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Studies

STUDIES

Increased ploidy of *Butomus umbellatus* in introduced populations is not associated with higher phenotypic plasticity to N and P

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Abstract

Separate introductions or post-introduction evolution may lead to multiple invader genotypes or cytotypes that differ in growth rates, biomass or chemical profile responses (phenotype) to a range of environments. If the invader has high trait plasticity to a range of resource levels, then sediment N or P enrichment may enhance invasiveness. However, the ways in which ploidy, plasticity, and available N or P interact are unknown for most species despite the potential to explain spread and impacts by invaders with multiple introduced lineages. We conducted a common garden experiment with four triploid and six diploid populations of *Butomus umbellatus*, collected from across its invasive range in the USA. Plants were grown under different N or P nutrient levels (4, 40, 200, 400 mg L^{-1} N; 0.4, 4, 40 mg L^{-1} P) and we measured reaction norms for biomass, clonal reproduction and tissue chemistry. Contrary to our expectation, triploid *B. umbellatus* plants were less plastic to variation in N or P than diploid *B. umbellatus* in most measured traits. Diploid plants produced 172 % more reproductive biomass and 57 % more total biomass across levels of N, and 158 % more reproductive biomass and 33 % more total biomass across P than triploid plants. Triploid plants had lower shoot:root ratios and produced 30 % and 150 % more root biomass than diploid plants in response to increases in N and P, respectively. Tissue chemistry differed between cytotypes but plasticity was similar; N was 8 % higher and C:N ratio was 30 % lower in triploid than diploid plants across levels of N and plant parts, and N was 22 % higher and C:N ratio 27 % lower across levels of P and plant parts. Our results highlight differences in nutrient response between cytotypes of a widespread invader, and we call for additional field studies to better understand the interaction of nutrients and ploidy during invasion.

Keywords: Biomass allocation; flowering rush (*Butomus umbellatus*); nutrient enrichment; phenotypic plasticity; plant invasion; polyploidy.

Introduction

Phenotypic responses to changing environmental conditions (i.e. phenotypic plasticity) explain the success of some invasive species during establishment and their subsequent spread to new areas (Davidson *et al.* 2011; Higgins and Richardson 2014; Turner *et al.* 2015). The ability to colonize and establish under varying conditions and in a range of environments can be highly advantageous for an invading species (Richards *et al.* 2005). This may be particularly important if the phenotypic response leads

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to a greater fitness advantage for the invader than the response of species in recipient communities (Leishman and Thomson 2005; Richardson and Pyšek 2006). Plasticity may also evolve in response to new environments encountered after introduction, for example along an invasion front (Chevin and Lande 2011), or through generation of novel genotypes as a result of genetic crossings between invaders originating from different locations (i.e. hybrid vigor; Williams *et al.* 2005; Lavergne and Molofsky 2007; Xu *et al.* 2010). The importance of phenotypic plasticity for defining niche breadth, especially with regards to successful plant invasions, has been well studied for more than a decade, with research focused on variation in trait plasticity to salinity (Richards *et al.* 2005; Meyerson *et al.* 2020), water availability (Leishman and Thomson 2005), competition (Peperkorn *et al.* 2005; Peacor *et al.* 2006), light (DeWalt *et al.* 2004; Siebenkäs *et al.* 2015), shade (Griffith and Sultan 2005), herbivory (Simoes and Baruch 1991; Schierenbeck *et al.* 1994; Agrawal *et al.* 2002; Ashton and Lerdau 2008; Bhattarai *et al.* 2017) and nutrients (Burns 2004; Hastwell and Panetta 2005; Leishman and Thomson 2005).

Nutrient enrichment, for example resulting from fertilizer use in agricultural areas, and its role in promoting plant invasions has been examined for a number of terrestrial (e.g. Huenneke *et al.* 1990; Lowe *et al.* 2002; Gross *et al.* 2005; Vasquez *et al.* 2008) and aquatic systems (e.g. Fan *et al.* 2013; Yu *et al.* 2018). Studies that examine plant phenotypic (e.g. growth rate, biomass allocation, tissue chemistry) variation in response to nutrient conditions (elevated *or* reduced) between congeneric species have led to a better mechanistic understanding of some plant invasions (Woo and Zedler 2002; Kolb and Alpert 2003; Godoy *et al.* 2011; Funk 2013) and the conditions under which some species, but not others, become invasive (Flanagan *et al.* 2015). In fact, understanding the role of trait plasticity in plant response to available nutrients (e.g. N, P) may be key to predicting areas at risk of invasion (Wang *et al.* 2017), assessing the performance of introduced species (Seabloom *et al.* 2015), managing invasive species (Blumenthal 2006) and understanding trophic interactions involving the invader (e.g. Room *et al.* 1989; Steinger and Müller-Schärer 1992; Center and Dray 2010). Although a number of traits have been associated with successful invaders, plasticity in nitrogen use efficiency, photosynthetic rate, biomass production and allocation (e.g. shoot:root ratio) and tissue chemistry (e.g. N, C:N) are consistently higher in invasive than non-invasive species across studies (Davidson *et al.* 2011). In fact, success of invaders in enriched systems may be due in part to their proportionally greater phenotypic response across a range of nutrient levels in comparison to native species (i.e. 'jack-of-all-trades' *sensu* Richards *et al.* 2006) (Huenneke *et al.* 1990; Lowe *et al.* 2002; Gross *et al.* 2005; Vasquez *et al.* 2008), their greater response to nutrient enhancement ('master-of-some') or interactions between nutrients and herbivory (Wright *et al.* 2014).

Evolutionary processes occurring during plant invasions can produce genetic structure and generate meaningful differences in invasiveness or response to management activities among and within populations (Bossdorf *et al.* 2005; Sax *et al.* 2005; Ward *et al.* 2008; Gaskin *et al.* 2013). The number and source of introductions, founder or bottleneck effects, hybridization and post-introduction adaptation (e.g. along latitudinal or productivity gradients) may all act to produce populations that diverge in key invasive traits such as reproductive mode or phenology (Kliber *et al.* 2005; Golani *et al.* 2007; Ward *et al.* 2008; Williams *et al.* 2014). For example, the aquatic plant *Alternanthera philoxeroides* has been introduced into the USA at least twice (Kay and Haller 1982), with introduced biotypes displaying differential susceptibility to herbicides (Kay 1992) and biological control agents (Pan *et al.* 2012), and responses to nutrients (N. E. Harms, unpubl. data). Another important aspect of genetic variability in invading populations concerns the relationship between chromosome number (ploidy), plasticity to environmental conditions (i.e. niche breadth) and invasiveness (Hahn *et al.* 2012). The role of increased ploidy in generating generalist phenotypes has been suggested a number of times (Levin 1983; te Beest *et al.* 2012; Hao *et al.* 2013), although there are an increasing number of cases where polyploids of invasive taxa tend to have reduced plasticity in important traits related to invasion success. For example, the invasive aquatic plant congeners *Ludwigia hexapetala and L. peploides* vary in ploidy (*L. hexapetala* is decaploid and L. peploides is diploid) and are invasive in the USA, but the colonizing form of the diploid *L. peploides* outperforms *L. hexapetala* at high nutrient levels, an important characteristic to consider for management where eutrophication occurs (Grewell *et al.* 2016). The success of invaders with multiple ploidy levels may also depend largely on interactions between ploidy, genome size and environmental variation (Meyerson *et al.* 2016, 2020). Despite considerable interest in evolutionary processes occurring during plant invasions, and the potential management benefit of better understanding relationships between invasion genetics and phenotypic plasticity to environmental variation, the interplay between genetic variation within invading populations and nutrient response and allocation has not been sufficiently studied (but see Riis *et al.* 2010; Kettenring *et al.* 2011; Hahn *et al.* 2012; Gioria and Osborne 2014).

