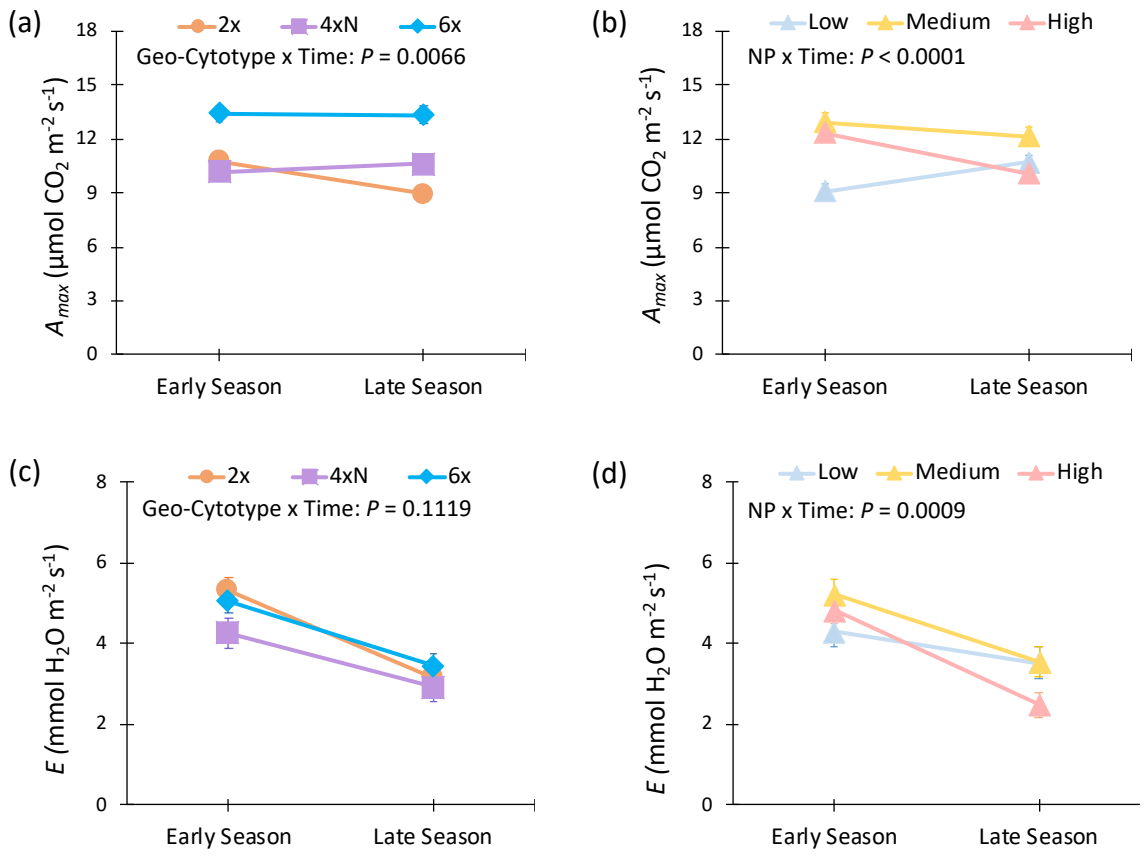

Figure 6: LSMean values for net photosynthetic rates (A_{net}, a,b) and transpiration rates (E, c,d) showing the significant effects of time (early season, late season) on native geo-cytotypes (a,c) and plants grown in different nutrient treatments (d,c). Significantly different mean values were determined by controlled contrasts between native cytotypes or nutrient treatments within each time point. Full statistical details can be found in Table 4 and the main text.

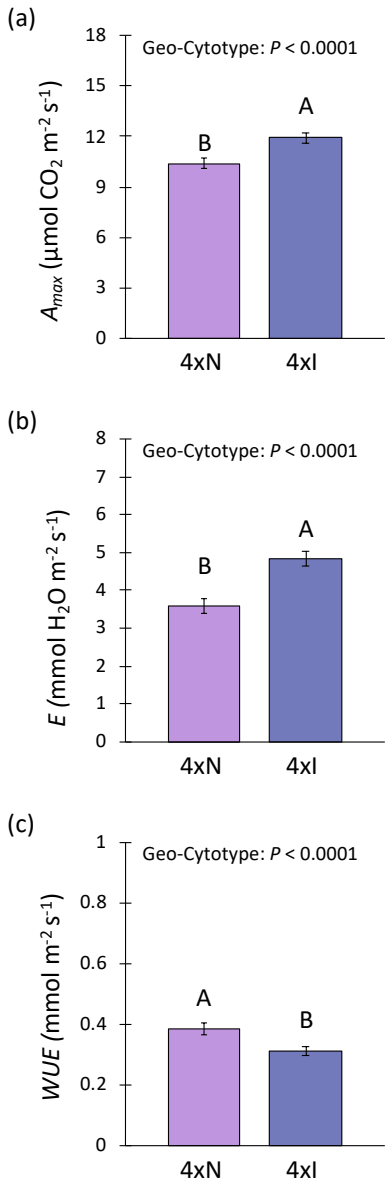

Figure 7: LSMean values for maximum photosynthetic activity (A_{max}, a), transpiration rate (E, b), and water use efficiency (WUE, c) for the controlled contrasts between invasive- (4xI) and native-tetraploids (4xN) within the significant model factor of plant geo-cytotype. Significantly different mean values determined by the controlled contrasts are noted with different letters, and error bars represent ± standard error. Full statistical details can be found in Table 4 and the main text.

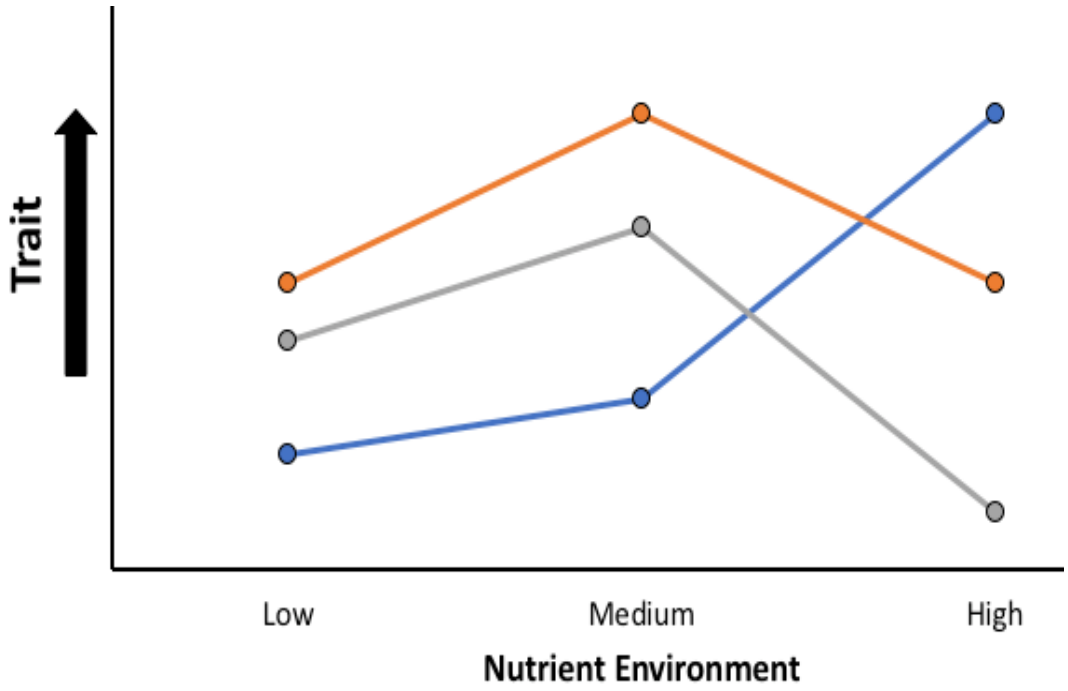

Figure S1: Hypothetical reaction norm plot for three maternal lines in response to nutrient level changes. Each maternal line is displaying patterns of phenotypic plasticity between nutrient level changes that would be masked by examining only changes from low to high treatments (Arnold et al. 2019).

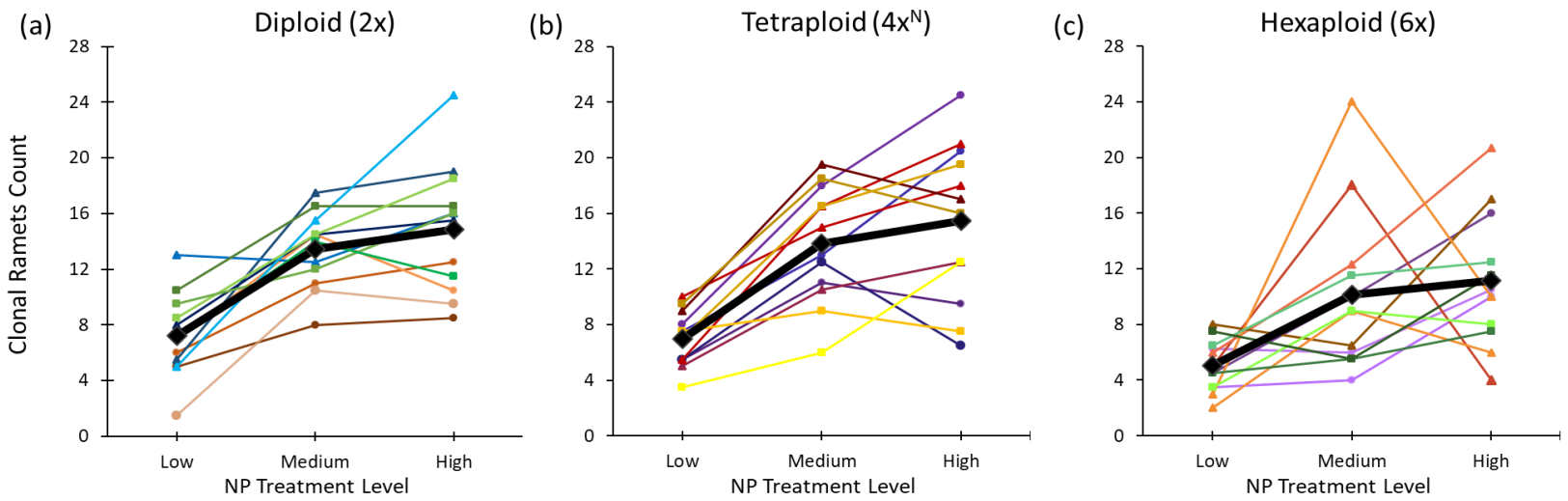

Figure S2: Reaction norm plots depicting phenotypic plasticity for clonal ramet production in diploids (a), native-tetraploids (b), and hexaploids (c). The colored symbols represent the averaged means of two individuals of the same maternal line at a given nutrient treatment, and the different symbol shapes (circle, triangle, square) are representative of the three different populations within a geo-cytotype. The colored lines connecting the NP treatments are the phenotypic plasticity reaction norm slopes, wherein a larger slope indicates more plasticity between treatment levels. The black symbols and lines represent the mean value and mean phenotypic plasticity reaction norm slopes, respectively, for a geo-cytotype. Full statistical details surrounding differences in plasticity between geo-cytotypes, nutrient level changes, their interaction, and maternal line can be found in Table 1.

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4 **Chapter 4:** From transcriptomes to traits: investigating the role of resource allocation tradeoffs in the invasion success of tetraploid *Solidago gigantea*[4]

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[4] Part of this chapter will be included in separate manuscript(s) that will be submitted to undecided journal(s). Erika Hersch-Green conceived the experiments, and Angela Walczyk, Erika Hersch-Green, and Carsten Külheim ran the experiments, analyzed and interpreted the data, and wrote and revised the manuscript.

