Perspectives in Plant Ecology, Evolution and Systematics 41 (2019) 125498

Contents lists available at ScienceDirect

Perspectives in Plant Ecology, Evolution and Systematics

journal homepage: www.elsevier.com/locate/ppees

Research article

Transgenerational effects of herbivory and soil nutrients transmitted via vegetative reproduction in the clonal plant *Alternanthera philoxeroides*

Bi-Cheng Dong[a], Peter Alpert[b], Fei-Hai Yu[a,c,*]

[a] School of Ecology and Nature Conservation, Beijing Forestry University, Beijing, 100083, China
[b] Biology Department, University of Massachusetts, Amherst, MA, 01003, USA
[c] Institute of Wetland Ecology & Clone Ecology/Zhejiang Provincial Key Laboratory of Plant Evolutionary Ecology and Conservation, Taizhou University, Taizhou, 318000, China

ARTICLE INFO

Keywords:
Asexual reproduction
Clonal plant
Insect herbivory
Nitrogen
Soil nutrients
Transgenerational effect

ABSTRACT

Epigenetic changes and maternal effects, collectively termed transgenerational effects, allow responses of organisms to environmental factors to be passed between generations. This is well-known in the case of sexual reproduction but little studied in asexual reproduction, which is often the primary mode of reproduction in clonal plants. To test for transgenerational effects via vegetative reproduction in the clonal herb *Alternanthera philoxeroides*, a first generation of clonal fragments were subjected to crossed nutrient and herbivory treatments, using the insect herbivore *Planococcus minor*. Stem and root fragments taken from these plants were then grown into a second vegetative generation and subjected to the same nutrient treatments. Adding nutrients increased total N concentration in first-generation fragments; herbivory increased total N slightly in shoots and decreased N slightly in roots. First-generation fragments given higher nutrients produced second-generation fragments with more final total dry mass and stem nodes. This effect was greater in second-generation fragments also given higher nutrients and greater in second-generation fragments derived from stems than in those derived from roots. Herbivory on first-generation fragments decreased growth of second-generation fragments slightly. This effect was greater if first-generation ramets had been given higher nutrients and greater in second-generation fragments derived from roots. Results strengthen evidence that transgenerational effects can be transmitted via vegetative reproduction in plants and show that such effects can be greater when resource availability is higher and can depend on the organ from which offsping are produced.

1. Introduction

Transgenerational effects comprise epigenetic changes or environmentally determined levels of provisioning or other characteristics of offspring that cause the response of an organism to the environment to affect the performance of its progeny (Wolf and Wade, 2009; Latzel and Klimešová, 2010). Transgenerational effects are widespread in both animals and plants (e.g., Jablonka and Raz, 2009; Zhang et al., 2013; Richter-Boix et al., 2014; Shama and Wegner, 2014; Walsh et al., 2014; Vu et al., 2015; Groot et al., 2016) and increasingly appear to offer an important means of maintaining or increasing fitness from generation to generation. In sexually reproducing plants, transgenerational effects include phenotypic responses to a wide range of environmental factors, and many of these responses can be shown to increase performance (Herman and Sultan, 2011; Latzel et al., 2014). For instance, transgenerational effects of herbivory (Agrawal, 2002), disturbance regime and nutrient availability (Latzel et al., 2009), light availability (Galloway and Etterson, 2007; McIntyre and Strauss, 2014), drought (Herman et al., 2012), salinity (Castro et al., 2013), and a fungal pathogen (Vivas et al., 2013) have been interpreted as adaptive. Transgenerational environmental effects may also contribute to the invasiveness of some annual, introduced plants (Fenesi et al., 2014).

Transgenerational effects might be expected to be especially important in species that mainly reproduce asexually and so have a low potential for adaptation though genetically based natural selection. This includes some clonal plant species (Schwaegerle et al., 2000; Latzel and Klimešová, 2010) and vegetatively propagated crops (Prentis et al., 2008; McKey et al., 2010). Transgenerational effects via vegetative reproduction could also help explain how some individual clones in clonal plant species become so invasive (Richards et al., 2012; Zhang et al., 2013; Shi et al., 2018). For instance, over three-fourths of the introduced plants of the aquatic, stoloniferous, highly invasive plant

* Corresponding author at: School of Nature Conservation, Beijing Forestry University, Beijing, 100083, China.
E-mail address: feihaiyu@126.com (F.-H. Yu).

https://doi.org/10.1016/j.ppees.2019.125498
Received 1 April 2019; Received in revised form 22 August 2019; Accepted 18 October 2019
Available online 22 October 2019
1433-8319/

Eichhornia crassipes on four continents appear to belong to a single clone from Peru (Zhang et al., 2010).

One way that transgenerational effects via sexual and vegetative reproduction might differ is in the relative importance of different types of mechanisms. The two main types of mechanisms that underlie transgenerational effects are generally considered to be epigenetic mechanisms such as methylation of DNA and modification of histones, and provisioning of offspring with resources such as carbohydrates or nutrients (Herman and Sultan, 2011; Holeski et al., 2012; Germain et al., 2013; Zas et al., 2013; Cortijo et al., 2014). Because even the smallest clonal fragments that can function as vegetative propagules in clonal plants tend to be larger in size and mass than their sexual propagules, the potential for provisioning of vegetative offspring might be expected to be relatively high.

Although transgenerational effects have been reported in asexually reproducing animals (Keiser and Mondor, 2013), there have been few direct tests for transgenerational effects via vegetative reproduction in plants. Latzel and Klimešová (2009) and Dong et al. (2018a) noted that clonal fragments of *Alternanthera philoxeroides* and *Plantago major* performed better in habitats more like those of the ramets from which they had been taken. González et al. (2017) similarly reported that stem fragments of *Trifolium repens* from parental plants grown with ample water availability produced larger plants than fragments from droughted parents and that the effect was greater if second-generation plants were grown with ample water. A number of other studies have provided less direct evidence (Pujalon et al., 2008; Latzel and Klimešová, 2009; Gao et al., 2010; Huber et al., 2014; Guarino et al., 2015; González et al., 2016, 2017; Latzel et al., 2016; Dong et al., 2017, 2018a,2018b, 2019; Latzel and Münzbergová, 2018; Shi et al., 2018).

Several studies point specifically to the possibility of transgenerational effects of herbivory via vegetative reproduction. Johansson (1994) found that ramets taken from populations of *Ranunculus lingua* with more herbivory had more resistance to herbivory; Monro and Poore (2004) reported intraclonal variation in defense against herbivores; and Lu and Ding (2012) observed greater ability for compensatory growth in plants of *Alternanthera philoxeroides* propagated from populations subject to higher levels of herbivory. However, few studies have explicitly tested for vegetative transgenerational effects in a clonal plant by manipulating herbivory factors (González et al., 2016; Dong et al., 2017, 2018b). Dong et al. (2017, 2018b) reported negative to neutral effects of herbivory on parents on the growth and physiological properties of vegetative offspring.

To provide an explicit test for positive as well as negative transgenerational effects via vegetative reproduction in clonal plants, with a focus on provisioning as a possible mechanism, we conducted a greenhouse experiment on the well-studied, widespread, invasive species *A. philoxeroides*. We manipulated nutrient availability as a factor that might increase provisioning and herbivory as a factor that might reduce it, and eliminated any confounding effects of clonal integration between generations by severing vegetative offspring before establishment. We asked: 1) Can exposure to increased resource availability increase performance of vegetative offspring? 2) Is this effect greater when offspring themselves experience higher levels of resource availability? 3) Can exposure to disturbance in the form of herbivory decrease performance of vegetative offspring? 4) Can treatment that allows recovery from herbivory within a generation also allow recovery from transgenerational effects of herbivory when applied in the next generation? 5) Do transgenerational effects differ between vegetative offspring derived from different organs such as stem nodes and roots?

2. Materials and methods

2.1. Species

Alternanthera philoxeroides (Mart.) Griseb., an amphibious, perennial, creeping herb in the Amaranthaceae, reproduces vegetatively by the production of roots and stem branches at stem nodes, which can thus function as ramets. Stem fragments as small as a single node, or ramet, can establish an extensive network of stems each up to 1.2 m long and bearing up to more than a dozen connected ramets within one growing season under favorable conditions (Wu and Raven, 2003; Dong et al., 2010, 2012, 2015).

A. philoxeroides is native to South America but has been introduced and become widespread and highly invasive in many countries (Holm et al., 1997; Julien et al., 2012). In China, *A. philoxeroides* is abundant in natural and constructed waterways, riparian habitats, and crop fields (Sainty et al., 1998; Pan et al., 2006) and occupies habitats with a wide range of levels of resources and disturbances including herbivory (Lu and Ding, 2012; Lu et al., 2013). Sexual reproduction by seeds is very rare, and the species is likely represented by a single clone in southern China (Xu et al., 2003; Ye et al., 2003; Li and Ye, 2006).

Planococcus minor (Maskell) is a generalist herbivore in the Pseudococcidae (Cox, 1989) with a wide distribution in subtropical and tropical regions (Williams and de Willink, 1992; Venette and Davis, 2004; Francis et al., 2012). Females are soft-bodied, wingless, covered with waxy filaments, and relatively sedentary; males are tiny, winged, and live only a few days (Francis et al., 2012; Roda et al., 2013). Females seek out the bases of leaves and buds of host plants, and feed from the phloem by inserting piercing and sucking mouthparts into plant tissues. Males also feed on plants during their first and second stages of development (Roda et al., 2013). Infestations of *P. minor* can cause leaves of *A. philoxeroides* to curl and wilt, and can reduce plant growth (Dong et al., 2017, 2018a,b).

Plants of *A. philoxeroides* were collected from a riparian agricultural area on 18–19 May 2011 in Taizhou, Zhejiang Province, China (28.87 °N, 121.01 °E), and vegetatively propagated in a greenhouse at Forestry Science Company, Ltd., in Beijing Forestry University. Individuals of *P. minor* were collected in the greenhouse; only female larvae were used.