We conducted a common garden experiment to examine genetic differences in growth, reproduction and tissue chemistry of the invasive wetland plant, *Butomus umbellatus*, grown hydroponically in increasing concentrations of either N or P. Although the relationship between plasticity of specific traits and fitness remains difficult to demonstrate without long-term demographic studies (Davidson *et al.* 2011), for clonally reproducing invaders such as *B. umbellatus*, biomass and reproduction (i.e. generation of vegetative propagules) are often used as fitness proxies (Pan and Price 2001; Eller and Brix 2012; Younginger *et al.* 2017). Literature reports suggest that triploid *B. umbellatus* plants have higher fitness under elevated nutrient conditions than diploid plants (Hroudová and Zákravský 1993; Madsen *et al.* 2016). However, we know little about biomass accumulation and allocation, clonal reproduction, tissue chemistry or plasticity differences in those traits between cytotypes. Therefore, the objectives of this study were to determine whether triploid *B. umbellatus* plants have higher performance than diploid plants across two nutrient (N and P) gradients and whether triploid plants have higher plasticity of measured traits to increasing nutrients. Specifically, we tested our predictions that (i) triploid plants would produce greater biomass (total, reproductive) than diploid plants across increasing N or P gradients overall, (ii) triploid plants would display increased plasticity to N or P and (iii) tissue chemistry (C, N, P, C:N, C:P, N:P) would similarly differ between cytotypes (diploid, triploid) in response to available nutrients.

Materials and Methods

Study system

Butomus umbellatus is a perennial Eurasian wetland plant species, with diploid and triploid cytotypes, introduced into North America multiple times during the last 100 years from different source areas (Anderson *et al.* 1974; Kliber *et al.* 2005).

Reproduction in *B. umbellatus* is primarily clonal (diploid and triploid plants), but can be sexual (diploid plants). Although viable seeds have been reported, they are not as important for spread in the USA as clonal propagules such as bulbils, small clonal propagules produced on roots and in leaf axils, or rhizome buds (Eckert *et al.* 2000, 2003). Although populations in the native European range are thought to be largely triploid, in North America the diploid cytotype is most common (Kliber *et al.* 2005). In 2013, a programme to develop biological control agents for *B. umbellatus* was initiated in collaboration between North American and European scientists (www.cabi.org/projects/project/56422). To date, there have been limited ecological comparisons between introduced *B. umbellatus* cytotypes in North America, but there is strong evidence that cytotype identity (i.e. triploid or diploid) is related to disease resistance (Harms *et al.* 2020), and growth and reproductive success (Eckert *et al.* 2000; Lui *et al.* 2005). Other than a single study in the native range (Hroudová and Zákravský 1993), no one has investigated the role of ploidy in plant response to increased nutrient availability for *B. umbellatus*.

Glasshouse experiment

To test for genetic-based differences in available nutrient-related growth and tissue chemistry plasticity in introduced *B. umbellatus* populations, we conducted a glasshouse common garden experiment. Plants were field-collected from 10 sites during 2016–17 (Fig. 1; **see Supporting Information—Table S1.1**), genotyped by Amplified Fragment Length Polymorphisms and grown in a common garden at the U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, MS, USA. Plants from four triploid (Genotype 1; G1), and six diploid (four Genotype 4, one Genotype 3 and one Genotype 5; G4, G3, G5) populations were repeatedly vegetatively propagated. Our selection of source populations for this work was made with the goal to include populations from a large geographic area, in order to capture genetic and phenotypic variation present in the USA. Genetic data in Gaskin *et al.* (2021) suggest that both diploid and triploid plants in the North American invasion reproduce clonally and not from seed via outcrossing. Thus, genetic diversity in North America is limited to only six genotypes (G1–G6). There was no genetic diversity within our diploid or triploid source populations (i.e. they were clonal), and thus all plants of a designated genotype are genetically identical.

Plants were grown outdoors in commercially available topsoil amended with slow-release fertilizer (Osmocote®; 15-9-2; Scotts Miracle-Gro, Marysville, OH, USA) and propagated from cuttings at least twice over 2 years to reduce maternal effects (Roach and Wulff 1987). Water was municipal-delivered and maintained 10–20 cm above the sediment surface in cultures. Approximately 1.5 weeks before transplanting for the experiment, potted plants were harvested, rinsed of debris and then prepared for repotting. For each population, rhizome fragments (~4 cm long) with leaves removed were floated in aerated water until replanting.

Six nutrient solutions were prepared, containing varying amounts of N or P **[see Supporting Information—Table S1.2]**. Solution compositions were based on a standard Hoagland's recipe (200 mg L^{-1} N; 40 mg L^{-1} P) (Hoagland and Arnon 1950), but modified to create levels of N or P that varied along a logarithmic scale for the experiment **[see Supporting Information—Supplement 1]** (similar to Garrish *et al.* 2010). To test for *B. umbellatus* response to N, four solutions were used in which [P] was held constant at 40 mg L^{-1} and [N] was modified (400, 200, 40, 4 mg L^{-1}). Likewise, three solutions were used in which [N] was constant at 40 mg L^{-1} and [P] altered (40, 4, 0.4 mg L^{-1}). All other micro- and macronutrients were the same between solutions. The range of nutrients used in this study, although designed to measure plant response across a gradient, fall within the range used in previous work with *B. umbellatus* (Manolaki *et al.* 2020).

