

ECOPHYSIOLOGICAL RESPONSES OF TWO HERBACEOUS SPECIES TO PRESCRIBED BURNING, ALONE OR IN COMBINATION WITH OVERSTORY THINNING¹

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The oak-rich deciduous forests of the central Appalachian Mountains of eastern North America have changed significantly since the onset of effective fire suppression early in the 20th century. Those changes have resulted in progressively decreasing light and nutrient supplies to herbaceous perennial understory species. Application of ecological restoration treatments such as reintroduction of frequent dormant-season fire and overstory thinning to pre-suppression density often increase light, soil temperature and moisture, and short-term nutrient availability to pre-suppression levels. To persist in this environment, perennial understory herbs must be able to acclimate phenotypically to the very different resource supply combinations present with and without fire suppression. As part of a larger study of the response of the long-lived herbaceous perennials *Desmodium nudiflorum* and *Panicum boschii* to ecosystem restoration treatments in Ohio mixed-oak forests, this study examined the ecophysiological effects of prescribed burning (B) and the combination of burning and thinning (T + B) in mixed-oak forests in southern Ohio. Control (C) plants had significantly lower maximum photosynthetic rate (A_{\max}) than those in the treated plots. The enhancement of A_{\max} averaged 26.7% and 52.7% in the B and T + B treatments, respectively. Plants from the T + B plots had higher quantum yield, stomatal conductance, and photosynthetic nutrient use efficiency than B and C plants. B plants had greater intrinsic water use efficiency (WUE) than plants in the C or T + B treatments. Light saturation point (LSP), light compensation point (LCP), and “dark” respiration (DR) did not differ among treatments. Photosynthetic parameters did vary significantly between the species, but no significant treatment \times species interactions were detected. Our results support the hypothesis that prescribed burning, especially when combined with overstory thinning, in these perennial herbs can result in phenotypic acclimation characterized by enhanced photosynthetic performance.

Key words: burning; herb; photosynthetic rate; thinning; water use efficiency.

The deciduous forests of the central Appalachian Mountains of eastern North America have been managed by humans for at least two millennia (Mann, 2005), while analysis of pollen and charcoal deposits in the region indicate that fire has been common in the forests of the region for at least twice that long (Delcourt and Delcourt, 1997). Despite major changes in the human population and the size–frequency distribution of fire over the last four millennia, the average fire return interval remained relatively stable until the rapid decline of Native American populations after European contact (Delcourt and Delcourt, 1997).

Effective fire suppression was established as early as 1930 in the Appalachian plateaus of Ohio (Sutherland, 1997) and 1940 in the Blue Ridge Mountains of North Carolina (Harmon, 1982). Fire return intervals increased significantly after that time; for example, Sutherland (1997) reports a post-suppression interval of 57 yr in southeastern Ohio, compared with a return interval of 7.5 yr prior to suppression. As a consequence of this policy, the unmanaged forests of the central and southern Appalachians have become denser, have increased detrital mass, and in some areas, have changed in tree species

composition (e.g., Iverson et al., 1997). It is unclear, however, whether this lengthy period of fire suppression has had a significant effect on the species composition or community structure of the herbaceous perennial assemblages that dominate the plant species diversity of these forests.

This is a particularly important issue because the great majority of the plant diversity present (at any given taxonomic level) in these ecosystems exists in the herbaceous, perennial understory, and the herbaceous stratum in these ecosystems is under the great pressure from herbivory by native mammals (especially white-tailed deer, *Odocoileus virginiana*), timber-harvesting activities, and development of the wildland–urban interface for human occupation.

From the perspective of an understory herb, a lengthy period of fire suppression results in reductions in light flux (due to increased woody stem density and basal area) and nutrient availability (due to greater competition for nutrients and possibly reduced decomposition). Similarly, ecological restoration treatments such as reintroduction of dormant-season fire at historical intervals and overstory thinning to return the woody plant assemblage to pre-suppression characteristics both may increase light, soil temperature and moisture, and short-term nutrient availability. Considerable phenotypic plasticity is probably essential for long-lived herbaceous perennials to persist through decades-long shifts in light, moisture, and nutrient availability.

Instantaneous leaf-level ecophysiological measurements have long been of interest to ecologists as they are often correlated with attributes of plant success and are assumed to determine long-term consequences of changes in abiotic/biotic factors affecting plant performance (McAllister et al., 1998).

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Many previous studies have demonstrated that ecophysiological characteristics change in response to natural ranges of altitude, moisture, light gradients, and soil N availability (Kubiske and Abrams, 1992; Dudley, 1996; Muraoka et al., 1997; Jonas and Geber, 1999), as well as to gaseous air pollutants in controlled growth environments (Loats and Rebbeck, 1999). Recent ecosystem level studies have addressed the manner in which different treatments, such as prescribed burning and restoration thinning, affect fine-scale differences in leaf ecophysiological responses such as photosynthesis, nutrient concentration, and instantaneous nutrient use efficiency (Reich et al., 1990; Kruger and Reich, 1997; Fleck et al., 1998; Lamontagne et al., 2002; Wallin et al., 2004).

In Appalachian hardwood forests, ecological restoration treatments designed to overcome the impact of almost a century of fire suppression (e.g., reintroduction of dormant-season fire at historic intervals, overstory thinning to pre-suppression structure) typically cause significant changes in microenvironmental and edaphic conditions. Overstory thinning reduces canopy leaf area index and increases light availability within a stand. Consequently, understorey perennials previously suppressed under low light might be expected to have enhanced photosynthetic rates as a direct result of the altered light environment. Low-severity, dormant-season fires typical of both natural and prescribed fires in this region typically have little impact on the forest floor light environment because little overstorey mortality results from such fires (Hutchinson et al., 2005). However, such fires generally increase post-fire nutrient availability, and plants may respond by increasing foliar nutrient concentrations, which might result in enhanced photosynthetic capacity (Reich et al., 1990). Attributing higher photosynthetic rates often observed after fire to higher nutrient availability remains controversial. Studies examining the relationship between foliar N and photosynthetic capacity within species are conflicting. Some report positive correlations (Field and Mooney, 1983; Reich and Walters, 1994; Gough et al., 2004), while others report weak or nonexistent relationships between foliar N and photosynthetic capacity (Thomas and Bazzaz, 1999).

