Am. Midl. Nat. (2022) 187:14–28

Impacts of Herbivory on Photosynthesis of Four Common Wisconsin Plant Species

NATHAN P. LEMOINE[1] AND MICHELLE L. BUDNY

Department of Biological Sciences, Marquette University, Milwaukee, Wisconsin 53201

ABSTRACT.—Overcompensation to herbivory is prevalent among plant species. However, we do not yet fully understand why plant species vary in their compensatory abilities. It is highly likely that overcompensation is determined by the ability of plants to elevate photosynthesis in response to herbivory, which is dictated by evolutionary exposure to grazing. Here, we tested the hypothesis that photosynthetic overcompensation should be predictable based on plant life form by simulating herbivore damage on four plant species: two common range grasses with long evolutionary exposure to grazing (*Andropogon gerardii*, *Bouteloua curtipendula*) and two common understory forbs that are resistant to, and therefore experience little, grazing (*Alliaria petiolata*, *Symplocarpus foetidus*). We measured leaf-level gas exchange in a high-resolution time series that extended throughout the growing season. We found no evidence of photosynthetic compensation for three of the four plant species. Interestingly, only *A. petiolata*, a highly invasive species, demonstrated increased photosynthesis and stomatal conductance following clipping. Further, the effects were short-lived, as both photosynthesis and stomatal conductance returned to baseline levels within 24 h. Our results suggest that elevated photosynthesis to herbivory might not be a general mechanism by which plants either resist or tolerate herbivory.

INTRODUCTION

Although commonly perceived as strictly negative, plant-herbivore interactions actually exist along a continuum from harmful to beneficial (McNaughton, 1983; Belsky, 1986). Beneficial interactions, wherein herbivory increases plant fitness (*i.e.*, overcompensation; Belsky, 1986; Tiffin, 2000), are less common than negative interactions, but still appear to be fairly prevalent. In some grasslands, mammalian herbivores stimulated aboveground and belowground primary production by promoting tillering and vegetative reproduction of dominant grasses (Milchunas and Lauenroth, 1993; Belsky, 1996; Frank *et al.*, 2002). Likewise, browsing releases some forbs from apical meristem dominance, enabling plants to produce several times more flowering stalks and fruits and seeds than unbrowsed plants (Paige and Whitham, 1987). Although it might appear overcompensation is restricted to mammalian herbivory, a recent meta-analysis identified 67 plant species, including both natural and agricultural species, that exhibit some degree of overcompensation following insect damage (Garcia and Eubanks, 2019) Some researchers now advocate using insect herbivores as a means to increase the marketable yield of crops (Poveda *et al.*, 2013, 2018). Therefore, overcompensation is an important and potentially widespread aspect of plant-herbivore interactions, but we do not yet fully understand the extent to which plant species vary in compensatory abilities.

Compensatory ability appears to vary with both grazer type and environmental context. In many cases, natural or simulated mammalian grazing can promote biomass growth, fruit production, or seed production. For example, simulated grazing increased fruit production, seed biomass, and root growth in *Sedum maximum* (Olejniczak, 2011). Simulated mammal

[1] Corresponding author: Department of Zoology, Milwaukee Public Museum, Wisconsin (zip code); E-mail:nathan.lemoine@marquette.edu

and insect damage also increased extrafloral nectary numbers in *Prunus avium* seedlings (Pulice and Packer, 2008). However, overcompensatory ability appears to depend on adequate access to resources. In eight North American prairie species, mycorrhizal inoculation was required for plants to compensate for grasshopper folivory (Kula *et al*., 2005). In *Leymus chinensis*, simulated herbivory increased aboveground biomass only under adequate water (Zhao *et al*., 2008). Importantly, as noted by Delaney (2008), most studies of overcompensation examine whole-plant responses, and there are considerably fewer tests of overcompensation at the physiological (*i.e.*, gas exchange) level.

Compensatory ability at the organism level is likely dictated, at least in part, by the impact of herbivory on photosynthesis (P_N). Given P_N drives both growth (Poorter *et al*., 1990) and production of inducible defenses (Zangerl *et al*., 1997), it is likely that the response of P_N to herbivory controls how effectively a plant compensates for tissue loss. The most widely observed mechanism of herbivory compensation is an increase in P_N on a per-area basis (Tiffin, 2000). Mechanisms for elevated P_N following herbivory vary, and include increased light availability following removal of upper canopy leaves (Anten and Ackerly, 2001), altered carbon source-sink dynamics (Retuerto *et al*., 2004), or elevated synthesis of RuBP and PEP carboxylase enzymes in undamaged tissues (Wareing *et al*., 1968). Regardless of the specific mechanism, P_N does not increase following herbivory in all species. For example, *Pascopyrum smithii* increased P_N by 10–20% following herbivory (Painter and Detling, 1981), as did *Agropyron cristatum* (Hamerlynck *et al*., 2016) and *Oenothera biennis* (Morrison and Reekie, 1995). Yet some species showed little to no effect of herbivory on P_N, such as *Bouteloua gracilis (*Detling *et al., 1979)*, *Agropyron spicatum (*Caldwell *et al., 1981)*, and *Pseudoroegnaria spicata (*Hamerlynck *et al., 2016)*. Notably, these contrasting results include two species within the genus *Agropyron* that exhibit very different physiological responses to herbivory. Clearly coexisting species vary in their ability to compensate for herbivory, and even congeners might not respond similarly to herbivory. We still lack information needed to develop a clear picture of how herbivory affects P_N in geographically widespread species.

Interspecific variability in overcompensation might arise from one of several, non-mutually exclusive factors. First, plants adopt different evolutionary strategies to maximize fitness in the presence of herbivores. Generally, plants can either resist or tolerate herbivory (Strauss and Agrawal, 1999), and the tradeoff between these two life history strategies might explain why some species exhibit elevated P_N following herbivory and others do not (Coley *et al*., 1985; de la Mata *et al*., 2017). In this context, palatable species with few defenses likely evolved the ability to tolerate herbivory via rapid regrowth, whereas unpalatable species that invest heavily in chemical and constitutive defenses might lack the spare resources needed to rapidly regrow following defoliation. Alternatively, plant species that evolved under intense grazing pressure typically exhibit larger P_N overcompensation than do congeners from regions with little historical grazing pressure (Nowak and Caldwell, 1984; Doescher *et al*., 1997; Hamerlynck *et al*., 2016). Therefore, evolutionary grazing exposure might explain interspecific variability in rates of P_N following herbivory. Finally, many studies of herbivory-induced changes in gas exchange use pot or hydroponic experiments. Field experiments often do not reproduce the same patterns observed in lab experiments (Nowak and Caldwell, 1984; Ashton and Lerdau, 2008), likely because abiotic conditions like soil moisture or nutrient availability constrain the ability of plants to exhibit P_N overcompensation (Zhao *et al*., 2008). Further, most compensation studies examine one species, and there are few field experiments that assess P_N overcompensation of multiple species simultaneously (Ramula *et al*., 2019).

