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Brenda J. Grewell

Caryn J. Futrell

Maria Iannucci

Rebecca E. Drenovsky

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RESEARCH ARTICLE

Resprouting potential of rhizome fragments from invasive macrophyte reveals superior colonization ability of the diploid congener

Brenda J. Grewell[1,*], Caryn J. Futrell[1], Maria T. Iannucci[1,2] and Rebecca E. Drenovsky[2]

[1]USDA-Agricultural Research Service, Invasive Species and Pollinator Health Research Unit, Department of Plant Sciences MS-4, University of California, Davis, Davis, CA 95616, USA, [2]Biology Department, John Carroll University, University Heights, OH 44118-4581, USA

*Corresponding author's e-mail address: bjgrewell@ucdavis.edu

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Abstract

Non-native aquatic *Ludwigia* species from a polyploid complex are among the world's most problematic invasive plants. These emergent, floating-leaved species respond to disturbance through fragmentation of shoots and/or rhizomes, spreading rapidly by hydrochorous dispersal and posing challenges for invasive plant management. While recruitment of clonal aquatic plant species from shoot fragmentation is well documented, regeneration from rhizome bud banks, although common, often is overlooked. It is further unclear how interactions among ploidy and resource availability influence regeneration success of rhizome fragments. We conducted a full factorial experiment in aquatic mesocosms to compare trait responses of *Ludwigia* congeners differing in ploidy (diploid, decaploid) grown from clonal rhizome fragments under contrasting soil nutrient availability (low, high). Similar to previous work with shoot fragments, the diploid congener had a higher relative growth rate and produced more biomass than the decaploid during this establishment stage of growth. High growth rates and biomass production were associated with greater rhizome N and P and reduced investment in below-ground structures. Comparing these results to previous shoot fragment studies with *Ludwigia*, rhizome fragments appear to have much greater growth potential, suggesting that management strategies should minimize disturbance to prevent fragmentation and dispersal of below-ground structures. Furthermore, rapid response to newly colonizing diploid invaders will be essential to minimizing spread, and reductions in nutrient loads to aquatic environments may be more effective towards controlling establishment of the diploid congener than the decaploid.

Keywords: Aquatic plants; bud banks; clonality; invasion ecology; polyploidy.

Introduction

Successful colonization of an invasive plant species is thought to result from biological traits enabling a plant to tolerate stresses, utilize limiting resources and colonize vacant niches in the new environment (Elton 1958). Identifying traits facilitating colonization and spread following plant introductions to novel environments continues to be a primary challenge in invasion ecology (Pyšek *et al.* 2009, 2015) that has significance for developing effective containment strategies prior to rapid population growth (Radosevich *et al.* 2003). These functional traits may include morphological, chemical, physiological and phenological attributes that interact with surrounding biotic and abiotic factors, with those traits displaying quantifiable

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responses to novel and changing environments being most relevant to formulating management strategies (Drenovsky *et al.* 2012).

Clonality is a plant trait that often is linked to invasiveness (Thompson *et al.* 1995; Pyšek 1997; Pyšek and Richardson 2007). However, many clonal plant species have not become invasive, and details on clonal traits leading to greater invasiveness merit further study (Keser *et al.* 2014). Environmental disturbances play a major role in producing clonal fragments. Under changing environmental conditions, plant regeneration from fragments is influenced by life history traits, including clonal propagation and regrowth capacity, combined with species-specific mechanistic traits supporting survival and growth (Klimeš *et al.* 1993; Li *et al.* 2013). Understanding these traits in invasive clonal plants is crucial, as their initial colonization, establishment and spread generally are facilitated by disturbance within plant communities.

Riverine wetland ecosystems are subject to regular disturbance events, such as flooding and bank erosion, that create gaps in riparian and aquatic vegetation and favour rapid establishment of herbaceous plants (Pyšek and Prach 1993; Barrat-Segretain *et al.* 1998; Santamaría 2002). These disturbances can result in rapid hydrochorous dispersal of asexual clonal shoot or rhizome fragments supporting bud banks. In fact, plant fragmentation following disturbance typically results in sprouting of previously dormant buds, realizing both new bud banks and seed banks (Klimešová and Klimeš 2007). Plant species that rapidly colonize areas following hydrologic disturbance typically have at least one fragment type with high regenerative capacity supporting their high colonization potential (Barrat-Segretain and Bornette 2000). Because spread of plants from asexual fragments often represents the first stage of a plant invasion (Bímová *et al.* 2003), there is growing recognition that clonal and bud bank traits should be given more prominence in trait-based analyses of the colonization, niche breadth and coexistence of plant species (Klimešová *et al.* 2016).

Cytological traits, including chromosome numbers and ploidy levels, also can play a role in invasion success of plant species, though they are rarely considered (Suda *et al.* 2015; Myerson *et al.* 2016). Polyploidization is a major force in plant evolution that can increase potential for rapid evolution of new or improved traits (Soltis and Soltis 2000; Soltis *et al.* 2009). Polyploidy can affect functional traits, including increased plant size (Levin 2002), stress tolerance (Schlaepfer *et al.* 2010) and phenotypic plasticity (Pandit *et al.* 2011; Hahn *et al.* 2012; te Beest *et al.* 2012), and collectively supports colonization and invasiveness (Weiss-Schneeweiss *et al.* 2013). However, some important exceptions comparing diploid versus polyploid congeners suggest polyploidy is not always an advantage (Buggs and Pannell 2007; Münzbergová 2007a,b; Černá and Münzbergová 2015; Grewell *et al.* 2016), and some diploid plant species are notably successful invaders in their naturalized ranges (i.e. *Ludwigia peploides*, *Hedera helix*). These diploid advantages may be rooted in functional traits, such as greater gas exchange rates in diploids versus polyploids, supporting carbon gain and, thus, growth (te Beest *et al.* 2012). Experimental research comparing ecological response of diploid and polyploid congeners to contrasting environments is rare (but see Wei *et al.* 2019), and compelling studies are needed (Soltis *et al.* 2010).

The growth of invasive wetland plants can be highly affected by local environmental conditions (Ehrenfeld 2010). Understanding how invasive plant functional traits, such as regeneration from rhizome fragments and ploidy, interact with contrasting environmental conditions can provide a foundation for early management intervention. Macrophytes rooted in sediment are able to uptake dissolved nutrients (e.g. ammonium and nitrate nitrogen; phosphorous) from both the water column and from sediments, though sediment-derived nutrients are the primary source affecting their growth (Barko and Smart 1983, 1986; McFarland *et al.* 1992; Carr and Chambers 1998). Hydrologic disturbances (e.g. flood–drought cycles, flow regulation, sediment deposition) influence key biogeochemical processes in river sediments (Boulton *et al.* 1998) and can increase sediment nutrient availability to wetland plants and facilitate wetland invasion (Olde Venterink *et al.* 2002; Zedler and Kercher 2010). Although polyploids are predicted to have wider niche breadths than their diploid ancestors, empirical comparisons of plant traits and ecological responses of diploid and polyploid congeners to contrasting environments such as soil nutrient availability are rare (Soltis *et al.* 2010), and thus further study is needed to support trait-based management approaches.