To test *B. umbellatus* phenotypic response to N or P, plants were grown hydroponically within a single glasshouse at

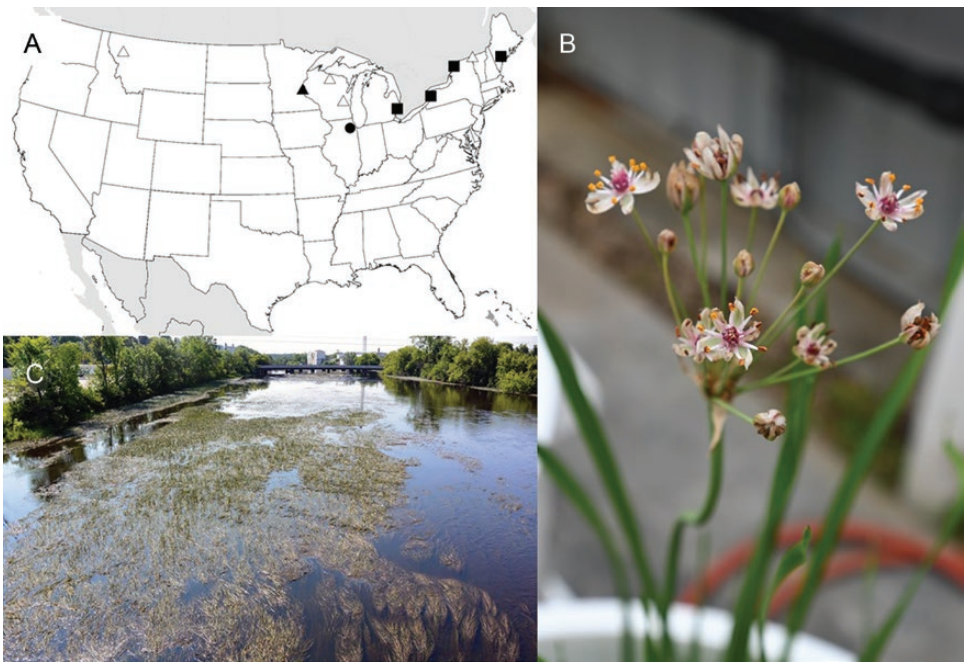

Figure 1. (A) Map of locations where *B. umbellatus* plants were collected for this work. Genotypes and ploidy are indicated by the symbols: White triangle = triploid G1, black circle = diploid G3, black square = diploid G4, black triangle = diploid G5. Also shown are (B) a close-up of a *B. umbellatus* inflorescence, and (C) a triploid *B. umbellatus* infestation in Oconto Falls, WI, USA.

the ERDC. At planting, plant propagules were weighed, then placed into net pots (12.7 mm diameter) filled with washed expanded clay rocks (8–16 mm diameter). Net pots were placed individually within white 4-L polyethylene food containers and charcoal-filtered tap water was added to each container. After 1 week, the water was drained and 1.5 L of nutrient solution was added to each container. One week after initial set-up, any plants that did not show signs of growth were replaced with new, pre-weighed propagules. We used six plant replicates per population for each treatment combination. Plants were grown for 8 weeks and nutrients were completely exchanged weekly. At harvest, plants were rinsed with reverse osmosis water, then separated into roots, shoots (leaves) and clonal reproductive (bulbils, rhizomes) tissues, placed into paper bags and dried at 60 °C. Only two plants produced inflorescences during the study, so sexual tissue biomass was not included in analyses. Tissues were weighed, then three randomly chosen replicates from each treatment combination were processed for tissue chemistry. Tissue chemistry analyses were performed at the Louisiana State University Agricultural Chemistry Laboratory, Baton Rouge, LA, USA. Nitrogen and carbon were determined by the modified Dumas method (CN 628 Dumas Analyzer; LECO, St. Joseph, MI, USA) and tissue phosphorus was determined by inductively coupled plasma (ICP) mass spectrometry (ARCOS; SPECTRO Analytical Instruments, Kleve, Germany) (Jones and Case 1990). For some samples, particularly those grown in the lowest N treatment (4 mg L^{-1} N), there was insufficient material for all nutrient analyses. In those cases, we prioritized analyses of C and N over P. From the results of plant tissue nutrient analyses, C:N ratio, C:P ratio and N:P ratio were calculated.

Statistical approach

We first tested whether *B. umbellatus* biomass phenotypes (total biomass, reproductive biomass, shoot:root ratio) differed in response to N and P using separate mixed-effects models (general linear model [GLM]). Fixed effects for the models were cytotype (two levels: diploid, triploid), nutrient level (four levels of N, three levels of P; N and P treatments were tested separately), the cytotype * nutrient level interaction and initial fresh weight of propagules. We did not have replication at the level of genotypes so examined variation in cytotype only. Thus, we assigned population as a random effect in all models and initial (at planting) fresh weight of plants was included as a covariate. A significant interaction involving cytotype indicated that diploid and triploid *B. umbellatus* differed in their plasticity of the measured trait to nutrient enrichment. To quantify the direction and magnitude of plasticity differences, we calculated effects sizes, as described below.

Additionally, we tested whether tissue chemistry differed between plant parts, cytotypes and nutrient levels. We first generated standardized *Z*-scores (i.e. variables with mean = 0 and standard deviation = 1) for chemistry variables (%N, %C, %P, C:N ratio, N:P ratio, C:P ratio) to control for differences in variance and measurement units of each variable (Gotelli and Ellison 2004). Next, to reduce dimensionality in the data set and decrease the likelihood of committing type I errors, we conducted a principal component analysis (PCA) on the *Z*-scores. Principal components (PCs) with eigenvalues > 1 were retained for analysis. The resulting PCs represented independent linear combinations of tissue chemistry variables and accounted for 71 % (two PCs for nitrogen nutrient treatment) and 70 % (two PCs for phosphorus nutrient treatment) of total variance present in the original variables. Tissue chemistry PCs were tested separately with mixed-effects models, using cytotype, plant part, nutrient level and all two- and three-way interactions as fixed effects, population as a random effect and initial fresh weight of flowering rush propagules as a covariate. As above, significant interactions involving cytotype (i.e. cytotype * nutrient, cytotype * plant part, cytotype * nutrient * plant part) indicated significant differences in phenotypic plasticity between diploid and triploid flowering rush plants. Because there was limited biomass produced in triploid plants under the lowest (4 mg L^{-1}) N treatment, we removed the low-N treatment from those analyses. For clarity, we limit discussion of tissue chemistry variables to those within each PC that were correlated ($r > 0.5$). Degrees of freedom for all mixed models were calculated following Kenward and Roger (1997). All statistical tests were performed with SAS ver. 9.4 (SAS Institute Inc., Cary, NC, USA, 2018).