We hypothesized that common species of Wisconsin would exhibit high interspecific variation in P_N following herbivory, and that these differences should be qualitatively related to species' ability to resist grazing. Specifically, we predicted unpalatable forest understory species would be less responsive than palatable prairie species. To test this hypothesis, we used field-based measurements of foliar gas exchange to quantify P_N overcompensation for two species in each category. Unpalatable, herbivory-resistant species were represented by *Alliaria petiolata* and *Symplocarpus foetidus.* Both of these species possess high concentrations of secondary chemical defenses. Inducible defenses in *A. petiolata* consist of glucosinolates (Cipollini *et al.*, 2005), and *S. foetidus* foliage contains constitutive defenses in the form of calcium oxalate crystals (Coté and Gibernau, 2012). As a result of these chemical defenses, both *A. petiolata* and *S. foetidus* are generally unpalatable to most mammals in North America (Averill *et al.*, 2016). The palatable species consisted of the C_4 grasses *Andropogon gerardii* and *Bouteloua curtipendula.* Both grass species are widely distributed throughout the plains and prairies of North America. Range grasses of North America typically lack strong chemical defenses (Mole and Joern, 1994) and also exhibit strong P_N plasticity following herbivory (Painter and Detling, 1981; Ingham and Detling, 1986; Doescher *et al.*, 1997; Hamerlynck *et al.*, 2016). For each species, we simulated herbivory via clipping and measured P_N (μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), and water-use efficiency (WUE, μmol CO_2 mol H_2O^{-1} m^{-2} s^{-1}) throughout a high-resolution time series, ranging from immediately following clipping through 3 wk of recovery. We expected that the palatable grasses would demonstrate rapid and sustained increases in P_N. We suspected increases in P_N would not be linked to higher photosynthetic efficiency (*i.e.*, WUE), because increased P_N would require higher g_s. If true, then g_s should increase, which would yield a constant WUE throughout the growing season.

METHODS

Study site and species.—We conducted our experiment at the University of Wisconsin–Milwaukee at Waukesha field station in Oconomowoc, Wisconsin (43.02N, –88.44W). The field station is an approximately 40 ha reserve containing a variety of natural and restored habitats, including oak savanna, jack pine forests, maple forests, oak forests, and tallgrass prairie. The tallgrass prairie is dominated by the grasses *A. gerardii*, *B. curtipendula*, and *Schizachyrium scoparium* and the forbs *Echinacea purpurea*, *E. paradoxa*, *Dalea spp.*, and Monardo *fistulosa.* The oak forest understory is dominated by *A. petiolata* and *Hesperis matronalis.* Both *Erythronium americanum* and *S. foetidus* are common spring ephemerals in the forest understory.

Simulated herbivory and gas exchange.—We initiated our experiment in the summer of 2020. Due to phenological differences, the experiment began in early May for *A. petiolata* and *S. foetidus* and in early July for *A. gerardii* and *B. curtipendula.* Despite different starting dates, experimental details were identical for all species. For each species, we identified 20 individuals separated by at least 0.5 m. Individuals were randomly assigned to either 'Control' or 'Clipped' treatments ($n = 10$ per treatment per species), the youngest fully expanded leaf was marked for gas exchange measurements. 'Clipped' plants had all other available leaves reduced by approximately 75% using scissors, whereas leaves of 'Control' plants were handled roughly but not trimmed. For the understory forbs, only 75% of leaf tissue was removed during clipping; the stem remained intact. For the range grasses, we clipped 75% of the aboveground biomass of every leaf (except for the leaf used for the measurement of gas exchange), leaving the base intact. We chose 75% as a level of herbivory that commonly yields a strong P_N response in other North American range grasses

(Hamerlynck *et al.*, 2016). This level of herbivory exceeds offtake by large mammals in many regions. At Konza Prairie, for example, bison regularly remove about 30% of grass biomass.

We measured P_N and g_s using a LICOR LI-6800 portable photosynthesis system. All measurements were taken between 9:00 and 17:00 h. Preliminary survey measurements found little influence of time of day on P_N of *A. petiolata* or *S. foetidus* during these hours (Fig. S1). Both temperature and vapor pressure deficit within the leaf chamber were set to match ambient conditions at the start of each new measurement. Reference CO_2 was held constant at 400 μmol mol^{-1} CO_2. Flow rate was a constant 400 μmol s^{-1}. Light was set to the saturating level as determined by preliminary A-Q curves (1000 μmol m^{-2} s^{-1} for *A. petiolata* and *S. foetidus*; 1200 μmol m^{-2} s^{-1} for *A. gerardii* and *B. curipendula; see* Figs. S2–S3).). Each leaf remained clamped in the chamber for a minimum of 1 min, and we logged gas exchange measurements when readings stabilized or after 5 min, whichever happened soonest.

Gas exchange measurements occurred in time series, with the first measurements taken immediately prior to clipping ('Preclipping') to account for any potential pre-existing biases among treatments. The leaf was left clamped in the chamber during clipping, and a second measurement was taken as soon as gas exchange stabilized following clipping/handling (usually within 5 min, '0 h'). A third measurement was taken 5 h later, and a fourth the following day. From then on, gas exchange was measured every week for 3 wk. Inclement weather prevented us from measuring gas exchange on *S. foetidus* at the 1 d interval.

Statistical analyses.—To test our hypothesis that herbivory induces P_N overcompensation in palatable, but not unpalatable, plant species, we estimated the percent change in P_N and g_s due to our herbivory treatments. We first modeled P_N and g_s using a Bayesian repeated-measures ANOVA with the structure:

$$y_{ij} \sim N(X_{ij}b + z_j, \sigma_y^2)$$

where y_{ij} was i^{th} observation of the j^{th} plant individual, X was a design matrix that included variables for clipping treatment, time period (as a categorical factor), leaf temperature (T_{leaf}), and leaf vapor pressure deficit (VPD), b was a matrix for coefficients in X, and z_j was a random intercept for the j^{th} plant individual (*i.e.*, the repeated-measure). To encourage regularization and prevent the erroneous overestimation of effect sizes, we placed weakly informative hierarchical priors on b and z (Lemoine *et al.*, 2016; Lemoine, 2019):

$$b \sim N(0, \sigma_b^2); \sigma_b^2 \sim N(0, 1)$$

$$z \sim N(0, \sigma_z^2); \sigma_z^2 \sim N(0, 1)$$

These hierarchical priors have the effect of making all estimates more conservative by pulling all coefficients towards zero, and therefore serve as *a priori* corrections for *post hoc* comparisons (Gelman *et al.*, 2012). All continuous variables (P_N, g_s, T_{leaf}, VPD) were standardized prior to analysis. We ran four Markov Chain Monte Carlo (MCMC) algorithms in parallel, allowing each chain to warm up for 10,000 iterations followed by sampling an additional 10,000 iterations (40,000 posterior draws total). Convergence was checked visually via density plots of parameters. Models were fit separately for each species. After model fitting, we calculated the estimated percent change in P_N and g_s due to clipping. Briefly, for every posterior draw, we calculated the predicted value for 'Control' and 'Clipped' plants at every time point. To remove any confounding environmental effects, we held T_{leaf} and VPD constant at 25 C and 1.5, respectively. These calculations yielded 40,000 estimates of P_N and

g_s for 'Control' and 'Clipped' plants at each time point. For each of the 40,000 estimates, we calculated the percent change due to clipping as 100(Clipped − Control)/Control.