2.2. Experimental design

The overall design of the experiment (Fig. 1) was to first subject a first generation of stem fragments to two herbivory treatments (present or absent) crossed with two soil nutrient treatments (additional nutrients added to the soil or additional nutrients not added) for 11 weeks. One set of replicates was then harvested for dry mass and chemistry, a second set was subjected to the herbivory and nutrient treatments for 9 more weeks before harvest for dry mass after a total of 20 weeks of treatment, and a third set was used as a source of second-generation stem and root fragments. In half of the replicates of the second set in the treatments with herbivory present, herbivores were removed at the start of the 9 additional weeks of treatment to create a third herbivory treatment (discontinued for 9 weeks after being present for 11 weeks). The discontinued treatment was used to test for ability for first-generation plants to recover from herbivory under the two nutrient treatments. The stem and root fragments taken from the third set of replicates were weighed for initial fresh mass, grown for 16 weeks under the same two nutrient treatments as given to first-generation plants, and harvested for dry mass to give the results for second-generation plants. Details of the design including numbers of replicates are given below.

On 7 August 2014, about 200 stem fragments each consisting of one node bearing two opposite leaves and 3 cm of proximal and distal stem were cut from stock plants. Fragments were individually grown in an equal mixture of quartz sand (0.5–1 mm particle size) and peat (Pindstrup Seedling; Pindstrup Mosebrug A/S, Pindstrup, Denmark) for one month, until most had produced new stems from axillary buds at the node. A subset of 140 fragments that had each grown a new stem approximately 15 cm long were then inidividually transplanted into pots that were 14 cm in diameter by 12 cm deep and filled with the soil mixture described above.

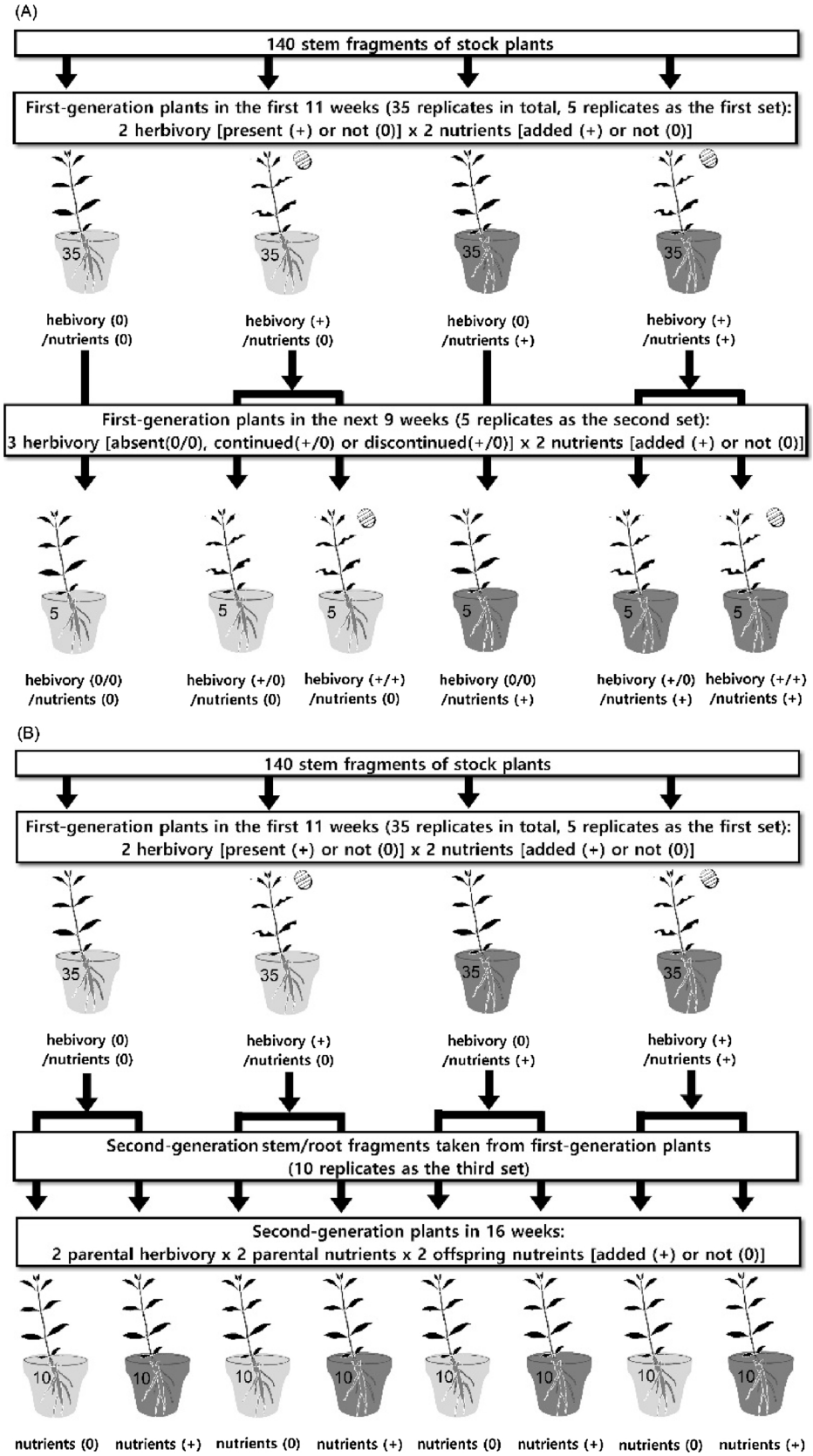

Fig. 1. Experimental scheme. (A) First-generation plants were grown from stem fragments of stock plants for 20 weeks under three herbivory treatments (present [+], absent [0], or discontinued after 11 weeks of being present [+/0]) crossed with two nutrient treatments (added [+] or not added [0]). (B) Second-generation plants were grown from stem and root fragments taken from first-generation plants after the initial 11 weeks of treatment and subjected for 16 weeks to two of the herbivory treatments (present or absent) crossed with the two nutrient treatments. Sets of first-generation plants were harvested after 11 and 20 weeks (5 replicates per treatment); second-generation plants were harvested after 16 weeks (10 replicates). See text for further explanation.

Pots were randomly assigned to one of four treatment combinations, two soil nutrient treatments (nutrients added or not) crossed with two herbivory treatments (herbivores added or not). For the nutrient addition treatment, 2 g L^{-1} soil of slow-release fertilizer (16 N: 9 P: 12 K: 2 Mg [by weight]; Osmocote Exact Standard 3–4 M, Scotts, Marysville, Ohio, USA) was added to a pot. For the herbivory present treatment, 7 larvae of *P. minor* were released on the leaves of a plant. The number and position of all larvae of *P. minor* were checked daily, and any larvae

that had moved onto plants in the herbivory absent treatment were removed. Very few larvae moved between treatments. In this and all subsequent sets of treatments, pots were randomly arranged and periodically repositioned to minimize possible effects of environmental heterogeneity. Enough tap water was supplied daily to keep the soil moist. Temperature in the greenhouse was 17.5 ± 0.5 °C (mean ± SE), as measured by a Hygrochron temperature logger (iButton DS1923; Maxim Integrated Products, USA). Photosynthetically active radiation at noon was approximately 300 μmol photons m^{-2} s^{-1}, as estimated by measuring lux with a digital light-meter (TES-1339; TES Electrical Electronic Corp., China) and multiplying by a conversion factor of 0.019 as recommended by Environmental Growth Chambers (http://www.egc.com/useful_info_lighting.php).

On 22 November 2014, five randomly chosen replicates were harvested for measurement of number of stem nodes and of dry mass and concentrations of and total nitrogen and nonstructural carbohydrates. Plants, excluding the original node derived from the parent, were divided into leaves, stems, and roots, dried at 70 °C for 48 h, weighed, and finely ground at a frequency of 30 Hz for 5 min with a MM400 Mixer Mill (Retsch GmbH, Haan, Germany). Approximately 500 mg of each sample was extracted in 80% ethanol at 80 °C for 30 min. The extract was then centrifuged at 4000 g for 10 min and the supernatant collected. This process was repeated three times. The three supernatants were then thoroughly mixed, and soluble sugar concentration determined by absorbance at 620 nm in a spectrophotometer. The starch in the pellet was measured using the perchloric acid/anthrone method (Morris, 1948; Luo et al., 2014). Total nonstructural carbohydrate concentration was estimated as the sum of soluble sugar and starch. This method of estimating nonstructural carbohydrates may underestimate actual values due to loss during drying and incomplete extraction of water-soluble carbohydrates, so we interpret these estimates with caution. A second subsample of about 8 mg was placed in an elemental analyzer (Vario EL III; Elementar, Hanau, Germany) to measure total N concentration; this measurement was conducted by the Analytical and Testing Center of the Institute of Botany of the Chinese Academy of Sciences in Beijing.

Ten other replicates were randomly selected to test effects of continuing and discontinuing herbivory on first-generation fragments. In five of the replicates with herbivores present, herbivory was allowed to continue on the fragments already subject to herbivory. In the other five, herbivory was discontinued by removing herbivores. Other treatments continued as before, and plants were kept in the same greenhouse. Air temperature was 14.5 ± 0.2 °C. Plants were harvested on 22 January 2015, and measured for number of new stem nodes and dry mass as described above.

The remaining 20 replicates were used to test effects of the initial 11-week treatments on the subsequent growth of second-generation plants. A 6-cm length of the largest root of each first-generation fragment and the sixth youngest node along the stem of the fragment were each weighed and individually planted in a pot as described. Each stem node had two leaves and 3 cm of proximal and distal stem, similar to the fragments used to grow the first-generation plants. Half of the replicates were then randomly assigned to each of the two nutrient treatments described above, giving 10 replicates per treatment. Treatments lasted from 22 November 2014 to 14 March 2015. Air temperature was 15.4 ± 0.2 °C. At harvest, number of new stem nodes and dry mass were measured as described above. The original mass taken from the first-generation fragments was excluded from analysis.