Variation in phenotypic plasticity

We took two approaches to compare plasticity in measured biomass and leaf chemistry traits between cytotypes. In the first approach we simply determined whether the cytotype * nutrient level interaction was significant ($P < 0.05$) in models described in the previous section. A significant interaction would indicate that phenotypic responses of cytotypes differed in relation to nutrient level. Our second approach, consisted of using effect size to quantify differences in direction and magnitude of response between cytotypes. To compare phenotypic plasticity between the two cytotypes, we calculated Hedge's g (J-corrected Cohen's d) (Borenstein *et al.* 2011; Davidson *et al.* 2011) from population means for each trait and nutrient level. This type of approach is valuable because it allows for a direct comparison with multiple traits standardized in units of standard deviation (Cook-Patton and Agrawal 2011) and unlike some other commonly used indices of phenotypic plasticity (e.g. Valladares *et al.* 2006), its values are not restricted from 0 to 1, so it accounts for both direction and magnitude of plasticity. We calculated g for each population and nutrient combination and used population-level g to test for differences in plasticity between cytotypes. First, d was calculated as:

$$d = \frac{\text{Mean}_{\text{max}} - \text{Mean}_{\text{min}}}{\text{SD}_{\text{pooled}}}$$

where Mean_{max} and Mean_{min} were the maximum and minimum mean values for each nutrient and population treatment. Pooled standard deviation was calculated as the square root of the mean of the two standard deviations (Cohen 1988):

$$\text{SD}_{\text{pooled}} = \frac{\sqrt{\text{SD}_{\text{max}} + \text{SD}_{\text{min}}}}{2}$$

We then corrected for small sample size by applying the J correction (Borenstein *et al.* 2011):

$$J = 1 - \frac{3}{(4df - 1)},$$

$$g = J * d,$$

with 10 degrees of freedom for biomass and four for tissue chemistry analyses. We present cytotype means and 95 % confidence intervals for g, generated from population-level calculations.

Results

Variation in biomass response to N and P between cytotypes

Despite our prediction that triploid plants would outperform diploid plants at increasing nutrient levels, diploid plants achieved greater total and reproductive biomass than triploid plants across nutrients (Figs 2 and 3; **see Supporting Information—Table S2.1**). Although both cytotypes performed similarly at low N, diploid plant biomass increased strongly with N (i.e. steep response curve), whereas triploid plant biomass increased, but at a lesser rate (resembling a 'master-of-some' scenario for the diploid *B. umbellatus* cytotype relationship between biomass variables and N; *sensu* Richards *et al.* 2006). Consequently, there were significant cytotype * N interactions for total biomass ($P < 0.001$), reproductive biomass ($P < 0.001$) and shoot:root ratio ($P = 0.02$) **[see Supporting Information—Table S2.2]**, indicating significant differences in plasticity between the two cytotypes for these traits. At 400 mg L^{-1} N, diploid plants produced nearly twice as much biomass overall and two and a half times more reproductive (bulbils) biomass, but 10 % less root biomass than triploid plants. Triploid plants produced 30 % more root biomass across N treatments, evident in the consistently lower calculated shoot:root ratios (Fig. 2E). Likewise, diploid plants consistently outperformed triploid plants in response to P additions (Fig. 3), resembling a 'jack-and-master' scenario for the relationship between biomass variables and P. We detected a significant cytotype * P interaction for total dry weight biomass ($P < 0.001$). Across levels of P, diploid plants produced 33 % more total biomass and 159 % more reproductive biomass than triploid plants ($P = 0.003$ and $P < 0.001$, respectively; **see Supporting Information—Tables S2.1 and S2.2**). However, shoot:root ratio of triploid plants was 68 % lower than diploid plants (Fig. 3E), a finding consistent with the higher root biomass allocation response to N.

The amount of variation in biomass response to N and P among populations depended on nutrient (Figs 2 and 3; **see Supporting Information—Table S2.3**). Triploid population-level variation was more than double diploid variation for reproductive biomass across N treatments (triploid coefficient of variation [CV] = 0.44, diploid CV = 0.18). However, diploid populations displayed 6 % (triploid CV = 0.16, diploid CV = 0.17) and 45 % (triploid CV = 0.08, diploid CV = 0.12) more variation in total biomass responses to N and P, respectively. Variation in shoot:root ratio was similar between cytotypes across levels of N (triploid CV = 0.37, diploid CV = 0.36), but across levels of

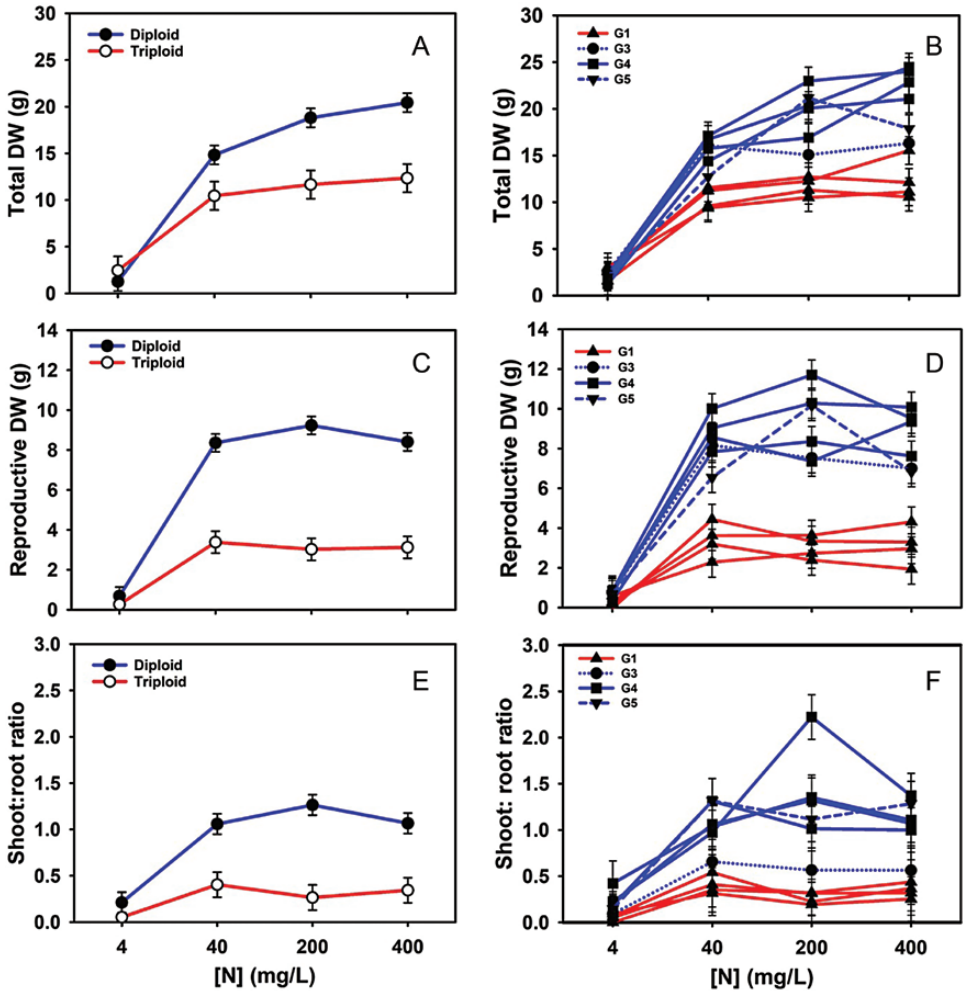

Figure 2. Reaction norms for *B. umbellatus* cytotypes (A, C, E) and populations (B, D, F). Reported are the means ± SE for growth and reproductive responses to four levels of nitrogen. Within cytotypes, we used four triploid populations of a single genotype and six diploid populations of three different genotypes (four G4, one G3 and one G5 population). DW = dry weight.