Invasive *Ludwigia* taxa from a polyploidy complex occur across a broad range of environmental conditions with contrasting sediment nutrient availability. Previous experimental studies of these taxa have focused on regeneration from shoot fragmentation (Hussner 2009, 2010; Thouvenot *et al.* 2013a; Glover *et al.* 2015; Grewell *et al.* 2016) and seeds (Ruaux *et al.* 2009; Gillard *et al.* 2017). However, bank erosion and other disturbances also produce and mobilize fragments of *Ludwigia* rhizomes. Given the high potential for resprouting from stored carbohydrate reserves in woody rhizome tissue (Klimešová *et al.* 2018), further investigation is necessary for improved mechanistic understanding of the invasiveness of these taxa. To our knowledge, there has been no previous evaluation of the potential role of rhizome fragments and their bud banks in colonization and spread of invasive *Ludwigia* species relative to critical resource gradients.

In a previous study of two invasive, congeneric *Ludwigia* species differing in ploidy level and generated from clonal shoot fragments, Grewell *et al.* (2016) found that diploid *L. peploides* subsp. *montevidensis* outperformed a polyploid congener when colonizing environments with contrasting light and nutrient availability. While the congeners had comparable performance with low nutrient availability, the diploid exhibited a superior growth rate and biomass accumulation than the polyploid under high nutrient availability, irrespective of light environment (Grewell *et al.* 2016). Here, we extend this work, focusing on a previously overlooked reproductive mode examining regeneration from partially to shallow-buried woody perennial rhizome fragments in early spring, at the time this process occurs under field conditions. We conducted a mesocosm experiment assessing growth, ecophysiological and other trait responses of polyploid *L. hexapetala* and its diploid progenitor *L. p.* subsp. *montevidensis* during establishment from rhizome fragments in contrasting sediment nutrient environments. We predicted that similar to work with shoot fragments, diploid *L. p.* subsp. *montevidensis* would effectively use available soil nutrients, and surpass the growth and biomass production of decaploid *L. hexapetala* during the initial stage of establishment.

Methods

Focal taxa

Two *Ludwigia* congeners from the polyploid *Ludwigia* sect. *Jussiaea* (Onagraceae) originating in South America (Wagner *et al.* 2007; Hoch *et al.* 2015) were the focus of this study: *L. peploides* subsp. *montevidensis* (diploid ($2n = 16$); creeping water primrose), and

L. hexapetala (decaploid (2*n* = 80); Uruguayan primrose-willow). Invasions by these species have been reported in California and the north-western USA (Okada *et al.* 2009), portions of the south-eastern USA (Hoch and Grewell 2012), across Europe (Thouvenot *et al.* 2013b), and into Australia and New Zealand. Both taxa are emergent floating-leaved macrophytes with a creeping growth habit, forming mats on the water surface (Rejmánková 1992). The two amphibious congeners are similar in appearance, with buoyant shoots bearing floating leaves; frequent rooting nodes along their stems; aerial, aerenchymatous roots for root aeration in saturated soils; 5(6)-merous yellow flowers; and woody capsules with seeds embedded in the fruit wall (Hoch and Grewell 2012).

Although long-established and invasive perennial populations of both taxa are found in shallow water at the edge of rivers and lakes under variable light, nutrient and flow conditions (Hussner 2010; Lambert *et al.* 2010; Thouvenot *et al.* 2013a), the niche breadth of mature *L. hexapetala* appears to be broader than that of *L. p.* subsp. *montevidensis*. *Ludwigia hexapetala* grows at deeper water depths than *L. p.* subsp. *montevidensis*, and under a broader range of soil moistures near the edges of rivers, lakes and ponds (B. J. Grewell, pers. obs.). This observation of well-established infestations also holds in the invaded European range, where standing biomass and production of *L. p.* subsp. *montevidensis* decrease with drying ecological conditions (Hussner 2009; Haury *et al.* 2014). In contrast, with hydrologic drawdown *L. hexapetala* is phenotypically plastic and can persist as a tall emergent macrophyte in adjacent terrestrial zones (Haury *et al.* 2014). Under field conditions, *L. hexapetala* can grow taller, more erect and with less branching than is typically observed in *L. p.* subsp. *montevidensis*. While many observations have been made of dense infestations, little has been reported on growth characteristics and niche breath of establishing asexual fragments during the colonization phase of invasions. Additionally, the two species differ in seasonal growth patterns. In California, *L. p.* subsp. *montevidensis* has a shorter growing season, producing flowers and seedpods beginning in late May and then slowing growth by mid- to late summer. *Ludwigia hexapetala* also begins to flower in May, but growth continues throughout the summer and the fall. Floating mats of *L. hexapetala* die back with frost or wash out with high winter flows, whereas live green biomass persists under water through winter. The taxa perennate via bud sprouting from dormant meristem tissues of persistent, lower woody stems and from perennial bud banks on rhizomes. Bud banks of clonal species include buds on intact plants and on transportable plant fragments (per Klimešová and Klimeš 2007).

Multiple regenerative modes are a notorious trait of aquatic and wetland plants, attributed to their architectural diversity (Willby *et al.* 2000), and shifts between various modes are a common plastic response to disturbances and other dynamic environmental conditions in aquatic ecosystems. These amphibious *Ludwigia* taxa are no exception. *Ludwigia p.* subsp. *montevidensis* and *L. hexapetala* have multiple reproductive modes: sexual reproduction from seed banks, clonal regeneration and spread from perennial bud banks, and/or regeneration from asexual shoot or rhizome fragments generated by hydrologic and anthropogenic disturbances. Shoot and rhizome fragments have buds at periodic nodes that sprout adventitious roots or shoots. Adventitious roots can be floating, or readily grow into damp sediment. Following bud bank classification by Klimešová and Klimeš (2007), *Ludwigia* taxa at our study sites produce hypogeogenous rhizomes below-ground and long-lived woody, epigeogenous rhizomes along river banks, with older parts of the rhizome buried in soil and younger parts exposed at the soil surface. We have observed allofragmentation and dispersal of both rhizome types following high river flow disturbance events in the study area. Molecular studies indicate little genetic variation within populations of *L. hexapetala*, suggesting reproduction is predominantly clonal and via fragmentation with hydrochorous spread of asexual fragments (Okada *et al.* 2009).

Experimental design

We evaluated growth responses of plants generated from bud banks of rhizome fragments in response to soil nutrient availability in both congeners. Due to limitations of mesocosm size and the above-ground growth expected from the rhizomes, the number of replicates was limited to six per ploidy level by nutrient treatment combination.

In early spring 2014, just prior to sprouting from bud banks under field conditions, rhizome fragments of *L. p.* subsp. *montevidensis* were collected along the Napa River tributary upstream of Lake Hennessey (Sage Creek, 38°29′23.7948″, –122°20′52.9944″). At this time, rhizome fragments of *L. hexapetala* were acquired in comparable habitat along the Laguna de Santa Rosa tributary channel upstream of its confluence with the mainstem Russian River below Wohler Pool (38°21′8.366″, –122°44′36.41″). Replicate sediment cores (4.7 cm diameter × 10 cm depth; *n* = 6) were collected at both population donor sites, and at six additional population sites invaded by *Ludwgia* spp. in the watersheds to assess sediment nutrient concentrations under field conditions and to establish a basis for experimental nutrient treatments **[see Supporting Information—Tables S1 and S2]**.