2.3. Data analysis

Two-way ANOVAs were used to test the effects of herbivory (absent or present, fixed factor) and soil nutrients (added or not, fixed factor) on the concentrations of soluble sugars, starch, total non-structural carbohydrates, and total N in the leaves, stems, and roots of the first-generation fragments and the initial fresh mass of the second-generation root and stem fragments after 11 weeks. Two-way ANOVAs were also used to test effects of herbivory (absent, continued, or discontinued) and nutrients (added or not) on the number of nodes, root to shoot ratio, and final dry leaf, stem, root, and total mass of first-generation fragments after 20 weeks. Three-way ANOVAs were used to test effects of herbivory and nutrient treatments of first-generation fragments and effect of nutrient treatment of second-generation fragments (fixed factor) on the number of nodes, root to shoot ratio, and final dry leaf, stem, and root mass of second-generation plants, separately for plants derived from stem fragments and for plants derived from root fragments. Four-way ANOVAs were added to include derivation of second-generation plants (stem or root fragment) as a factor. Differences between individual means were tested with linear contrasts. Data except for root to shoot ratio were transformed to the natural log before analysis to meet requirements for homoscedasticity and normality. All analyses were conducted using SPSS 22.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. First-generation fragments

3.1.1. Effects of nutrients and herbivory treatments

First-generation fragments showed clear effects of herbivory and nutrient treatments on net accumulation of new dry mass, number of stem nodes, and allocation of mass between roots and shoots after 11 weeks (Fig. 2). Fragments with added nutrients had about twice as much mass and twice as many stem nodes as fragments without added nutrients. Fragments subjected to herbivory had about 30% less total new mass and 25–40% fewer new nodes than fragments without herbivory. There were no significant interactive effects of herbivory and nutrients (Fig. 2).

After 11 weeks of treatment, the concentrations of soluble sugars, starch, and total N in the leaves, stems, and roots of first-generation fragments also showed some significant effects of herbivory and nutrient treatments (Fig. 3). Addition of nutrients decreased concentrations of starch and total non-structural carbohydrates by 25–45% in leaves, stems and roots, and increased concentrations of N by 40–250%, with especially large increases in stems. Effects of herbivory differed between shoots and roots. In leaves and stems, herbivory had neutral to positive effects on concentrations of non-structural carbohydrates, soluble sugars, starch, and N (Fig. 3A–H). In roots, herbivory decreased concentrations of soluble sugars and N, and increased concentration of starch and total non-structural carbohydrates in plants without added nutrients (Fig. 3I–L).

Effects of herbivory and nutrients on the concentrations of N and non-structural carbohydrates and on the mass of first-generation fragments combined to result in large differences in the total amounts of N and carbohydrates contained in fragments given different treatments (Table S3). Fragments given added nutrients contained about four times as much N as those not given added nutrients, and fragments subject to herbivory had about 40% less N than those not subject to herbivory. Total amount of soluble sugars varied only about half as much between treatments as did N but showed a qualitatively similar pattern. Amount of starch per fragment did not show the same pattern. Fragments contained about an order of magnitude more starch than soluble sugars; starch was mostly in roots.

3.1.2. Effects of continued and discontinued herbivory treatments

After 20 weeks of treatment, first-generation fragments with continued herbivory had produced 30–50% less net new total dry mass and 15–40% fewer new stem nodes than plants with no herbivory (Fig. 4). Adding nutrients had increased production of mass by 40–130% and increased production of nodes by 2–4 times. The strength of herbivory effects was roughly similar to that after 11 weeks (Fig. 3), though fragments treated for 20 weeks had about twice as much total mass and

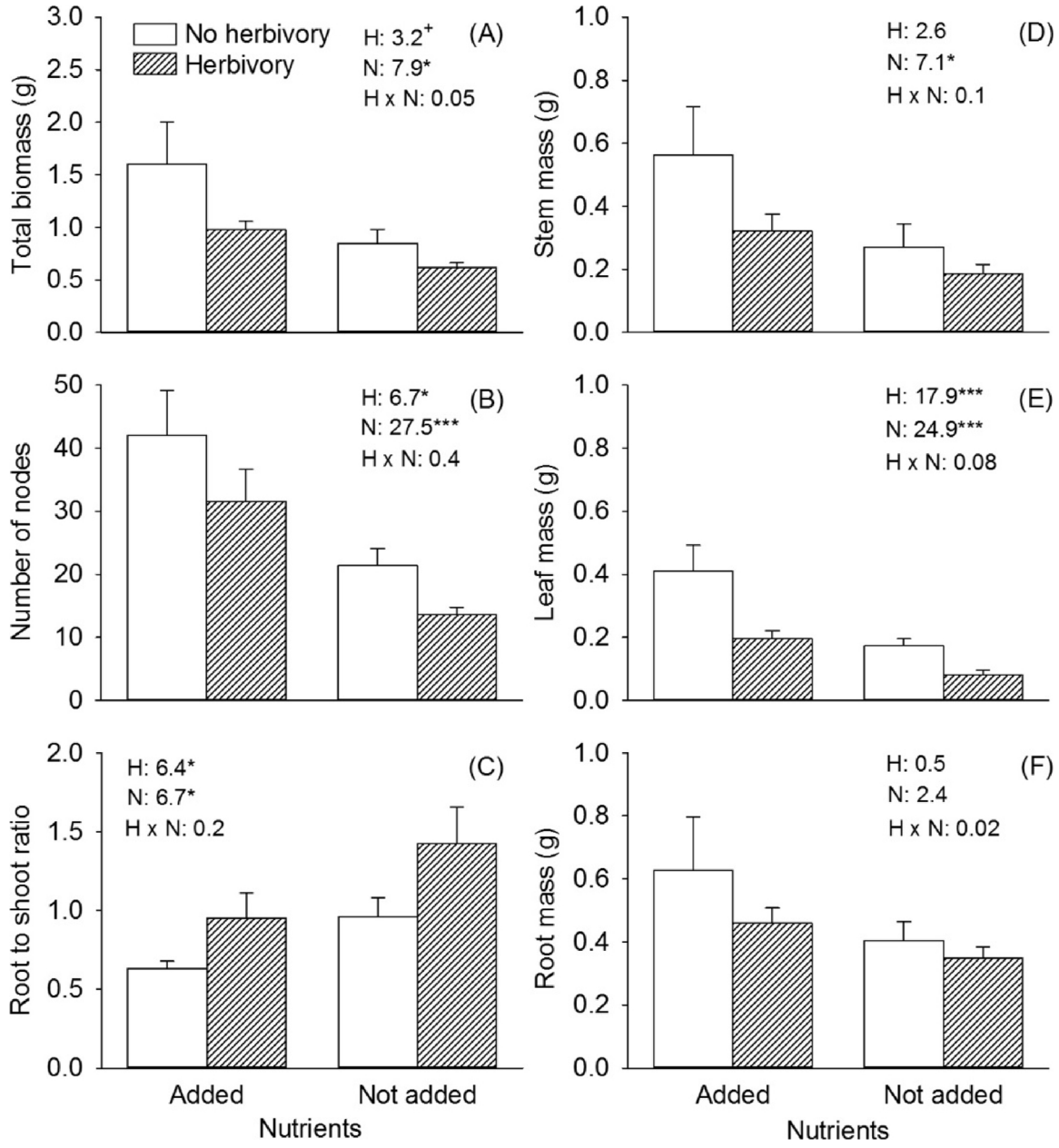

Fig. 2. Effects of herbivory and nutrient treatments on mean (+ SE) final total mass, leaf mass, stem mass, root mass, root to shoot ratio, and number of nodes produced by first-generation plants of *Alternanthera philoxeroides* after 11 weeks. Labels show ANOVAs ($F_{1,16}$) of effects of herbivory (H), nutrients (N), and H × N, with symbols for *P*: no symbol > 0.1; + 0.05-0.1; * 0.01-0.05; ** 0.001-0.01; *** < 0.001.

nodes as those harvested after 11 weeks.

First-generation fragments given added nutrients were able to recover from herbivory, but fragments not given added nutrients were not (Fig. 4). After 20 weeks with added nutrients, total mass and number of nodes were similar in fragments on which herbivory had been discontinued after 11 weeks and in fragments never subject to herbivory. Without added nutrients, mass and size of fragments with discontinued herbivory were little greater than those of plants subject to continued herbivory. Recovery of production after discontinuation of herbivory appeared mainly due to stem and leaf growth; even in plants with added nutrients, there was little difference in root mass between plants with continued and discontinued herbivory, and root to shoot ratio remained low after discontinuation of herbivory.

3.2. Second-generation fragments

3.2.1. Effects of first-generation nutrients and herbivory treatments

The initial fresh mass of second-generation stem fragments was at least 30% greater in those taken from first-generation fragments given added nutrients and no herbivory than in those taken from other first-generation fragments (Fig. 5). Effects of treatments of first-generation fragments on the initial fresh mass of second-generation root fragments were qualitatively similar but smaller.