General ecophysiological responses of tree seedlings and saplings to resource changes produced by various ecosystem restoration treatments have been well characterized (Reich et al., 1990; Fleck et al., 1998; Gilbert et al., 2003; Clemente et al., 2005). In contrast, the responses of long-lived perennial forest herbs remain poorly quantified, despite the growing view that these species are uniquely suitable as indicators of ecosystem health. In this present study, we examined the effects of prescribed burning alone or combined with overstorey thinning on two perennial herbaceous species commonly found in mixed-oak forests in southern Ohio. The objectives of this study were (1) to evaluate the effects of prescribed fire and thinning on photosynthetic responses and plant resource use strategies, (2) to compare the ecophysiological responses of a dicot with those of a monocot, and (3) to relate leaf-level photosynthetic responses to morphological and nutritional responses, such as specific leaf area and foliar nutrient concentrations.

MATERIALS AND METHODS

Study sites and plant species—This study was conducted in the mixed-oak forests of Zaleski State Forest (82°37' W, 39°35' N) and the Raccoon

Ecological Management Area (REMA) (82°39' W, 39°20' N) on the unglaciated Allegheny Plateau of southeastern Ohio, USA. The climate of the region is cool, temperate, and continental with a mean annual temperature of 11.3°C and mean annual precipitation of 1024 mm. The sites are underlain predominantly by sandstones and shales of Pennsylvanian Age. Soils are mostly loams and silt loams that are acidic and well drained (Sutherland and Hutchinson, 2003). These sites are typical of oak-dominated forests within the central and southern Appalachians. Additional study site details are provided by Boerner et al. (2005).

Two perennial herbaceous species, *Desmodium nudiflorum* (L.) DC (Fabaceae) and *Panicum boscii* Gould & C.A. Clark (Poaceae), were selected at each site based on the following criteria: (1) they provided a comparison between a dicot and a monocot of ecological responses to different restoration treatments, and (2) they were abundant in both study areas and occupied habitats across a light and nutrient gradient.

Desmodium nudiflorum, a native perennial dicot, occurred naturally over a wide range of light and nutrient conditions in both study areas, with the greatest frequency in more open and drier areas. Leaves typically emerge in early to mid-May. Mature plants produce a whorl of trifoliate leaves, each 5–10 cm long. Plants usually have one infertile and one or two fertile stems, which can grow 50 cm or higher. In the field, flowering starts in early August. Each flower is short-lived, but within an inflorescence, flowers can develop sequentially. Loments form in late August, usually producing 2–3 seeds per loment. Each plant can produce 2–50 seeds. Half of the seeds commonly have underdeveloped embryos (the embryo is flat and shorter than half of the seed length). A fully developed seed weighs ~10 mg. Fruit coats are covered with abundant sticky trichomes, which facilitate seed distribution by sticking to animal fur.

Panicum boscii, a perennial grass, was one of the most common grasses in both study areas. It usually colonizes disturbed, open, and relatively dry areas such as ridges and canopy gaps; however, it does occasionally occur in shaded areas. This species occurs most frequently in distinct patches. Leaf blades are 4–10 cm long and 1–4 cm wide. It has large spikelets, hairy nodes, and clasping leaves. Flowering typically starts in early June. Seeds become mature in early July, and mean seed mass is ~1 mg.

Sampling design—This study was part of Ohio Hills Fire and Fire Surrogates (FFS) research site (National Fire and Fire Surrogates Network Study). The original experimental design had randomly assigned control (C), burn (B), thin (T), and thin plus burn (T + B) units of 20–30 ha within three areas of southern Ohio (Tar Hollow State Forest, Zaleski State Forest, and the Raccoon Ecological Management Area [REMA]). These sites were thinned during late fall 2000 and early spring 2001 and burned in early April 2001 and again in April 2005. Thinning from below removed an average of 27.9% of the basal area and left an average of 20.9 m²/ha in residual basal area; this increased the percentage of open sky from approximately 8% (C and B only) to approximately 15% (T and T + B) (D. Yaussy, USDA Forest Service, personal communication). The low-intensity dormant-season spring fires were heterogeneous within and among sites, but flames rarely exceeded 2 m in height. Fire consumed unconsolidated leaf litter and fine woody fuels while leaving the majority of the coarse woody fuels only charred. We sampled three units (C, B, T + B) in two of the study sites (Zaleski and REMA). We randomly chose six 20 × 50 m² study plots in each of the six treatment units. From early June through late July 2005, 3–4 *D. nudiflorum* plants and 2–3 *P. boscii* plants in each sample plot were selected for gas exchange measurements and later harvests. The plants were selected using the following criteria: (1) plants were at least 8 m apart to avoid cluster effects and (2) newly emergent *D. nudiflorum* seedlings and individuals with two or more infertile stems were avoided. *Desmodium nudiflorum* was sampled in all 36 plots, whereas *P. boscii* was found and sampled in 27 of the 36 plots.