Epigeogenous rhizome fragments were excavated from sediment with shovels, transported in insulated coolers to the laboratory and refrigerated overnight prior to planting. Rhizomes were trimmed to 40 cm lengths that each had seven visible bud nodes with evidence of prior season rooting. Initial fresh weights of replicates varied between taxa but were very similar within taxa (decaploid rhizome fragments, 40.8 ± 2.2 g; diploid rhizome fragments, 13.6 ± 0.8 g).

Twelve rhizomes from each species were randomly assigned to one of two soil nutrient treatments. Target nutrient levels in experimental treatments were determined based on the range of nutrients found at sites naturalized by these *Ludwigia* taxa in the source watersheds **[see Supporting Information—Table S2]**. The low nutrient soil contained a 90:10 ratio of sterilized sand to potting soil (Scotts Miracle-Gro, Marysville, OH, USA) with 4.0 ppm carbon (C), 0.37 ppm nitrogen (N) and 11 ppb extractable phosphorus (P) in the prepared soil mix, comparable to invaded sites with low available sediment nutrients on the mainstem Russian River. The high nutrient soil contained a 90:10 ratio of the same potting mix and sand which we supplemented with potassium nitrate (0.12 g kg^{-1}), potassium sulfate (0.082 g kg^{-1}), dolomite lime (1.95 g kg^{-1}), gypsum (0.50 g kg^{-1}) and superphosphate (0.88 g kg^{-1}) to achieve 26.0 ppm C, 1.9 ppm N and 196 ppb P in the prepared high nutrient soil mix, comparable to the most eutrophic sites invaded by *Ludwigia* spp. in the region **[see Supporting Information—Table S2]**. Experimental soil mixes were analysed for initial soil nutrient conditions (*n* = 6 per treatment) to confirm desired composition. Soils sampled from population sites and experimental soil mixes were analysed for total N by micro-Dumas combustion on a Perkin Elmer 2400 CHNS/O analyser (Perkin Elmer, Waltham, MA, USA), and for Olsen's extractable P using a sodium bicarbonate solution (Olsen *et al.* 1954) and analysis by spectrophotometry using the molybdenum-ascorbic acid method (Murphey and Riley 1962).

Following treatment assignment, rhizomes were individually planted (n = 24) in 52-L rectangular plastic tubs (63.2 cm × 40.6 cm × 21.6 cm; Quantum 2516-8, Quantum Storage Systems, Miami, FL, USA). The rhizomes were partially buried just below the sediment surface as observed under field conditions, and they were held in place with two wire anchor hoops to avoid loss during initial inundation. An additional ten, 40-cm rhizome fragments of each congener were evaluated for initial mass and total non-structural carbohydrate (TNC) concentrations using a spectrophotometric assay for reducing sugars (Nelson 1944) following enzyme digestion (Swank *et al.* 1982). These initial results were used to evaluate dynamics of carbon (C) storage reserves at the end of the experiment.

Rhizome fragment plantings were conditioned in a greenhouse in moist soil and under ambient spring light conditions for 3 weeks until sprouting from bud banks had occurred. On 30 April 2014 when each treatment had new emergent shoots that were ~10 cm long, the tubs were moved from the greenhouse and arranged in a randomized block design (2 congeners × 2 nutrient treatments × 6 replicates) on brick stands within four large outdoor, circular fibreglass aquatic mesocosms (9500 L volume; 0.9 m depth × 3.7 m diameter). Water levels were slowly raised in the mesocosms to full levels by morning of 1 May 2014 so that the sediment surfaces in the tubs were submersed 30 cm below the water surface. Mesocosms experienced natural light and temperature fluctuations. Water levels were maintained at 90 cm depth in mesocosms to maintain a shallow inundation depth of 30 cm above the rooting medium. Water was continuously circulated within each mesocosm to provide oxygen and minimize algal growth.

Rhizome fragment response measurements

After more than 4 weeks of growth, photosynthetic rate (A_{sat}) and stomatal conductance (g_s) were measured using a Li-Cor 6400 portable infrared CO_2 gas analyser (Li-Cor Biosciences, Lincoln, NE, USA). Due to the size of the floating plant mats and the fragility of floating shoots, we did not assess replicate plants in the middle of the mesocosms, in order to avoid disturbance and allofragmentation prior to harvest and final growth measurements. Therefore, gas exchange was only recorded for four replicates from each species and nutrient treatment combination. For each measurement, three subsamples were taken at 10-s intervals once the chamber reached equilibrium. Subsample measurements were averaged before analysis. The Li-Cor 6400 was used with a closed top chamber with a red-blue light source. Chamber conditions were set at 1000 µmol m^{-2} s^{-1} photon flux density, 400 µmol s^{-1} flow rate and 400 µmol mol^{-1} CO_2 concentration; humidity was maintained between 40 and 50 % by increasing or decreasing air flow through the desiccant chamber. The marked leaf sections measured in the cuvette were harvested to determine leaf area, and measurements were re-calculated based on actual leaf area, as appropriate.

After 5 weeks of growth in the mesocosms, the experimental plants were harvested. At harvest, the longest shoot was identified as the main shoot. Total shoot length, and numbers of primary and secondary branches along the shoot were measured on the main shoot. Below-ground biomass, consisting of roots and rhizomes, was washed to remove soil, sieved through a No. 20 mesh sieve to obtain fine roots. All biomass was refrigerated until it could be separated by organ. Below-ground biomass was divided into rhizomes and sediment roots. Above-ground biomass was divided into floating roots, white roots and floral parts, whereas stems and leaves remained together, representing shoot biomass. All samples were processed within 7 days of harvest. Samples were dried at 70 °C for 48 h and then weighed. Relative growth rate (RGR) was calculated ($RGR = (\ln(w_2) - \ln(w_1))/(t_2 - t_1)$), in which w_1 and w_2 are biomass at initial time t_1 and final time t_2, respectively. Leaf tissue was analysed for total N concentration by micro-Dumas combustion on a Costech CN analyser and for total P on a Perkin Elmer ICP-OES following dry ashing and acid dissolution. Post-harvest rhizome tissue samples were analysed for TNC using a spectrophotometric assay for reducing sugars following enzyme digestion using the method previously described.

Statistical analyses

Response variables were grouped by five functional trait types: biomass and allocation, plant architecture, photosynthetic traits, leaf tissue nutrients and carbon storage reserves. All variables were then evaluated for compliance with parametric model assumptions. Data sets for each trait group were then analysed using multivariate analysis of variance (MANOVA) in a general linear model (GLM) and evaluated for significance using the Wilks' lambda test statistic. In all models, ploidy level (diploid, decaploid), soil nutrient level (low, high) and block (reflecting mesocosm pool) were included as main effects, and the interaction of ploidy * soil nutrient level was included. Overall MANOVAs for all trait groups were significant **[see Supporting Information—Table S3]**, so we proceeded with and report protected univariate analysis of variance (ANOVA) models. The Shapiro–Wilks test for normality and the Levene's test for equal variance were used to test univariate model assumptions. When data for a variable showed unequal variance, a weighted ANOVA model was run using the inverse of the variance (Kutner *et al.* 2004). No data transformations were made. *Post hoc* tests on least square means using Tukey–Kramer multiple comparisons tests were performed. The above-mentioned statistics were run using SAS v9.4. To investigate relationships between ecophysiological response traits and treatment groups, we analysed the data by principal component analysis using CANOCO v.5.