After 16 weeks of nutrient treatments, second-generation stem fragments showed strong effects of both the first- and the second-generation treatments (Fig. 6, Tables 1 and S2). Total net production of new dry mass was about 5 times greater and net production of new stem nodes was about 4 times greater in second-generation stem fragments given added nutrients than in those not given added nutrients. Production of new mass was also 60–150% higher and production of new

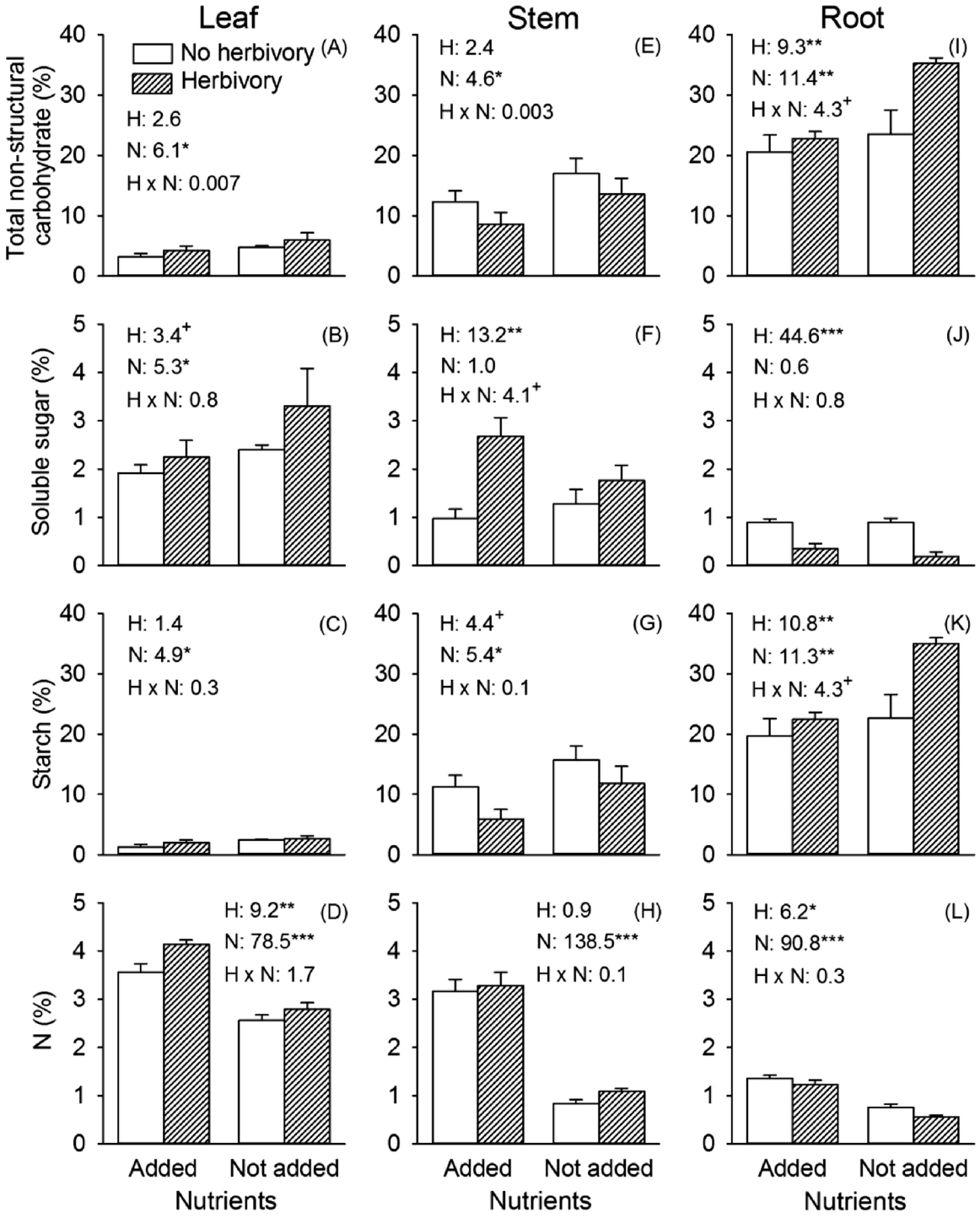

Fig. 3. Effects of herbivory and nutrient treatments on concentrations (mean + SE) of total non-structural carbohydrates, soluble sugars, starch, and N in leaves, stems, and roots of first-generation plants of *Alternanthera philoxeroides* after 11 weeks. Labels show ANOVAs (*F*, with df = 1,16 for stem and for N, and 1,14 for others) of effects of herbivory (H) and nutrients (N), with symbols for *P*: no symbol > 0.1; + 0.05-0.1; * 0.01-0.05; ** 0.001-0.01; *** < 0.001.

nodes 20–100% higher in second-generation fragments taken from first-generation fragments given added nutrients than in those taken from first-generation fragments not given added nutrients. Effects of first-generation nutrient treatments on number of stem nodes and all components of mass were greater in second-generation ramets given added nutrients than in those not given added nutrients.

Effects of first-generation herbivory on the final mass and number of nodes of second-generation stem fragments were relatively small, but mostly qualitatively consistent among measures (Fig. 6). Herbivory on first-generation fragments generally decreased growth of second-generation stem fragments slightly if both first- and second-generation fragments were given added nutrients.

Effects of first- and second-generation treatments on second-generation root fragments (Fig. 7, Tables 1 and S2) differed from effects on second-generation stem fragments (Fig. 6, Tables 1 and S2) in several regards. First, effects of first- and second-generation nutrient treatments on second-generation root fragments did not interact. Second, first-generation herbivory tended to decrease growth of second-generation root fragments when either first- or second-generation fragments were given added nutrients. Third, when neither generation was given added

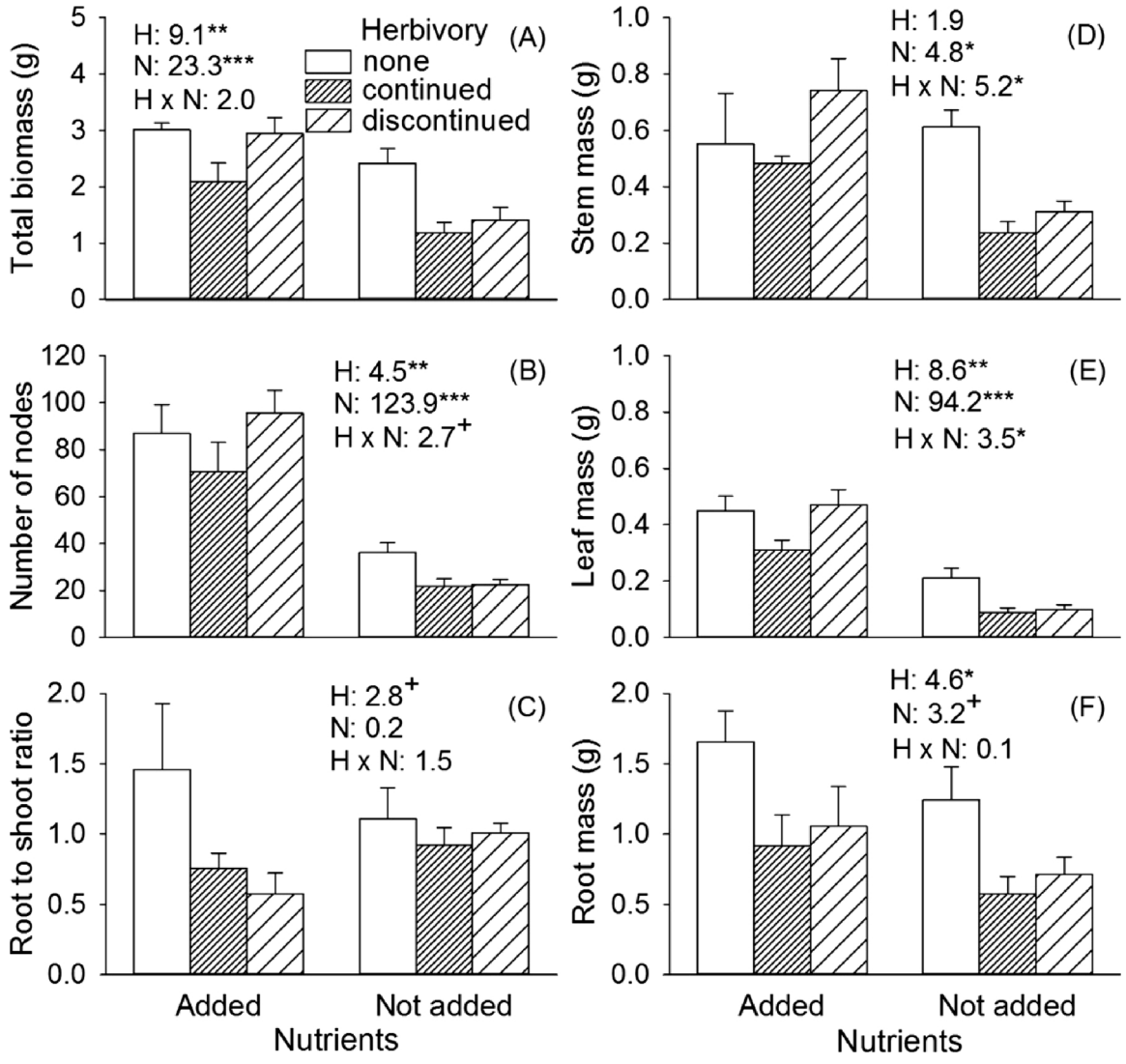

Fig. 4. Effects of added soil nutrients and continued and discontinued herbivory on mean (+ SE) final total mass, leaf mass, stem mass, root mass, root to shoot ratio, and number of nodes produced by first-generation plants of *Alternanthera philoxeroides* after 20 weeks. Labels show ANOVAs (*F*) of effects of herbivory (H, df = 2, 24), nutrients (N, df = 1, 24), and H × N (df = 2, 24), with symbols for *P*: no symbol > 0.1; + 0.05-0.1; * 0.01-0.05; ** 0.001-0.01; *** < 0.001.

nutrients, first-generation herbivory had a small positive effect on the production of new nodes and leaf mass by second-generation root fragments. Fourth, the negative effect of first-generation herbivory on root to shoot ratio persisted in second-generation root fragments.