4.1 Abstract

- *Solidago gigantea* is a noxious invader in parts of Europe and Asia, but only in its tetraploid form. Phenotypic changes associated with polyploidy are positively correlated with the invasive success of some plant species, but the genetic and molecular effects of polyploidy on invasion dynamics are comparatively less known. Here, we examined whether gene expression patterns unique to polyploids give an invasive edge over diploids, whether invasive populations experienced local adaptation to their non-native habitats through gene expression patterns, and whether these potentially different expression patterns depend upon the nutrient environment.
- We performed RNA-sequencing on diploid, native-tetraploid, and invasive-tetraploid populations of *S. gigantea* grown in either low or high nitrogen (N) and phosphorus (P) treatments. Additionally, we determined whether growth (above- and belowground biomass) and foliar chemistry (mono-, di-, and sesquiterpenes; C:N ratios) traits differed between geo-tetraploids and/or the nutrient environment.
- A total of 101,169 transcripts were sequenced from diploid and tetraploid *S. gigantea*. Differential gene and enrichment analyses revealed that: 1) native-tetraploids downregulate gene groups related to photosynthesis and terpene production and upregulate gene groups related to growth, and development relative to diploids when grown in low NP condition and 2) native- and invasive tetraploids marginally differ in the expression and associated values of growth

and defensive traits, with the exception of native-tetraploids having smaller belowground biomass than invasive-tetraploids.

- Overall, we found evidence that tetraploids regulate gene expression differently than diploids, potentially as a means of conserving and/or re-allocating resources in nutrient-limited environments. Given that polyploids have larger genome sizes than diploids, polyploids might be downregulating costly transcripts and their associated traits in favor of supplementing resources to costly genomic maintenance. Furthermore, the lack of differences between native- and invasive tetraploid populations at the trait and gene expression level for growth and foliar chemistry attributes might indicate that not enough evolutionary time has passed for differences to emerge and/or that some selection pressures are similar in both native and invasive habitats

4.2 Introduction

Invasive species pose a global ecological and economic threat, with over $100 billion being spent annually in the United States alone to combat biological invasions (Pimentel, Zuniga, and Morrison, 2005; Pfennigwerth and Kuebbing, 2012). The biology and ecology of plant invasions have been studied extensively over the last several decades, and this has provided a wealth of information pertaining to which plant traits (Pyšek and Richardson, 2008; Drenovsky, Khasanova, and James, 2012) and ecological conditions (De Roy et al., 2013; Lembrechts et al., 2018) likely contribute to invasive success. However, whether specific genetic attributes of plants make them successful as invaders remains less understood (Guo et al., 2018; Manoharan et al., 2019).

Polyploidy (whole genome duplication) is a highly prevalent genomic attribute in invasive plant species (Pandit, Pocock, and Kunin, 2011; Te Beest et al., 2012). While many of the phenotypic traits that typically characterize polyploids, such as larger sizes (Ramsey and Schemske, 2002; Knight and Beaulieu, 2008; Simón-Porcar et al., 2017) and self-compatibility (Van Kleunen and Johnson, 2007; Petanidou et al., 2012), have been identified as invasive plant traits (Pyšek and Richardson, 2008; Drenovsky, Khasanova, and James, 2012; Te Beest et al., 2012), the molecular and genetic attributes underlying the invasive success of polyploids are comparatively less understood (Hegarty and Hiscock, 2008; Te Beest et al., 2012; Xu, Ge, and Wang, 2019). Given that polyploidization is a large-scale mutation that disrupts an organism's genomic, transcriptomic, and epigenetic features (Comai, 2005; Otto, 2007; Parisod, 2012; Doyle and Coate, 2019), it is thought that the altered gene expression patterns in polyploids play

an especially strong role in the invasion success of some polyploid species (Te Beest et al., 2012; Suda et al., 2015). These changes in gene expression patterns between diploids and polyploids tend to occur in three main ways. First, multiple gene copies can lead to dosage effects where the production of a gene product increases with the number of duplicated genes present (Comai, 2005; Jackson and Chen, 2010; Doyle and Coate, 2019). These dosage-dependent expression patterns have been reported in multiple species with a diploid-polyploid complex (DeMaggio and Lambrukos, 1974; Guo, Davis, and Birchler, 1996; Visger et al., 2019), such as in *Tolmiea spp.* where the expression levels of certain genes have been found to increase with allele number in tetraploids only (Visger et al., 2019). Second, expression patterns can change via the neo- or sub-functionalization of genes, which can occur if duplicate gene copies and/or repetitive gene sequences accumulate mutations that evolve into new or varied functions and/or if genes become silenced following polyploidization (Comai, 2005; Jackson and Chen, 2010; Doyle and Coate, 2019). Examples of novel gene-silencing patterns have been reported in both polyploid plant (Duarte et al., 2006; Liu and Adams, 2010; Wendel et al., 2018) and fish taxa (Ren et al., 2017) relative to related diploids. For instance, novel gene expression patterns were found in an allopolyploid triploid fish species (*Ctenopharyngodon idella x Megalobrama amblycephala)* in comparison to its diploid progenitors, owing to notable and novel gene silencing patterns induced by whole genome duplication (Ren et al., 2017). Finally, the acquisition of multiple gene copies through either the duplication of a single species' genome (i.e., autopolyploid) and/or the combination of two or more species genomes (i.e., allopolyploidy) can make the genome less susceptible to genetic drift and increase genetic diversity (Meirmans and Van

Tienderen, 2013), potentially allowing polyploids to accumulate more variation in gene expression patterns and phenotypic novelty than diploids (Te Beest et al., 2012; Van de Peer, Mizrachi, and Marchal, 2017; Rejlová et al., 2019). Given this rationale, some gene expression patterns between diploids and polyploids *should* differ, and numerous studies have provided support for this hypothesis (Galitski et al., 1999; Gottlieb, 2003; Chen, 2007; Miller, Zhang, and Chen, 2012; Liqin et al., 2019; Visger et al., 2019), especially for plant functional traits such as photosynthesis (Visger et al., 2019) and leaf area (Liqin et al., 2019). Yet, we still know very little about the functional role these differential expression patterns between diploids and polyploids play in the context of tolerating and/or adapting to different environmental conditions and how this might have supported the invasion success of polyploids.

Biologically available nitrogen (N) and phosphorus (P) are essential in the composition of major plant molecules (e.g., ATP, chlorophyll, RuBisCO, proteins, lipids) and the processes in which these molecules are used (e.g., growth, photosynthesis, and reproduction; Evans, 1983, 1989; Elser et al., 2007; Elser, Acquisti, and Kumar, 2011). Nucleic acids (i.e., DNA, RNA) also represent substantial N and P sinks within a cell, as both DNA and RNA require N and P for their synthesis and structure (Elser, Acquisti, and Kumar, 2011; Raven, 2013). Plants with large genomes (e.g. polyploids) are suspected to have greater elemental N and P demands and to be more limited when environmental resources are scarce than those with smaller genomes (e.g., diploids; Lewis, 1985; Leitch and Bennett, 2004; Cavalier-Smith, 2005; Hessen et al., 2010; Guignard et al., 2016). Recent studies support this hypothesis, in that polyploids appear to exhibit enhances in growth and fitness when grown in high versus low nutrient

environments, while diploids show little change in response to the nutrient environment (Šmarda et al., 2013; Guignard et al., 2016; Bales and Hersch-Green, 2019; Walczyk and Hersch-Green, 2019). Because environmentally available N and P tends to be both limited (Koerselman and Meuleman, 1996; Elser et al., 2007; Du et al., 2020) and spatially heterogenous (García-Palacios, Maestre, and Gallardo, 2011; Zhang et al., 2014), plants with intrinsically greater nutrient demands are more likely to have experienced selective pressures to (a) reduce their nutrient requirements and/or (b) evolve strategies to conserve and/or acquire more nutrients (Grossman and Rice, 2012; Fernández-Martínez et al., 2019).

Given that the transcriptome is more plastic than the genome (Hessen et al., 2010; Dal Santo et al., 2013; Raven, 2013; Liscovitch-Brauer et al., 2017), polyploids might be able to conserve N and P by downregulating genes associated with costly functional traits in nutrient-poor environments. While there are currently no studies as of yet explicitly testing this hypothesis, studies have demonstrated that gene expression for key functional traits (e.g., photosynthesis, cell wall construction, auxin metabolism) can depend upon the individual effects of ploidy level (Galitski et al., 1999; Miller, Zhang, and Chen, 2012; Liqin et al., 2019; Visger et al., 2019) and nutrient availability (O'Rourke, McCabe, and Graham, 2020; Tiwari et al., 2020; O'Rourke and Graham, 2021). Photosynthetic, growth, reproductive, and defensive traits are likely candidates for differential gene expression between diploids and polyploids in nutrient limited conditions as they are comprised of multiple complex gene networks (Boch et al., 1998; Choi et al., 2003; Comai, 2005; Migocka and Papierniak, 2011) and have large nutrient and energy demands (Güsewell, 2004; Ågren, 2008). To conserve elemental costs,

polyploid plants might experience trade-offs in the regulation and phenotypic expression of these costly traits dependent upon nutrient availability. For example, polyploids might down-regulate genes related to defense in favor of maintaining the expression of growth genes in certain environmental contexts, such as nutrient-poor conditions.

Such expression trade-offs might be useful in biological invasions, as invading plants are typically exposed to novel ecological selection pressures upon introduction (Von der Lippe and Kowarik, 2008; Marco et al., 2010). For example, establishing invaders may experience increased disturbances (e.g., mowing; Song et al., 2018), new climatic conditions (e.g., precipitation, temperature; Atwater, Ervine, and Barney, 2018) and/or new competitors (Zwerschke et al., 2018) that were not present in their native habitats. These ecological factors could act as barriers to establishment if the invaders cannot survive, establish themselves, and/or reproduce in their non-native habitat (Mooney and Cleland, 2001). Alternatively, novel selection pressures could result in the emergence of adaptive traits and associated gene expression patterns unique to invading populations (Prentis et al., 2008; Whitney and Gabler, 2008; Crooks and Rilov, 2009; Novo et al., 2015). These altered gene expression patterns and associated traits could become selectively favored in invasive populations over time as a form of post-introduction adaptation if they coincide with increased fitness and/or competitive ability (Lee, 2002; Rius et al., 2015; Wu, Li, and Wang, 2020). For instance, some hypotheses suggest that a release from strong selection pressures that are only present in a species' native environment can result in novel resource allocations strategies and/or traits in invasive populations that increase competitiveness against local flora (Blossey and

Notzold, 1995; Callaway and Aschehoug, 2000; Joshi and Vrieling, 2005; Zhang and Jiang, 2006).

Herbivory is one such selective pressure that typically has a strong, negative effect on plant fitness (Becerra, 2007; Fornoni, 2011; Valverde et al., 2015) and can favor costly strategies and adaptations to reduce (e.g., chemical and mechanical defenses) and/or mitigate (e.g., maintain or increase fitness once damaged) the negative consequences of herbivory (Bazzaz et al., 1987; Baldwin, 1998; Mutikainen et al., 2002; Mitchell et al., 2016). Many invasive plant species 'escape' specialist herbivores upon their re-location from native to novel habitats (*situ* Keane and Crawley, 2002), and it is thought that successful invasive plants evolve unique resource tradeoffs in the absence of specialized enemies that shift investment from defense to growth as a strategy for outcompeting local plants (Evolution of Increased Competitive Abilities (EICA), *in situ* Blossey and Notzold, 1995). This hypothesis has received partial support from numerous studies showing that invasive populations display increased feeding preference/damage by insects (i.e., reduced defensive ability; Hull-Sanders et al., 2007; Zou, Rogers, and Siemann, 2008; Rotter, Vallejo-Marin, and Holeski, 2019; Egbon et al., 2020), a reduction in anti-herbivory traits (i.e., reduced defensive ability; Blair and Wolfe, 2004; Huang et al., 2010; Stevenson, Nicolson, and Wright, 2017), and/or greater values for growth traits (i.e., increased competitive ability; Zou, Rogers, and Siemann, 2007; González-Teuber et al., 2017; Rotter, Vallejo-Marin, and Holeski, 2019; Egbon et al., 2020) relative to native populations. However, there are comparatively fewer studies providing support for this hypothesis at the genetic and transcriptomic level (but see Broz et al., 2009; Feng et al., 2009; Prentis et al., 2010; Zhang et al., 2020).