Following gas exchange measurements, each whole plant was carefully excavated. Leaf area was determined using a LI-COR 3100 leaf area meter (LI-COR, Lincoln, Nebraska, USA), and leaves were oven-dried at 70°C for 2 d and weighed. Specific leaf area (SLA) for each individual plant was calculated as the total leaf area divided by total leaf dry biomass. Dried foliage (lamina) was ground and then digested using H₂SO₄ and H₂O₂. Nitrogen concentration of the digest was determined using the microplate method (Sims et al., 1995). Leaf N concentration per area (N_{area}) was expressed as the ratio of leaf N on a mass basis divided by SLA.

Gas exchange measurements—Plants were sampled at Zaleski from 25 June to 8 July and at REMA from 8 July to 28 July 2005. Gas exchange rates were measured on fully expanded top leaves (or top laminas of a randomly

selected trifoliate leaf for *D. nudiflorum*). One leaf (lamina) per individual for 3–4 individuals per species was sampled within each plot. Gas exchange was measured midday (10:00 to 15:00) with a LI-COR 6400 portable photosynthesis system (LI-COR). Air was supplied by a constant flow rate, and CO₂ concentration was controlled by the mixer in the LI-COR system console. A built-in LED red light source was used to produce different irradiances during each measurement. Light response curves were generated with photon flux densities (PPFDs) of 1000, 600, 200, 100, 40, 20, 0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 400 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂ and at $27^\circ \pm 2^\circ\text{C}$. In each trial, a leaf was placed in the chamber and allowed to acclimate to the maximum light level, 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Each subsequent measurement was delayed 2–6 min for equilibration before the light level was changed. Previous studies have shown that some plants need 30 min or more to acclimate to new light level (Kursar and Coley, 1993); however, short reaction times (<5 min) are common for many understory species under low light (Pooter and Oberbauer, 1993; Thomas and Bazzaz, 1999). Previous work in this area has shown that stomatal conductance of these two species at 1000 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light level generally fell within the range 0.07–0.3 $\text{mol}\cdot\text{H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (J. Huang, unpublished data). Therefore, if a stomatal conductance of a selected plant was <0.07, that plant was discarded and a different plant was selected and measured.

Curve fitting and parameter calculations—Nonrectangular hyperbolae (Lambers et al., 1998) and Mitscherlich functions (Potvin et al., 1990; Peek et al., 2002; Heschel, 2004) are widely used in fitting light response curves. However, we found the Mitscherlich function tended to yield unacceptably low quantum use efficiency estimates. Therefore, light response curves fitted to our data using the model of Hanson et al. (1987) in a modified Gauss–Newton nonlinear iterative method (SAS Institute, 2001) are presented. This approach yielded quantum efficacy within a reasonable range (Singsaas, 2001) as well as small sums of squares of residuals. The maximum photosynthetic rate (A_{max}), quantum yield (QE), light compensation point (LCP), and “dark” respiration rate (DR) were calculated from each light response curve according to Hanson et al. (1987). Because “dark” respiration rates were estimated from the best-fit model, they may differ to some degree from those estimated from direct measurements at night in the field. However, even such field measurements may vary from true dark respiration given variations in nighttime light fluxes from moonlight and cloud cover. The light saturation point (LSP) was estimated as the light level at 90% of A_{max} (Thomas and Bazzaz, 1999). Intrinsic water use efficiency (WUE) was estimated from the ratio of net photosynthetic rate (A) to stomatal conductance (g_i) instead of transpiration rate (E) (Osmond et al.) because A/g_i usually provides a more consistent estimate of water use efficiency than A/E (Muraoka et al., 1998). Photosynthetic nitrogen use efficiency (NUE) was calculated as $A_{\text{max}}/\text{leaf [N]}$.

Statistical analysis—After fitting the light response curve for each plant, light curve parameters (A_{max} , QE, LCP, DR, and LSP) were estimated. These parameters, together with stomatal conductance (g_i) and intrinsic water use efficiency (A/g_i) at photosynthetic photon flux density [PPFD] = 1000 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and NUE, were averaged for each plot, and plot means were then analyzed as response variables using mixed-model (PROC MIXED) analysis of variance (ANOVA) (SAS Institute, 2001). In these analyses, site was considered a random effect while species, treatment, and the interaction between these two factors were included as fixed effects. Plots, the experiment unit, were considered replications. Least-square means were used to test for significant species and treatment differences.

Where treatment and treatment-by-species effects were significant ($P \leq 0.05$), a posteriori least-squares mean separations with Tukey–Kramer correction were used to test for differences among treatments. Normality and homogeneity of variance of the residuals were tested using PROC UNIVARIATE in SAS (SAS Institute, 2001).

We acknowledge that the spatial design might be considered pseudoreplication. However, given the homogeneity of the forest stand (Sutherland and Hutchinson, 2003), the similarity of the soil characteristics among the treatment units (Sutherland and Hutchinson, 2003), and the heterogeneity of microenvironment and soil conditions after the application of the treatments (Boerner and Brinkman, 2004), we believe the individual sampling plots were spatially independent of one another, with no cluster effects or spatial autocorrelation issues. The spatial independence of sample plots and the appropriateness of this statistical design has been demonstrated in these study sites in previous studies (Boerner and Brinkman, 2004; Boerner et al., 2005). Linear regression was used in each treatment to examine the relationship between SLA, N_{area} , and A_{max} .