We hypothesized a clipping-induced increase in P_N, coupled with no change in g_s, would increase WUE for palatable species throughout the growing season. As a test of this hypothesis, we first estimated population-level WUE as the slope of the P_N-g_s relationship, expressed as μmol CO_2 mol^{-1} H_2O m^{-2} s^{-1}. For each species at each time point, we fit a linear regression with a hierarchical shrinkage parameter:

$$P_N \sim N(X\ b, \sigma_y^2)$$

$$b \sim N(0, \sigma_b^2); \sigma_b^2 \sim N(0, 1)$$

where P_N was net CO_2 assimilation. X was a design matrix that contains variables for g_s, clipping treatment, T_{leaf}, and VPD, and b was a matrix for coefficients in X. To allow for different WUE (*i.e.*, slopes) for 'Control' and 'Clipped' treatments, X also included the g_s:clipping interaction. Continuous variables (g_s, T_{leaf}, VPD) were standardized prior to analyses, such that the slopes describe WUE at average temperature and air moisture content. Post-analysis, slopes were back-transformed to the original scale. As above, models were executed with four MCMC chains in parallel, with 10,000 warmup iterations followed by 10,000 sampling iterations for each chain. Differences in WUE were compared for each time period.

Analyses and graphics were implemented in Python v3.7.8. Graphics required the *matplotlib* and *seaborn* modules, and data prep and analyses used *numpy*, *scipy*, *patsy*, and *pandas* modules. Bayesian models were written in STAN and fit using CmdStan v2.25, accessed from Python via *cmdstanpy*. Posterior density plots were generated by the *arviz* module. All results are expressed in terms of means ± Bayesian credible intervals (CI_{95}).

RESULTS

Photosynthesis (net CO_2 assimilation).—Contradicting our hypothesis, neither grass species demonstrated P_N overcompensation. Photosynthesis was strongly, positively related to ambient temperature for *A. gerardii* ($Pr(T_{leaf} > 0) = 0.992$), but not for *B. curtipendula* ($Pr(T_{leaf} > 0) = 0.573$). Neither species experienced P_N inhibition by ambient air moisture, as evidenced by the lack of negative relationship between P_N and VPD for both *A. gerardii* ($Pr(VPD < 0) = 0.683$) and *B. curtipendula* ($Pr(VPD) = 0.869$). Moreover, neither species demonstrated ontogenetic shifts in P_N, as would be evidenced by changes in P_N throughout the growing season after statistically controlling for T_{leaf} and VPD (Fig. 1). P_N remained between 14–20 μmol CO_2 m^{-2} s^{-1} throughout the experiment for both *A. gerardii* and *B. curtipendula*, and at no time point did P_N of clipped plants statistically differ from controls for either *A. gerardii* or *B. curtipendula* (Table 1, Fig. 1).

Our hypothesis was further contradicted by patterns in P_N overcompensation of the unpalatable understory species, which showed much greater variability in P_N than did C_4 grasses. For example, temperature had a strong, positive effect on *A. petiolata* ($Pr(T_{leaf} > 0) = 1.000$), but not *S. foetidus* ($Pr(T_{leaf} > 0) = 0.493$). Likewise, increasing VPD suppressed P_N of *A. petiolata* ($Pr(VPD < 0) = 0.971$), but not *S. foetidus* ($Pr(VPD < 0) = 0.493$). *S. foetidus* also exhibited no discernable ontogenetic trends in P_N, as values remained between 6–10 μmol CO_2 m^{-2} s^{-1} throughout the experiment (Fig. 1). In contrast, P_N of *A. petiolata* declined by ~25% throughout the growing season, from approximately 15 μmol CO2 m^{-2} s^{-1} during the Pre-Clipping stage to approximately 11 μmol CO_2 m^{-2} s^{-1} by the third week of the experiment (Fig. 1). Surprisingly, clipping had a strong, compensatory effect on P_N of *A.*

petiolata. Initially, P_N was elevated in both *A. petiolata* and *S. foetidus*, likely due to random differences in allocation of plants to treatments (Table 1, Fig. 1). Immediately following clipping, there was no detectable change in P_N for either species (Table 1, Fig. 1). Within 5 hr, P_N of 'Clipped' *A. petiolata* exhibited a 17% (Bayesian CI_{95}: 7.07%–28.71%) increase in P_N relative to 'Controls', although the signal of clipping was lost by the next day (Table 1, Fig. 1). During the same time frame, *S. foetidus* did not show any plasticity in P_N, and in both species, all differences were eliminated by the second week of measurement. Therefore, only a single, highly unpalatable species demonstrated any P_N overcompensation, and effects were quite short-lived.

Stomatal conductance.—We predicted that clipping would increase g_s throughout the growing season. Our hypothesis was refuted for three of the four species studied here. In *A. gerardii*, *B. curtipendula*, and *S. foetidus*, g_s was not affected by either temperature ($Pr(T_{leaf} > 0) = 0.581$, 0.822, 0.730, respectively) or VPD ($Pr(VPD < 0) = 0.280$, 0.536, 0.858. respectively). Likewise, none of the three species presented any obvious ontogenetic patterns in g_s. Stomatal conductance was lowest for *S. foetidus*, ranging from 0.00–0.15 mol H2O m^{-2} s^{-1} across the experiment (Fig. 2). Both *A. gerardii* and *B. curtipendula* had the second lowest g_s, which remained relatively fixed at about 0.16 mol H_2O m^{-2} s^{-1} (Fig. 2). None of these three species showed any significant effect of clipping on g_s, although g_s of clipped *S. foetidus* was moderately elevated relative to controls in the second week (Table 1, Fig. 2). However, g_s of *S. foetidus* was quite low, which magnifies small changes into large percent differences. As a result, percent change of *S. foetidus* was estimated imprecisely for most weeks (Table 1). Even during the second week, the Bayesian CI_{95} for the percent change in g_s due to clipping ranged from –7.72% to 67.35%, suggesting that this effect is highly uncertain and cannot be stated with confidence or precision.

As with P_N, g_s of *A. petiolata* was highly dynamic. Overall, *A. petiolata* possessed a higher g_s than for any other species. At the initial time point, g_s of *A. petiolata* was at its lowest (~0.25 mol H20 m^{-2} s^{-1}), though still 66% higher than for either *A. gerardii* or *B. curtipendula* and 150% higher than g_s of *S. foetidus* (Fig. 2). These differences were magnified during the growing season, as g_s of *A. petiolata* neared 0.40 mol H_2O m^{-2} s^{-1} by the second week before declining rapidly to initial conditions in the third week (Fig. 2). Unlike the other three species, g_s of *A. petiolata* also was highly sensitive to the environment; temperature had a strong positive influence on g_s ($Pr(T_{leaf} > 0) = 1.000$), whereas increasing VPD reduced g_s ($Pr(VPD < 0) = 1.000$). Supporting our hypothesis, clipping of *A. petiolata* briefly elevated g_s relative to controls by 16.94%, although the effect was relatively uncertain (Bayesian CI_{95}: 1.01%–35.97%, Table 1, Fig. 2).