While we know that polyploidy and the environment can independently influence gene expression patterns and a plant's ability to tolerate local ecological conditions, our understanding of how polyploidy, natural selection, and the nutrient environment jointly influence gene expression patterns in such ways that enhance invasion success is limited. The aim of this study is two-fold: to determine (1) if polyploids utilize resource allocation trade-offs at both the trait and molecular level that differ from diploids and/or are dependent upon the nutrient environment to conserve N and P, and (2) if a species' non-native habitat can select for allocation patterns that favor investment into growth over defensive genes and traits in invasive relative to native populations. We chose to vary N and P availability because polyploids are suspected to have greater N and P demands than diploids (Lewis, 1985; Leitch and Bennett, 2004; Cavalier-Smith, 2005; Hessen et al., 2010; Guignard et al., 2016) and because invasive habitats might have greater nutrient availabilities due to anthropogenic-caused nutrient enrichment in urban environments where biological invasions typically begin (Penuelas et al., 2013; Fowler et al., 2015; Goyette et al., 2016; Asabere et al., 2018). Here, we examine gene expression patterns, growth traits, and foliar chemical profiles of three geo-cytotypes of *Solidago gigantea* collected from their native and/or invasive ranges grown in low and high NP soils to address the following two hypotheses: (**H1**) To mitigate nutrient costs, polyploids down-regulate and decrease investment into costly traits such as growth, photosynthesis, defense, and/or reproduction relative to diploids when grown in low NP conditions. (**H2**) Selection has favored the downregulation and decreased investment into defensive traits and the up-regulation and increased investment into growth, photosynthetic, and/or

reproductive traits in invasive versus native tetraploid populations, and these patterns are predicted to be stronger in nutrient-limited conditions

4.3 Methods

Plant Material:

Solidago gigantea Aiton (Asteraceae, Giant Goldenrod) is an insect-pollinated, perennial aster native to North America but is present as a noxious invader in parts of Europe and Asia (Schlaepfer et al., 2008a). Three cytotypes displaying distinct spatial segregation occur within its native range: diploid (2n = 2x = 18) populations are found along the Atlantic coast, tetraploid populations (2n = 4x = 36) are found within the Great Lakes region, and hexaploid populations (2n = 6x = 54) are found within the Great Plains region (Schlaepfer et al., 2008a; Hull-Sanders et al., 2009), but invasive populations are primarily tetraploid (Schlaepfer et al., 2008a; Schlaepfer et al., 2008b). During the summers of 2017 and 2019, we collected seeds from 21 wild native populations spanning *S. gigantea*'s native range and verified the ploidy level of maternal plants using flow cytometry (Walczyk and Hersch-Green, 2022). In fall 2018, seeds from 15 wild invasive populations of *S. gigantea* were collected near Zurich, Switzerland and sent to Michigan Technological University (Department of Biological Sciences, Houghton, MI, USA). We selected three diploid, four invasive-tetraploid, and four native-tetraploid populations from these seed sources to be used in this study. The ploidy level of all plants used in this study were verified following a flow cytometry method described by Verloove, Zonneveld, and Semple (2017); details can be found in Chapter 1: Appendix 1.

RNA Sequencing

Experimental Design- A total of 24 seedlings each belonging to one of 14 unique maternal lines collected from 11 wild populations (N = 3 diploid, N = 4 invasive-

tetraploid, N = 4 native-tetraploid) were germinated into 1.5L square pots containing a 50:50 mixture of vermiculite to Sun Grow Mix 1 potting soil (Sun Grow Horticulture, Agawam, Massachusetts, USA) in a greenhouse at Michigan Technological University. At 8 weeks of growth, plants were randomly assigned to either low or high N:P treatment groups (N = 4 per geo-cytotype per treatment group), based on the range of soil N and P measured at seed collection sites (see Walczyk and Hersch-Green, 2022 for details). The potting soil already contained 110 ppm N (μg N · g^{-1}) and 25 ppm P (μg P · g^{-1}) which we designated as the low treatment, and we added nutrients to the high treatments so that they contained a total 165 ppm N (μg N · g^{-1}) and 37.5 ppm P (μg P · g^{-1}). Treatments were administered as 40mL solutions of ammonium nitrate and potassium monophosphate (high treatments) or water (low treatments). All plants also received an additional 20 mL solution of potassium sulfate (100 ppm; μg K · g^{-1}) and micronutrients (Fertilome chelated liquid iron and other micronutrients; Voluntary Purchasing Groups, Bonham, Texas, USA). We rotated pots weekly to prevent non-random effects of variable greenhouse conditions, and the experiment concluded after 15 weeks of growth.

RNA Extraction and Library Preparation- At 15 weeks of growth, leaves of the same ontogeny (youngest fully mature leaf) were harvested from each of the 24 plants, weighed to the nearest milligram, and immediately frozen in liquid nitrogen before being stored at -80°C. Frozen leaves were ground to a powder using a micro-pestle and RNA was then extracted using the Qiagen RNAeasy Plant Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Extracted RNA samples were quantified

using a Qubit 4 Fluorometer (Thermo-Fisher Scientific, Waltham, Massachusetts, USA). Total RNA quantity among samples ranged from 1144 ng to 9540 ng.

Sequencing on PacBio and Illumina Sequencers- To create a reference transcriptome, we combined equal RNA concentrations from the 24 individuals into a single pooled RNA sample and submitted it to the University of California, Davis for Iso-Seq library preparation and sequencing on a PacBio Sequel II (Pacific Biosciences, Menlo Park, California, USA) with 10 kb reads.

cDNA libraries for mRNA sequencing were prepared on all 24 individual samples using NEBNext Ultra II RNA Library Prep Kit for Illumina (E7775) and NEBNext Multiplex Oligos for Illumina Index Primer Set #1 and #2 (E7335S and E7500S; New England Biolabs, Ipswich, Massachusetts, USA) according to the manufacturer's instructions. Because long-reads are needed for PacBio genome assembly, we increased fragment size to aid in transcriptome assembly by selecting insert fragment lengths of 200- to 500-bp by adjusting the number and length of PCR cycles based on the recommendations within the manufacturer's protocol. Final library concentrations were quantified on a Qubit 4 Fluorometer and ranged from 27.2 ng to 954 ng. Sequencing on all 24 samples was run on 2 lanes on an Illumina HiSeq 4000 (Illumina Inc., San Diego, California, USA) at the University of California, Davis in the forward and reverse direction with 150 bp paired end reads. To ensure that mapping quality was the same for diploids and tetraploids, we used the software Qualimap (Konstantin et al. 2015) to preform quality control on mapping coverage depth and quality for each cytotype. Mapping depth and quality metrics did not differ between diploids and tetraploids (mean

mapping depth for 2x = 16.9, for 4x = 18.1; mean mapping quality for 2x = 13.7, for 4x = 14.6).

Reference and Transcriptome Assemblies- All assembly and mapping steps were performed on Michigan Technological University's high-performance computing infrastructure *Superior*, using the *WildForGen* shared server. PacBio long-reads from the pooled sample were clustered and aligned into a reference transcriptome for *S. gigantea* using CD-HIT-EST (Li et al. 2001), with the sequence identity threshold set to 0.98 with all other parameters set to default. A .bed file containing a single annotation of each transcript was then created and contained three columns describing the 1) transcript identification, 2) a starting point of 1bp length, and 3) the end site as the base pair length of the transcript.

Next, raw reads from all 24 samples were trimmed of adapters and low-quality reads in both directions using the software "BBDuk" within the BBtools platform (Bushnell, Rood, and Singer, 2017; www.sourceforge.net/projects/bbmap/), using standard parameters plus a force-trim to remove the first 15 bp of each read. These right and left trimmed reads were first assessed for quality using the FastQC (Andrews, 2015) and MultiQC (Ewels et al., 2016) software before being mapped to the assembled reference transcriptome.

Read Mapping- Trimmed paired .fastq files from each of the 24 plants were then mapped onto the reference transcriptome using the software BWA (Li and Durbin, 2009). Output .sam files were converted to a .bam files, sorted, and indexed to the reference transcriptome via a looped shell code command using SAMtools v1.13 (Li et al., 2009).

We mapped reads to each individual sample's transcriptome and extracted the raw counts per transcript and individual using the Bedcov software within SAMtools v1.13 (Li et al., 2009).

Functional Annotation and Transcript Classification- A BLAST alignment to obtain the functional annotation and gene ontogeny (GO) of transcripts was performed using Blast2GO Basic v.6.0.3 (Götz et al., 2008).

Gene Expression and Enrichment Analyses- Statistical analyses of differentially expressed genes was performed using the Bioconductor package DESeq2 v. 3.15 (Love, Huber, and Anders, 2014) in R v.4.2.0 (R Core Team, 2022), which uses large matrices of multi-conditional expression data in its statistical analyses (Anders and Huber, 2010). Differential expression analyses were preformed between the following six contrasted groups: (1) Diploids grown in low NP vs diploids grown in high NP ($\alpha = 0.01$),(2) native-tetraploids grown in low NP vs native-tetraploids grown in high NP ($\alpha = 0.01$), (3) diploids vs native-tetraploids grown in low NP ($\alpha = 0.01$), (4) diploids vs. native-tetraploids grown in high NP ($\alpha = 0.01$), (5) native-tetraploids vs invasive-tetraploids grown in low NP ($\alpha = 0.05$), and (6) native-tetraploids vs invasive tetraploids grown in high NP ($\alpha = 0.05$). Derived lists of differentially expressed genes were sorted by false discovery rate (FDR) adjusted significance of less than 0.01, separated into up-or down-regulated transcripts, and then submitted to Blast2GO Basic v.6.0.3 (Götz et al., 2008) for gene enrichment analyses using Fischer's Exact Test.

Differential Expression and Enrichment Analysis Data Visualization- We used the "EnhancedVolcano" (Blighe, Rana, and Lewis, 2022) in R version 4.2.0 to make volcano

plots from the differential gene expression data from the DESeq2 results. Blast2GO Basic v.6.0.3 (Götz et al., 2008) was used to create bar graphs showing the top 15 most significant GO ID terms between comparison groups.

Growth and Foliar Chemistry Traits

Experimental Design- A total of 80 seedlings of randomized half-sibling maternal lines originating from four separate native and invasive tetraploid populations were germinated into 2L round pots containing a 50:50 mixture of vermiculite to Sun Grow Mix 1 potting soil (Sun Grow Horticulture, Agawam, Massachusetts, USA) in a greenhouse. At 8 weeks of growth, plants were randomly assigned to the same low or high NP concentrations and treatment regimens used in the RNA sequencing experimental design (i.e., low NP = 110 ppm N ($\mu g\ N \cdot g^{-1}$) and 25 ppm P ($\mu g\ P \cdot g^{-1}$); high NP = 165 ppm N ($\mu g\ N \cdot g^{-1}$) and 37.5 ppm P ($\mu g\ P \cdot g^{-1}$). Pots were rotated weekly, and the experiment concluded after 19 weeks of growth.

Biomass- Plants were divided into their above- and belowground parts at harvest (week 19 of growth), dried at 60℃ for 48hr (aboveground) or 72hr (belowground) in a drying oven and then were weighed to the nearest gram.

Leaf Material and Terpene Extraction - Leaves of the same ontology (youngest fully developed leaf on a plant) from a total of 50 plants (N = 26 native-tetraploid, N = 24 invasive-tetraploid) grown in low or high NP treatments, and comprised of randomized maternal lines originating from 4 separate native- and invasive tetraploid populations were used for secondary chemistry determination. The wet mass of collected leaves were weighted to the nearest milligram, flash frozen with liquid nitrogen, and then stored in a -

80°C freezer. Terpene extractions followed the methods of Hull-Sanders et al. (2009), in which frozen leaves were ground to a find powder and immediately transferred to a glass vial containing 6 mL of 70% hexane-30% ethyl acetate and 0.26mg of the internal standard nonadecane. The contents of the vials were centrifuged for 10min at 3000 RPM and reduced to a 3 mL volume under a gentle stream of ultra-high purity N_2 has using an Organomation 12-position nitrogen evaporator (Organomation, Berlin, Massachusetts, USA). We then transferred 1.5mL of the concentrated sample to scintillation vials for analysis via gas chromatography-mass spectrometry (GC-MS).