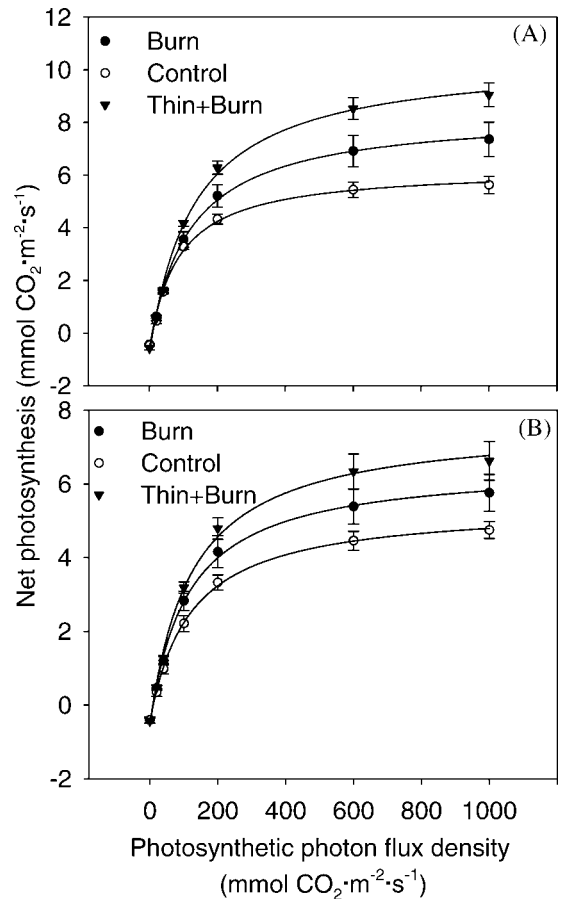


Fig. 1. Net photosynthesis of (A) *Desmodium nudiflorum* and (B) *Panicum boscii* in relation to photosynthetic photon flux density. Symbols represent means with standard errors. For *D. nudiflorum*, $N = 12$ for each treatment. For *P. boscii*, $N = 7, 10, 10$ in the control, burn, and thin + burn treatments, respectively.

RESULTS

Net photosynthesis (A) and stomatal conductance (g_i)—In both *D. nudiflorum* and *P. boscii*, thinning combined with burning (T + B) greatly enhanced net photosynthesis and stomatal conductance (g_i), compared with the control plants (Figs. 1, 2). Burning alone (B) increased net photosynthesis and stomatal conductance to a lesser extent (Figs. 1, 2). The B plants exhibited greater variation in net photosynthesis and stomatal conductance than did either the C or T + B treatment plants of both species (Figs. 1, 2). Across all treatments *D. nudiflorum* had higher net photosynthetic rate and stomatal conductance than *P. boscii*.

The effects of the ecosystem restoration treatments on intrinsic WUE and g_i at light saturation condition (PPFD = 1000 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) were similar in the two species (Fig. 3). Because there were no significant species-by-treatment interactions for either WUE ($P = 0.762$) or g_i ($P = 0.139$), plants of the two species were pooled for analysis of treatment effects. Plants from the T + B plots had the highest g_i , while plants from the other two treatments did not differ significantly from one another (Fig. 3). Plants from the B plots had higher intrinsic WUE than plants from either the T + B plots or the

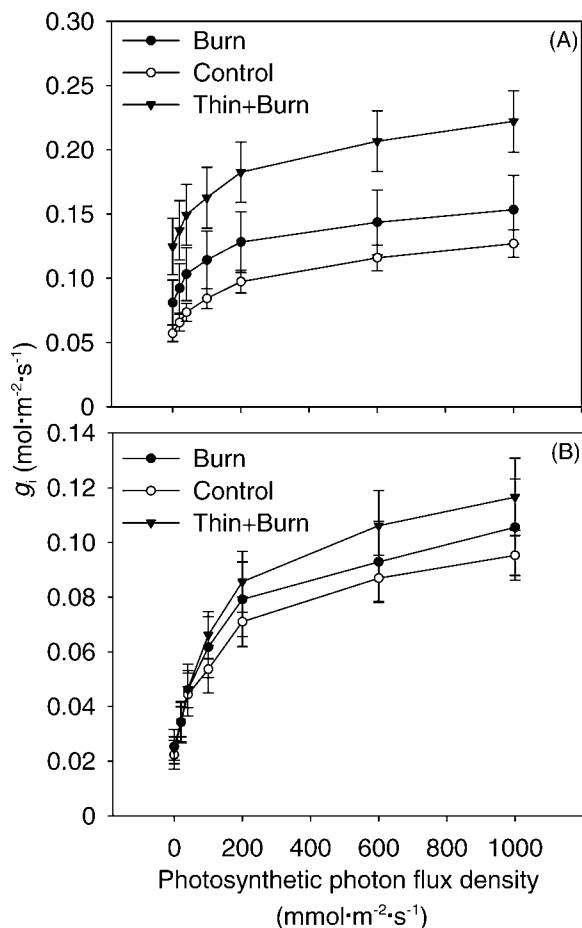


Fig. 2. Stomatal conductance of (A) *Desmodium nudiflorum* and (B) *Panicum boscii* in relation to photon flux density. See Fig. 1 for sample information.

controls, and the latter two did not differ significantly from one another (Fig. 3). Over all treatments, *P. boscii* had lower stomatal conductance ($P < 0.001$) and greater WUE ($P = 0.023$) than that of *D. nudiflorum*.

Light responses of photosynthetic parameters—The photosynthetic parameters A_{\max} , QE, PNUE, and LSP varied significantly among treatments and between species (Table 1 and Fig. 4). As was the case for WUE and g_i , there were no significant species-by-treatment interactions in the ANOVA (Table 1), and therefore the two species were pooled for analysis of treatment effects.