Results

Growth, biomass and allocation

Total biomass increased with greater sediment nutrient availability for both taxa; however, the diploid *L. p.* subsp. *montevidensis* displayed a higher RGR (ploidy * nutrient interaction: $F_{1,17} = 12.12$, $P = 0.0029$; Fig. 1A) and produced more total biomass in response to nutrient availability than decaploid *L. hexapetala* (ploidy * nutrient interaction: $F_{1,17} = 41.00$, $P < 0.0001$; Fig. 1B). Based on the initial ANOVA, ploidy ($F_{1,17} = 7.77$, $P = 0.0126$) and sediment nutrient availability ($F_{1,17} = 288.53$, $P < 0.0001$; Fig. 1C) were significant factors influencing below-ground biomass allocation of plants regenerated from rhizome fragments. However, after accounting for multiple comparisons, differences were due solely to sediment nutrient availability. Rhizome biomass increased in both species with higher sediment nutrient availability (ANOVA: ploidy $F_{1,17} = 21.8$, $P = 0.0002$; nutrients $F_{1,17} = 8.18$, $P = 0.0109$; ploidy * nutrient interaction and block, not significant), but the proportion of total biomass allocated to below-ground growth was low, as both taxa shifted their allocation above-ground.

Plant architecture

Polyploid *L. hexapetala* regenerated from rhizome fragments produced longer primary shoots than its diploid congener (ploidy: $F_{1,17} = 4.20$, $P = 0.056$; Fig. 1D). Both species strongly increased

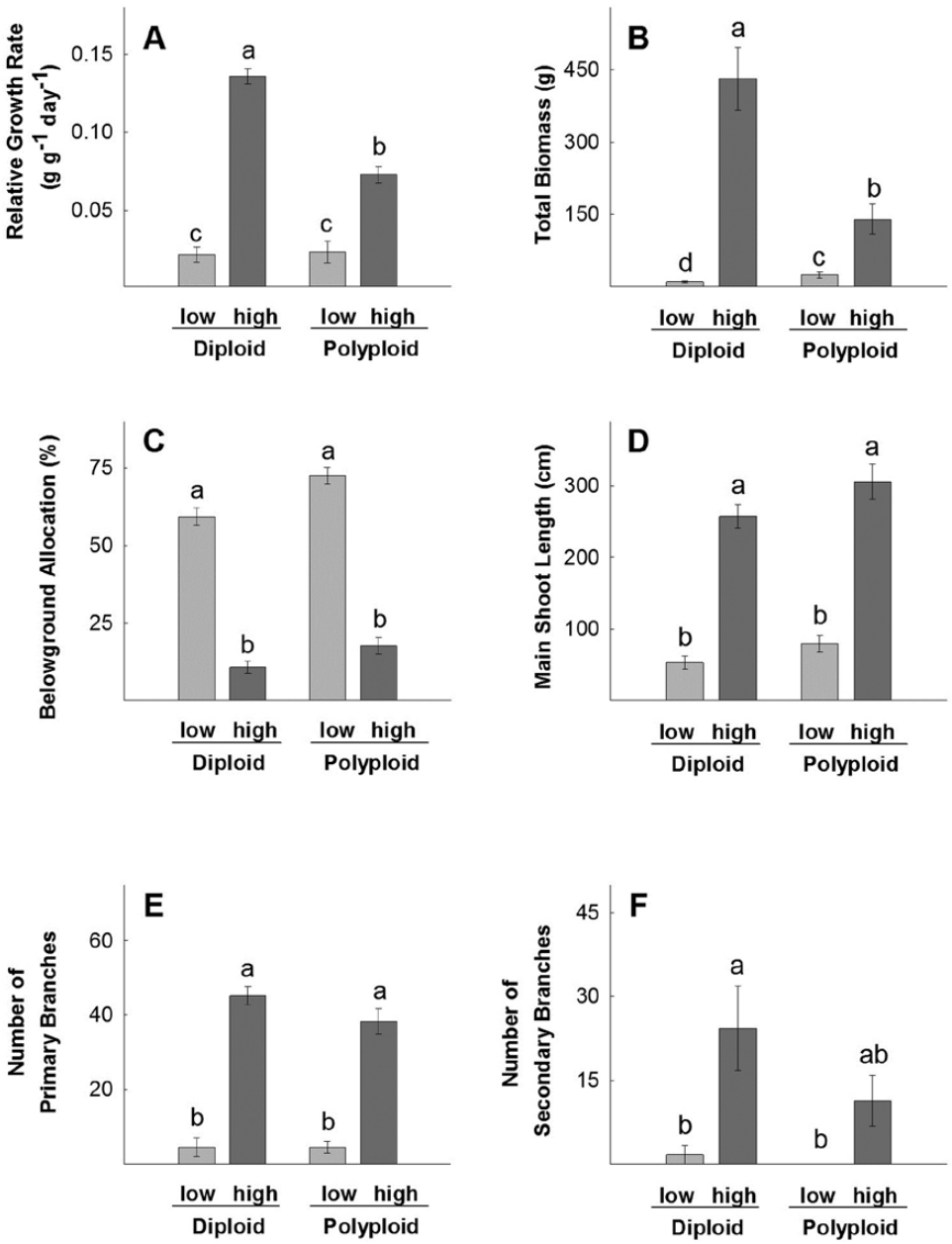

Figure 1. Means (±SE) for relative growth rates (A), biomass production (B), below-ground allocation of biomass (C) and plant architecture traits (D–F) in two taxa, *Ludwigia peploides* subsp. *montevidensis* (diploid) and *L. hexapetala* (decaploid) grown from rhizome fragments in response to soil nutrient (low, high) treatments. Letters above bars correspond to the results of multiple comparison tests.

main shoot length (nutrient: $F_{1, 17} = 202.13$, $P < 0.0001$; Fig. 1D) and the number of primary branches along the main shoot (nutrient: $F_{1, 17} = 240.79$, $P < 0.0001$; Fig. 1E) under high sediment nutrient availability. The number of secondary branches was also influenced by sediment nutrient level (nutrient: $F_{1, 3} = 13.34$, $P = 0.002$; Fig. 1F). While absolute numbers of branches produced by the taxa grown from rhizomes at contrasting nutrient levels were similar, observations and the ANOVA results suggest the comparatively long primary shoot of *L. hexapetala* was the main difference in architecture that contrasted with the more dense and shorter branches of the diploid *L. p.* subsp. *montevidensis*. However, the magnitude of the differences in shoot length between ploidy levels was not sufficiently different per the *post hoc* tests.

Photosynthetic measurements

Plants from both ploidy levels showed a significant increase in photosynthetic rate (nutrient: $F_{1, 17} = 24.38$, $P = 0.0008$; Fig. 2A) and stomatal conductance (nutrient: $F_{1, 17} = 10.67$, $P = 0.0097$; Fig. 2B) with increased sediment nutrient availability. The ploidy * nutrient interaction was not significant, as maximum values of both gas exchange trait responses were similar for both species under high nutrient conditions.