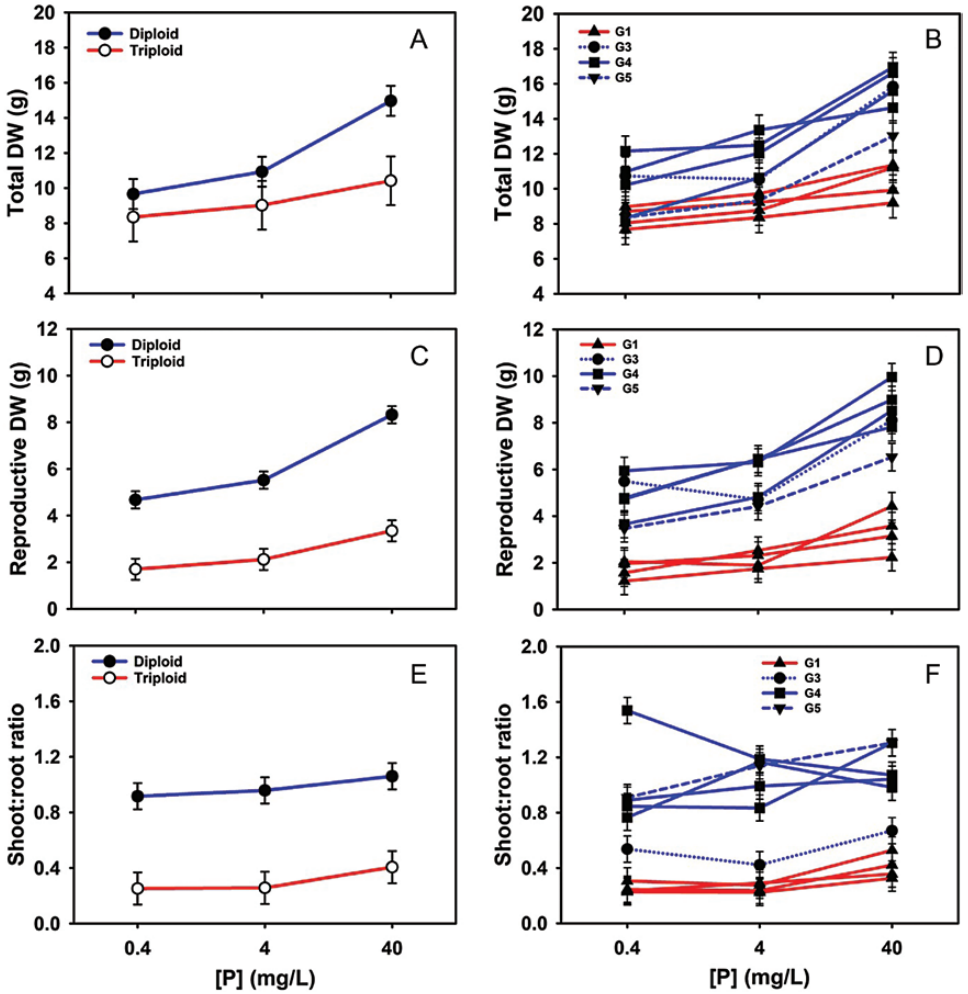

Figure 3. Reaction norms for *B. umbellatus* cytotypes (A, C, E) and populations (B, D, F). Reported are the means ± SE for growth and reproductive responses to three levels of phosphorus. Within cytotypes, we used four triploid populations of a single genotype and six diploid populations of three different genotypes (four G4, one G3 and one G5 population). DW = dry weight.

P diploid populations were nearly twice as variable (triploid CV = 0.16, diploid CV = 0.30). Increased variation among diploid populations may be due to the increased genetic diversity of diploid cytotypes in the introduced range. Although there is a sole triploid genotype currently known in North America, there are a number of diploid genotypes. The G4 *B. umbellatus* genotype is the most common diploid *B. umbellatus* genotype in North America (Gaskin *et al.* 2021) and all G4 populations (n = 4) had similar responses to nutrient enrichment (Figs 2 and 3). The lone G5 population (Forest Lake, MN, USA) had a similar response curve to the other diploid populations. An interesting finding is that the Springbrook Pond, IL population (G3) had traits of both diploid and triploid populations. For instance, G3 plants were more similar to triploid than other diploid plants in shoot:root ratio, but not other measured responses to high levels of P or N (Figs 2 and 3). G3 plants responded to increased N and P with higher reproductive output and total biomass production than triploid plants and were similar in that respect to other diploid populations. Thus, G3 plants had an intermediate response to increased nutrients when compared with triploid and other diploid populations. However, we acknowledge that because of our sample size for diploid genotypes (n = 1 population for each G3 and G5), we were unable to fully investigate variation at the genotype level.

Variation in tissue chemistry in response to N and P

Tissue chemistry varied by cytotype, level and type of nutrient availability, and in allocation to plant parts (Fig. 4; **see Supporting Information—Tables S2.1 and S2.5**). We generated two PCs ($PC1_N$, $PC2_N$) that explained 39 % and 32 %, respectively, of variation in tissue chemistry (combined 71 %) in response to different levels of N (**see supporting information Table S2.4**). $PC1_N$ was negatively correlated to tissue phosphorus (r = –0.61) and positively correlated with C:P ratio (r = 0.89) and N:P ratio (r = 0.90). $PC2_N$ was positively correlated with tissue nitrogen (r = 0.86) and negatively correlated with C:N ratio (r = –0.83). Similarly, two PCs ($PC1_P$, $PC2_P$) that explained 40 % and 29 % of variation in tissue chemistry (combined 69 %) were generated from phosphorus-response variables. $PC1_P$ was positively correlated with N (r = 0.60), N:P ratio (r = 0.85) and C:P ratio (r = 0.87), and negatively correlated with C:N ratio (r = –0.51). $PC2_P$ was negatively correlated with N (r = –0.77) and positively with C:N ratio (r = 0.79). Overall, the pattern for both sets of PCs was as follows: $PC1_N$ was most related to tissue phosphorus, $PC2_N$ and $PC2_P$ were most related to tissue nitrogen and $PC1_P$ was related to both tissue nitrogen and phosphorus.