Water use efficiency.—Given the lack of responsiveness in P_N and g_s for *A. gerardii*, *B. curtipendula*, and *S. foetidus*, as well as the similar patterns of P_N and g_s for *A. petiolata*, it is unsurprising that WUE was relatively constant. Throughout the growing season, WUE of *A. gerardii* and *S. foetidus* increased weakly, but not significantly, whereas WUE of *A. petiolata* declined by over 85% between the start and end of the experiment (Fig. 3). The decline in WUE of *A. petiolata* likely reflects the combination of an ontogenetic drop in P_N coupled with an increase in g_s (Figs. 1,2). Clipping had relatively little effect on WUE of any species. In *A. gerardii*, WUE of control plants was marginally or significantly higher in control plants than in clipped plants at 1 d (Control: 83.69 ± 23.37, Clipped: 38.78 ± 18.58 μmol CO_2 mol H_2O m^{-2} s^{-1}), 2 wk (Control: 75.51 ± 13.72, Clipped: 47.38 ± 15.79 μmol CO_2 mol H_2O m^{-2} s^{-1}), and 3 wk (Control: 86.83 ± 23.97, Clipped: 53.37 ± 23.92 μmol CO_2 mol H_2O m^{-2} s^{-1}) (Table 1, Fig. 3). Likewise, clipping appeared to reduce WUE of *B. curtipendula* at 1 wk (Control: 68.83 ± 25.85, Clipped: 26.49 ± 19.66 μmol CO_2 mol H_2O m^{-2} s^{-1}), but not at any

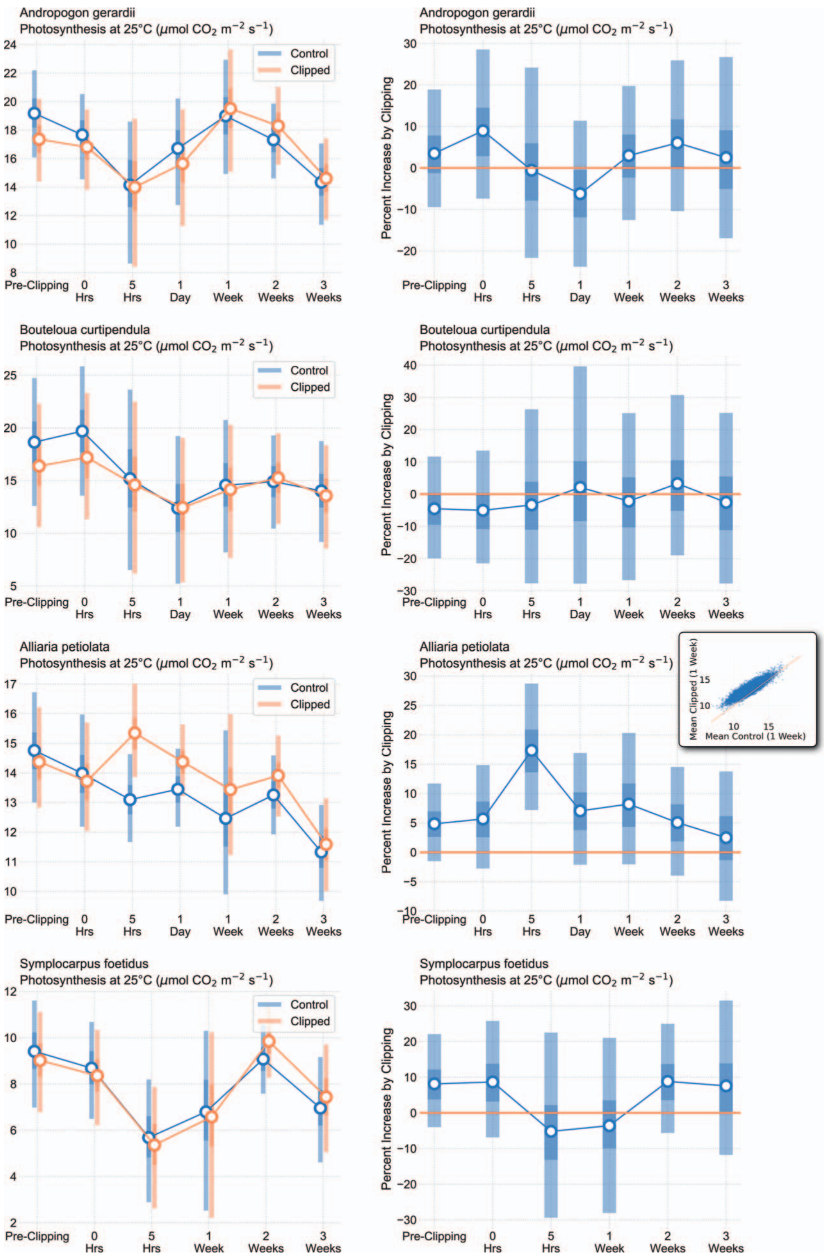

FIG. 1.—Photosynthesis (P_N) as measured by net CO2 assimilation (μmol CO_2 m^{-2} s^{-1}) throughout the experiment for the two palatable (*A. gerardii* and *B. curtipendula*) and unpalatable (*A. petiolata* and *S.foetidus*) species. Left column shows the modeled means after correcting to a standard temperature and VPD. Points show modeled means, light shading and dark shading show the Bayesian CI_{95} and Bayesian CI_{50} of the mean, respectively. Right column shows the percent change in P_N due to clipping at each time point for each species. Points are modeled means, light and dark shading show the Bayesian

TABLE 1.—Bayesian probabilities for contrasts of percent change in P_N, g_s, and WUE due to clipping for each species at each time point. Rows in bold show significant (at Pr > 0.95, Pr < 0.05), or marginally significant (at Pr > 0.90; Pr < 0.10), contrasts. These probabilities represent the probability that PN in the clipped treatment was greater than in the control treatment. Therefore, Pr > 0.95 means there is a 95% chance that P_N was greater in the clipped treatment than the control treatment, and Pr < 0.05 means there is a 95% chance that P_N was greater in the control treatment than in the clipped treatment

	Pr(Clipping > Control): P_N	Pr(Clipping > Control): g_s	Pr(Clipping > Control): WUE
A. gerardii			
Preclipping	0.686	0.284	0.257
0 h	0.852	0.414	0.154
5 h	0.456	0.208	0.278
1 d	0.232	0.236	**0.033**
1 wk	0.643	0.492	0.488
2 wk	0.746	0.528	**0.069**
3 wk	0.564	0.293	**0.096**
B. curtipendula			
Preclipping	0.223	0.212	0.715
0 h	0.206	0.206	0.423
5 h	0.126	0.127	0.753
1 d	0.617	0.618	0.125
1 wk	0.421	0.419	**0.044**
2 wk	0.338	0.336	0.484
3 wk	0.367	0.372	0.293
A. petiolata			
Preclipping	**0.927**	0.810	0.255
0 h	**0.899**	0.555	0.236
5 h	**1.000**	**0.982**	0.309
1 d	**0.931**	0.727	0.762
1 wk	**0.934**	0.819	0.591
2 wk	0.861	0.880	0.464
3 wk	0.658	0.179	0.254
S. foetidus			
Preclipping	**0.907**	0.840	0.820
0 h	0.867	0.887	0.852
5 h	0.307	0.305	0.205
1 d	Measurements missing due to inclement weather		
1 wk	0.371	0.304	0.131
2 wk	0.879	**0.918**	0.546
3 wk	0.762	0.508	0.130

other time point (Table 1, Fig. 3). Given the lack of consistent pattern in WUE, coupled with the lack of change in the original parameters P_N and g_s for both *A. gerardii* and *B. curtipendula*, it is difficult to attribute these differences between clipping treatments to anything other than random variation.