GC-MS Profiling of Foliar Terpenes- Terpene concentrations were quantified with a Trace 1310 Gas Chromatograph coupled with an ITQ 1100 Ion Trap MS (ThermoFischer Scientific, Waltham, Massachusetts, USA) at the Great Lakes Research Center at Michigan Technological University using the method of Hull-Sanders et al. (2009). Compounds were separated using a Rtx-5MS (30m x 0.25mm; DF = 0.25um) Low-Bleed GC/MS column (fused silica; Restek. Bellefonte, Pennsylvania, USA) with the injector temperature set to 250°C. Chemical analysis of three technical replicates per concentrated leaf sample was run at an initial oven temperature of 35°C held constant for three minutes before being increased to 280°C at the rate of 10°C per minute. Terpene peaks were grouped by retention time and identified using the NIST2000 software database and Mass Spectrometry Data Center (Chemdata.nist.gov) and then cross-checked against other studies reporting the retention times and identification of terpenes in *Solidago* species (Johnson, Hull-Sanders, and Meyer, 2007; Hull-Sanders et al., 2009; Dobjanschi et al., 2019). We measured total terpene concentrations for mono-, di-, and sesquiterpenes by first averaging the concentration values for each terpene across the three technical

replications and then summing the concentration values for each terpene group within each individual sample. A total of 9 samples (N = 5 invasive-tetraploids, N = 4 hexaploids) were excluded from the final dataset due to low quality peaks on the GC-MS.

Foliar C:N Ratios- We quantified foliar [C] and [N] per milligram leaf tissue by grinding and homogenizing three leaves from two individuals of the same maternal line collected at the 15th week of growth for each geo-tetraploid by nutrient combination (N = 10 per geo-tetraploid per treatment, total N = 40). Approximately 5mg of each combined and ground sample was then analyzed on an elemental analyzer (Costech Analytical Technologies Inc., Valencia, California, USA) at the University of Minnesota. The C:N ratio was calculated by dividing foliar [C] by foliar [N]. A single native-tetraploid sample did not successfully run and was excluded from the total dataset.

Statistical Analyses: Statistical models comparing biomass and foliar chemistry traits consisted of a combination of the following fixed-effect independent factors: geo-tetraploid ($4x^N$ = native-tetraploid, $4x^I$ = invasive-tetraploid), NP-treatment (LL= low, HH = high), and/or population (nested within 'geo-tetraploid). Data transformations were made as needed to meet model assumptions of normality and homoscedasticity and are noted below. We used the following post-hoc analyses to test for differences among means: 1) Student's t-test (comparisons between two levels) for significant single model factors, and 2) controlled contrasts for significant interactions among model factors. Statistical analyses for non-sequencing data were performed using JMP Pro version 16 (SAS Institute, Cary, North Carolina, USA).

We used seven separate ANOVA models to test whether aboveground biomass (N = 80), belowground biomass (N = 80), total terpene concentrations (N = 42, log-transformed), monoterpene concentrations (N = 42, log-transformed), diterpene concentrations (N = 42, log-transformed), sesquiterpene concentrations (N = 42, log-transformed), and C:N ratios (N = 40) values were dependent upon geo-tetraploid, NP treatment, their interaction, and/or population of origin.

4.4 Results

Transcriptome Assembly – The general statistics of each assembled transcriptome for the diploids, native-tetraploid, and invasive-tetraploid *S. gigantea* plants grown in either low or high NP treatments are given in Table 1. The clustered transcriptome had a total of 101,169 transcripts.

Do gene expression patterns differ within geo-cytotypes between low and high NP treatments? – After mapping raw reads to the reference transcriptome, read counts were compared between low versus high NP treatment conditions in diploids, native-tetraploids, and invasive-tetraploids.

Diploids low versus high NP: A total of 1,064 differentially expressed (DE) genes were found within the comparison of diploids grown in high versus low NP treatments. Of these DE genes, 518 were up-regulated, and 546 were down-regulated in the high NP treatment relative to the low treatment (Figure 1a). GO ID terms that were significantly more highly expressed included *gene expression*, *sexual reproduction*, and *nucleotide metabolic processes*, while significant GO ID terms for lower expressed genes were comparatively fewer and included *cellular metabolic processes* and *regulation of cellular processes* (Table 2; Figure 2a).

Native-tetraploids low versus high NP: A total of 161 DE genes were found within the comparison of native-tetraploids grown in high versus low NP treatments. Of these DE genes, 43 were up-regulated, and 118 were down-regulated in the high NP treatment relative to the low treatment (Figure 1b). There were a few GO ID terms that were

significantly more highly expressed which included *organelle*, *cellular metabolic processes*, and *regulation of cellular processes* terms, while there were more significant GO ID terms for lower expressed genes which included *cellular N-compound biosynthetic processes, phosphate-containing compound metabolic processes*, and *responses to biotic and abiotic stress* (Table 2; Figure 2b).

Invasive-tetraploids low versus high NP: A total of 249 DE genes were found within the comparison of invasive-tetraploids grown in high versus low NP treatments. Of these DE genes, 136 were up-regulated, and 113 were down-regulated in the high NP treatment relative to the low treatment (Figure 1c). GO ID terms that were more highly expressed tended to be related metabolic processes and other highly expressed GO terms included *glucoside synthesis processes* and *responses to stimuli*. GO ID terms related to defense and growth had lower expression and included *defense responses*, *cellular N-compound biosynthetic processes*, and *response to biotic and external biotic stimu*li (Table 2; Figure 2c).

Do gene expression patterns differ between geo-cytotypes within low or high NP treatments? - After mapping raw reads to the reference transcriptome, read counts were compared between 1) diploids versus native-tetraploids and 2) invasive- versus native-tetraploids under both low and high NP conditions.

Diploids versus native-tetraploids in low NP: Under low NP conditions, a total of 13,051 DE genes were found within the comparison of native-tetraploids versus diploids. Of these DE genes, 6,277 were up-regulated, and 6,774 were down-regulated in the native-tetraploids relative to the diploids (Figure 3a). In general, native-tetraploids tended to

have more highly expressed GO terms than diploids (Table 3), which included *cellular processes*, *growth and development*, and *responses to abiotic stress*. Lower expressed GO ID terms tended to be related to nutrient-costly processes and included *terpene biosynthesis processes*, *carbon fixation*, *NADPH regeneration*, and *NADP metabolic processes* (Table 3; Figure 4a).

Diploids versus native-tetraploids in high NP: Under high NP conditions, a total of 7,282 DE genes were found within the comparison of native-tetraploids versus diploids. Of these DE genes, 3,726 were up-regulated, and 3,556 were down-regulated in the native-tetraploids relative to the diploids (Figure 3b). Similar to the low NP treatments, native-tetraploids tended to have more highly expressed GO ID terms than diploids (Table 3), including *sexual reproduction*, *cellular metabolic processes*, and *responses to external biotic stimuli*. Lower expressed GO ID terms included *terpene biosynthesis processes*, *purine nucleotide biosynthesis processes*, and the *chloroplast* (Table 3; Figure 4b).

Invasive- versus native-tetraploids in low NP: Under low NP conditions, a total of 761 DE genes were found within the comparison of invasive versus native-tetraploids. Of these DE genes, 478 were up-regulated, and 283 were down-regulated in the native-tetraploids relative to the invasive-tetraploids (Figure 5a). GO ID terms that were more highly expressed in native-tetraploids tended to be related to nitrogen metabolic processes and included *organonitrogen compound biosynthetic*, *pyridine nucleotide metabolic processes*, *regulation of N-compound metabolic processes*, in addition to *fruit development*. Lower expressed GO ID terms included *responses to abiotic stress* and *cellular responses to hormone stimuli* (Table 4; Figure 6a).

Invasive- versus native-tetraploids in high NP: Under high NP conditions, a total of 489 DE genes were found within the comparison of invasive versus native-tetraploids. Of these DE genes, 249 were up-regulated, and 240 were down-regulated in the native-tetraploids relative to the invasive-tetraploids (Figure 5b). GO ID terms that were more highly expressed in native-tetraploids included *responses to N-compounds* and *responses to chemicals.* However, native-tetraploids tended to have more down-regulated GO ID terms than invasive-tetraploids in high NP treatments (Table 4), which included *defense responses*, *growth and development*, *N-compound transport*, and *the regulation of N-compound metabolic processes* (Table 4; Figure 6b).

Do growth traits and/or foliar chemistry differ between native and invasive tetraploids dependant upon NP availability? –

Growth traits: All plants had significantly greater above- and belowground biomass when grown in high versus low NP treatments (LSMean ± SE for aboveground biomass in low NP = 1.05 ± 0.11g, high NP = 2.20 ± 0.11g; for belowground biomass in low NP = 2.27 ± 0.17g, high NP = 3.94 ± 0.17g; Table 5 A, B). While native- and invasive tetraploids responded similarly to the nutrient environment, invasive tetraploids had significantly greater belowground biomass than native-tetraploids regardless of the NP environment (LSMean ± SE for native-tetraploids =2.76 ± 0.16g, invasive-tetraploids = 3.46 ± 0.18g; Table 5B). No other model factors or interactions among model factors had a significant effect on above- nor belowground biomass (Table 5 A, B).

Foliar terpene concentrations: A list of all GC-MS identified terpenes and other chemical components in *S. gigantea* leaves can be found in Table 6. We did not detect any

significant differences in foliar terpenes concentrations between native and invasive tetraploids, as no model factors or interactions among model factors had a significant effect on the foliar concentrations of total terpenes, monoterpenes, diterpenes, and sesquiterpenes (Table 5 C-F).

C:N ratios: Only a plant's population of origin had a significant effect on foliar C:N ratios; no other model factors or interactions among model factors had a significant effect (Table 5G).

4.5 Discussion

The prevalence of polyploidy in invasive plant taxa strongly suggests that some attributes associated with whole genome duplication aid in invasion success (Pandit, Pocock, and Kunin, 2011; Te Beest et al., 2012). Phenotypic traits present in some polyploids but not their diploid progenitors (e.g., self-compatability, larger biomasses; Ramsey and Schemske, 2002; Knight and Beaulieu, 2008; Simón-Porcar et al., 2017), have been found among some of the most noxious invasive species (Pyšek and Richardson, 2008; Drenovsky, Khasanova, and James, 2012; Te Beest et al., 2012). However, our understanding of how the molecular and genetic attributes of polyploids might impact invasion dynamics is comparatively lacking (Hegarty and Hiscock, 2008; Te Beest et al., 2012; Xu, Ge, and Wang, 2019). Here, we aim to address this gap by investigating whether gene expression patterns of tetraploid *S. gigantea* "pre-adapt" them to be more invasive than diploids by either reducing the expression and associated nutrient investment into costly traits in adverse nutrient conditions and/or by upregulating genes and traits groups associated with greater competitive ability and/or reproductive fitness. Furthermore, we also investigated whether selection post-introduction favors alternate gene expression patterns and investment into costly traits in invasive- versus native tetraploid populations as a means of adapting to the abiotic and biotic conditions of the non-native habitat. We found 1) evidence that tetraploids can regulate gene expression as a potential means of conserving nutrients and energy in nutrient-limited environments, and 2) an overall lack of differential expression for growth and defensive traits between native- and invasive-tetraploids. We discuss these findings in the broader contexts of ecology and invasion biology below.