3.2.2. Correlations between initial provisioning and final growth

Dividing the mean contents of N and non-structural carbohydrates in leaves plus stems (Table S3) by mean number of stem nodes in the first-generation fragments at 11 weeks (Fig. 3) gave a rough estimate of

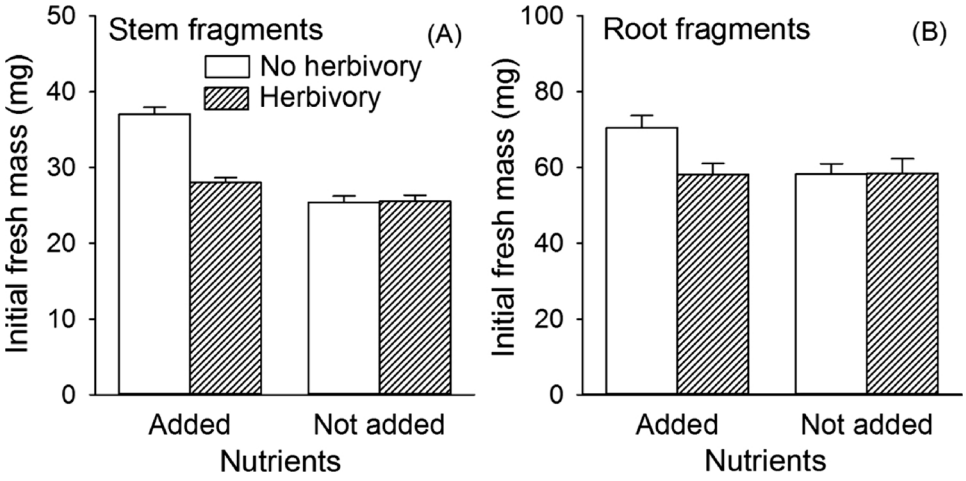

Fig. 5. Effects of first-generation nutrient and herbivory treatments on the initial fresh mass (mean + SE) of stem and root fragments taken from first-generation plants to grow second-generation plants. ANOVA of stem fragments: nutrients – $F_{1,76} = 78.5$, $P < 0.001$; herbivory – $F_{1,76} = 31.3$, $P < 0.001$; interaction – $F_{1,76} = 32.5$, $P < 0.001$. ANOVA of root fragments: nutrients – $F_{1,76} = 3.4$, $P = 0.07$; herbivory – $F_{1,76} = 3.5$, $P = 0.07$; interaction – $F_{1,76} = 3.8$, $P = 0.05$.

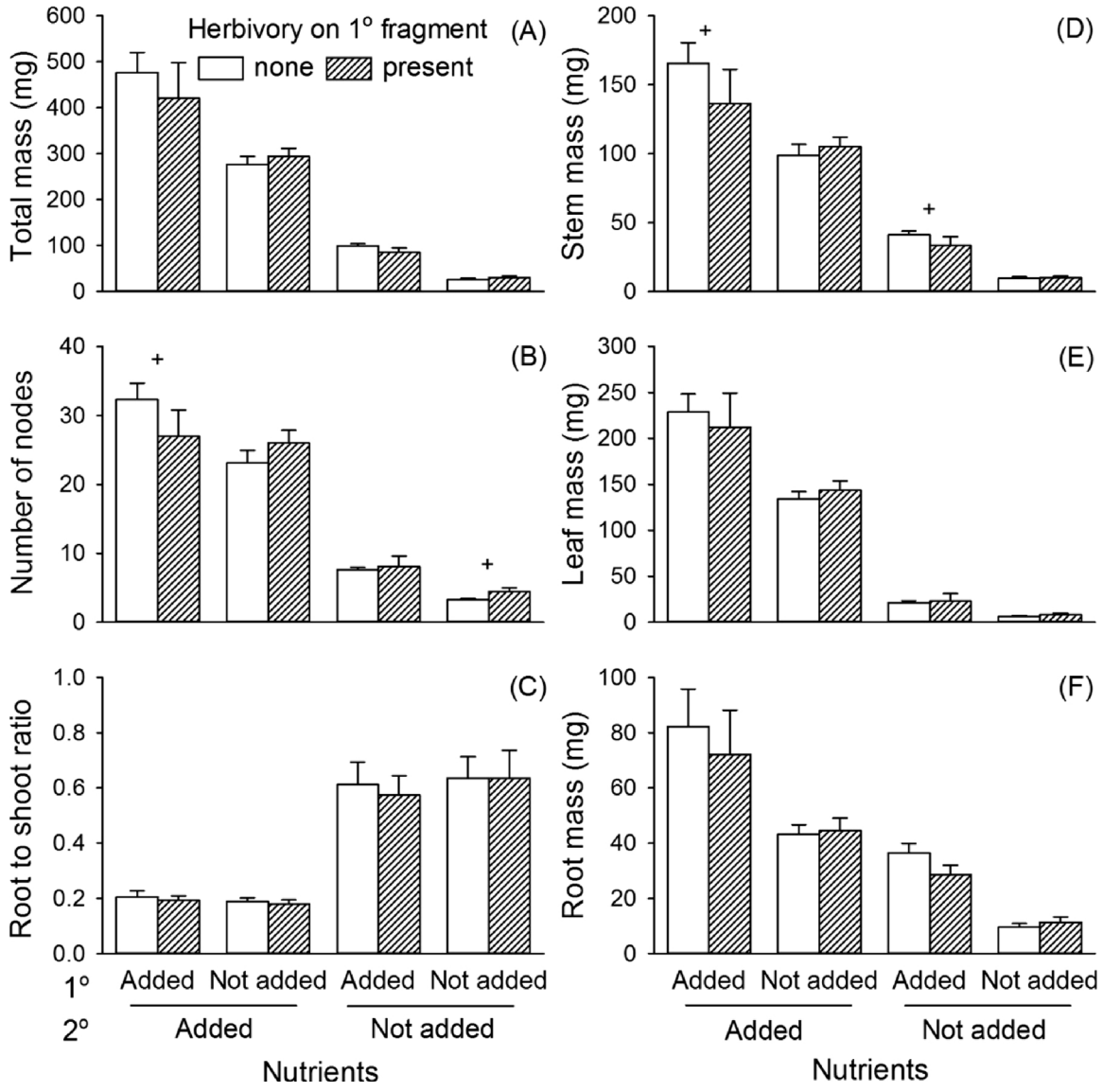

Fig. 6. Effects of herbivory and nutrient treatments of first-generation (1°) plants and nutrient treatments of second-generation (2°) plants on mean (+ SE) final total mass, leaf mass, stem mass, root mass, root to shoot ratio, and number of nodes produced by second-generation plants derived from stem fragments in *Alternanthera philoxeroides*. Symbols show *P* that means did not differ between herbivory treatments within nutrient treatments: no symbol > 0.1; + 0.05-0.1. See Table 1 for ANOVAs.

the provisioning of the second-generation stem fragments in the different first-generation treatments (Table S4). Estimated provisioning with N was about twice as great in treatments with added nutrients as in treatments where nutrients were not added. Within the treatment with added nutrients, provisioning was about one-fourth greater when herbivory was absent than when it was present. Within the treatment with no added nutrients, provisioning with N did not differ between herbivory treatments. These differences were similar to the differences in final mass between second-generation fragments within each second-generation nutrient treatment. For example, within the second-generation treatment with added nutrients (Fig. 6), final total dry mass was about two-thirds greater in second-generation fragments taken from first-generation fragments given added nutrients than from those not given them. Within the first-generation treatment with added nutrients, final mass was about 15% greater when first-generation herbivory had been present than when it had not.

Estimated provisioning with non-structural carbohydrates did not match final mass of second-generation stem fragments as closely as did provisioning with N. Mainly, provisioning with carbohydrates was about two-thirds greater in first-generation treatments without than with added nutrients (Table S4), whereas final mass of second-generation fragments was much lower after first-generation treatments without than with added nutrients (Fig. 6). Provisioning as measured by initial fresh mass of second-generation stem fragments (Fig. 5) also failed to closely match their final mass (Fig. 6). Accordingly, including initial fresh mass as a covariate in ANCOVAs of growth of second-generation fragments (Table S1) changed apparent effects of first-generation treatments only from those in ANOVAs without initial fresh mass as a factor (Table 1), the main change being stronger interactive effects of first-generation herbivory and nutrient treatments on the final dry mass and number of nodes in second-generation stem fragments.

4. Discussion

Results clearly showed that exposure to high resource availability in one generation can increase the performance of the next generation of vegetative offspring in a clonal plant. Second-generation stem and root fragments taken from first-generation clonal fragments given added soil

Table 1
ANOVAs of effects of herbivory and nutrient treatments of first-generation plants and nutrient treatments (2˚ nutrients) of second-generation plants on production of mass and ramets and allocation by second-generation plants derived from stem and root fragments in *Alternanthera philoxeroides*. Values give *F*; symbols show *P*: no symbol > 0.1; + 0.05-0.1; * 0.01-0.05; ** 0.001-0.01; *** < 0.001. Values for which $P <$ 0.05 are in bold. See Figs. 6 and 7 for data.

	Total mass	Number of nodes	Root: shoot	Stem mass	Leaf mass	Root mass
Stem fragments:						
Herbivory (H)	0.9	0.2	0.1	1.7	0.4	1.0
Nutrients (N)	**95.5*****	**34.7*****	0.1	**85.0*****	**41.7*****	**54.7*****
2 ˚Nutrients	**567.6*****	**506.4*****	**97.3*****	**452.6*****	**606.9*****	**107.5*****
H x N	3.8 $^{+}$	**5.9***	0.1	**5.7***	2.0	2.2
H x 2 ˚N	0.13	1.8	0.01	0.01	0.02	0.02
N x 2 ˚N	**28.6*****	**14.9*****	0.5	**33.1*****	**9.1****	**13.5*****
H x N x 2 ˚N	0.005	0.1	0.04	0.01	0.03	0.03
Root fragments:						
Herbivory (H)	**5.7***	0.6	**4.3***	**8.7****	1.2	0.04
Nutrients (N)	**26.0*****	**19.7*****	1.2	0.5	**56.6*****	**33.7*****
2 ˚Nutrients	**140.1*****	**149.6*****	**97.4*****	**95.3*****	**255.3*****	**10.4****
H x N	0.2	**8.9****	0.2	0.1	3.2^{+}	1.9
H x 2 ˚N	3.5^{+}	2.0	1.0	0.3	**7.0****	1.6
N x 2 ˚N	0.1	0.04	0.04	0.02	2.9^{+}	0.001
H x N x 2 ˚N	**4.5***	**7.4****	0.02	3.8^{+}	**5.8***	1.6

nutrients accumulated up to two and half times as much new dry mass and two times as many new stem nodes as those taken from first-generation fragments not given added nutrients. These effects were almost as great as those of the first-generation nutrient treatments on the first-generation fragments themselves. This appears to be one of the few positive, direct tests for positive transgenerational effects via vegetative reproduction in clonal plants, and probably via asexual reproduction in plants more generally (Schwaegerle et al., 2000; González et al., 2017; Dong et al., 2018a, 2019).