←

CI_{95} and Bayesian CI_{50} of the mean, respectively. Inset shows a scatterplot of the correlation between 'Control' and 'Clipped' means for *A. petiolata* at 1 wk to illustrate that the confidence intervals can overlap, but the means can remain significantly different, due to correlation in the estimates (*i.e.*, when P_N is low for clipped, it is similarly lower for control)

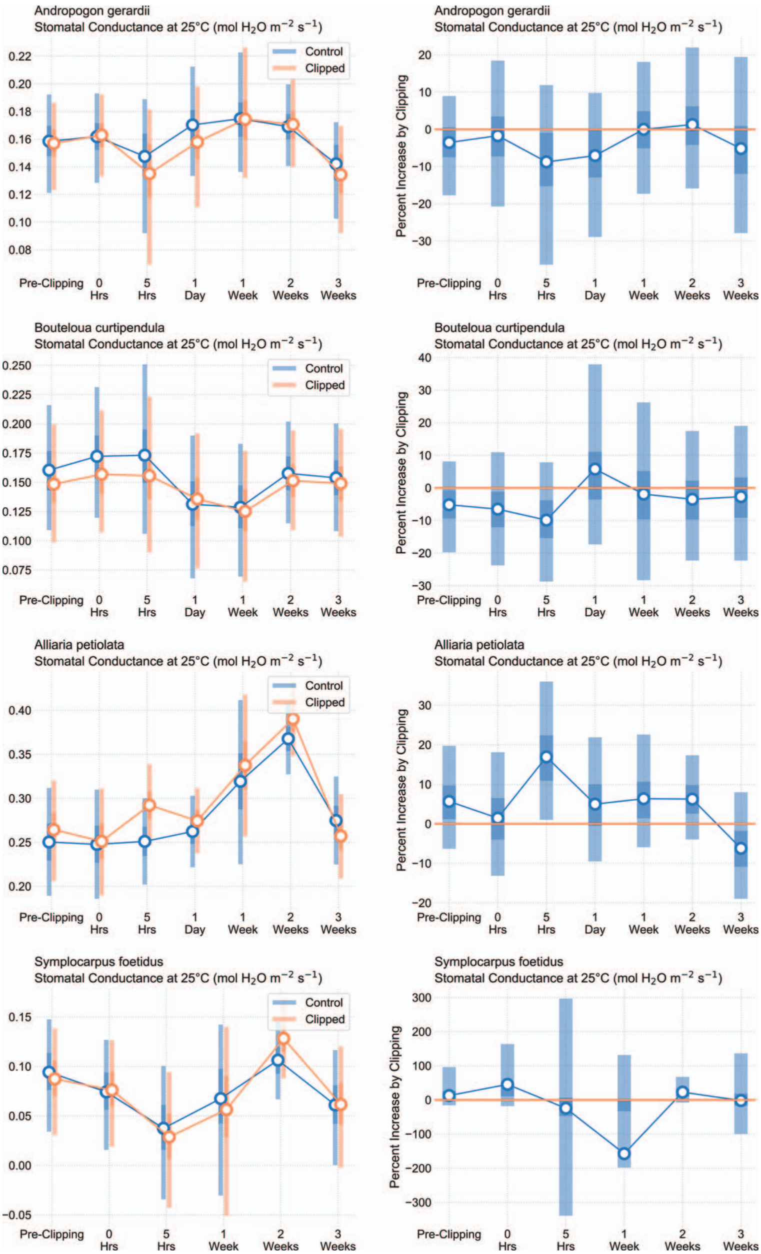

FIG. 2.—Stomatal conductance (g_s) as measured by net H_2O assimilation (mol H_2O m^{-2} s^{-1}) throughout the experiment for the two palatable (*A. gerardii* and *B. curtipendula*) and unpalatable (*A. petiolata* and *S. foetidus*) species. Left column shows the modeled means after correcting to a standard temperature and VPD. Points show modeled means, light shading and dark shading show the Bayesian CI$_{95}$ and Bayesian CI$_{50}$ of the mean, respectively. Right column shows the percent change in P_N due to clipping at each time point for each species. Points are modeled means, light and dark shading show the Bayesian CI$_{95}$ and Bayesian CI$_{50}$ of the mean, respectively

DISCUSSION

We found little support for our hypothesis that P_N overcompensation is related plant life form, such that grasses would be more plastic with respect to P_N than understory forbs. Indeed, only *A. petiolata*, arguably the least palatable of the four species examined here, exhibited any degree of P_N overcompensation, and did so only for a short 24 h window following defoliation. Increased P_N appears to have been driven by stomatal opening and subsequent elevated gas exchange, as *A. petiolata* also increased g_s and maintained a constant WUE. This confirms our second hypothesis that elevated P_N would be driven by increased stomatal conductance. The patterns reported here raise some interesting questions: Is the inability of grasses in this study to increase P_N consistent with other studies, or caused by a paucity of grazers at our study site? Why did only *A. petiolata* exhibit elevated P_N following herbivory?

Given that many rangeland grasses increase P_N after defoliation (Detling *et al.*, 1979; Painter and Detling, 1981; Ingham and Detling, 1986), we were surprised that P_N of neither *A. gerardii* nor *B. curtipendula* responded to mechanical clipping. This was especially surprising, since both species evolved under heavy grazing pressure (Peden *et al.*, 1974; Plumb and Dodd, 1993) and previous studies report a 25% increase in PN for *B. gracilis* (Detling *et al.*, 1979) and over 100% for *B. curtipendula* (Ingham and Detling, 1986), although P_N of *A. gerardii* is typically unaffected by clipping, as reported here (Wallace and Svejcar, 1987). The P_N responsiveness of *Bouteloua spp.* relative to *A. gerardii* supports observations that *B. curtipendula* tolerates grazing better than most other tallgrass species of Wisconsin, including *A. gerardii* (Neiland and Curtis, 1956). However, we were unable to reproduce the positive impact of herbivory on P_N in *B. curtipendula*, likely because there are two key differences between our study and earlier work. First, our study site lacks bovid grazers and is instead dominated by white-tailed deer, which typically avoid consuming grasses (McMahan, 1964). Therefore, grasses at our site have not experienced intense herbivory within the past 50 y, and short-term grazing history can affect how grasses compensate for damage. For example, Smith (1998) found that ramets of *B. curtipendula* from ungrazed pastures were less tolerant of herbivory than ramets from grazed pastures. The absence of grazers at our site might predispose grasses to be less responsive to damage, but few comparative studies exist that identify the genetic and evolutionary constraints on P_N responses to herbivory. Second, so far as we know, only one study has conducted field tests of P_N responsiveness to herbivory, as we did here (Nowak and Caldwell, 1984). Most previous tests of herbivory and P_N grew plants hydroponically in ideal nutrient solutions (Detling *et al.*, 1979; Painter and Detling, 1981) or in nutrient-rich potting soils (Ingham and Detling, 1986; Wallace and Svejcar, 1987; Smith, 1998; Hamerlynck *et al.*, 2016). Field soils, by contrast, are often nutrient- or water-limited, and both factors affect how P_N responds to herbivory (Zhao *et al.*, 2008). As a result, field experiments often fail to reproduce the effects of herbivory demonstrated in the lab (Nowak and Caldwell, 1984). Therefore, it seems likely that the responsiveness of *A. gerardii* and *B. curitpendula* in natural environments, especially those without regular grazing, is lower than what would be expected from laboratory studies.