Patterns of gene expression allows polyploids to cut and re-distribute costs – The greater material costs associated with large polyploid genomes are predicted to reduce plant investment into costly traits related to growth, competition, and reproduction, especially in nutrient poor environments, as a means of compensating for the costliness of the genome (Šmarda et al., 2013; Guignard et al., 2016; Faizullah et al., 2021). We found some evidence of such a strategy in *S. gigantea*, as tetraploids down-regulated more genes associated with photosynthesis and terpene production than diploids in nutrient-limiting conditions (Table 3). Photosynthesis is particularly N and P demanding cellular process that works in tandem with plant growth (Evans, 1989). Leaf N is primarily distributed into either the thylakoid membrane and pigment proteins, such as chlorophyll (4 N atoms per molecule; Evans, 1989) in the Light Reactions and/or the soluble pigments, such as RUBISCO, found in the Calvin-Benson Cycle (Evans, 1989; Hessen et al., 2010; Hohmann-Marriott and Blankenship, 2011). P is also integral for photosynthetic functioning, as it is a major structural component of ATP, NADP, and many key enzymes driving the Calvin-Benson Cycle, such as RUBISCO (Evans, 1989; Hessen et al., 2010; Hohmann-Marriott and Blankenship, 2011). Hence, it is possible that the genome size of polyploids was directly linked to a tradeoff that prioritized N and P investment into the structure and maintenance of the genome over the macromolecules needed in photosynthesis (Kelly, 2018; Majda, Beisser, and Boenigk, 2021). Not only would N and P be conserved through the reduction of photosynthetic activity, but it would also be saved through the act of not transcribing the RNA of associated genes. While studies exploring the relationship between genomic material costs, photosynthetic functioning, and nucleic acid investment are limited, a few recent studies have found

nutrient availability can induce tradeoffs involving genome size and photosynthesis at the molecular level. For example, studies have found evidence for reduced genomic materials costs in both plant species with poor photosynthetic N-use efficiency (Kelly, 2018) and in photosynthetic protists (Majda, Beisser, and Boenigk, 2021) through their tendency of having fewer costly codon pairs (i.e., AT pairs = 7N, GC pairs= 8N). This implies that selection might act to reduce genomic material costs in some photosynthetic species.

We also found that tetraploids downregulated more genes associated with mono-, di-, and sesquiterpene biosynthesis relative to diploids when grown in both low and high NP conditions (Table 3, Figure 3), which was consistent with our finding of decreased terpene concentrations in polyploids reported in Chapter 2. Terpenes are a large class of carbon-based compounds that serve a variety of functions within a plant, including defense against generalist herbivores, bacteria, and fungi in plants (Ninkuu et al., 2021). In addition to being a large carbon-sink within an organism, terpene production requires N for terpene synthase activity and P within the structuring of ATP, NADP, and the diphosphates required to synthesize the various carbon skeletons that characterize terpenes (Bohlmann, Meyer-Gauen, and Croteau, 1998; Bustamante et al., 2020). However, the effect of P-limitation on foliar terpene production is mixed, as studies have found foliar terpene concentrations to increase (Blanch et al., 2012), decrease (Bustamante et al., 2020), or remain the same (Blanch et al., 2008) in low versus high P-conditions. Because we did not sequence the transcriptomes of plants grown in only N- or only P-limited environments, we do not know whether the downregulation of terpene synthesis genes in polyploids seen here was driven by N or P limitation.

Some gene groups were more up-regulated in tetraploids than in diploids under low NP-conditions (Table 3), potentially indicating a tradeoff between resource sources when nutrients were limited. For instance, gene groups related to development, reproduction, DNA replication, and translation were more up-regulated in tetraploids in low NP conditions, and tetraploids also had significantly larger above- and belowground biomasses than diploids (Table 3). Two different trade-off strategies might have been utilized by tetraploid *S. gigantea* in these limited conditions. First, tetraploids might have reduced investment into foliar defense and/or photosynthesis in favor of investing it into plant development and growth, as evidenced by the up-regulated development genes and the larger biomasses in tetraploids. Second, the up-regulated of gene groups related to DNA replication and translation further the hypothesis that organisms with larger genomes experience tradeoffs between investment into the genome versus other costly factions (Faizullah et al., 2021; Wang et al., 2021). Differential gene expression was also found between diploids and tetraploids grown in high-NP conditions, with tetraploids up-regulated notably more gene groups than diploids when compared to the low treatments (Table 3). This pattern could also be the result of tetraploid release from nutrient constraints in the high NP treatments that allowed the plant to invest more equally into other costly gene functions and traits (Table 3). Together, the specific patterns of differentially expressed genes being dependent upon ploidy level and the nutrient environment provide strong evidence to support the hypothesis that large genomes impose material costs that constrain functioning unless a plant is either a) released from the nutrient constraints and/or b) can invoke internal tradeoffs to reallocate resources

away from less important nutrient sinks and towards the genome and other high priority functions.

Growth and defensive traits were similar in native and invasive tetraploid populations - Differences in the selective environment between a species' native and invasive habitats can result in traits and life strategies unique to each of the two ranges (Prentis et al., 2008; Whitney and Gabler, 2008; Crooks and Rilov, 2009; Novo et al., 2015). Growth and defensive traits are suspected to be especially susceptible to such regional differences (Blossey and Notzold, 1995; Callaway and Aschehoug, 2000). Traits related to growth (e.g., biomass accumulation; growth rate) are important components of plant competition because the size and/or speed at which a plant grows can have consequences on its ability to acquire resources (Craine and Dybzinski, 2013; Schwartz, Gibson, and Young, 2015), as larger and/or faster growing plants tend to be at a competitive advantage for reaching above and belowground resources (Aerts, 1999; Tilman, 2007; Craine and Dybzinski, 2013; Goldberg et al., 2017). Defensive traits allow a plant to resist damage and/or maintain fitness when a plant is subject to attack from herbivores and other antagonists (Mitchell et al., 2016). Both growth and defensive traits are costly (Bazzaz et al., 1987; Baldwin, 1998; Mitchell et al., 2016), and investments into one nutrient sink over the other might be shaped by the selective environment. Specifically, a lack of specialist enemies in a species' invasive habitat is hypothesized to have evolved resource allocation tradeoffs favoring investment into growth traits over some defensive traits (Blossey and Notzold, 1995). Given that *S. gigantea* has multiple specialist enemies endemic to its native range (e.g., *Trirhabda borealis, T. virgata, Eurosta solidaginis, Epiblema scudderiana, Rhopalomyia solidaginis*; Messina, 1982;

Tooker and De Moraes, 2008; Morrell and Kessler, 2017), we expected to see evidence of evolved competitive ability in invasive-tetraploid populations manifested as greater trait values and gene expression patterns for growth traits and reduced trait values and gene expression patterns for defensive traits. We did not find evidence to support our prediction, as invasive-tetraploids populations did not down-regulate more defensive genes, nor did they up-regulate more growth genes relative to their native counterparts (Table 4). Furthermore, we did not detect any differences in aboveground biomass values nor foliar terpene concentrations between the native and invasive tetraploids (Table 5). Our results contradict two other studies comparing foliar terpene concentrations between geo-cytotypes of *S. gigantea*: Johnson, Hull-Sanders, and Meyer (2007) found invasive populations to have significantly more diterpenes than native populations, while Hull-Sanders et al. (2009) found diploids and invasive-tetraploids to have reduced secondary chemicals. However, the lack of ploidy-level identification within both native- and invasive population in Johnson, Hull-Sanders, and Meyer (2007), and an error misidentifying both native- and invasive tetraploids as diploids in Hull-Sanders et al. (2009, 2015) makes comparisons to our present results unhelpful. Studies involving other focal species report a similar lack of differential growth (Felker-Quinn, Schweitzer, and Bailey, 2013; Shelby et al., 2016) and/or defensive (Franks et al., 2008; Cripps et al., 2009; Felker-Quinn, Schweitzer, and Bailey, 2013) traits between native and invasive populations of a species to what we have found here. Because there are far fewer studies exploring these differences from the genetic level, it is difficult to gauge how our findings compare to other gene expression studies, as some of these studies have found invasive populations to both up-regulate (Manoharan et al., 2019) and down-regulate

(Broz et al., 2009) gene groups related to defense. Thus, although we observed that native- and invasive tetraploid populations varied little in their growth and defensive traits and gene expression patterns, the evolution of different growth versus defensive investment patterns between such populations are likely to be species-specific, require sufficient evolutionary time, and/or depend on the degree of abiotic and biotic differences between native- and invasive habitats. Furthermore, the lack of differential responses reported here could have also occurred if invasive populations are exposed to consistent damage from generalist enemies (Joshi and Vrieling, 2005), if active herbivory was needed to induce defensive responses at the genetic level (Diaz, 2018), and/or if not enough evolutionary time has passed for the two populations to show differences in these traits.

Potential evidence for local adaptation in native and invasive tetraploid populations – While we did not find evidence of local adaptation through our comparisons of plant growth and defensive traits, we did find some differential expression patterns for other gene groups and associated traits that might represent evolved differences between native- and invasive tetraploid populations. Specifically, native-tetraploids upregulated more gene groups related to N-metabolic processes than invasive-tetraploids in low NP treatments (Table 4). These N-metabolic processes refer to a variety of physiological processes related to the creation of N-containing metabolites, such as amino acids (Beatty et al., 2016). This suggests, that when N and P are limited, native populations are using more N in metabolic pathways relative to invasive populations. It is possible that this increase in N-usage is translated into enhancements for other competitive, defensive, and/or reproductive traits that we were unable to phenotypically measure here. For

example, native populations might use the higher expression of these metabolic pathways to invest more N into current-season reproductive efforts than invasive-populations. On the other hand, invasive populations may have experienced selective pressures to invest less resources into current-sexual reproduction, given that invaders typically experience severe decreases in genetic diversity and population sizes via bottleneck effects (Estoup et al., 2016). Instead, invasive populations might have experienced selective pressures that prioritized investment into belowground structures, such as root systems and clonal ramets, for future reproductive efforts and over-wintering (Lubbe, Klimešová, and Henry, 2021). Our additional finding of larger belowground biomasses in invasive populations provides some support for this idea (Table 5), but more studies explicitly testing for different N-uptake, -utilization, and -investment strategies between native and invasive populations would are needed (but see Funk, 2013; Knauf et al., 2021). Furthermore, it is also possible that observed differences in gene expression patterns are the artifacts population-level selection and is not fully applicable to an entire cytotype's native or invasive region. We were able to include native-tetraploid samples that originated from a variety of source populations spanning *S. gigantea*'s tetraploid-range. However, we were unable to capture a similar sampling of range in the invasive populations as they all originated from a small region in Switzerland. Future studies comparing native and invasive polyploid populations of any species would benefit from encompassing a wide distribution of populations in both native and invasive ranges.

4.6 Tables

Table 1: Basic statistics from transcriptome assembly from Illumina HiSeq 4000 sequenced RNA extracts of twenty-four *Solidago gigantea* plants.

Plant ID	Ploidy Level	NP Treatment	Average read length (bp)	% GC	Total Sequences (millions)
HG 8	2x	High	118 bp	42%	7.1
HG 8	2x	Low	118 bp	42%	19
NF 10	2x	High	112 bp	42%	24.3
NF 10	2x	Low	118 bp	42%	20.4
TVP 3	2x	High	114 bp	42%	22.2
TVP 3	2x	Low	118 bp	41%	21.5
TVP 9	2x	High	118 bp	41%	22.3
TVP 9	2x	Low	116 bp	41%	20.7
CHP 4	4xN	High	113 bp	42%	27.8
CHP 4	4xN	Low	113 bp	42%	26.1
CIHA 10	4xN	High	118 bp	42%	24.1
CIHA 10	4xN	Low	118 bp	41%	21.2
DF 4	4xN	High	118 bp	42%	17.1
DF 4	4xN	Low	104 bp	42%	15.8
KAS 10	4xN	High	118 bp	42%	19.8
KAS 10	4xN	Low	118 bp	43%	18.8
Pop 14-4	4xI	High	100 bp	41%	20
Pop 14-4	4xI	Low	118 bp	41%	21.3
Pop 15-1	4xI	High	113 bp	42%	25.1
Pop 15-1	4xI	Low	117 bp	42%	19.9
Pop 2-3	4xI	High	110 bp	41%	23.9
Pop 2-3	4xI	Low	117 bp	42%	16.4
Pop 3-3	4xI	High	117 bp	41%	19.1
Pop 3-3	4xI	Low	118 bp	42%	22.9

Table 2: Results of GO enrichment analysis between diploids, native-tetraploids, and invasive-tetraploids grown in low versus high NP treatments. The differential enrichment patterns of parent terms for plants grown in high NP treatments relative to those grown in low NP treatments are depicted below and are denoted by arrows (↑ = mostly upregulated; ↓ = mostly downregulated, ↑↓ = some nested child terms are both up- and down-regulated, no arrow = no differences in regulation).