The potential ecological significance of positive vegetational transgenerational effects in clonal plants is two-fold. First, fragmentation of clones into single ramets, as simulated here, could combine with these effects to produce vegetative propagules with relatively high fitness. Clonal species in aquatic habitats, including *A. philoxeroides*, may tend to have relatively high rates of fragmentation and to disperse vegetative offspring relatively widely (Barrat-Segretain, 1996; Riis and Sand-Jensen, 2006; Bornette and Puijalon, 2011; Dong et al., 2012). High resource availability in one location could thus promote spread into other locations and, for example, increase the invasiveness of some introduced, aquatic, clonal plant species. Second, transgenerational effects could be an alternative to genetic variation as a source of local acclimation in large, individual clones (Li and Ye, 2006; Latzel and Klimešová, 2010). This need not depend on fragmentation and could interact with ongoing transfers of resources or signals between generations when parents and offspring within clones remain physically connected and physiologically integrated, as is common in clonal plant species (de Kroon and van Groenendael, 1997; Song et al., 2013; Dong et al., 2015).

Vegetative transgenerational effects in *A. philoxeroides* depended on the environment of the offspring. Second-generation stem fragments showed greater positive effects of adding nutrients to first-generation fragments if the second-generation fragments were also given added nutrients than if they were not. Sexually transmitted transgenerational effects in plants can likewise depend on the environment of the offspring (Yang et al., 2015; Groot et al., 2016). One interesting question is whether transgenerational effects tend to be greater when the environment of offspring is more favorable, as found here; or when the environment of offspring is less favorable, as seems equally plausible (Uller et al., 2013). Previous findings on transgenerational effects via seeds provide evidence both for the first (Fenesi et al., 2014; Dechaine et al., 2015) and for the second possibility (Herman et al., 2012). Plants with a high maximum growth rate such as *A. philoxeroides* can become quickly limited by resource availability when resource levels are low, in which case positive transgenerational effects that promote initial growth may also disappear relatively quickly (Engqvist and Reinhold, 2016; Dong et al., 2018a).

Nutrient treatments affected allocation of mass within each generation but caused no transgenerational effects on allocation. First- and second-generation plants each had higher root to shoot ratios when not given added nutrients than when given them, consistent with the commonly observed pattern of increased allocation to organs needed to acquire limiting resources (Thornley, 1972; Bloom et al., 1985; Hilbert, 1990). However, first-generation nutrient treatments did not affect root to shoot ratio in second-generation plants. This may be related to the fact that transgenerational effects can have both benefits and costs. For example, persistence of responses to the parental environment could have negetative effects on offspring when their environment differs from the environment of their parents (Engqvist and Reinhold, 2016; Dong et al., 2018a,b).

Persistence of responses to the parent environment can also have costs when the environment is constant. Negative effects of herbivory on first-generation plants persisted between vegetative generations, though they were smaller in the second-generation than in the first-generation plants. Like effects of added nutrients, transgenerational effects of herbivory depended upon the environment of the offspring. Previous studies of transgenerational effects of herbivory have emphasized effects on defense or resistance (Johansson, 1994; Monro and Poore, 2004; Lu and Ding, 2012; Holeski et al., 2012), but the previous work on *A. philoxeroides* (Dong et al., 2017) indicated that non-structural carbohydrates and N content played a more important role than defensive compounds such as phenols and tannins in transgenerational effects. Previous studies of transgenerational effects of other types of disturbance or stress have variously found negative (Huber et al., 2014) or positive effects (Herman et al., 2012; Castro et al., 2013; Germain et al., 2013; Vivas et al., 2013) on measures related to performance. One possibility is that transgenerational effects of stress via reducing provisioning of offspring may generally be negative, whereas epigenetic effects may often be positive. Reduced provisioning may directly decrease survival and growth and delay development of offspring in *A. philoxeroides* (Dong et al., 2017, 2018a,b, 2019). In contrast, epigenetic modifications may allow clonal offspring to maintain adaptive phenotypes, at least when environmental conditions are relatively constant (González et al., 2016, 2017).

Conditions that permitted recovery from herbivory within the first generation did not eliminate transgenerational effects of herbivory. As measured by final total dry mass, first-generation fragments recovered from herbivory only when given added nutrients, whereas effects of first-generation herbivory on second-generation root fragments

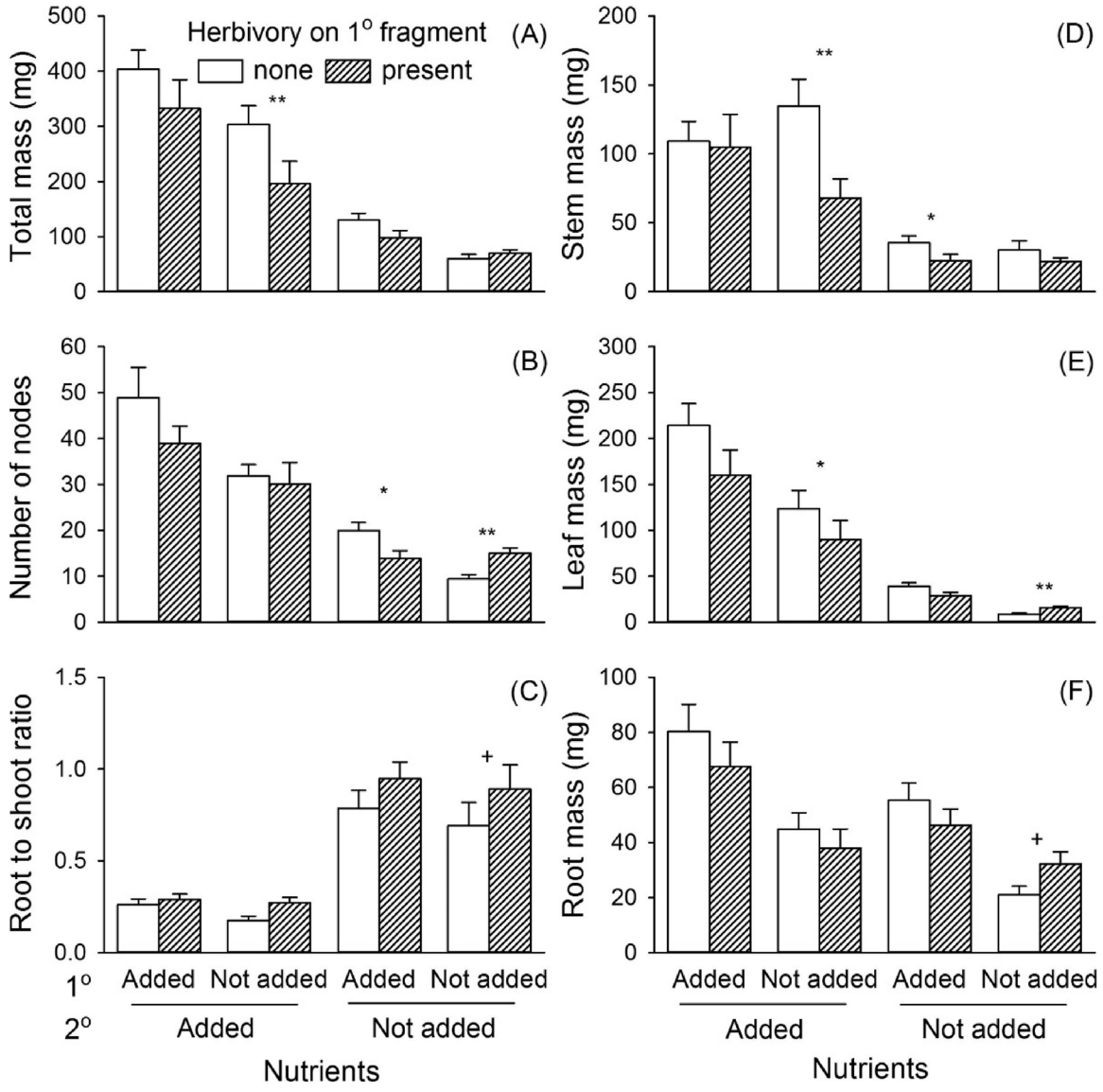

Fig. 7. Effects of herbivory and nutrient treatments of first-generation (1°) plants and nutrient treatments of second-generation (2°) plants on mean (+ SE) final total mass, leaf mass, stem mass, root mass, root to shoot ratio, and number of nodes produced by second-generation plants derived from root fragments in *Alternanthera philoxeroides*. Symbols show *P* that means did not differ between herbivory treatments within nutrient treatments: no symbol > 0.1; + 0.05-0.1; * 0.01-0.05; ** 0.001-0.01. See Table 1 for ANOVAs.

persisted only when second-generation fragments were given added nutrients. There was some parallel between recovery within and between generations in that shoot but not root mass recovered within the first generation and effects of herbivory persisted more in second-generation root than in second-generation stem fragments. There appear to be no previous studies that compare recovery from disturbance or stress within and between generations.

Different clonal plant species reproduce vegetatively via different organs (Klimešová and de Bello, 2009; Herben and Klimešová, 2015), and some species reproduce by more than one organ (Sosnová et al., 2010; Engelhardt et al., 2014). Results clearly showed that transgenerational effects in *A. philoxeroides* differed depending on whether second-generation plants originated from stem or from root fragments of first-generation plants. Second-generation plants derived from stem fragments showed stronger effects of first-generation nutrient treatments than those derived from root fragments. The reverse was true for effects of first-generation herbivory treatments. Since all fragments were likely derived from a single clone, this indicates that transgenerational effects via vegetative reproduction within the same genotype can differ between vegetative offspring produced from different organs. One plausible explanation is that effects of the parental environment on provisioning may differ between different organs, as was found in the case of stems and roots in this study. Differential transgenerational effects on offspring derived from different organs could also be due to differences in concentrations of secondary metabolites such as tannins and phenolics (Dong et al., 2017), hormonal effects (Hisano et al., 2016), or epigenetic effects on early development (Aceituno et al., 2008).