Leaf chemistry

Leaf N concentrations were the same for both species grown from rhizome fragments in contrasting nutrient environments [see Supporting Information—Table S3]. Leaf P concentration

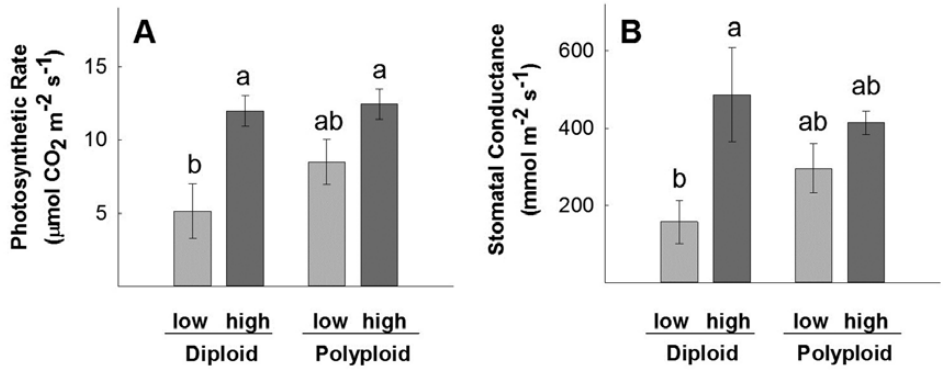

Figure 2. Means (±1 SE) for photosynthetic and stomatal conductance rates (A, B) in *Ludwigia peploides* subsp. *montevidensis* (diploid) and *L. hexapetala* (decaploid) rhizome fragments in response to soil nutrient (low, high) treatments. Letters above bars correspond to the results of multiple comparison tests.

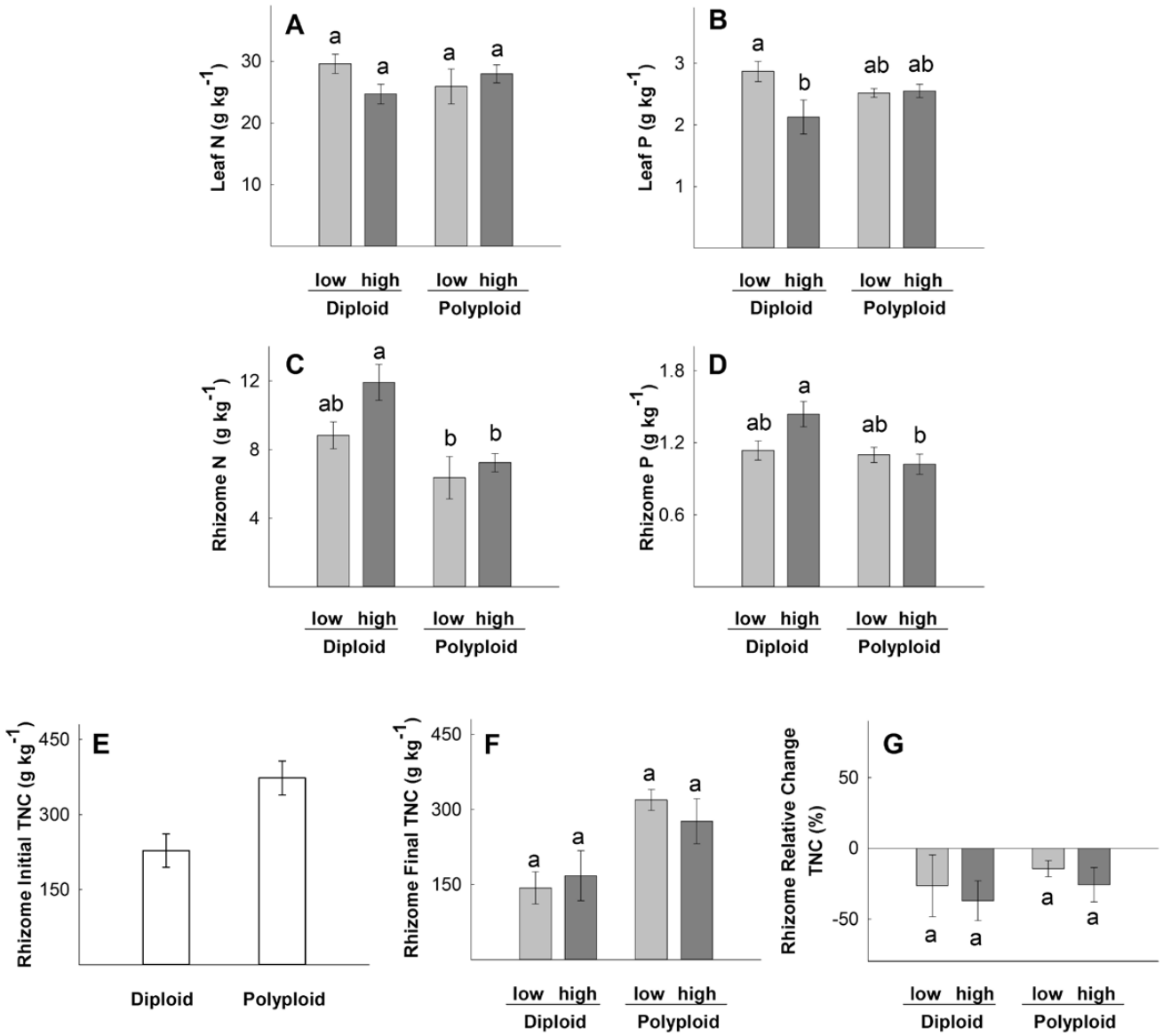

Figure 3. Means (±1 SE) for leaf (A, C) and rhizome (B, D) chemistry (N, P) and carbon storage reserve traits (E–G) in two taxa, *Ludwigia peploides* subsp. *montevidensis* (diploid) and *L. hexapetala* (decaploid) grown from rhizome fragments in response to soil nutrient (low, high) treatments. Letters above bars correspond to the results of multiple comparison tests.

decreased in *L. p.* subsp. *montevidensis* with increased sediment nutrient availability but showed little change in *L. hexapetala* (ploidy * nutrient interaction: $F_{1, 17} = 5.85$, $P = 0.0271$; Fig. 3B).

Carbon and nutrient storage reserves

Rhizome N was significantly greater in diploid *L. p.* ssp. *montevidensis* (ploidy: $F_{1, 17} = 12.71$, $P = 0.0024$; Fig. 3C), particularly at high sediment nutrient concentrations (nutrient: $F_{1, 17} = 5.34$, $P = 0.0337$; Fig. 3C). Rhizome P was greatest in *L. p.* ssp. *montevidensis* at high sediment nutrients and lowest in *L. hexapetala* at high nutrients (ploidy * nutrient interaction: $F_{1, 17} = 4.84$, $P = 0.042$; Fig. 3D).

At the start of the experiment, both species had significant reserves of TNC in rhizome fragments collected for the experiment, although carbon storage reserves were ~60 % higher in decaploid rhizomes (Fig. 3E). At the end of the experiment, both taxa had depleted these carbon reserves to support growth, but decaploid *L. hexapetala* had significantly more carbon storage reserves than the diploid ($F_{1, 17} = 8.99$, $P = 0.0081$; Fig. 3F and G).

Ecophysiological trait relationships to plant growth

Twelve functional traits explained 83.15 % of the variation (Fig. 4). Greater rhizome N and P concentrations supported greater growth, which was also associated with greater branching and shoot length. Higher gas exchange rates were associated with higher leaf N concentration and greater rhizome TNC. Shifting allocation below-ground was associated with plants grown at lower sediment nutrient availability and lower growth, shoot branching and shoot length.