Category	GO ID	Parent Term	No. Child Terms	2x: Low v. High	4xN: Low v. High	4xI: Low v. High
Cellular Processes	GO:0007049	Cell Cycle	4	↑		
	GO:0048869	Cellular Development Process	3	↓	↓	↓
	GO:0050794	Regulation of Cellular Processes	11	↓	↑↓	↓
Defense	GO:0006952	Defense Response	13		↓	↓
Growth and Development	GO:0032502	Development Process	13		↓	↓
	GO:0040007	Growth	2			↓
	GO:0009888	Tissue Development	6			↓
Macromolecule Synthesis	GO:0009059	Macromolecule Biosynthesis Process	4	↑	↓	↓↑
	GO:0010556	Regulation of Biological Process	5	↑	↓	↓↑
Metabolism and Energetics	GO:0044237	Cellular Metabolic Process	4	↓	↓↑	↓↑
	GO:0019222	Regulation of Metabolic Process	1	↓↑	↓	↓

Nitrogen	GO:0044271	Cellular Nitrogen Compound Biosynthetic Process	2	↑	↓	↑
	GO:0009117	Nucleotide Metabolic Process	9	↑		↑
	GO:1901566	Organonitrogen Compound Biosynthesis Process	6	↑		
	GO:0019362	Pyridine Nucleotide Metabolic Process	4	↑		↑
	GO:0051171	Regulation of Nitrogen Compound Metabolic Process	14	↑	↓	↓↑
	GO:0006351	Transcription, DNA-templated	9	↓↑	↓	↓↑
Cell and Organelle	GO:0016020	Membrane	14		↓	↓↑
	GO:0070925	Organelle Assembly	5	↑		
	GO:0043226	Organelles	11	↓↑	↓↑	↓↑
Phosphorus	GO:0006796	Phosphate Containing Compound Metabolic Process	3		↓	↓
Photosynthesis	GO:0009507	Chloroplast		↑		
Reproduction	GO:0019953	Sexual Reproduction	16	↑		
Response to Abiotic Stimuli	GO:0009725	Cellular Response to Hormone	4		↓	
	GO:0009628	Response to Abiotic Stress	9	↑	↓	↓↑
	GO:0042221	Response to Chemical	5		↓	↓
	GO:0009607	Response to Biotic Stimulus	1		↓	↓

Response to Biotic Stimuli	GO:0043207	Response to External Biotic Stimulus	8		↓	↓
RNA	GO:0010467	Gene Expression	7	↑	↓	↓↑
	GO:0051252	Regulation of RNA Metabolic Process	11	↓↑	↓	↓↑
	GO:0010468	Regulation of Gene Expression	6	↑	↓	↓↑

Table 3: Results of GO enrichment analysis between diploids versus native-tetraploids grown in low or high NP treatments. The differential enrichment patterns of parent terms in native-tetraploids relative to diploids are depicted below and are denoted by arrows (↑ = mostly upregulated; ↓ = mostly downregulated, ↑↓ = some nested child terms are both up- and down-regulated, no arrow = no differences in regulation).

Category	GO ID	Parent Term	No. Child Terms	Low: 2x v. 4xN	High: 2x v. 4xN
Cellular Processes	GO:0006884	Cell Volume Homeostasis	1	↑	↑
	GO:0048869	Cellular Development Process	3	↑	↑
	GO:0051726	Regulation of Cell Cycle	4	↑	↑
	GO:0050794	Regulation of Cellular Processes	11	↑	↑
Defense	GO:0032870	Cellular Response to Hormone Stimulus	4	↓↑	↑
	GO:0006952	Defense Response	13	↑	↓↑
	GO:0046246	Terpene Biosynthetic Process	1	↓	↓
	GO:0016114	Terpenoid Biosynthetic Process	2	↓↑	↓↑
	GO:0043693	Monoterpene Biosynthetic Process	1	↓	↓
	GO:0016099	Monoterpenoid Biosynthetic Process	1	↓↑	↑
	GO:0016102	Diterpenoid Biosynthetic Process	2	↓	↓
	GO:0051762	Sesquiterpene Biosynthetic Process	3	↓	↓
	GO:0016114	Tetraterpenoid Biosynthetic Process	3	↓	
	GO:0009963	Positive Regulation of Flavonoid Biosynthetic Process	1	↓	
	GO:0019748	Secondary Metabolic Process	1	↑	↑
	GO:0010026	Trichomes	2	↑	↑
DNA	GO:0005694	Chromosome	10	↑	↑

	GO:1902969	Mitotic DNA Replication	5	↑	↑
	GO:0006275	Regulation of DNA Replication	6	↑	↑
	GO:0016570	Histone Modification	11	↑	↑
Growth and Development	GO:0032502	Developmental Process	12	↑	↓↑
	GO:0009888	Tissue Development	6	↑	↑
	GO:0048367	Shoot System Development	11	↑	↑
Macromolecule Synthesis	GO:0009059	Macromolecule Biosynthetic Process	4	↑	↑
	GO:0009057	Macromolecule Catabolic Process	1	↑	↑
	GO:0016491	Oxidoreductase Activity	1	↓	
	GO:0010556	Regulation of Biological Process	5	↑	↑
Metabolism and Energetics	GO:0140657	ATP-Dependent Activity	1	↑	↑
	GO:0044237	Cellular Metabolic Process	4	↓↑	↑
	GO:0019222	Regulation of Metabolic process	1	↑	↑
Nitrogen	GO:0044271	Cellular Nitrogen Compound Biosynthetic Process	2	↑	↑
	GO:0071705	Nitrogen Compound Transport	1	↓	↓
	GO:0006164	Purine Nucleotide Biosynthetic Process	3		↓
	GO:0019362	Pyridine Nucleotide Metabolic Process	4	↑	↓↑
	GO:0051171	Regulation of Nitrogen Compound Metabolic Process	14	↑	↑
	GO:0006351	Transpiration, DNA-templated	9	↑	↑
Cell and Organelle	GO:0005618	Cell Wall	11	↑	↑
	GO:0043226	Organelles	11	↑	↓↑

	GO:0070925	Organelle Assembly	5	↓↑	↓↑
	GO:1902116	Regulation of Organelle Assembly	3	↑	↑
Phosphorus	GO:0006740	NADPH Regeneration	3	↓	
	GO:0006796	Phosphate Containing Compound Metabolic Process	3	↓	
	GO:0051174	Regulation of Phosphorus Metabolic Process	5	↑	↑
Photosynthesis	GO:0015977	Carbon Fixation	12	↓	
	GO:0009507	Chloroplast	7		↓
	GO:0006739	NADP Metabolic Process	1	↓	
	GO:0010233	Phloem Transport	2		↓
	GO:0009853	Photorespiration	1	↓	
	GO:0010119	Regulation of Stomatal Movement	2	↑	↑
	GO:0009579	Thylakoid	2	↓	↓
	GO:0010148	Transpiration	1	↑	↑
Reproduction	GO:0009908	Flower Development	6	↑	↑
	GO:0010154	Fruit Development	1	↑	↑
	GO:0009555	Pollen Development	6	↓	
	GO:0019953	Sexual Reproduction	16	↓↑	↑
Response to Abiotic Stimuli	GO:0071214	Cellular Response to Abiotic Stimulus	7	↑	↑
	GO:0104004	Cellular Response to Environmental Stimulus	1	↑	↑
	GO:0009725	Cellular Response to Hormones	4	↑	↑
	GO:0033554	Cellular Response to Stress	4	↑	↑
	GO:0009649	Entrainment of Circadian Clock	1	↓	
	GO:0009628	Response to Abiotic Stimulus	8	↑	↑

Response to Biotic Stimuli	GO:0051702	Biological Process Involved in Symbiotic Interaction	2	↓	
	GO:0043207	Response to External Biotic Stimulus	8	↓↑	↑
RNA	GO:0010467	Gene Expression	7	↑	↑
	GO:0051252	Regulation of RNA Metabolic Process	11	↑	↑
	GO:0010468	Regulation of Gene Expression	6	↑	↑
	GO:0006417	Regulation of Translation	9	↑	↑
	GO:0006401	RNA Catabolic Process	5	↑	↑
	GO:0016070	RNA Metabolic Process	22	↑	↑
	GO:0006412	Translation	3	↑	↑

Table 4: Results of GO enrichment analysis between invasive- versus native-tetraploids grown in low or high NP treatments. The differential enrichment patterns of parent terms in native-tetraploids relative to invasive-tetraploids are depicted below and are denoted by arrows (↑ = mostly upregulated; ↓ = mostly downregulated, ↑↓ = some nested child terms are both up- and down-regulated, no arrow = no differences in regulation).

Category	GO ID	Parent Term	No. Child Terms	Low: 4xI v. 4xN	High: 4xI v. 4xN
Defense	GO:0006952	Defense Response	13		↓
	GO:0032870	Cellular Response to Hormone Stimulus	4	↓	
Growth and Development	GO:0032502	Development Process	12		↓
	GO:0022622	Root System Development	11		↓
Macromolecules Synthesis	GO:0009059	Macromolecule Biosynthetic Process	4	↓↑	↓
	GO:0009057	Macromolecule Catabolic Process	1		↓
	GO:0010556	Regulation of Biological Process	5	↓↑	↓
Metabolism and Energetics	GO:0044237	Cellular Metabolic Process	4	↓↑	↓
	GO:0019222	Regulation of Metabolic Process	2	↓↑	↓
Nitrogen	GO:0044271	Cellular Nitrogen Compound Biosynthetic Process	2	↓↑	↓
	GO:0071705	Nitrogen Compound Transport	2		↓
	GO:1901566	Organonitrogen Compound Biosynthetic Process	2	↑	
	GO:0019362	Pyridine Nucleotide Metabolic Process	4	↑	
	GO:0051171	Regulation of Nitrogen Compound Metabolic Process	14	↑	↓

	GO:1901698	Response to Nitrogen Compound	2		↑
	GO:0006351	Transcription, DNA-templated	9	↑	
Cell and Organelle	GO:0043226	Organelles	11	↓↑	↑
Photosynthesis	GO:0009507	Chloroplast	7		↓
Reproduction	GO:0010154	Fruit Development	1	↑	
Response to Abiotic Stimuli	GO:0009628	Response to Abiotic Stimulus	8	↓	↓
	GO:0042221	Response to Chemical	5		↑
RNA	GO:0010467	Gene Expression	7	↓↑	↑
	GO:0010468	Regulation of Gene Expression	6	↑	↓
	GO:0016070	RNA Metabolic Process	22	↑	↓

Table 5: ANOVA results showing the effects of geo-tetraploid (native-tetraploids = $4x^N$, invasive-tetraploids = $4x^I$), soil NP treatments (LL= low, HH=high), their interaction, and geo-cytotype nested within population on A) Aboveground biomass, B) belowground biomass, C) total foliar terpene concentrations (log-transformed), D) foliar monoterpene concentrations (log-transformed), E) foliar diterpene concentrations (log-transformed), F) foliar sesquiterpene concentrations (log-transformed), and G) C:N ratios. Overall model results are reported in the footnotes, bold values indicate a significant effect at $\alpha = 0.05$. Student's t-tests ($\alpha = 0.05$) were used to determine significant differences between means when geo-tetraploid or NP treatment were significant.