Control plants had significantly lower A_{\max} than those in the treated plots. Maximum photosynthetic rate in plants from the B plots averaged 31.1% greater in *D. nudiflorum* and 24.9% greater in *P. boscii* than in the control plot plants (Fig. 4A). Similarly, plants in the T + B plots had A_{\max} that was 60.9% greater than controls in *D. nudiflorum* and 46.6% greater than controls in *P. bosci* (Fig. 4A). Quantum yield, a measure of photosynthetic efficiency when light is limited, was significantly greater in the T + B treatment than in either the B and or C treatments, which did not differ from one another (Fig. 4B). The “dark” respiration rate, light compensation point, and saturation point did not differ significantly among treatments

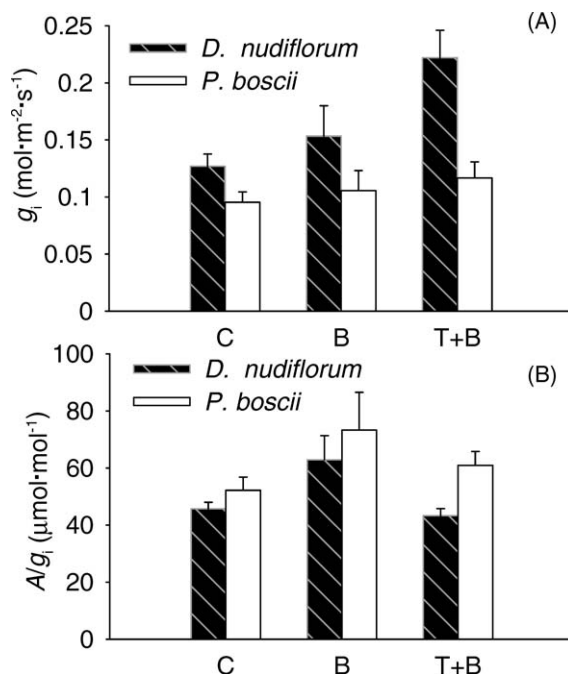


Fig. 3. (A) Stomatal conductance (g_i) and (B) intrinsic water use efficiency (A/g_i) of two herbaceous species in response to ecosystem restoration treatments. Abbreviations along the x -axis represent control (C), burning alone (B), and burning combined with thinning (T + B). Bars represent means with standard errors. For *Desmodium nudiflorum*, $N = 12$ plots for each treatment. For *Panicum boscii*, $N = 7, 10, 10$ in the C, B, and T + B treatments, respectively.

(Fig. 4C–E). The instantaneous PNUE, an index of the efficiency of N use during the biochemical reactions of photosynthesis (Field and Mooney, 1983), was significantly higher in T + B plants than B or C plants (Fig. 4F).

Compared to *P. boscii*, *D. nudiflorum* had significantly higher A_{\max} (7.20 ± 0.36 vs. 5.87 ± 0.30 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), QE (0.058 ± 0.002 vs. 0.045 ± 0.003 $\mu\text{mol CO}_2\cdot\text{mol}^{-1}$ photons), and PNUE (114.64 ± 4.77 vs. 81.23 ± 6.16 $\mu\text{mol CO}_2\cdot\text{mol}^{-1}\cdot\text{N}\cdot\text{s}^{-1}$). Light saturation points and DR did not differ significantly between these two species (Table 1).

Relationship between A_{\max} , specific leaf area, and leaf [N]—In *D. nudiflorum*, A_{\max} was significantly and negatively correlated with SLA in plants given the B and T + B treatments, but not among the control plants ($P = 0.001$ in B and $P = 0.066$ in T + B plants; Fig. 5A). When the three treatments were pooled, A_{\max} was again negatively correlated with SLA ($P < 0.001$). Similarly, we observed that SLA was negatively correlated with total plant biomass (data not shown). In contrast, in *P. boscii* A_{\max} and SLA were not significantly correlated when treatments were considered separately (Fig. 5B) and were only marginally correlated when three treatments were pooled ($P = 0.067$).

In *D. nudiflorum*, A_{\max} was positively correlated with N_{area} in B and T + B plants, but not in control plants ($P = 0.027$ in B and $P = 0.019$ in T + B plants; Fig. 5C). When treatments were pooled for *D. nudiflorum*, A_{\max} was positively and significantly correlated with N_{area} ($P < 0.001$). For *P. boscii*, a significant relationship between A_{\max} and N_{area} was only detected with T +

TABLE 1. Three-factor ANOVA for stomatal conductance rate, intrinsic water use efficiency, light response curve parameters, and instantaneous N use efficiency among different treatments and species. Numbers presented are *F* ratios. Tukey-Kramer was used to adjust *P* values. **P* < 0.05, ***P* < 0.01.

Source	df	<i>g</i> _i	WUE	<i>A</i> _{max}	QE	LCP	DR	LSP	PNUE
Species	1	18.61**	6.64**	14.44**	21.87**	3.09	0.45	4.42*	23.24**
Treatment	2	5.36**	3.95*	16.78**	6.60**	1.75	3.02	1.57	7.10**
Species × treatment	2	2.05	0.47	0.68	1.28	2.50	0.87	1.79	3.07
Error	56								

Notes: *g*_i, stomatal conductance rate at photosynthetic photon flux density [PPFD] = 1000 mmol·m⁻²·s⁻¹; WUE, intrinsic water use efficiency at PPFD = 1000 mmol·m⁻²·s⁻¹; *A*_{max}, maximum photosynthetic rate; QE, quantum yield; LCP, light compensation point; DR, dark respiration rate; LSP, light saturation point, which was estimated as the light level at 90% of *A*_{max}; PNUE, photosynthetic nitrogen use efficiency.