Discussion

The regenerative ability of clonal fragments that become dispersal units and transport bud banks can greatly influence plant population dynamics and potentially have positive effects on plant survival (Klimešová and Klimeš 2007; Klimešová *et al.* 2018). The ability to spread and regenerate from plant fragments is especially important to the success of plant species invading riverine and other aquatic habitats with frequent disturbance regimes. Improved understanding of underlying plant traits that support recolonization of disturbance-generated fragments may contribute to improved management strategies to control their invasive spread (Bímová *et al.* 2003; Dong *et al.* 2010; Grewell *et al.* 2016). The two *Ludwigia* congeners that we investigated were able to resprout, survive and grow successfully from bud banks of rhizome fragments. However, the availability of soil nutrient resources and ploidy level affected measured plant trait responses. Despite significant initial carbon storage reserves in decaploid rhizomes, the experiment reported here provides further evidence that polyploidy does not always yield superior performance at the initial establishment phase of plant growth from asexual rhizome fragments. The responses observed in this work, based on single populations of each congener, are similar to work sourcing shoot fragment material from other *Ludwigia* populations (Grewell *et al.* 2016; B. J. Grewell, unpubl. data), which documented more prolific growth of the diploid congener during the early colonizing stage.

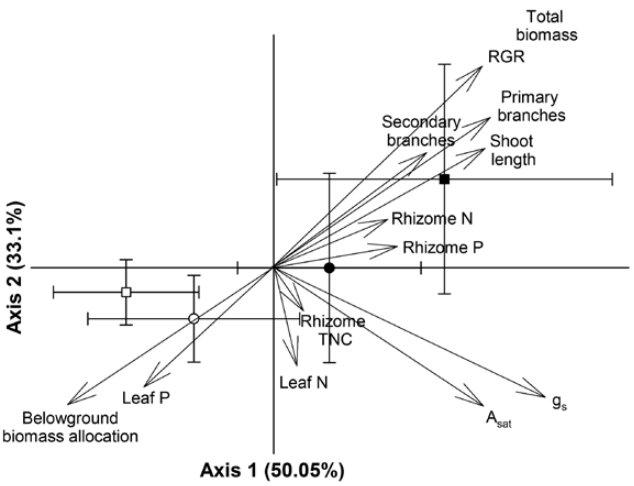

Figure 4. Principal component analysis of functional trait responses in two taxa, *Ludwigia peploides* subsp. *montevidensis* (diploid, square symbols) and *L. hexapetala* (decaploid, circle symbols) grown from rhizome fragments in response to soil nutrient treatments (low nutrients, white symbols; high nutrients, black symbols).

The RGR and biomass production of both species were higher with increased soil nutrient availability, but these responses were much stronger in the diploid congener, *L. p.* subsp. *montevidensis*. Based on results from our principal component analysis, the high RGR and biomass allocation production responses in the diploid plants were likely driven synergistically by rhizome nutrient reserves and changes in biomass allocation. Investment in rhizome N and P was positively associated with all growth variables, whereas a shift to below-ground biomass was associated with low growth and biomass production. From an economic perspective, investing in above-ground tissues increased the rate of return as measured in growth in *L. p.* subsp. *montevidensis* (Drenovsky and James 2010).

Clonal structures, such as rhizomes, serve as resource storage organs that can support fitness and performance of plants subjected to disturbance or temporal changes in growing conditions (Suzuki and Stuefer 1999; Dong *et al.* 2010; Dong *et al.* 2011). Carbon storage reserves of plants fluctuate with life cycle and seasonal dynamics and are metabolized to support resprouting, growth and maintenance (Suzuki and Steufer 1999). In our experiment, decaploid rhizomes had twice the non-structural carbohydrate reserves than diploids at the start of the experiment. Although not a significant difference based on *post hoc* Tukey's tests due to low replication, the diploids showed a trend of potentially being more efficient in utilizing stored reserves for biomass production. Certainly, carbohydrate dynamics are complex and can serve multiple functions in plants following disturbances. However, it is possible that slower metabolic rates associated with polyploidization (Levin 1983) may underlie the observed trends in carbon utilization efficiencies between the diploid and decaploid. Our *ex situ* experiments provide an estimate of regeneration potential of invasive *Ludwigia* congeners following disturbance and fragmentation in environments with contrasting soil nutrient availability. However, the regenerative capacity of bud banks under field conditions can be expected to vary depending on growth and plasticity of the plant and the timing and severity of disturbance affecting the population. Compared to previous work on the regeneration potential of *Ludwigia* shoot fragments (Glover *et al.* 2015; Grewell *et al.* 2016), growth of both congeners was more prolific in response to nutrient availability when grown from rhizome fragments. Similarly, in a study of four invasive *Reynoutria* (syn. *Fallopia*, Polygonaceae; knotweeds) that also grow along river margins, Bímová *et al.* (2003) also found that regeneration from rhizome fragments was more efficient than that from shoot fragments. The fact that these similar growth responses are observed in plants from distant taxonomic

lineages (Myrtales vs. Caryophyllales) provides strong evidence for this trait response which has previously received little attention in invasive clonal plant species. Following disturbance, dispersal and resprouting from rhizome fragment bud banks can potentially be a significant mechanism for the successful establishment and spread of invasive other rhizomatous species of riverine wetland plants.

Conclusions and Management Implications

Understanding how growth strategies and functional traits of invasive plant species, such as regeneration from rhizome bud banks and ploidy level, interact with contrasting environmental conditions can provide a foundation for early management intervention. Traditional ideas regarding plant strategies presume high seed production and short generation times to be adaptive responses to disturbance (Grime 2001). In the case of these clonal plant species, regeneration from clonal fragment bud banks with stored carbon reserves is also a successful response strategy, in which repeated to severe disturbance regimes (e.g. flood, fire, herbivory) are the norm (Van der Meijden *et al.* 1988; Klimešová and Klimeš 2003, 2007). *Ludwigia* species in riverine wetlands have long evolved in response to frequent disturbance stresses.

Despite clear differences in trait responses between taxa differing in ploidy level, both *Ludwigia* taxa in our experiments exhibited high regeneration ability when grown from rhizome fragments in high sediment nutrient environments. Management strategies to prevent bank erosion and other disturbances that produce and mobilize rhizome fragments are warranted. Biocontrol organisms that directly deplete below-ground storage reserves or significantly reduce translocation of carbon to below-ground storage organs could potentially enhance integrated management strategies. Particularly under field conditions, the highly invasive decaploid *L. hexapetala* also invests heavily in shoot elongation that can enhance foraging ability for limited resources in heterogeneous environments. For these decaploids, management options such as mechanical removal or biocontrol agents that reduce shoot biomass should improve management.

Our experiments also suggest diploid *L. p.* subsp. *montevidensis* has a superior ability to maximize resource uptake, use and allocation across contrasting resource gradients in comparison to its decaploid congener *L. hexapetala*. Management strategies should prioritize rapid response to newly colonizing diploid invaders, and reductions in nutrient loads to aquatic environments may be a more effective component of a comprehensive management strategy towards controlling establishment of the diploid congener than the decaploid.