Source	df	MS	F	Prob > *F*	Student's t-test
A) Aboveground biomass					
Geo-tetraploid (T)	1	0.01	0.03	0.8609	
NP Treatment (NP)	1	26.39	56.22	**<0.0001**	HH > LL
T x NP	1	0.70	1.50	0.2251	
Population [Geo-tetraploid]	6	1.25	2.67	**0.0216**	
Model Error	70	0.47			
B) Belowground biomass					
Geo-tetraploid (T)	1	8.06	8.06	**0.0059**	$4x^I > 4x^N$
NP Treatment (NP)	1	55.76	53.56	**<0.0001**	HH > LL
T x NP	1	1.85	1.77	0.1872	
Population [Geo-tetraploid]	6	1.92	1.84	0.1038	
Model Error	70	1.04			
C) Total Terpenes					
Geo-tetraploid (T)	1	0.82	1.35	0.2548	
NP Treatment (NP)	1	6.15	10.09	*0.0034	
T x NP	1	0.02	0.03	0.8655	
Population [Geo-tetraploid]	6	0.96	1.57	0.1880	
Model Error	31	0.61			
D) Total Monoterpenes					
Geo-tetraploid (T)	1	0.79	1.24	0.2746	
NP Treatment (NP)	1	6.08	9.51	*0.0043	
T x NP	1	0.00	0.01	0.9353	
Population [Geo-tetraploid]	6	1.10	1.72	0.1490	
Model Error	31	0.64			
E) Total Diterpenes					
Geo-tetraploid (T)	1	1.02	1.02	0.2685	
NP Treatment (NP)	1	5.37	6.66	*0.0148	
T x NP	1	1.91	2.36	0.1343	

Population [Geo-tetraploid]	6	0.93	1.15	0.3570
Model Error	31	0.81		
F) Total Sesquiterpenes				
Geo-tetraploid (T)	1	0.32	0.29	0.5816
NP Treatment (NP)	1	2.99	2.77	0.1063
T x NP	1	0.94	0.87	0.3590
Population [Geo-tetraploid]	6	0.57	0.52	0.7855
Model Error	31	1.08		
G) C:N Ratio				
Geo-tetraploid (T)	1	179.75	4.09	0.0525
NP Treatment (NP)	1	7.54	0.17	0.6817
T x NP	1	85.65	1.95	0.1734
Population [Geo-tetraploid]	6	127.74	2.91	**0.0242**
Model Error	29	43.97		

Overall model for aboveground biomass: $R^2 = 0.51$, $F_{9,79} = 8.22$ $P < 0.0001$, N = 80
Overall model for belowground biomass: $R^2 = 0.52$, $F_{9,79} = 8.36$ $P < 0.0001$, N = 80
Overall model for total foliar terpene concentrations: $R^2 = 0.39$, $F_{9,40} = 2.16$, $P = 0.0544$, N = 41
Overall model for total foliar monoterpene concentration: $R^2 = 0.39$, $F_{9,40} = 2.19$, $P = 0.0508$, N = 41
Overall model for total foliar diterpene concentration: $R^2 = 0.33$, $F_{9,40} = 1.70$, $P = 0.1313$, N = 41
Overall model for total foliar sesquiterpene concentration: $R^2 = 0.17$, $F_{9,40} = 0.70$, $P = 0.7033$, N = 41
Overall model for foliar C:N ratio: $R^2 = 0.45$, $F_{9,38} = 2.68$, $P = 0.0213$, N = 39
* = overall model not significant

Table 6: List of foliar terpenes in *Solidago gigantea* identified via GC-MS.

Chemical Name	RT
Monoterpenes	
α-Pinene	7.37
β-Phellandrene	8.17
α-pinene (isomer)	8.46
Limonene	9.10
Camphene	13.17
Diterpenes	
SolidagoicAcid (A or B)	25.1
SolidagoicAcid (A or B)	25.8
Sesquiterpenes	
Aromadendrane	14.60
α-Gurjuene	14.85
Germacrene-D	15.01
(+)-Epi-bicycosesquiphellandrene	15.82
Other Compounds	
1-Heptene, 6-methyl	6.56
α-copaene	15.82
Trans-androsterone	19.75
1-ethyl-3-propyladamate	25.2
Unknown Compounds	
Unknown 1	19.08
Unknown 2	22.38
Unknown 3	26.14
Unknown 4	27.92
Unknown Methyl Ester	27.50

4.7 Figures

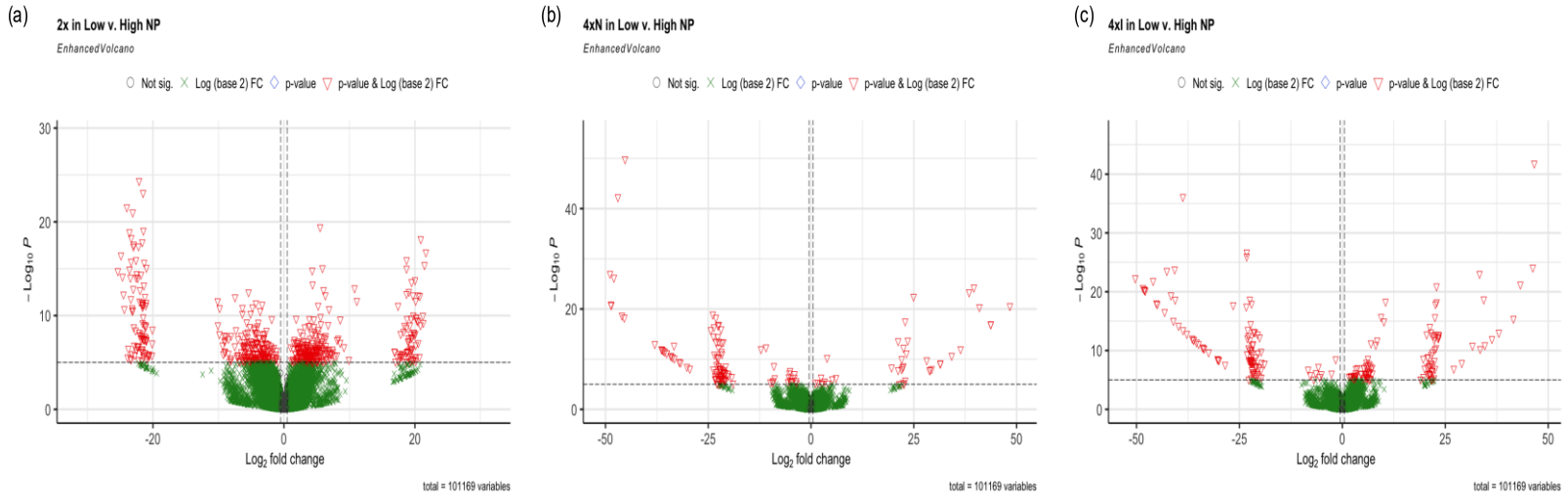

Figure 1: Volcano plot of fold-change expression level (x-axis) against -Log10P (y-axis) showing the comparison of differentially expressed genes (DEGs) between a) diploids, b) native-tetraploid, and c) invasive-tetraploids grown in high versus low nitrogen+phosphorus treatments. Each point represents a transcript, and those with significantly different expression are in red. The red scatters to the right of "0" along the x-axis indicate differentially upregulated DGEs, while the red scatters to the left of "0" indicate differentially downregulated DGEs.

Figure 2: Results of enrichment analyses of gene ontology (GO) terms in a) diploids, b) native-tetraploid, and c) invasive-tetraploids grown in high versus low nitrogen+phosphorus treatments using Fisher's Exact test available in the Blast2Go software. The top 15 most downregulated (teal) and upregulated (maroon) GO terms are reported, see Table 2 for more details.

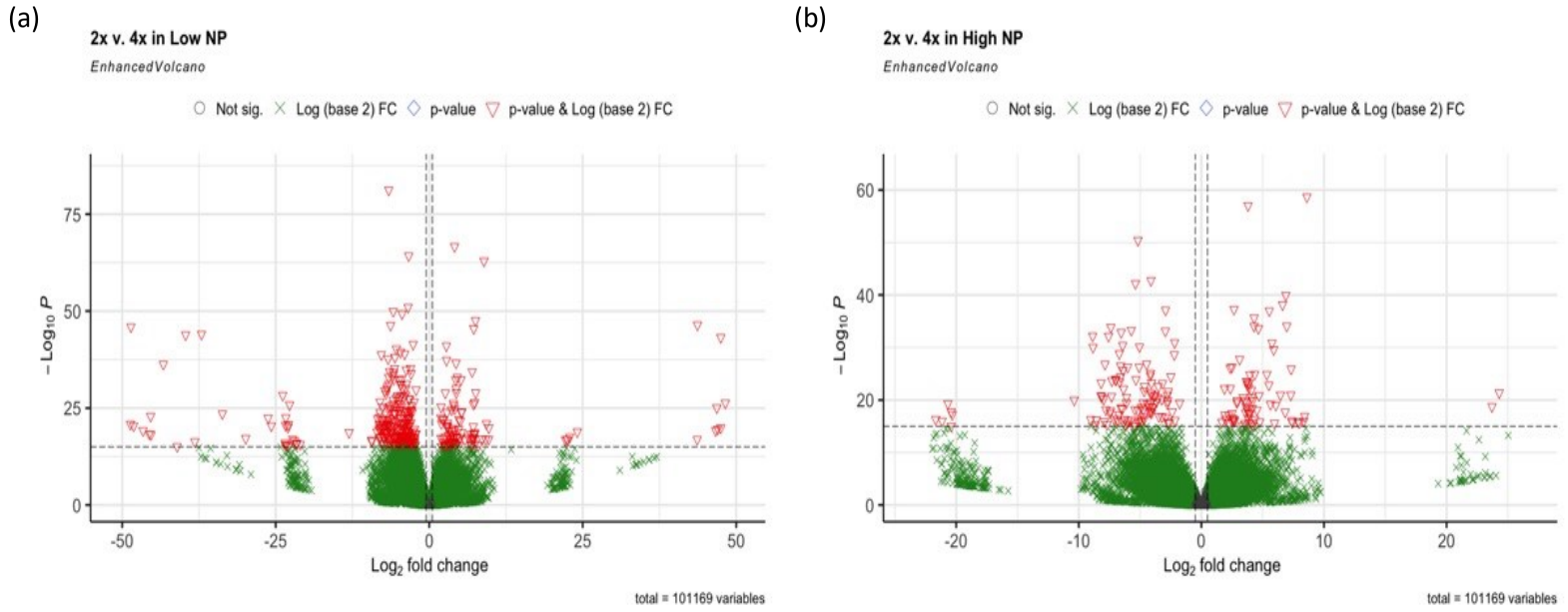

Figure 3: Volcano plot of fold-change expression level (x-axis) against -Log10P (y-axis) showing the comparison of differentially expressed genes (DEGs) between native-tetraploids versus diploids in a) low and b) high nitrogen+phosphorus treatments. Each point represents a transcript, and those with significantly different expression are in red. The red scatters to the right of "0" along the x-axis indicate differentially upregulated DGEs, while the red scatters to the left of "0" indicate differentially downregulated DGEs.