Results provide evidence that provisioning with N can be a mechanism for vegetatively transmitted transgenerational effects. Relative differences in the estimated, initial content of N in second-generation stem fragments taken from different first-generation treatments closely matched the relative differences in the final total dry mass of these fragments within second-generation treatments. Differences in initial content of non-structural carbohydrates or in initial total fresh mass of second-generation fragments did not match their final dry mass as closely. In contrast, Germain et al. (2013) found that increased performance of seeds from drought-stressed parents was more related to

seed mass than to N content. Zas et al. (2013) similarly concluded that transgenerational effects on the dry mass of seedlings of a pine were mostly explained by provisioning as measured by seed mass. Provisioning as a mechanism for transgenerational effects can probably itself either be due simply to resource supply to the parent or to epigenetic regulation of allocation (Herman and Sultan, 2011; Zas et al., 2013), and disentangling these effects could be challenging.

In sum, results here show that transgenerational effects can be transmitted via vegetative reproduction in plants independently of continued physiological integration through maintenance of physical connections. Moreover, these effects can depend upon the environment of the offspring and are sometimes greater when the environment is more favorable. Effects can be positive or negative and can be induced by resource availability or by disturbance. Multiple effects can interact, and conditions that reverse intragenerational effects do not necessarily reverse transgenerational effects. Effects can differ depending on from which organ an offspring is produced. In future studies it could be interesting to compare differences in transgenerational effects transmitted via provisioning and physiological integration.

Transgenerational effects transmitted via sexual reproduction in plants are known to play a wide range of ecologically important roles. Such effects can increase tolerance of stress (Herman et al., 2012; Castro et al., 2013; Germain et al., 2013; Vu et al., 2015) and of disturbances such as herbivory (Holeski et al., 2012), increase ecological processes such as productivity (Latzel et al., 2013), and enhance evolutionary potential (Dechaine et al., 2015). Results here provide an initial indication that transgenerational effects in clonally produced plants could have a similarly pervasive influence on their ecology.

Funding

This work was supported by grants from the National Natural Science Foundation of China (Nos. 31500331, 31670428) and the Fundamental Research Funds for the Central Universities (2015ZCQ-BH-01).

Acknowledgements

We are grateful to Zhi-Wen Ma for assistance with the experiment.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ppees.2019.125498.

References

Aceituno, F.F., Moseyko, N., Rhee, S.Y., Gutiérrez, R.A., 2008. The rules of gene expression in plants: organ identity and gene body methylation are key factors for regulation of gene expression in *Arabidopsis thaliana*. BMC Genomics 9, 438.
Agrawal, A.A., 2002. Herbivory and maternal effects: mechanisms and consequences of transgenerational induced plant resistance. Ecology 83, 3408–3415.
Barrat-Segretain, M.H., 1996. Strategies of reproduction, dispersion, and competition in river plants: a review. Vegetatio 123, 13–37.
Bloom, A.J., Chapin, F.S., Mooney, H.A., 1985. Resource limitation in plants-an economic analogy. Annu. Rev. Ecol. Syst. 16, 363–392.
Bornette, G., Puijalon, S., 2011. Response of aquatic plants to abiotic factors: a review. Aquat. Sci. 73, 1–14.
Castro, B.M., Moriuchi, K.S., Friesen, M.L., Badri, M., Nuzhdin, S.V., Strauss, S.Y., Cook, D.R., von Wettberg, E., 2013. Parental environments and interactions with conspecifics alter salinity tolerance of offspring in the annual *Medicago truncatula*. J. Ecol. 101, 1281–1287.
Cortijo, S., Wardenaar, R., Colomé tatché, M., Gilly, A., Etcheverry, M., Labadie, K., Caillieux, E., Hospital, F., Aury, J.M., Wincker, P., 2014. Mapping the epigenetic basis of complex traits. Science 343, 1145–1148.
Cox, J.M., 1989. The mealybug genus *Planococcus* (Homoptera: Pseudococcidae). *Bulletin of the British Museum* (*Natural History*). Entomology 58, 1–78.
Dechaine, J.M., Brock, M.T., Weinig, C., 2015. Maternal environmental effects of competition influence evolutionary potential in rapeseed (*Brassica rapa*). Evol. Ecol. 29, 77–91.
de Kroon, H., van Groenendael, J., 1997. The Ecology and Evolution of Clonal Growth in Plants. Backhuys Publishers, Leiden.
Dong, B.C., Alpert, P., Guo, W., Yu, F.H., 2012. Effects of fragmentation on the survival and growth of the invasive, clonal plant *Alternanthera philoxeroides*. Biol. Invasions 14, 1101–1110.
Dong, B.C., Alpert, P., Zhang, Q., Yu, F.H., 2015. Clonal integration in homogeneous environments increases performance of *Alternanthera philoxeroides*. Oecologia 179, 393–400.
Dong, B.C., Fu, T., Luo, F.L., Yu, F.H., 2017. Herbivory-induced maternal effects on growth and defense traits in the clonal species *Alternanthera philoxeroides*. Sci. Total Environ. 605–606, 114–123.
Dong, B.C., van Kleunen, M., Yu, F.H., 2018a. Context-dependent parental effects on clonal offspring performance. Front. Plant Sci. 9, 1824.
Dong, B.C., Meng, J., Yu, F.H., 2019. Effects of parental light environment on growth and morphological responses of clonal offspring. Plant Biol. 21, 1083–1089.
Dong, B.C., Wang, M.Z., Liu, R.H., Luo, F.L., Li, H.L., Yu, F.H., 2018b. Direct and legacy effects of herbivory on growth and physiology of a clonal plant. Biol. Invasions 20, 3631–3645.
Dong, B.C., Yu, G.L., Guo, W., Zhang, M.X., Dong, M., Yu, F.H., 2010. How do internode length, position and presence of leaves affect survival and growth of *Alternanthera philoxeroides* after fragmentation? Evol. Ecol. 24, 1447–1461.
Engqvist, L., Reinhold, K., 2016. Adaptive trans-generational phenotypic plasticity and the lack of an experimental control in reciprocal match/mismatch experiments. Methods Ecol. Evol. 7, 1482–1488.
Engelhardt, K.A.M., Lloyd, M.W., Neel, M.C., 2014. Effects of genetic diversity on conservation and restoration potential at individual, population, and regional scales. Biol. Conserv. 179, 6–16.
Fenesi, A., Dyer, A.R., Gered, J., Sandor, D., Ruprecht, E., 2014. Can transgenerational plasticity contribute to the invasion success of annual plant species? Oecologia 176, 95–106.
Francis, A.W., Kairo, M.T., Roda, A.L., Liburd, O.E., Polar, P., 2012. The passionvine mealybug, *Planococcus minor* (Maskell) (Hemiptera: Pseudococcidae), and its natural enemies in the cocoa agroecosystem in Trinidad. Bio. Control 60, 290–296.
Galloway, L.F., Etterson, J.R., 2007. Transgenerational plasticity is adaptive in the wild. Science 318, 1134–1136.
Gao, L., Geng, Y., Li, B., Chen, J., Yang, J., 2010. Genome-wide DNA methylation alterations of *Alternanthera philoxeroides* in natural and manipulated habitats: implications for epigenetic regulation of rapid responses to environmental fluctuation and phenotypic variation. Plant Cell Environ. 33, 1820–1827.
Germain, R.M., Caruso, C.M., Maherali, H., 2013. Mechanisms and consequences of water stress-induced parental effects in an invasive annual grass. Int. J. Plant Sci. 174, 886–895.
González, A.P.R., Chrtek, J., Dobrev, P.I., Dumalasová, V., Fehrer, J., Mráz, P., Latzel, V., 2016. Stress-induced memory alters growth of clonal offspring of white clover (*Trifolium repens*). Am. J. Bot. 103, 1567–1574.
González, A.P.R., Dumalasová, V., Rosenthal, J., Skuhrovec, J., Latzel, V., 2017. The role of transgenerational effects in adaptation of clonal offspring of white clover (*Trifolium repens*) to drought and herbivory. Evol. Ecol. 31, 345–361.
Groot, M.P., Kooke, R., Knoben, N., Vergeer, P., Keurentjes, J.J.B., Ouborg, N.J., Verhoeven, K.J.F., 2016. Effects of multi-generational stress exposure and offspring environment on the expression and persistence of transgenerational effects in *Arabidopsis thaliana*. PLoS One 11, e0151566.
Guarino, F., Cicatelli, A., Brundu, G., Heinze, B., Castiglione, S., 2015. Epigenetic diversity of clonal white poplar (*Populus alba* L.) populations: could methylation support the success of vegetative reproduction strategy? PLoS One 10, e0131480.
Herben, T., Klimešová, J., 2015. Clonal and bud bank traits: patterns across temperate plant communities. J. Veg. Sci. 26, 243–253.
Herman, J.J., Sultan, S.E., 2011. Adaptive transgenerational plasticity in plants: case studies, mechanisms, and implications for natural populations. Front. Plant Sci. 2, 102.
Herman, J.J., Sultan, S.E., Horgan-Kobelski, T., Riggs, C., 2012. Adaptive transgenerational plasticity in an annual plant: grandparental and parental drought stress enhance performance of seedlings in dry soil. Integr. Comp. Biol. 52, 77–88.
Hilbert, D.W., 1990. Optimization of plant root:shoot ratios and internal nitrogen concentration. Ann. Bot. 66, 91–99.
Hisano, H., Matsuura, T., Mori, I.C., Yamane, M., Sato, K., 2016. Endogenous hormone levels affect the regeneration ability of callus derived from different organs in barley. Plant Physiol. Biochem. 99, 66–72.
Holeski, L.M., Jander, G., Agrawal, A.A., 2012. Transgenerational defense induction and epigenetic inheritance in plants. Trends Ecol. Evol. 27, 618–626.
Holm, L.G., Doll, J., Holm, E., Pancho, J., Herberger, J., 1997. World Weeds: Natural Histories and Distribution. John Wiley and Sons, NY.
Huber, H., Visser, E.J.W., Clements, G., Peters, J.L., 2014. Flooding and fragment size interact to determine survival and regrowth after fragmentation in two stoloniferous *Trifolium* species. AoB Plants 6 plu024.
Jablonka, E., Raz, G., 2009. Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. Q. Rev. Biol. 84, 131–176.
Johansson, M.E., 1994. Life history differences between central and marginal populations of the clonal aquatic plant *Ranunculus lingua*: a reciprocal transplant experiment. Oikos 70, 65–72.
Julien, M., Sosa, A., Chan, R., Schooler, S., Traversa, G., 2012. *Alternanthera philoxeroides* (Martius) Grisebach – alligator weed, *Alternanthera philoxeroides* (Martius) Grisebach – alligator weed. In: Julien, M., McFadyen, R., Cullen, J. (Eds.), Biological Control of Weeds in Australia. CSIRO Publishing, Collingwood, pp. 43–51.
Keiser, C.N., Mondor, E.B., 2013. Transgenerational behavioral plasticity in a parthenogenetic insect in response to increased predation risk. J. Insect Behav. 26, 603–613.