Supporting Information

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

File S1. Supplementary Tables S1, S2, S3 and data analysis code.pdf

File S2. Plant functional trait response data.xls

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Contributions by the Authors

B.J.G., R.E.D. and M.T.I. conceived the study and designed the experiment; all authors contributed to execution of the experiment; R.E.D., C.J.F., B.J.G. and M.T.I. analysed the data; and B.J.G. and R.E.D. wrote and revised the manuscript with assistance of C.J.F.

Conflict of Interest

None declared.

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Literature Cited

Barko JW, Smart RM. 1983. Effects of organic matter additions to sediment on the growth of aquatic plants. *Journal of Ecology* **71**:161–175

Barko JW, Smart RM. 1986. Sediment-related mechanisms of growth limitations in submersed macrophytes. *Ecology* **67**:1328–1340.

Barrat-Segretain MH, Bornette G. 2000. Regeneration and colonization abilities of aquatic plant fragments: effect of disturbance seasonality. *Hydrobiologia* **421**:31–39.

Barrat-Segretain MH, Bornette G, Vilas-Boas HA. 1998. Comparative abilities of vegetative resprouting among aquatic plants growing in disturbed habitats. *Aquatic Botany* **60**:201–211.

Bímová K, Mandák B, Pyšek P. 2003. Experimental study of vegetative regeneration in four invasive *Reynoutria* taxa (Polygonaceae). *Plant Ecology* **166**:1–11.

Boulton AJ, Findlay S, Marmonier P, Stanley EH, Valett HM. 1998. The functional significance of the hyporheic zone in streams and rivers. *Annual Review of Ecology and Systematics* **29**:59–81.

Buggs RJA, Pannell JR. 2007. Ecological differentiation and diploid superiority across a moving ploidy contact zone. *Evolution* **61**:125–140.

Carr GM, Chambers PA. 1998. Macrophyte growth and sediment phosphorous and nitrogen in a Canadian prairie river. *Freshwater Biology* **39**:525–536.

Černá L, Münzbergová Z. 2015. Conditions in home and transplant soils have differential effects on the performance of diploid and allotetraploid *Anthericum* species. *PLoS One* **10**:e0116992.

Dong BC, Liu RH, Zhang Q, Li HL, Zhang MX, Lei GC, Yu FH. 2011. Burial depth and stolon internode length independently affect survival of small clonal fragments. *PLoS One* **6**:e23942.

Dong BC, Yu GL, Guo W, Zhang MX, Dong M, Yu FH. 2010. How internode length, position and presence of leaves affect survival and growth of *Alternanthera philoxeroides* after fragmentation? *Evolution and Ecology* **24**:1447–146.

Drenovsky RE, Grewell BJ, D'Antonio CM, Funk JL, James JJ, Molinari N, Parker IM, Richards CL. 2012. A functional trait perspective on plant invasion. *Annals of Botany* **110**:141–153.

Drenovsky RE, James JJ. 2010. Designing invasion-resistant plant communities: the role of plant functional traits. *Rangelands* **32**:32–37.

Ehrenfeld JG. 2010. Ecosystem consequences of biological invasions. *Annual Review of Ecology and Systematics* **41**:59–80.

Elton CS. 1958. *The ecology of invasions by animals and plants*. London: Methuen.

Gillard M, Grewell BJ, Futrell CJ, Deleu C, Thiébaut G. 2017. Germination and seedling growth of water primroses: a cross experiment between two invaded ranges with contrasting climates. *Frontiers in Plant Science* **8**:1677.

Glover R, Drenovsky RE, Futrell CJ, Grewell BJ. 2015. Clonal integration in *Ludwigia hexapetala* under different light regimes. *Aquatic Botany* **122**:40–46.

Grewell BJ, Skaer Thomason MJ, Futrell CJ, Iannucci M, Drenovsky RE. 2016. Trait responses of invasive aquatic macrophyte congeners: colonizing diploid outperforms polyploid. *AoB Plants* **8**:plw014; doi:10.1093/aobpla/plw014.

Grime JP. 2001. *Plant strategies, vegetation processes, and ecosystem properties*. Chichester, UK: Wiley.

Hahn MA, van Kleunen M, Müller-Schärer H. 2012. Increased phenotypic plasticity to climate may have boosted the invasion success of polyploid *Centaurea stoebe*. *PLoS One* **7**:e50284.

Haury J, Druel A, Cabral T, Paulet Y, Bozec M, Coudreuse J. 2014. Which adaptations of some invasive *Ludwigia* spp. (Rosidae, Onagraceae) populations occur in contrasting hydrological conditions in Western France? *Hydrobiologia* **737**:45–56.

Hoch PH, Grewell BJ. 2012. *Ludwigia*. In: Baldwin BG, Goldman DH, Keil DJ, Patterson R, Rosatti TJ, Wilkin DH, eds. *The Jepson Manual: vascular plants of California*, 2nd edn. Berkeley, CA: University of California Press, 948–949.

Hoch PC, Wagner WL, Raven PH. 2015. The correct name for a section of *Ludwigia* L. (Onagraceae). *PhytoKeys* **50**:31–34.

Hussner A. 2009. Growth and photosynthesis of four invasive aquatic plant species in Europe. *Weed Research* **49**:506–515.

Hussner A. 2010. Growth response and root system development of the invasive *Ludwigia grandiflora* and *Ludwigia peploides* to nutrient availability and water level. *Fundamentals of Applied Limnology* **177**:189–196.

Keser LH, Dawson W, Song YB, Yu FH, Fischer M, Dong M, van Kleunen M. 2014. Invasive clonal plant species have a greater root-foraging plasticity than non-invasive ones. *Oecologia* **174**:1055–1064.

Klimeš L, Klimešová J, Osbornova J. 1993. Regeneration capacity and carbohydrate reserves in a clonal plant *Rumex alpinus*: effect of burial. *Vegetatio* **109**:153–160.

Klimešová J, Klimeš L. 2003. Resprouting of herbs in disturbed habitats: is it adequately described by Bellingham–Sparrow's model? *Oikos* **103**:225–229.

Klimešová J, Klimeš L. 2007. Bud banks and their role in vegetative regeneration – a literature review and proposal for simple classification and assessment. *Perspectives in Plant Ecology, Evolution and Systematics* **8**:115–129.

Klimešová J, Martínková J, Ottaviani G, Field K. 2018. Belowground functional plant ecology: towards and integrated perspective. *Functional Ecology* **32**:2115–2126.

Klimešová J, Tackenberg O, Herben T. 2016. Herbs are different: clonal and bud bank traits can matter more than leaf–height–seed traits. *New Phytologist* **210**:13–17.

Kutner H, Nachtsheim CJ, Neter J, Li W. 2004. *Applied linear statistical models*, 5th edn. New York: McGraw Hill/Irwin.

Lambert E, Dutartre A, Coudreuse J, Haury J. 2010. Relationships between the biomass production of invasive *Ludwigia* species and physical properties of habitats in France. *Hydrobiologia* **656**:173–186.

Levin D. 1983. Polyploidy and novelty in flowering plants. *The American Naturalist* **122**:1–25.

Levin D. 2002. *The role of chromosomal change in plant evolution*. Oxford: Oxford University Press.

Li X, Shen Y, Huang Q, Fan Z, Huang D. 2013. Regeneration capacity of small clonal fragments of the invasive *Mikania micrantha* H.B.K.: effects of burial depth and stolon internode length. *PLoS One* **8**:e84657.