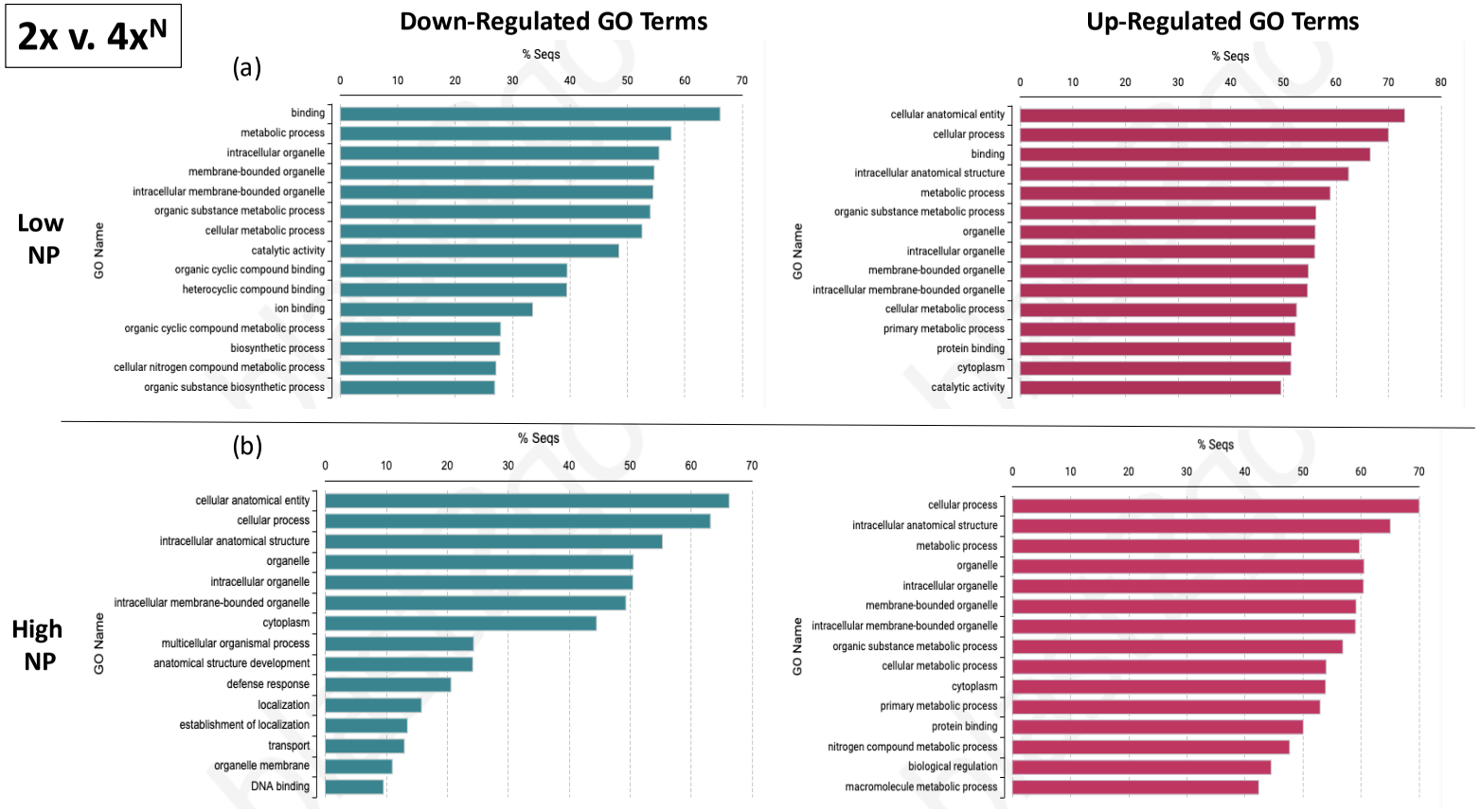

Figure 4: Results of enrichment analyses of gene ontology (GO) terms in native-tetraploids versus diploids in a) low and b) high nitrogen+phosphorus treatments using Fisher's Exact test available in the Blast2Go software. The top 15 most downregulated (teal) and upregulated (maroon) GO terms are reported, see Table 3 for more details.

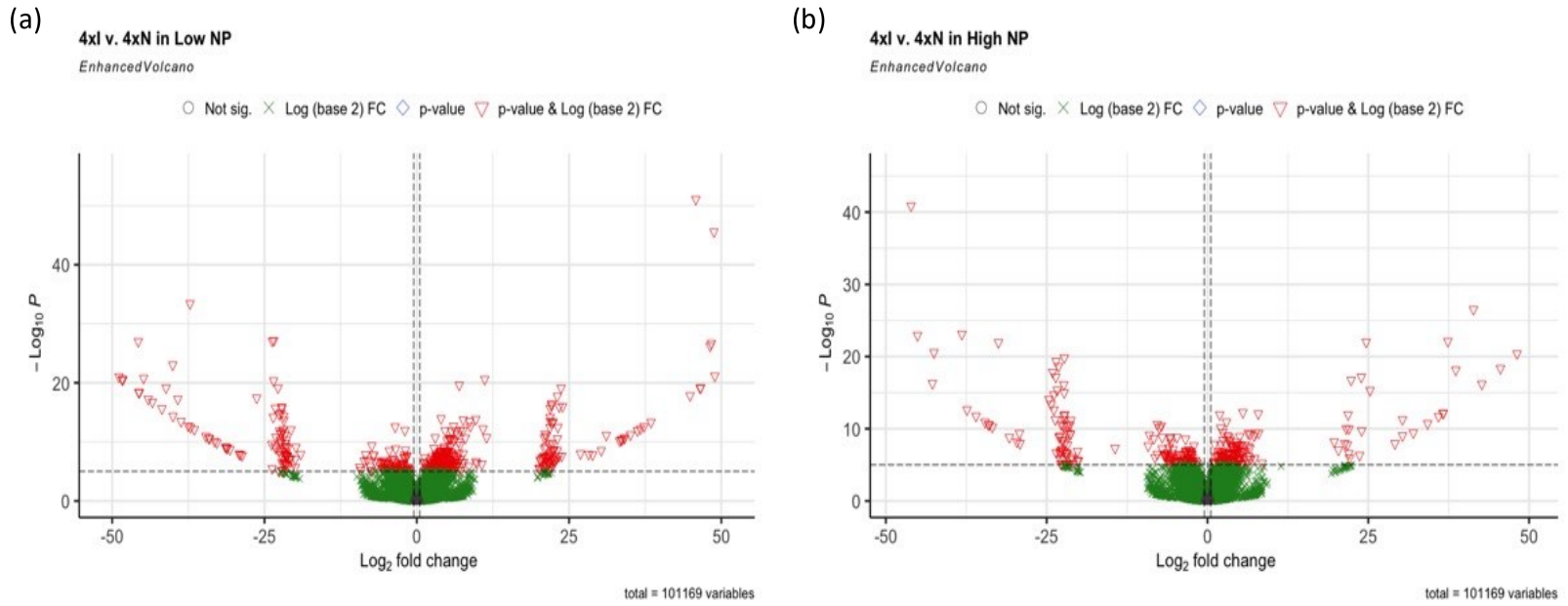

Figure 5: Volcano plot of fold-change expression level (x-axis) against -Log10P (y-axis) showing the comparison of differentially expressed genes (DEGs) between native-tetraploids versus invasive-tetraploids in a) low and b) high nitrogen+phosphorus treatments. Each point represents a transcript, and those with significantly different expression are in red. The red scatters to the right of "0" along the x-axis indicate differentially upregulated DGEs, while the red scatters to the left of "0" indicate differentially downregulated DGEs.

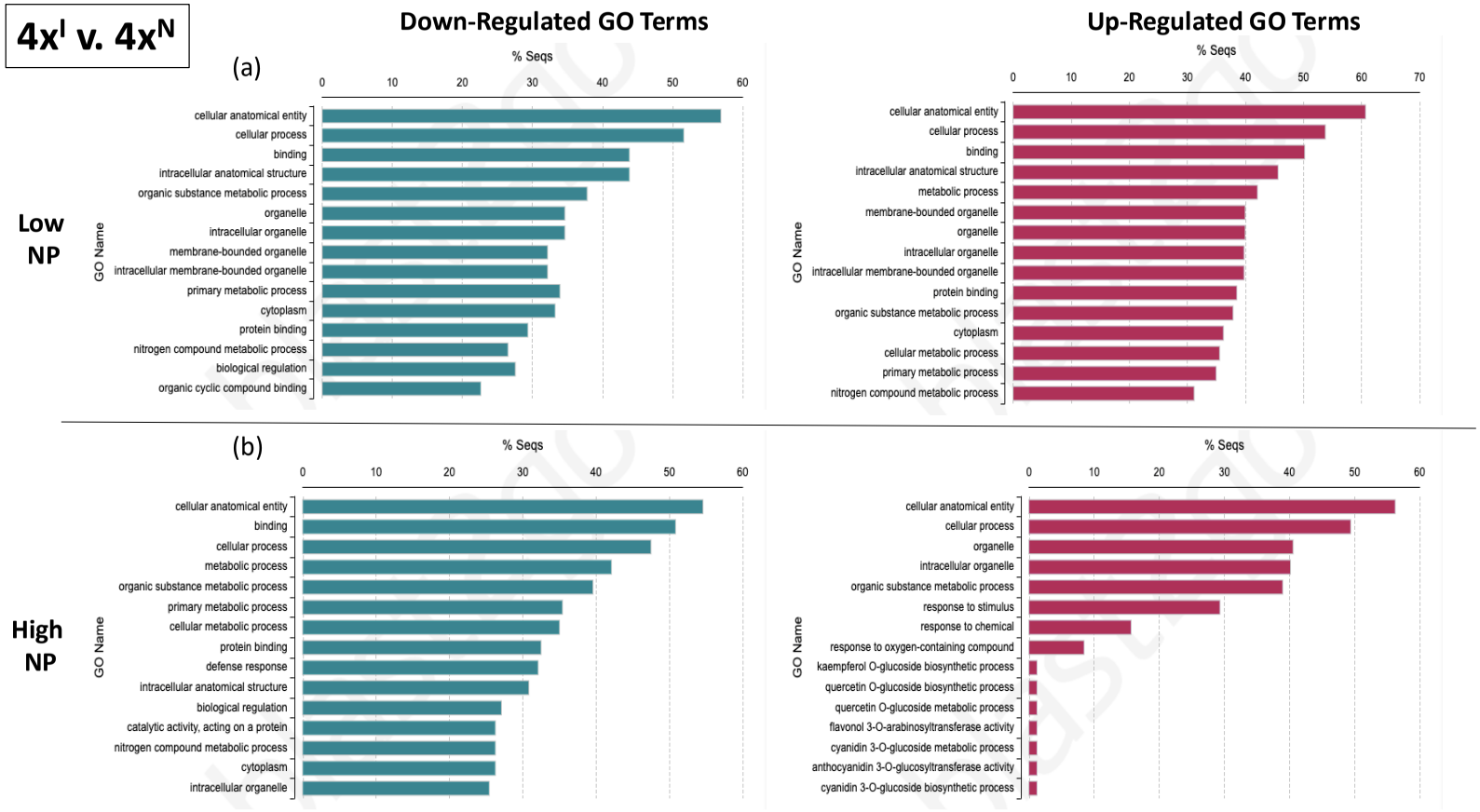

Figure 6: Results of enrichment analyses of gene ontology (GO) terms in native-tetraploids versus invasive-tetraploids in a) low and b) high nitrogen+phosphorus treatments using Fisher's Exact test available in the Blast2Go software. The top 15 most downregulated (teal) and upregulated (maroon) GO terms are reported, see Table 4 for more details.

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Conclusion

The overall goal of this dissertation was to evaluate how genome size and polyploidy influence tolerances to nutrient availability as a means of better understanding their ecological and evolutionary roles in the context of cytotype-dynamics and biological invasions. Here, I used diploid, native-tetraploid, invasive-tetraploid, and hexaploid *Solidago gigantea* in a series of greenhouse, potted-field, and RNA sequencing studies to address this goal. In chapter 1, I found morphological and physiological differences between diploid and native-tetraploids, regardless of nutrient and water availability. These results suggest that tetraploids might be better competitors, and thus better potential invaders, than diploids in a range of environmental contexts. In chapter 2, I found evidence of polyploids having greater genomic material costs than diploids, and that polyploids might have evolved strategies to mitigate these costs over both long and short periods of time. In chapter 3, I found polyploids to be more phenotypically plastic than diploids for some growth traits, but only when exposed to very large gains in NP availability. I also found a lack of plasticity between native- and invasive-tetraploids, suggesting that not enough evolutionary time has passed for differences to evolve, severe bottlenecks limited the range of phenotypic expression in invasive populations, and/or the selective environments in both native- and invasive habitats are similar. Finally, in chapter 4, I found that native-tetraploids down-regulate photosynthetic and defensive but up-regulate developmental and stress response gene groups relative to diploids in low NP conditions. This suggests that polyploids might be able to downregulate costly transcripts and their associated traits in favor of supplementing resources to costly genomic maintenance. Furthermore, while differences between native- and invasive tetraploids

populations was marginal, the up-regulation of N-metabolic gene groups in native-tetraploids suggests that invasive populations use comparatively less N. Taken together, these studies show that there are some material costs associated with genome size, and these costs might limit the ecological success of polyploids in nutrient poor conditions. However, mechanisms selectively favored to reduce these costs could lessen the selective pressures favoring small genomes. These studies also highlight the importance that the soil nutrient environment could play in the invasive success of *S. gigantea* and other polyploid invaders, as anthropogenic-caused nutrient enrichment may create environments that release polyploid invaders from nutrient constraints and allow for enhanced investment into fitness and competitive traits.

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