However, it is possible that we underestimated the response of photosynthesis to herbivory, because we used artificial grazing. Simulated herbivory is commonly used in compensation studies, because it can be reliably imposed and standardized among replicates (Schat and Blossey, 2005; Ashton and Lerdau, 2008; Olejniczak, 2011). The downside to mechanical clipping is that it does not mimic the damage style of natural herbivory and lacks chemicals present in saliva that can stimulate plant defenses. As a result artificial herbivory often fails to provoke the same response in plants as natural herbivory (Lehtilä and Boalt,

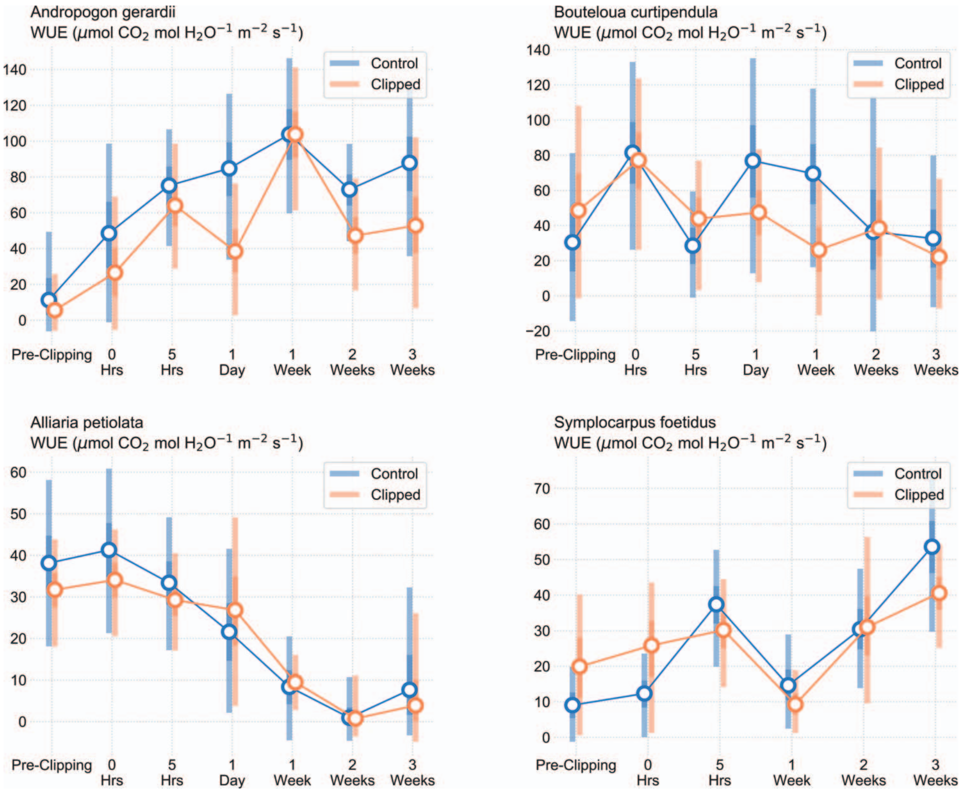

FIG. 3.—Water use efficiency (WUE, μmol CO_2 mol H_2O m^{-2} s^{-1}) as estimated from the slope of the P_N–g_s relationship throughout the experiment for the two palatable (*A. gerardii* and *B. curtipendula*) and unpalatable (*A. petiolata* and *S. foetidus*) species. Points show slope means, light shading and dark shading show the Bayesian CI_{95} and Bayesian CI_{50} of the slope, respectively, as estimated from 40,000 posterior draws

2008). Nonetheless, artificial herbivory does consistently stimulate plant responses to herbivory, albeit lower in magnitude than natural herbivory (Paige, 1999; Lehtilä and Boalt, 2008). All in all, it is likely that our artificial clipping treatment would have stimulated photosynthetic overcompensation, if slightly weaker than would occur by browsing, in the species studied here.

Although *A. gerardii* and *B. curtipendula* were less tolerant of clipping than expected, *A. petiolata* was more responsive than expected given its extreme resistance to herbivory. Resistance and tolerance to herbivory often, but not always, demonstrate tradeoffs (Strauss *et al.*, 2002). We expected *A. petiolata* and *S. foetidus*, which are extremely resistant to herbivory, to be less tolerant than the grasses. However, resistance *per se* is likely not the determinant of how P_N responds, given that *S. foetidus* did not overcompensate despite also being unpalatable. We believe that the difference between *A. petiolata* and *S. foetidus* is explained by their different resistance mechanisms; *S. foetidus* possesses constitutive defenses in the form of calcium oxalate crystals (Coté and Gibernau, 2012), whereas *A. petiolata* instead induces production of glucosinolates, peroxidases, and trypsin inhibitors via jasmonic acid and ethylene pathways (Cipollini *et al.*, 2005). Both jasmonic acid and ethylene are upregulated in response to herbivory and control stomatal conductance (Glick *et al.*, 2007; Von Dahl and Baldwin, 2007; Ullah *et al.*, 2018), but typically work to inhibit P_N and g_s, rather than enhance it (Havko *et al.*, 2020). It is possible that *A. petiolata* increased photosynthesis in order to drive the production of secondary metabolites. However, many grasses that lack inducible defenses also show marked increases in both P_N and g_s (Caldwell *et al.*, 1981; Ingham and Detling, 1986; Doescher *et al.*, 1997). Although the precise physiological mechanism remains unknown, P_N overcompensation in *A. petiolata* derives from increased stomatal opening, given higher gas exchange of both CO_2 and H_2O. It also remains unknown whether elevated P_N following herbivory is a common mechanism underlying multiple different responses. Our results lend support to recent observations that resistance and tolerance need not necessarily trade off (Kariñho-Betancourt and Núñez-Farfán, 2015).

Our results provide tantalizing evidence that P_N overcompensation might be more prevalent among nonnative species than in native species. Of the four species tested here, only *A. petiolata* is nonnative and is considered one of the 'world's worst 100 invaders' (Parker *et al.*, 2013). Other comparative studies have documented similarly strong compensatory P_N to deer browsing in *A. petiolata* relative to native species, although the effect of deer was indirect via increased light availability by browsing taller competitors (Heberling *et al.*, 2017). Non-native species typically possess greater P_N overcompensation than native counterparts (Caldwell *et al.*, 1981; Nowak and Caldwell, 1984), and, in some cases, can regrow biomass faster than native species following herbivory (Croy *et al.*, 2020). However, these patterns are not consistent across all species, as nonnative tropical shrubs of Hawaii were no more tolerant of herbivory than native shrubs (Lurie *et al.*, 2017). Such contradictions are to be expected, given the persistent debate about whether invasive species do (Davidson *et al.*, 2011) or do not (Godoy *et al.*, 2011; Palacio-López and Gianoli, 2011) possess more plastic phenotypes than native species. In the case of *A. petiolata*, it is unlikely that elevated P_N following herbivory provides a competitive advantage, given that its high resistance to herbivory precludes P_N overcompensation. It is the high resistance to herbivory that accounts for the spread of *A. petiolata* throughout North America, rather than the ephemeral stimulation of P_N. The two may even be related, if P_N exhibits rapid, but unsustained, increases to drive the production of induced defenses.