McFarland DG, Barko JW, McCreary NJ. 1992. Effects of sediment fertility and initial plant-density on growth of *Hydrilla verticillata* (LF) Royle and *Potamogeton nodosus* Poiret. *Journal of Freshwater Ecology* **7**:191–200.

Münzbergová Z. 2007a. No effect of ploidy level in plant response to competition in a common garden experiment. *Biological Journal of the Linnean Society* **92**:211–219.

Münzbergová Z. 2007b. Population dynamics of diploid and hexaploid populations of a perennial herb. *Annals of Botany* **100**:1259–1270.

Murphey J, Riley JP. 1962. A modified single-solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* **27**:31–36.

Myerson LA, Cronin JT, Bhattarai GP, Brix H, Lambertini C, Lucanová, Rhinehart S, Suda J, Pyšek P. 2016. Do ploidy level and nuclear genome size and latitude of origin modify the expression of *Phragmites australis* traits and interactions with herbivores? *Biological Invasions* **18**:2531–2549.

Nelson N. 1944. A photometric adaptation of the Somogyi method for determination of glucose. *Biological Chemistry* **153**:375–380.

Okada M, Grewell BJ, Jasieniuk M. 2009. Clonal spread of invasive *Ludwigia hexapetala* and *L. grandiflora* in freshwater wetlands of California. *Aquatic Botany* **91**:123–129.

Olde Venterink H, Davidsson TE, Kiehl K, Leonardson L. 2002. Impact of drying and re-wetting on N, P, and K dynamics in a wetland soil. *Plant and Soil* **243**:119–130.

Olsen SR, Cole CV, Watanabe FS, Dean LA. 1954. *Estimation of available phosphorus in soils by extraction with sodium bicarbonate*. USDA Circular 939. Washington, DC: U.S. Government Printing Office.

Pandit MK, Pocock MJO, Kunin WE. 2011. Ploidy influences rarity and invasiveness in plants. *Journal of Ecology* **99**:1108–1115.

Pyšek P. 1997. Clonality and plant invasions. In: de Kroon H, van Groenendael J, eds. *The ecology and evolution of clonal plants*. Leiden, The Netherlands: Backhuys, 405–427.

Pyšek P, Jarošik V, Pergl J, Randall R, Chytrý M, Kühn I, Tichý L, Danihelka J, Chrtekjun J, Sádlo J. 2009. The global invasion success of Central European plants is related to distribution characteristics in their native range and species traits. *Diversity and Distributions* **15**:891–903.

Pyšek P, Manceur AM, Alba C, McGregor KF, Pergl J, Štajerová K, Chytrý M, Danihelka J, Kartesz J, Klimešová J, Lučanová M, Moravcová L, Nishino M, Sádlo J, Suda J, Tichý´ L, Kühn I. 2015. Naturalization of central European plants in North America: species traits, habitats, propagule pressure, residence time. *Ecology* **96**:762–774.

Pyšek P, Prach K. 1993. Plant invasions and the role of riparian habitats: a comparison of four species alien to central Europe. *Journal of Biogeography* **20**:413–420.

Pyšek P, Richardson D. 2007. Traits associated with invasiveness in alien plants: where do we stand? In: Nentwig W, ed. *Biological invasions*. Berlin: Springer, 97–125.

Radosevich SR, Stubbs MM, Ghersa CM. 2003. Plant invasions: pattern and process. *Weed Science* **51**:254–259.

Rejmánková E. 1992. Ecology of creeping macrophytes with special reference to *Ludwigia peploides* (H.B.K.) Raven. *Aquatic Botany* **43**:283–299.

Ruaux B, Greulich S, Haury J, Berton J-P. 2009. Sexual reproduction of two alien invasive *Ludwigia* (Onagraceae) on the middle Loire River, France. *Aquatic Botany* **90**:143–148.

Santamaría L. 2002. Why are most aquatic plants widely distributed? Dispersal,clonal growth, and small-scale heterogeneity in a stressful environment. *Acta Oecologia* **23**:137–154.

Schlaepfer DR, Edwards PJ, Billeter R. 2010. Why only tetraploid *Solidago gigantea* (Asteraceae) became invasive: a common garden comparison of ploidy levels. *Oecologia* **163**:661–673.

Soltis DE, Albert VA, Leebens-Mack J, Bell CD, Paterson AH, Zheng C, Sankoff D, Depamphilis CW, Wall PK, Soltis PS. 2009. Polyploidy and angiosperm diversification. *American Journal of Botany* **96**:336–348.

Soltis DE, Buggs RJA, Doyle JJ, Soltis PS. 2010. What we still don't know about polyploidy. *Taxon* **59**:1387–1403.

Soltis PS, Soltis DE. 2000. The role of genetic and genomic attributes in the success of polyploids. *Proceedings of the National Academy of Sciences of the United States of America* **97**:7051–7057.

Suda J, Meyerson LA, Leitch IJ, Pyšek P. 2015. The hidden side of plant invasions: the role of genome size. *The New Phytologist* **205**:994–1007.

Suzuki J-I, Stuefer JF. 1999. On the ecological and evolutionary significance of storage in clonal plants. *Plant Species Biology* **14**:11–17.

Swank JC, Below FE, Lambert RJ, Hageman RH. 1982. Interaction of carbon and nitrogen metabolism in the productivity of maize. *Plant Physiology* **70**:1185–1190.

te Beest M, Le Roux JJ, Richardson DM, Brysting AK, Suda J, Kubesová M, Pysek P. 2012. The more the better? The role of polyploidy in facilitating plant invasions. *Annals of Botany* **109**:19–45.

Thompson K, Hodgson JG, Rich T. 1995. Native and alien invasive plants: more of the same? *Ecography* **18**:390–402.

Thouvenot L, Haury J, Thiebaut G. 2013a. Seasonal plasticity of *Ludwigia grandiflora* under light and water depth gradients: an outdoor mesocosm experiment. *Flora* **208**:430–437.

Thouvenot L, Haury J, Thiebaut G. 2013b. A success story: water primroses, aquatic plant pests. *Aquatic Conservation-Marine and Freshwater Ecosystems* **23**:790–803.

Van der Meijden E, Wijn M, Verkaar HJ. 1988. Defence and regrowth, alternative plant strategies in the struggle against herbivores. *Oikos* **51**:355–363.

Wagner WL, Hoch PC, Raven PH. 2007. Revised classification of the Onagraceae. *Systematic Botany Monographs* **83**:1–240.

Wei N, Cronn R, Liston A, Ashman TL. 2019. Functional trait divergence and trait plasticity confer polyploid advantage in heterogeneous environments. *The New Phytologist* **221**:2286–2297.

Weiss-Schneeweiss H, Emadzade J, Jang T-S, Schneeweiss GM. 2013. Evolutionary consequences, constraints and potential of polyploidy in plants. *Cytogenetic and Genome Research* **140**:137–150.

Willby NJ, Abernethy VJ, Demars BOL. 2000. Attribute-based classification of European hydrophytes and its relationship to habitat utilization. *Freshwater Biology* **43**:43–74.

Zedler JB, Kercher S. 2010. Causes and consequences of invasive plants in wetlands: opportunities, opportunists, and outcomes. *Critical Reviews in Plant Sciences* **23**:431–452.