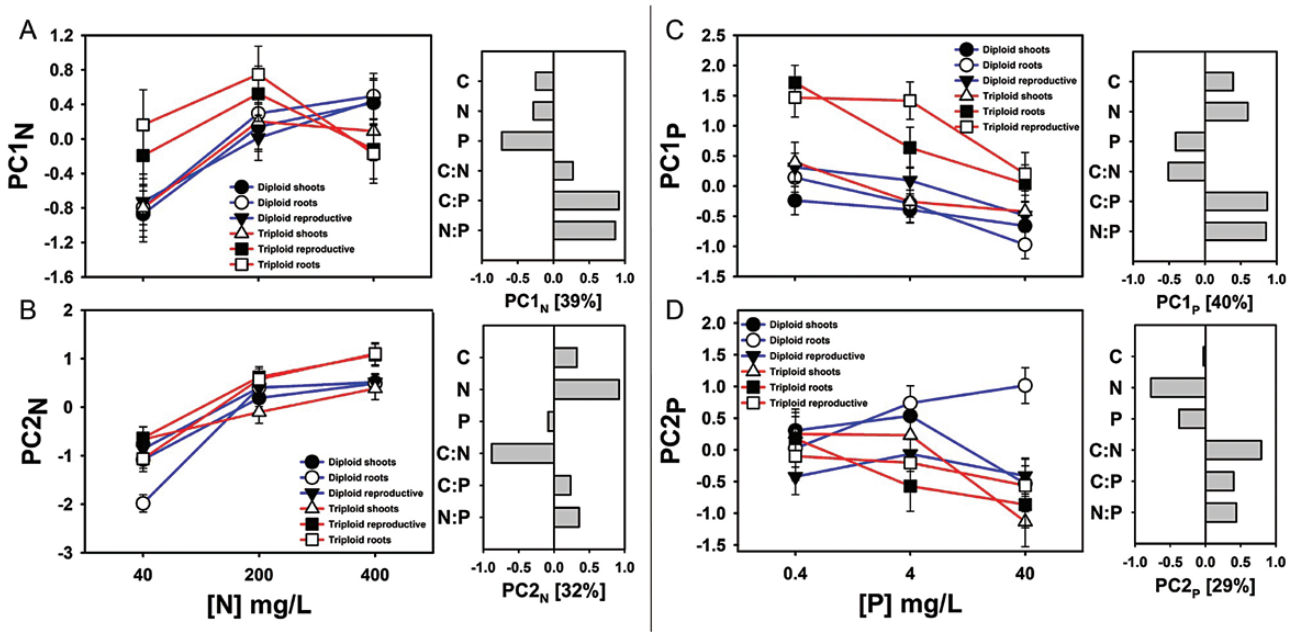

Figure 4. Mean ± SE tissue chemistry PCs for flowering rush diploid or triploid plant parts, grown in increasing levels of nitrogen (A, B) or phosphorus (C, D). The individual variable loadings for the PCs are shown in adjacent panels.

We found differences in tissue chemistry PCs between cytotypes, although many differences depended on nutrient level and plant part (Fig. 4; **see Supporting Information—Table S2.5**). Overall, tissue nitrogen ($PC2_N$, $PC1_P$) was higher in triploid plants than diploid plants across nitrogen ($PC2_N$; $P = 0.03$) or phosphorus ($PC1_P$; $P = <0.001$) treatments, and C:N ratio was consistently lower in diploid versus triploid plants (Fig. 4). Not surprisingly, N:P ratio ($PC1_N$, $PC1_P$) generally increased with nitrogen ($P = 0.003$) and decreased with phosphorus ($P < 0.001$) nutrients for both cytotypes, although based on means of untransformed data, N:P ratios in triploid plants declined 89 % between 200 and 400 mg L^{-1} N, whereas N:P in diploid plants increased 236 % over the same nutrient interval.

Although root N and P concentrations in both cytotypes increased with N, triploid plants typically had higher root concentrations overall (Fig. 4B). Triploid plants had 38 % higher P (0.64 % dry weight P) and 13 % higher N (3.67 % dry weight N) but 32 % lower C:N ratio overall in roots. In response to variation in P, roots in triploid plants had higher concentrations of N (46 % higher), P (52 % higher) and C (4 % higher), but a 34 % lower C:N ratio than diploid plants. In shoots, diploid plants had 5 % higher C and 24 % higher C:N ratio, but lower N and P (both 11 % lower). In response to P, reproductive tissue chemistry was similar between cytotypes for C and P but triploid plants had 18 % higher N and 15 % lower C:N than diploid plants.

Phenotypic plasticity in response to N and P

We detected differences in plasticity to nutrients, as a biomass response, between the triploid and diploid cytotypes (Fig. 5). Contrary to our prediction, diploid plants displayed two times the plasticity for total biomass (diploid $g = 5.35$, triploid $g = 2.77$) and more than double the plasticity in reproductive biomass (diploid $g = 4.87$; triploid $g = 1.84$) in response to N. Plasticity in shoot:root ratio was nearly three times higher for diploid plants ($g = 1.92$) in response to N than triploid plants ($g = 0.62$). In response to

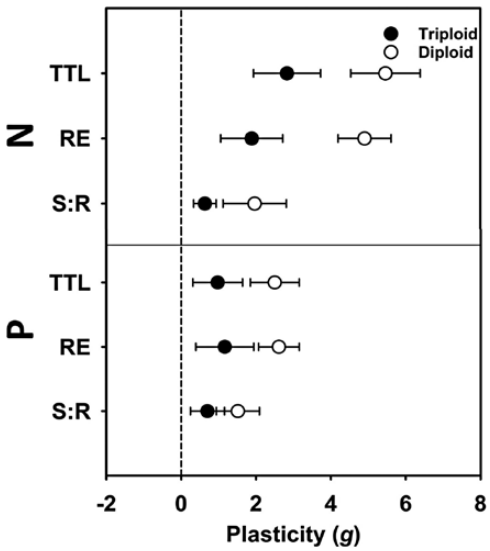

Figure 5. Mean ± 95 % CI for phenotypic plasticity (Hedge's *g*) of introduced *B. umbellatus* cytotypes in response to N or P enrichment. TTL = total biomass, RE = reproductive biomass, S:R = the ratio of shoot (leaf) to root biomass.

P, diploid plant plasticity was double that of triploid plants for total biomass (diploid $g = 1.85$, triploid $g = 0.97$), reproductive biomass (diploid $g = 2.61$, triploid $g = 1.16$) and shoot:root ratio (diploid $g = 1.51$, triploid $g = 0.70$).