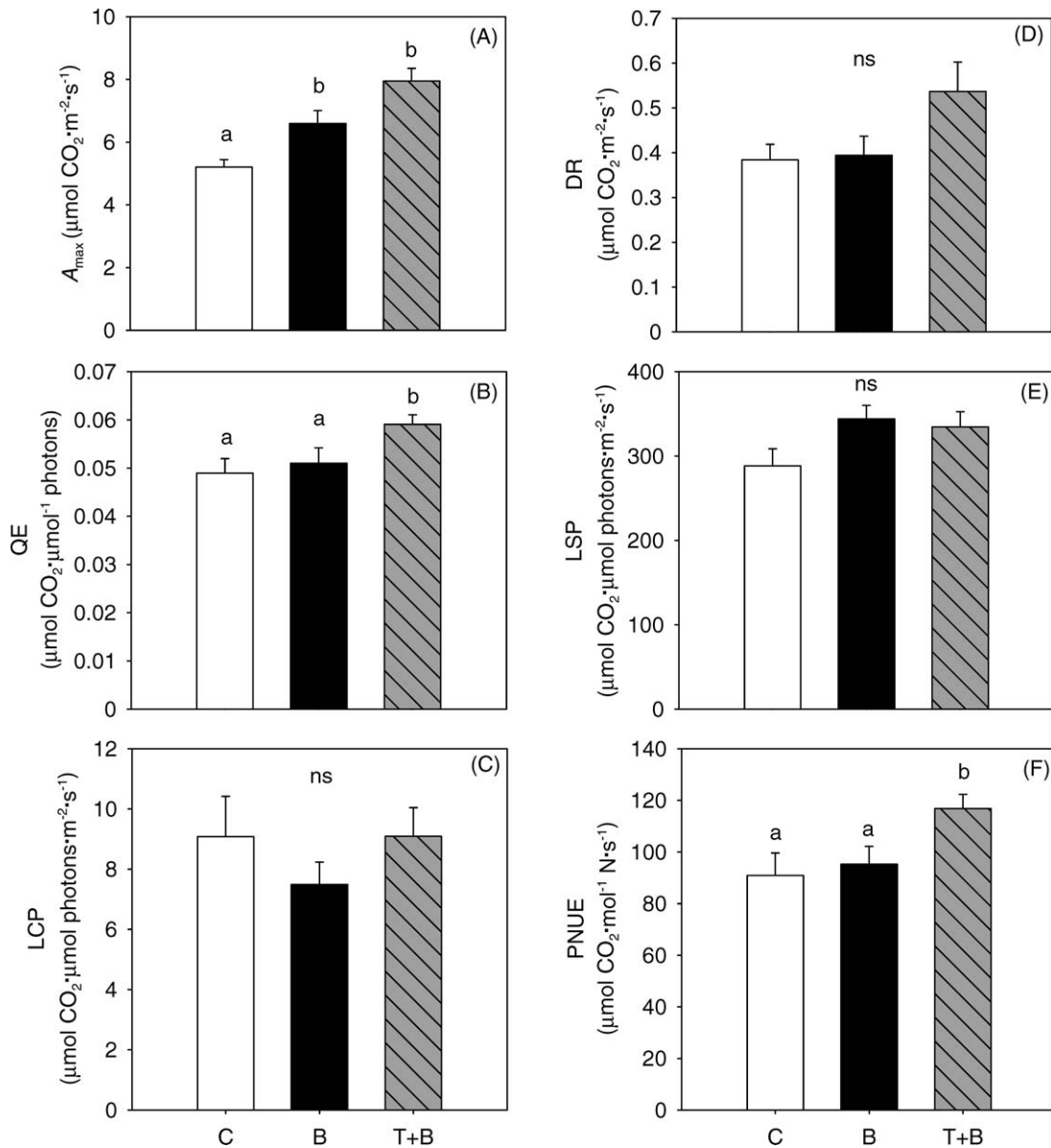


Fig. 4. Photosynthetic light responses (maximum photosynthetic rate [*A*_{max}], quantum yield [QE], light compensation point [LCP], “dark” respiration [DR], light saturation point [LSP], and photosynthetic nitrogen use efficiency [PNUE]) of two herbaceous species to ecosystem restoration treatments. Abbreviations along the *x*-axis represent control (C), burning alone (B), and burning combined with thinning (T + B). Bars represent means with standard errors. Means with the same lowercase letter did not differ significantly based on Tukey-Kramer pairwise comparisons at the $\alpha = 0.05$ level of significance; “ns” represents no significant differences among treatments.