In summary we tested the hypothesis that variation in P_N overcompensation would be qualitatively related to palatability and the ability of a species to resist grazing, with unpalatable forest understory species being less responsive than palatable prairie species. We found no evidence supporting this hypothesis. Three of the four species did not exhibit P_N overcompensation in any form, and the only plant here to express P_N plasticity was also the least palatable. Therefore, more investigation of the metabolic and physiological mechanisms underpinning P_N overcompensation, and the ecological consequences, could potentially explain competitive hierarchies in plant communities, plant invasions, and the evolution of plant defense strategies.

Acknowledgments.—We would like to thank the University of Wisconsin–Milwaukee at Waukesha faculty and field station staff, Teresa Schueller and Marlin Johnson, for allowing us to work at the site and giving us an endless supply of natural history knowledge. This work was funded by National Science Foundation Division of Environmental Biology 1941390 to NPL. Author's contributions as follows: NPL and MLB designed the experiment. MLB conducted the experiment. NPL analyzed the data. NPL wrote the manuscript with feedback from MLB.

Availability of data and material: https://doi.org/10.6084/m9.figshare.13724116.v1;
Code availability: https://doi.org/10.6084/m9.figshare.13724116.v1

LITERATURE CITED

ANTEN, N. P. R. AND D. D. ACKERLY. 2001. Canopy-level photosynthetic compensation after defoliation in a tropical understorey palm. *Funct. Ecol.*, **15**:252–262.

ASHTON, I.W. AND M.T. LERDAU. 2008. Tolerance to herbivory, and not resistance, may explain differential success of invasive, naturalized, and native North American temperate vines. Divers. Distrib., **14:**169–178.

AVERILL, K. M., D. A. MORTENSEN, E. A. H. SMITHWICK AND E. POST. 2016. Deer feeding selectivity for invasive plants. *Biol. Invasions*, **18**:1247–1263.

BELSKY, A. J. 1986. Does herbivory benefit plants? *Am. Nat.*, **127**:870–892.

———. 1996. Western juniper expansion: is it a threat to arid northwestern ecosystems. *J. Range Manag.*, **49**:53–59.

CALDWELL, M. M., J. H. RICHARDS, D. A. JOHNSON, R. S. NOWAK AND R. S. DZUREC. 1981. Coping with herbivory: photosynthetic capacity and resource allocation in two semiarid Agropyron bunchgrasses. *Oecologia*, **50**:14–24.

CIPOLLINI, D., J. MBAGWU, K. BARTO, C. HILLSTROM AND S. ENRIGHT. 2005. Expression of constitutive and inducible chemical defenses in native and invasive populations of Alliaria petiolata. *J. Chem. Ecol.*, **31**:1255–1267.

COLEY, P. D., J. P. BRYANT AND F. S. CHAPIN. 1985. Resource availability and plant antiherbivore defense. *Science (80-.)*, **230**:895–899.

COTÉ, G. G. AND M. GIBERNAU. 2012. Distribution of calcium oxalate crystals in floral organs of araceae in relation to pollination strategy. *Am. J. Bot.*, **99**:1231–1242.

CROY, J. R., L. A. MEYERSON, W. J. ALLEN, G. P. BHATTARAI AND J. T. CRONIN. 2020. Lineage and latitudinal variation in Phragmites australis tolerance to herbivory: implications for invasion success. *Oikos*, **129**:1341–1357.

DAVIDSON, A. M., M. JENNIONS AND A. B. NICOTRA. 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecol. Lett.*, **14**:419–431.

DE LA MATA, R., S. HOOD AND A. SALA. 2017. Insect outbreak shifts the direction of selection from fast to slow growth rates in the long-lived conifer Pinus ponderosa. *Proc. Natl. Acad. Sci.*, **114**:7392–7396.

DELANEY, K.J. 2008. Injured and uninjured leaf photosynthetic responses after mechanical injury on *Nerium oleander* leaves, and *Danaus plexippus* herbivory on *Asclepias curassavica* leaves. *Plant Ecol.*, **199**:187–200.

DETLING, J. K., M. I. DYER AND D. T. WINN. 1979. Net photosynthesis, root respiration, and regrowth of Bouteloua gracilis following simulated grazing. *Oecologia*, **41**:127–134.

DOESCHER, P. S., T. J. SVEJCAR AND R. G. JAINDL. 1997. Gas exchange of Idaho fescue in response to defoliation and grazing history. *J. Range Manag.*, **50**:285–289.

FRANK, D. A., M. M. KUNS AND D. R. GUIDO. 2002. Consumer control of grassland plant production. *Ecology*, **83**:602.

GARCIA, L. C. AND M. D. EUBANKS. 2019. Overcompensation for insect herbivory: a review and meta-analysis of the evidence. *Ecology*, **100**:1–14.

GELMAN, A., J. HILL AND M. YAJIMA. 2012. Why we (usually) don't have to worry about multiple comparisons. *J. Res. Educ. Eff.*, **5**:189–211.

GLICK, B. R., Z. CHENG, J. CZARNY AND J. DUAN. 2007. Promotion of plant growth by ACC deaminase-producing soil bacteria. *Eur. J. Plant Pathol.*, **119**:329–339.

GODOY, O., F. VALLADARES AND P. CASTRO-DÍEZ. 2011. Multispecies comparison reveals that invasive and native plants differ in their traits but not in their plasticity. *Funct. Ecol.*, **25**:1248–1259.

HAMERLYNCK, E. P., B. S. SMITH, R. L. SHELEY AND T. J. SVEJCAR. 2016. Compensatory photosynthesis, water-use efficiency, and biomass allocation of defoliated exotic and native bunchgrass seedlings. *Rangel. Ecol. Manag.*, **69**:206–214.

HAVKO, N. E., M. R. DAS, A. M. MCCLAIN, G. KAPALI, T. D. SHARKEY AND G. A. HOWE. 2020. Insect herbivory antagonizes leaf cooling responses to elevated temperature in tomato. *Proc. Natl. Acad. Sci.*, **117**:2211–2217.

INGHAM, R. E. AND J. K. DETLING. 1986. EFFECTS OF DEFOLIATION AND NEMATODE CONSUMPTINO ON GROWTH and leaf gas exchange in Bouteloua curtipendula. *Oikos*, **46**:23–28.

KARIÑHO-BETANCOURT, E. AND J. NÚÑEZ-FARFÁN. 2015. Evolution of resistance and tolerance to herbivores: testing the trade-off hypothesis. *PeerJ*, **3**:e789.

KULA, A., D.C. HARTNETT AND G.W.T. WILSON. 2005. Effects of mycorrhizal symbiosis on tallgrass prairie plant-herbivore interactions. *Ecol. Lett.*, **8**:61–69.