Plasticity of tissue chemistry varied between plant parts but we observed no consistent cytotype differences (Fig. 6). Plasticity of chemistry N and C:N ratio PCs was highest for shoot ($PC2_N$; diploid $g = 2.86$, triploid $g = 1.99$) and root ($PC2_N$: diploid $g = 2.39$, triploid $g = 1.94$) tissues in response to N, and shoots ($PC1_P$: diploid $g = 1.08$, triploid $g = 1.14$; $PC2_P$: diploid $g = 1.12$, triploid $g = 1.30$) in response to P. Plasticity of the P PC ($PC1_N$) was highest

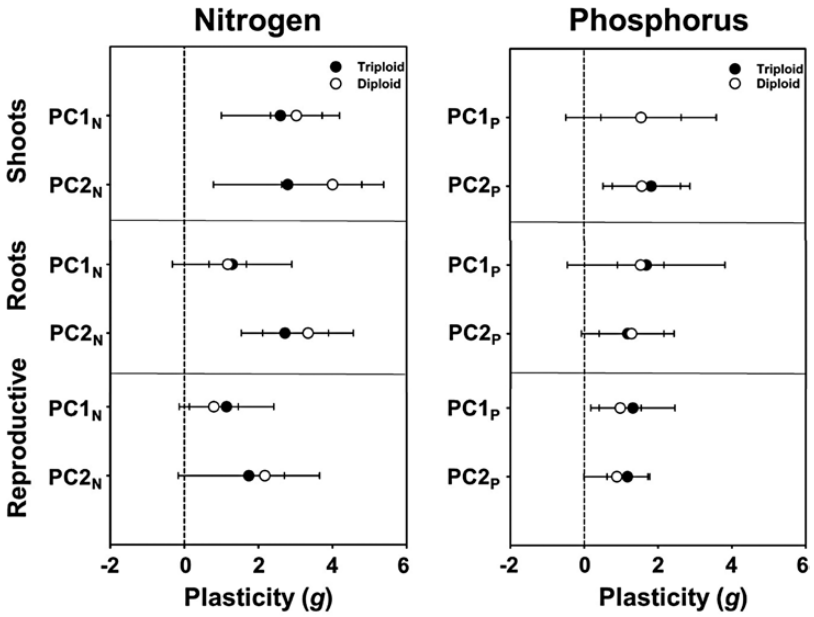

Figure 6. Plasticity (Hedge's *g*) in tissue chemistry PCs of introduced *B. umbellatus* cytotypes in response to P or N enrichment. Points are mean values and error bars are 95 % confidence intervals.

(diploid $g = 2.16$, triploid $g = 1.85$) in shoots in response to N. For both cytotypes, plasticity in shoot $PC1_N$ (P, C:P ratio, N:P ratio), shoot and root $PC2_N$ (N, C:N ratio), shoot and reproductive $PC2_P$ (N, C:N ratio) and reproductive $PC1_P$ (N, C:P ratio, N:P ratio) were greater than zero (Fig. 6).

Discussion

Our results demonstrate that different cytotypes present in *B. umbellatus* populations within North America display significant phenotypic variation in traits, and support a growing list of studies that have shown genetic-based variation in character traits among invader populations that correlate with invasion success (Eckert *et al.* 2000; Lavergne and Molofsky 2007). For instance, the weed *Phyla canescens* has been introduced into several areas outside its native range, but the traits thought to be most important for its success vary between invaded areas, with high seed output in Australia but vigorous clonal/vegetative growth in France (Xu *et al.* 2010). In the current study, and contrary to our prediction, increased ploidy in *B. umbellatus* was not associated with greater plasticity to increased nutrients and, although there was considerable variation among diploid populations, diploid populations consistently outperformed triploid populations across two nutrient gradients. Although less plastic for measured traits in response to variation in nutrient levels, triploid *B. umbellatus* has invaded aquatic systems across the USA and Canada, creating expansive monocultures and causing negative economic (e.g. water delivery), recreational (Boutwell 1990), ecological (Parkinson *et al.* 2010) and human health (e.g. cercarial dermatitis; Parkinson *et al.* 2010) impacts. In triploid plants, all traits except below-ground biomass plateaued in our moderately low N treatment (40 mg L^{-1}). At higher N concentrations, the only significant response observed in triploid plants occurred as an increase in root biomass and related lower shoot:root ratio. This is surprising because allocation of resources to underground biomass should typically occur when nutrients are scarce (Poorter *et al.* 2012). However, below-ground biomass and competitive ability can be positively correlated (Aerts *et al.* 1991; Cahill *et al.* 2000), so an increase in below-ground biomass may be adaptive and thus important in explaining invasion success and negative impacts observed in recipient communities. Furthermore, as below-ground competition is expected to be highest in nutrient-limited environments (Tilman 1989; Cahill 1999), triploid *B. umbellatus* plants with low shoot:root ratios may be better adapted than diploid plants to compete for soil resources in low nutrient environments. Studies that address the question of whether polyploids of invasive species perform better in low-resource environments than their diploid relatives are relatively rare, but polyploidy in general may lead to increased performance in extreme conditions, such as at high altitudes or xeric environments (del Pozo and Ramirez-Parra 2015), and increase tolerance to nutrient or water stress (Deng *et al.* 2012; Allario *et al.* 2013; but see discussion of the Large Genome Constraint Hypothesis below for a contrasting viewpoint).

Increased biomass, reproductive, or chemical plasticity in response to nutrient gradients has been shown in other systems to be indicative of invasiveness (Burns 2004), or resistance to invasion (Peacor *et al.* 2006), and is typically associated with higher ploidy (Levin 1983; Leitch and Leitch 2008; te Beest *et al.* 2012) and smaller genome size (e.g. Knight *et al.* 2005; Hessen *et al.* 2010). Although the Large Genome Constraint Hypothesis posits that the metabolic costs of replicating excess DNA may make polyploidy maladaptive for an organism, *especially* in extreme environments (Knight *et al.* 2005), costs of polyploidy are likely outweighed by the benefits, which may include positive effects of gene redundancy on masking deleterious alleles and adaptive functional divergence of the replicate genes (te Beest *et al.* 2012). In the current study, diploid plants displayed higher plasticity of measured traits to a nutrient gradient. Greater biomass and reproductive plasticity to nutrients may partially explain why their distributional range in North America is broader than the range for triploid *B. umbellatus* (widely distributed across north-eastern and Midwestern states as compared to the Pacific Northwest). With one exception, diploid and triploid plants are not known to co-occur in the USA, but this study raises the

question of potential outcomes of co-invasion by cytotypes in the same area and whether diploid plants will outperform triploid plants in field locations, ultimately displacing triploid plants and becoming the dominant cytotype. Investigations into competitive interactions between genotypes of *B. umbellatus*, particularly along resource gradients, may lead to further predictions on which habitats are at risk for invasion. Though it has not been reported, a possible explanation for the success of both cytotypes in the USA, and that they do not frequently co-occur, is that they occupy habitats with differing nutrient availability.