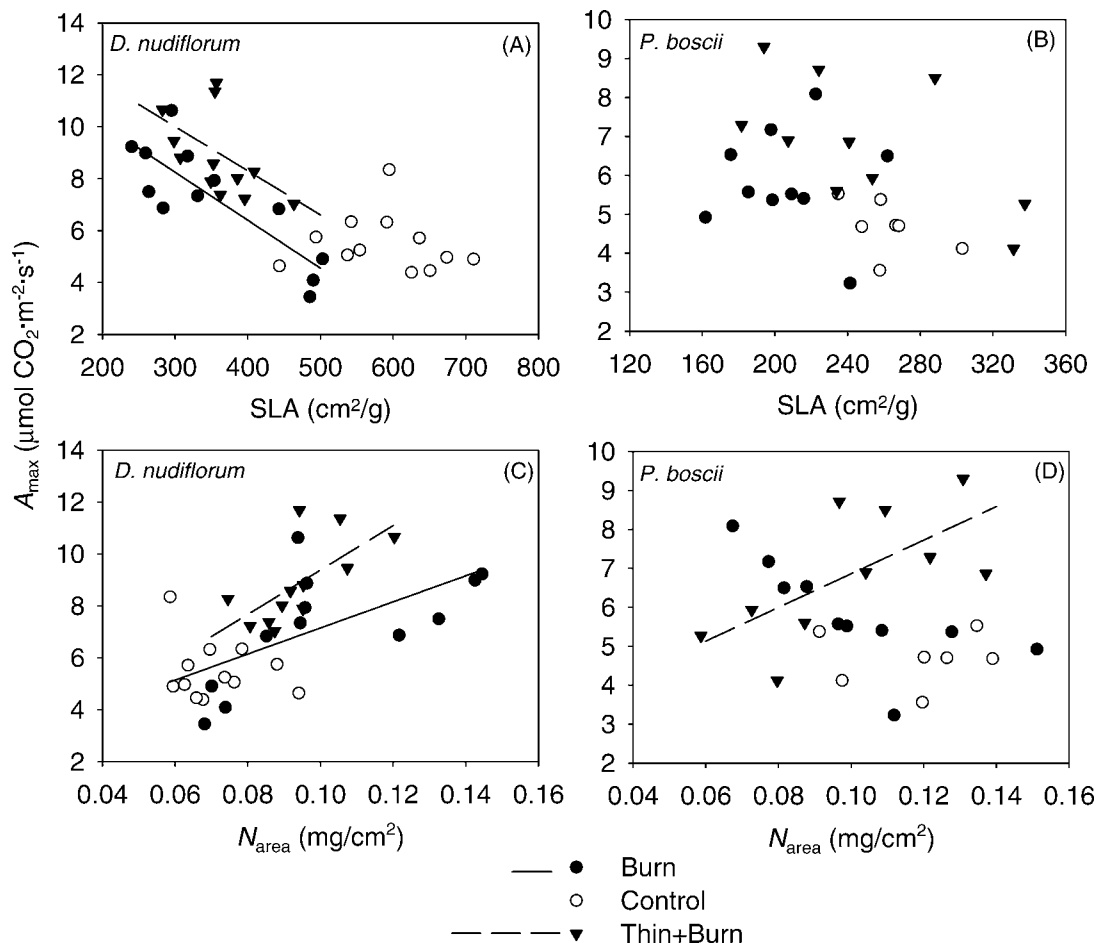


Fig. 5. Relationship between specific leaf area (SLA), leaf N concentration per area (N_{area}) and maximum photosynthetic rate (A_{\max}) for two herbaceous species among different ecosystem restoration treatments. (A) A_{\max} and SLA in *Desmodium nudiflorum*. Burn: $A_{\max} = 13.8 - 0.0185 \text{ SLA}$, $R^2 = 0.7$, $P = 0.001$; Control: A_{\max} is not correlated with SLA; Thin + Burn: $A_{\max} = 15.1 - 0.0174 \text{ SLA}$, $R^2 = 0.299$, $P = 0.066$. (B) A_{\max} and SLA in *P. boscii*. No significant correlations were found in any treatment. (C) A_{\max} and N_{area} in *D. nudiflorum*. Burn: $A_{\max} = 2.13 + 50.2 N_{\text{area}}$, $R^2 = 0.399$, $P = 0.027$; Control: A_{\max} is not correlated with N_{area} ; Thin + Burn: $A_{\max} = 0.84 + 85.4 N_{\text{area}}$, $R^2 = 0.441$, $P = 0.019$. (D) A_{\max} and N_{area} in *Panicum boscii*. Thin + Burn: $A_{\max} = 2.54 + 43.2 N_{\text{area}}$, $R^2 = 0.448$, $P = 0.034$. No significant correlations were found in the Burn and the Control treatments.

B plants (Fig. 5D); when treatments were pooled, A_{\max} and N_{area} were not significantly correlated ($P = 0.566$).

DISCUSSION

Response to silvicultural treatments—Prescribed burning alone (B) or in combination with thinning (T + B) resulted in enhanced photosynthetic performance of two common woodland herbaceous plant species, relative to control plants, and the combination treatment stimulated photosynthesis more than burning alone (52.7% vs. 26.7%). Similarly, we also observed that mean plant biomass was approximately 82% and 113% greater in *D. nudiflorum* from the B and T + B treatments, respectively, than in control plants (Huang, 2007). Moreover, both seed number and mean seed mass were significantly greater in plants from the manipulated sites than that in control plants (Huang, 2007). The positive influence of fire alone and in combination with thinning on plant photosynthesis, plant biomass, seed size, and total seed production may facilitate the

persistence and abundance of this herbaceous species in the post-fire years.

Light is a limiting resource for many understory herbaceous species. The T + B treatment greatly enhanced canopy openness (average of ~8% in control and B, average of ~15% in T + B; T. Hutchinson, USDA Forest Service, unpublished data). Increased photosynthetic and leaf conductance rates of both species within the T + B plots would only have been unexpected if one or both were strongly adapted to shade conditions. We did not expect either species to be strongly adapted to shade because both species (and particularly *P. boscii*) tend to be somewhat more abundant in areas of less dense canopy cover.

As low-intensity, dormant-season prescribed fire usually has little effect on large canopy trees in this ecosystem type, restoration burning is not likely to increase light availability to understory plants as much as thinning (Hutchinson et al., 2005). However, prescribed fire does remove forest floor litter and can influence short-term soil N availability. Reich et al. (1990) demonstrated post-fire enhancement of leaf photosynthesis in tree seedlings in a mixed-oak forest in central

Wisconsin. The authors considered this post-fire stimulation to be a consequence of enhanced leaf N concentration resulting from increased soil N availability. However, fire decreased both N and P availability in our study area (Boerner et al., 2004; Huang, 2007). Therefore, the positive photosynthetic responses to burning in our study cannot be explained by enhanced soil N availability. In addition, though burning alone enhanced A_{\max} in both species, the response varied greatly among plots and between study sites. We suggest that the patchy and variable enhancement of photosynthesis in these two species was most likely the result of changes in forest floor microclimate (e.g., soil temperature, soil moisture, litter depth) that tend to be spatially very heterogeneous after the low-severity fires.