LEHTILÄ, K. AND E. BOALT. 2008. The use and usefulness of artificial herbivory in plant-herbivore studies. p. 257–2I5. *In:* Function W. W. Weisser and E. Siemann, (eds). Insects and Ecosystem. Springer Berlin Heidelberg, Berlin, Heidelberg

LEMOINE, N. P. 2019. Moving beyond noninformative priors: why and how to choose weakly informative priors in Bayesian analyses. *Oikos*, **128**:912–928.

———, A. M. HOFFMAN, A. J. FELTON, L. E. BAUR, F. CHAVES, J. GRAY, Q. YU AND M. D. SMITH. 2016. Underappreciated problems of low replication in ecological field studies. *Ecology*, **97**:2554–2561

MCMAHAN, C. A. 1964. Comparative food habits of deer and three classes of livestock. *J. Wildl. Manage.*, **28**:798–808.

MCNAUGHTON, S. J. 1983. Compensatory plant growth as a response to herbivory. *Oikos*, **40**:329–336.

MILCHUNAS, D. G. AND W. K. LAUENROTH. 1993. Quantitative effects of grazing on vegetation and soils over a global range of environments. *Ecol. Monogr.*, **63**:327–366.

MOLE, S. AND A. JOERN. 1994. Feeding behavior of gramnivorous grasshoppers in response to host-plant extracts, alkaloids, and tannins. *J. Chem. Ecol.*, **20**:3097–3109.

MORRISON, K. D. AND E. G. REEKIE. 1995. Pattern of defoliation and its effect on photosynthetic capacity in Oenothera biennis. *J. Ecol.*, **83**:759–767.

NEILAND, B. M. AND J. T. CURTIS. 1956. Differential responses to clipping of six prairie grasses in Wisconsin. *Ecology*, **37**:355–365.

NOWAK, R. S. AND M. M. CALDWELL. 1984. A test of compensatory photosynthesis in the field: implications for herbivory tolerance. *Oecologia*, **61**:311–318.

OLEJNICZAK, P. 2011. Overcompensation in response to simulated herbivory in the perennial herb Sedum maximum. *Plant Ecol.*, **212**:1927–1935.

PAIGE, K. N. AND T. G. WHITHAM. 1987. Overcompensation in response to mammalian herbivory: the advantage of being eaten. *Am. Nat.*, **129**:407–416.

———. 1999. Regrowth following ungulate herbivory in Ipomopsis aggregate: geographic evidence for overcompensation. Oecologia, **118:**316–323.

PAINTER, E. L. AND J. K. DETLING. 1981. Effects of defoliation on net photosynthesis and regrowth of western wheatgrass. *J. Range Manag.*, **34**:68–71.

PALACIO-LÓPEZ, K. AND E. GIANOLI. 2011. Invasive plants do not display greater phenotypic plasticity than their native or non-invasive counterparts: a meta-analysis. *Oikos*, **120**:1393–1401.

PARKER, J. D., M. E. TORCHIN, R. A. HUFBAUER, N. P. LEMOINE, C. ALBA, D. M. BLUMENTHAL, O. BOSSDORF, J. E. BYERS, A. M. DUNN, R. W. HECKMAN, M. HEJDA, V. JAROŠÍK, A. R. KANAREK, L. B. MARTIN, S. E. PERKINS, P. PYŠEK, K. A. SCHIERENBECK, C. SCHLODER, R. VAN KLINKEN, K. J. VAUGH, W. WILLIAMS AND L. M. WOLFE. 2013. Do invasive species perform better in their new ranges? *Ecology*, **94**:985–994

PEDEN, D. G., G. M. VAN DYNE, R. W. RICE AND R. M. HANSEN. 1974. The trophic ecology of Bison bison L. on shortgrass plains. *J. Appl. Ecol.*, **11**:489–497.

PLUMB, G. E. AND J. L. DODD. 1993. Foraging ecology of bison and cattle on a mixed prairie: implications for natural area management. *Ecol. Appl.*, **3**:631–643.

POORTER, H., C. REMKES AND H. LAMBERS. 1990. Carbon and nitrogen economy of 24 wild species differing in relative growth rate. *Plant Physiol.*, **94**:621–627.

POVEDA, K., M. I. GOMEZ JIMENEZ AND A. KESSLER. 2013. The enemy as ally: herbivore-induced increase in crop yield. *Ecol. Appl.*, **23**:515–522.

PULICE, C.E. AND A. A. PACKER. 2008. Herbivory induces extrafloral nectary production in *Prunus avium. Funct. Ecol.,* **22**:801–807.

ULLAH, A., H. MANGHWAR, M. SHABAN, A. H. KHAN, A. AKBAR, U. ALI, E. ALI AND S. FAHAD. 2018. Phytohormones enhanced drought tolerance in plants: a coping strategy. *Environ. Sci. Pollut. Res.*, **25**:33103–33118. Environmental Science and Pollution Research.

RAMULA, S., K.N. PAIGE, T. LENNARTSSON AND J. TUOMI 2019. Overcompensation: a 30-year perspective. *Ecology*, **100**:1–6.

RETUERTO, R., B. FERNANDEZ-LEMA, RODRIGUEZ-ROILOA AND J. R. OBESO. 2004. Increased photosynthetic performance in holly trees infested by scale insects. *Funct. Ecol.*, **18**:664–669.

SCHAT, M. AND B. BLOSSEY. 2005. Influence of natural and simulated leaf beetle herbivory on biomass allocation and plant architecture of purple loosestrife (*Lythrum salicaria* L.). *Environ. Entomol.*, **34**:906–914.

SMITH, S. E. 1998. Variation in response to defoliation between populations of Bouteloua curtipendula var. caespitosa (Poaceae) with different livestock grazing histories. *Am. J. Bot.*, **85**:1266–1272.

STRAUSS, S. Y. AND A. A. AGRAWAL. 1999. The ecology and evolution of plant tolerance to herbivory. *Trends Ecol. Evol.*, **14**:179–185.

———, J. A. RUDGERS, J. A. LAU AND R. E. IRWIN. 2002. Direct and ecological costs of resistance to herbivory. *Trends Ecol. Evol.*, **17**:278–285.

TIFFIN, P. 2000. Mechanisms of tolerance to herbivore damage: what do we know? *Evol. Ecol.*, **14**:523–536.

VON DAHL, C. C. AND I. T. BALDWIN. 2007. Deciphering the role of ethylene in plant-herbivore interactions. *J. Plant Growth Regul.*, **26**:201–209.

WALLACE, L. L. AND T. J. SVEJCAR. 1987. Mycorrhizal and clipping effects on Andropogon gerardii photosynthesis. *Am. J. Bot.*, **74**:1138–1142.

WAREING, P. F., M. M. KHALIFA AND K. J. TREHARNE. 1968. Rate-limiting processes in photosynthesis at saturating light intensities. *Nature*, **220**:453–457.

ZANGERL, A. R., A. M. ARNTZ AND M. R. BERENBAUM. 1997. Physiological price of an induced chemical defense: Photosynthesis, respiration, biosynthesis, and growth. *Oecologia*, **109**:433–441.

ZHAO, W., S. P. CHEN AND G. H. LIN. 2008. Compensatory growth responses to clipping defoliation in Leymus chinensis (Poaceae) under nutrient addition and water deficiency conditions. *Plant Ecol.*, **196**:85–99.

SUBMITTED: 11 MAY 2021 ACCEPTED: 11 OCTOBER 2021