The implications of variation in growth and biomass allocation plasticity between genotypes or cytotypes extend beyond nutrient uptake and possible competitive interactions with other plant species to potentially impact trophic interactions. Performance of herbivores is influenced by host plant abundance (biomass available for food), nutritional status (N, C:N) (Scriber and Slansky 1981; Awmack and Leather 2002; Wilson *et al.* 2007; Harms and Cronin 2019), allocation patterns of nutrients within plant parts (Eatough Jones *et al.* 2008; Hunter 2016), defensive chemistry (Throop and Lerdau 2004; Nybakken *et al.* 2018) and interactions between plants and higher tropic levels (i.e. mediated by plant volatiles; Turlings and Erb 2018), all of which are affected by plant phenotypic response to nutrients. Greater growth or tissue chemistry plasticity in response to herbivory may increase plant performance through tolerance (i.e. reduced effects of herbivory on plant fitness) or resistance (e.g. reduced performance of the herbivore through interaction with plant chemical defences) (Yoshizuka and Roach 2011). Variation in induced resistance (the defensive reaction by a plant to damage from an herbivore or pathogen) may be particularly important for invasive plants because the magnitude and type of induction can depend on the identity, type or origin of herbivore encountered (Liu *et al.* 2018; Zhang *et al.* 2018). Herbivores or pathogens introduced for management (i.e. biological control) will be particularly susceptible to variation in plant plasticity because biological control agents are restricted to a single host species.

Nutrient availability via the bottom-up cascade from environment to host plant to herbivore can ultimately determine success of biological control (Room and Thomas 1985; Room *et al.* 1989; Wheeler and Center 1996; Center and Dray 2010; Hunter 2016; Uyi *et al.* 2016). For example, fertilizer application was needed to increase tissue N in giant salvinia (*Salvinia molesta*) to levels that supported egg production and larval development of the biological control agent, *Cyrtobagous salviniae* (Room and Thomas 1985). Similarly, increasing N availability in nutrient solution led to higher tissue N and lower tissue C:N ratio in *A. philoxeroides*, accelerated larval development and larger adults of the biological control agent *Agasicles hygrophila* (Harms and Cronin 2019). Thus, an understanding of relationships between environment and plant nutrients is important when testing efficacy of potential biological control agents or evaluating establishment and control failures at field sites (Room and Thomas 1985; Wheeler and Center 1997). In general, herbivore performance is positively correlated with tissue N and negatively with tissue C:N ratio (Awmack and Leather 2002), a pattern which has also been found for a generalist herbivore on *B. umbellatus* (Harms and Walter 2021). In the current study, N and C:N ratio varied between cytotypes in several instances; C:N ratio was lower in triploid plants overall in response to N and P enrichment. Thus, triploid *B. umbellatus* plants with lower C:N ratio may be more susceptible to herbivory than diploid plants and may therefore be more affected by biological control, if introduced. In support of this argument, Harms *et al.* (2020) found that triploid *B. umbellatus* plants were more susceptible than diploid plants to infection by generalist pathogens, at least under controlled experimental conditions. Additionally, Harms and Walter (2021) found decreased N, and increased C:N ratio, of diploid versus triploid *B. umbellatus* plants likely contributed to reduced herbivore performance in laboratory assays. Mindful of potential differences in plant growth and phenotype between greenhouse and field conditions (e.g. Poorter *et al.* 2016), we suggest that additional studies be undertaken to better understand nutrient conditions in field infestations of diploid and triploid *B. umbellatus*.

For genetically diverse invaders, genetic identity and associated phenotypic responses to environmental conditions can be important for understanding ecological impacts resulting from establishment and spread (Lee 2002; terHorst and Lau 2015) or for making management decisions to curb future impacts (Gaskin *et al.* 2011). In the wetland invader, *B. umbellatus*, we found differences in phenotypic plasticity related to enrichment with N or P. In contrast to a previous report (Hroudová and Zákravský 1993), we found that triploid *B. umbellatus* plants underperformed relative to diploid plants in all biomass categories except roots and were significantly less plastic in response to available nutrients. Whether disproportionate allocation of biomass to roots is advantageous for triploid plants and thus contributing to invasion success in the USA is unknown. However, the clear differences between cytotypes in biomass allocation and chemistry suggest that cytotypes can occupy different habitats and may require unique management approaches.

Supporting Information

The following additional information is available in the online version of this article—

Table S1.1. Populations used in the current study to detect differences in *Butomus umbellatus* cytotype biomass and chemistry responses to gradients of N or P.

Table S1.2. Nutrient solutions used in the current experiment. Approximate concentrations (mg L^{-1}) of macronutrients given. Micronutrients followed the standard Hoagland's nutrient recipe (Hoagland and Arnon 1950).

Table S2.1. Least squared means and standard errors for diploid and triploid flowering rush plant biomass and tissue chemistry variables in relation to nutrient solution (both biomass and tissue chemistry) and plant part (tissue chemistry only). Means were calculated using mixed-effects models (GLM). Fixed effects for the biomass (total dry weight, shoot:root ratio, reproductive dry weight) models were cytotype (two levels: diploid, triploid), nutrient level (four levels of N, three levels of P; N and P treatments were tested separately), the cytotype * nutrient level interaction and initial fresh weight of propagules. We did not have replication at the level of genotypes so examined variation in cytotype only. Thus, we assigned population as a random effect in all models and initial (at planting) fresh weight of plants was included as a covariate. For tissue chemistry (%C, %N, %P, C:N ratio, C:P ratio, N:P ratio), we used similar mixed models as above. Fixed effects were cytotype (two levels: diploid, triploid), nutrient level (four levels of N, three levels of P; N and P treatments were tested separately), plant part (shoot, root, reproductive parts) and all two- and three-way interactions. As above, population was a random effect in all models and initial (at planting) fresh weight of plants was included as a covariate.

Table S2.2. Test statistics from mixed-effects models for growth and reproductive biomass responses of diploid and triploid *Butomus umbellatus* plants to N and P. Models are described above.

Table S2.3. Coefficients of variation (CV), calculated for diploid and triploid cytotype biomass variables, using population means.

Table S2.4. Individual variable loadings for each *Butomus umbellatus* tissue chemistry principal component (PC). PCs with eigenvalues > 1 were retained for use in mixed models.

Table S2.5. Mixed model test statistics for tissue chemistry principal components (PCs) of diploid and triploid *Butomus umbellatus* plant response to N and P. Tissue chemistry PCs were tested separately with mixed-effects models, using cytotype, plant part, nutrient level and all two- and three-way interactions as fixed effects, *B. umbellatus* population as a random effect and initial fresh weight of flowering rush propagules as a covariate. Degrees of freedom for all mixed models were calculated following Kenward and Roger (1997).

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Conflict of Interest

None declared.

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Contributions by the Authors

N.E.H. performed the research, secured the funding, collected data and conducted data analysis. N.E.H., J.T.C. and J.F.G. interpreted the data and wrote the manuscript.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request or at https://doi.org/10.6084/m9.figshare.14935725.

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