Klimešová, J., de Bello, F., 2009. CLO-PLA: the database of clonal bud bank traits of Central European flora. J. Veg. Sci. 20, 511–516.
Latzel, V., Allan, E., Silveira, A.B., Colot, V., Fischer, M., Bossdorf, O., 2013. Epigenetic diversity increases the productivity and stability of plant populations. Nat. Commun. 4, 2875.
Latzel, V., González, A.P.R., Rosenthal, J., 2016. Epigenetic memory as a basis for intelligent behavior in clonal plants. Front. Plant Sci. 7, 1354.
Latzel, V., Janeček, Š., Dolezal, J., Klimešová, J., Bossdorf, O., 2014. Adaptive transgenerational plasticity in the perennial *Plantago lanceolata*. Oikos 123, 41–46.
Latzel, V., Hájek, T., Klimešová, J., Gómez, S., 2009. Nutrients and disturbance history in two *Plantago* species: maternal effects as a clue for observed dichotomy between resprouting and seeding strategies. Oikos 118, 1669–1678.
Latzel, V., Klimešová, J., 2009. Fitness of resprouters versus seeders in relation to nutrient availability in two *Plantago* species. Acta Oecol. 35, 541–547.
Latzel, V., Klimešová, J., 2010. Transgenerational plasticity in clonal plants. Evol. Ecol. 24, 1537–1543.
Latzel, V., Münzbergová, Z., 2018. Anticipatory behavior of the clonal plant *Fragaria vesca*. Front. Plant Sci. 9. https://doi.org/10.3389/fpls.2018.01847.
Li, J., Ye, W.H., 2006. Genetic diversity of alligator weed ecotypes is not the reason for their different responses to biological control. Aquat. Bot. 85, 155–158.
Lu, X.M., Ding, J.Q., 2012. History of exposure to herbivores increases the compensatory ability of an invasive plant. Biol. Invasions 14, 649–658.
Lu, X.M., Siemann, E., Shao, X., Wei, H., Ding, J.Q., 2013. Climate warming affects biological invasions by shifting interactions of plants and herbivores. Glob. Change Biol. 19, 2339–2347.
Luo, F.L., Chen, Y., Huang, L., Wang, A., Zhang, M.X., Yu, F.H., 2014. Shifting effects of physiological integration on performance of a clonal plant during submergence and de-submergence. Ann. Bot. 113, 1265–1274.
McKey, D., Elias, M., Pujol, B., Duputie, A., 2010. The evolutionary ecology of clonally propagated domesticated plants. New Phytol. 186, 318–332.
Monro, K., Poore, A.G.B., 2004. Selection in modular organisms: is intraclonal variation in macroalgae evolutionarily important? Am. Nat. 163, 564–578.
McIntyre, P.J., Strauss, S.Y., 2014. Phenotypic and transgenerational plasticity promote local adaptation to sun and shade environments. Evol. Ecol. 28, 229–246.
Morris, D.L., 1948. Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Science 107, 254–255.
Pan, X.Y., Geng, Y.P., Zhang, W.J., Li, B., Chen, J.K., 2006. The influence of abiotic stress and phenotypic plasticity on the distribution of invasive *Alternanthera philoxeroides* along a riparian zone. Acta Oecol. 30, 333–341.
Prentis, P.J., Wilson, J.R.U., Dormontt, E.E., Richardson, D.M., Lowe, A.J., 2008. Adaptive evolution in invasive species. Trends Plant Sci. 13, 288–294.
Pujalon, S., Piola, F., Bornette, G., 2008. Abiotic stresses increase plant regeneration ability. Evol. Ecol. 22, 493–506.
Richards, C.L., Schrey, A.W., Pigliucci, M., 2012. Invasion of diverse habitats by few Japanese knotweed genotypes is correlated with epigenetic differentiation. Ecol. Lett. 15, 1016–1025.
Richter-Boix, A., Orizaola, G., Laurila, A., 2014. Transgenerational phenotypic plasticity links breeding phenology with offspring life-history. Ecology 95, 2715–2722.
Riis, T., Sand-Jensen, K., 2006. Dispersal of plant fragments in small streams. Freshw. Rev. 51, 274–286.
Roda, A., Francis, A., Kairo, M.T., Culik, M., 2013. *Planococcus minor* (Hemiptera: Pseudococcidae): bioecology, survey and mitigation strategies, *Planococcus minor* (Hemiptera: Pseudococcidae): bioecology, survey and mitigation strategies. In: Pena, J.E. (Ed.), Potential Invasive Pests of Agricultural Crops. CABI publishing, UK, pp. 288.
Schwaegerle, K.E., McIntyre, H., Swingley, C., 2000. Quantitative genetics and the persistence of environmental effects in clonally propagated organisms. Evolution 54, 452–461.
Sainty, G., McCorkelle, G., Julien, M., 1998. Control and spread of alligator weed *Alternanthera philoxeroides* (Mart.) Griseb., in Australia: lessons for other regions. Wetl. Ecol. Manage. 5, 195–201.
Shama, L.N.S., Wegner, K.M., 2014. Grandparental effects in marine sticklebacks: transgenerational plasticity across multiple generations. J. Evol. Biol. 27, 2297–2307.
Shi, W., Chen, X.J., Gao, L.X., Xu, C.Y., Qu, X.K., Bossdorf, O., Yang, J., Geng, Y.P., 2018. Transient stability of epigenetic population differentiation in a clonal invader. Front. Plant Sci. 9, 1851.
Sosnová, M., van Diggelen, R., Klimešová, J., 2010. Distribution of clonal growth forms in wetlands. Aquat. Bot. 92, 33–39.
Song, Y.B., Yu, F.H., Keser, L.H., Dawson, W., Fischer, M., Dong, M., van Kleunen, M., 2013. United we stand, divided we fall: a meta-analysis of experiments on clonal integration and its relationship to invasiveness. Oecologia 171, 317–327.
Thornley, J.H.M., 1972. A balanced quantitative model for root:shoot ratios in vegetative plants. Ann. Bot. 36, 431–441.
Uller, T., Nakagawa, S., English, S., 2013. Weak evidence for anticipatory parental effects in plants and animals. J. Evol. Biol. 26, 2161–2170.
Venette, R., Davis, E., 2004. Mini risk assessment – passionvine mealybug: *Planococcus minor* (Maskell) [Pseudococcidae: Hemiptera]. National Cooperative Agricultural Pest Survey (CAPS) Target Pests CAPS PRA: *Planococcus minor*.
Vivas, M., Zas, R., Sampedro, L., Solla, A., 2013. Environmental maternal effects mediate the resistance of maritime pine to biotic stress. PLoS One 8, e70148.
Vu, W.T., Chang, P.L., Moriuchi, K.S., Friesen, M.L., 2015. Genetic variation of transgenerational plasticity of offspring germination in response to salinity stress and the seed transcriptome of *Medicago truncatula*. BMC Evol. Biol. 15, 59.
Walsh, M.R., Whittington, D., Funkhouser, C., 2014. Thermal transgenerational plasticity in natural populations of Daphnia. Integr. Comp. Biol. 54, 822–829.
Williams, D.J., de Willink, M.C.G., 1992. Mealybugs of Central and South America. CAB International, London.
Wolf, J.B., Wade, M.J., 2009. What are maternal effects (and what are they not)? Philos. Trans. R. Soc. B: Biol. Sci. 364, 1107–1115.
Wu, Z.Y., Raven, P.H., 2003. Flora of China Vol. 5 Science Press and Missouri Botanical Garden Press, Beijing, China, and St. Louis, Missouri, USA.
Xu, C.Y., Zhang, W.J., Fu, C.Z., Lu, B.R., 2003. Genetic diversity of alligator weed in China by RAPD analysis. Biodivers. Conserv. 12, 637–645.
Yang, F., Yang, X.J., Baskin, J.M., Baskin, C.C., Cao, D.C., Huang, Z.Y., 2015. Transgenerational plasticity provides ecological diversity for a seed heteromorphic species in response to environmental heterogeneity. Perspect. Plant Ecol. 17, 201–208.
Ye, W.H., Li, J., Cao, H.L., Ge, X.J., 2003. Genetic uniformity of *Alternanthera philoxeroides* in South China. Weed Res. 43, 297–302.
Zas, R., Cendan, C., Sampedro, L., 2013. Mediation of seed provisioning in the transmission of environmental maternal effects in maritime pine (*Pinus pinaster* Aiton). Heredity 111, 248–255.
Zhang, Y.Y., Fischer, M., Colot, V., Bossdorf, O., 2013. Epigenetic variation creates potential for evolution of plant phenotypic plasticity. New Phytol. 197, 314–322.
Zhang, Y.Y., Zhang, D.Y., Barrett, S.C.H., 2010. Genetic uniformity characterizes the invasive spread of water hyacinth (*Eichhornia crassipes*), a clonal aquatic plant. Mol. Biol. 19, 1774–1786.