Stomatal conductance responses were similar to those of A_{\max} . The combination of thinning and burning resulted in significantly higher conductance rates, whereas burning alone did not. This response is likely due to increased water availability resulting from decreases in both canopy interception and plant transpiration resulting from thinning the canopy (Swift et al., 1993; Brèda et al., 1995). Intrinsic water use efficiency was consistently highest in plants in the burn-only plots, confirming previous reports (Fleck et al., 1995, 1996). Relative to the control conditions, B plots are warmer (due to the blackened forest floor surface) while T + B plots are both warmer and moister (due to reduced canopy interception and transpiration). We suggest that the plants in the burned units are experiencing greater water stress (at least relative to CO_2 uptake) than are plants in the control and T + B plots.

Species differences—These two herbaceous species represent different life histories, leaf structure, and foliar nutrient concentrations. *Desmodium nudiflorum* is a legume with high SLA, mass-based N concentrations (N_{mass}), and intermediate shade tolerance. In contrast, *P. boscii* is a shade-intolerant monocot with relatively small SLA and low N_{mass} . *Desmodium nudiflorum* had higher A_{\max} , stomatal conductance, QE, and NUE than *P. boscii*. Empirical studies have demonstrated that high-SLA species generally have higher leaf water content per unit dry mass, lower concentrations of cell wall compounds and C per unit leaf mass, and N_{mass} , mass-based A_{\max} , and PNUE (Pooter and Evans, 1998; Reich et al., 1998). The lower SLA of *P. boscii* may also contribute to its greater WUE. Low-SLA species usually have thick, dense leaves, which increases the distance through which water must diffuse to leave the leaf, leading to water conservation (McDowell, 2002). Taken together, these differences suggest that *P. boscii* is likely to be better suited to more open, dry conditions than *D. nudiflorum*.

Overall, *D. nudiflorum* had more and greater responses to the restoration treatments than did *P. boscii*; thus, *D. nudiflorum* had more plasticity in response to changes in the light environment than *P. boscii*. One component of this greater plasticity of *D. nudiflorum* may involve its symbiotic relationship with N-fixing bacteria. We observed far more nodules on the roots of *D. nudiflorum* from the T + B and B treatments than on the roots of control plants (Huang, 2007), and this suggests a greater potential for N fixation in the managed sites. Greater N fixation in the nodules may lead to increased leaf N and therefore enhanced photosynthetic capacity.

Relationship between photosynthetic capacity, SLA, and leaf N—Empirical studies have shown a strong positive

correlation between light-saturated rate of photosynthesis and N content across a range of species (Field and Mooney, 1986; Evans, 1989; Reich et al., 1994). An increase in A_{\max} associated with foliar [N] can result from either increased stomatal conductance or increased carboxylation capability (Brown et al., 1996). After studying more than 100 species in six distinct biomes of the Americas, Reich et al. (1999) concluded that N_{mass} , SLA, and A_{\max} were all positively correlated with each other. However, other investigators have reported mixed results in characterizations of the relationship between foliar N status and A_{\max} within a given species (Murthy et al., 1996; Schoettle and Smith, 1999). Our results indicate that A_{\max} increased with N_{area} in the more phenotypically plastic *D. nudiflorum* but not in less plastic *P. boscii*. Similarly, we observed that stomatal conductance was positively correlated with N_{area} in *D. nudiflorum* but not in *P. boscii*. This suggests either that *P. boscii* has higher resistance to CO_2 diffusion to Rubisco-active sites in the chloroplast or, alternately, that this species allocates a relatively low proportion of its N to the photosynthetic apparatus at any given foliar [N] (Seemann et al., 1987; Wullschleger, 1993; Brown et al., 1996).

The relationship between SLA and A_{\max} differed between these two species. *Desmodium nudiflorum* had higher SLA, and, surprisingly, it also had higher area-based and mass-based A_{\max} even though N_{area} was lower than *P. boscii*. This is probably due to higher photosynthetic N use efficiency in *D. nudiflorum* than *P. boscii*. Our results showed a strong and significant negative relationship between SLA and A_{\max} in *D. nudiflorum* ($P < 0.001$) but only a marginally significant, relatively weak negative trend in *P. boscii* ($P = 0.076$). Because A_{\max} was positively correlated with plant biomass in *D. nudiflorum*, it is not surprising that SLA was negatively correlated with plant biomass. This again reinforces the conclusion that *D. nudiflorum* has greater phenotypic plasticity than *P. boscii* because *D. nudiflorum* can adjust SLA, leaf thickness, and N_{area} in response to changes in the light environment to a much greater extent than can *P. boscii*.

Both of these perennial herbs have persisted in these forests through the changes brought about by almost a century of fire suppression. *Panicum boscii* has persisted by occupying those limited areas where the light environment and forest floor microclimate have remained more like what one would have seen prior to fire suppression such as more open and drier areas. *Desmodium nudiflorum* has remained somewhat more cosmopolitan in its distribution within these forests. Both species responded positively to the combined thinning and burning treatment, with the response of *D. nudiflorum* greater in both magnitude and in the range of ecophysiological processes affected. Continued monitoring of the abundance and fecundity of these two perennials as the restoration treatments continue is critical to determining whether these relatively short-term responses will translate to increased fitness and abundance over time.

Mixed-oak forest occupies more of North America east of the Mississippi River today than does any other natural or quasi-natural ecosystem type, and the large majority of the plant species (genus, family) diversity present in this ecosystem type occurs in the herbaceous understory. Despite a rich literature on the influences of human activities on tree species, relatively little is known about the impacts on the herbaceous perennials that dominate this stratum (Gilliam and Roberts, 2003). The strong, positive physiological responses to

ecosystem restoration manipulations demonstrated here supply a measure of confidence that the diversity that characterized these assemblages prior to European contact can be preserved for the longer term.

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