

Leaf- and shoot-level plasticity in response to different nutrient and water availabilities

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Summary Phenotypic plasticity in response to environmental variation occurs at all levels of organization and across temporal scales within plants. However, the magnitude and functional significance of plasticity is largely unexplored in perennial species. We measured the plasticity of leaf- and shoot-level physiological, morphological and developmental traits in nursery-grown *Populus deltoides* Bartr. ex Marsh. individuals subjected to different nutrient and water availabilities. We also examined the extent to which nutrient and water availability influenced the relationships between these traits and productivity. *Populus deltoides* responded to changes in resource availability with high plasticity in shoot-level traits and moderate plasticity in leaf-level traits. Although shoot-level traits generally correlated strongly with productivity across fertilization and irrigation treatments, few leaf-level traits correlated with productivity, and the relationships depended on the resource examined. In fertilized plants, leaf nitrogen concentration was negatively correlated with productivity, suggesting that growth, rather than enhanced leaf quality, is an important response to fertilization in this species. With the exception of photosynthetic nitrogen-use efficiency, traits associated with resource conservation (leaf senescence rate, water-use efficiency and leaf mass per area) were uncorrelated with short-term productivity in nutrient- and water-stressed plants. Our results suggest that plasticity in shoot-level growth traits has a greater impact on plant productivity than does plasticity in leaf-level traits and that the relationships between traits and productivity are highly resource dependent.

Keywords: adaptive plasticity, leaf initiation, photosynthesis, *Populus deltoides*, productivity, relative growth rate, resource availability, seasonal patterns.

Introduction

A remarkable feature of plants is their ability to respond plastically to changes in their environment (Grime et al. 1986, Sultan 2000, Givnish 2002). Although plasticity is assumed to be

adaptive, tests of adaptive plasticity (defined here as the capacity of a single genotype to produce different functionally appropriate phenotypes in different environments; Sultan 1995) are rare and tend to focus on one or a few traits, usually at one scale of organization. Few studies have investigated the effects of resource availability on the contributions of leaf- and plant-level plasticity to fitness or productivity (Blais and Lechowicz 1989, Kitajima 1994, Sims et al. 1994, Williams et al. 1995, Coleman et al. 1998, Poorter 1999, Montgomery 2004, Pons and Anten 2004, Heschel et al. 2004, Monclus et al. 2006). Understanding the functional significance of trait responses to environmental heterogeneity is critical for identifying which ecophysiological traits are likely to be subject to adaptive evolution (Ackerly et al. 2000) and for identifying predictors of productivity (e.g., Monclus et al. 2006). This information is also essential for modeling responses of natural and agricultural systems to human influences (Funk and Lerdaу 2004).

Many studies examining the functional significance of plasticity in various traits have focused on herbaceous annuals or short-lived perennials (but see Pigliucci et al. 1997, Pons and Anten 2004, Casper et al. 2005 and Valladares et al. 2005), because it is relatively easy to measure fitness in short-lived species (Geber and Griffen 2003). It has been suggested that plasticity is more likely to increase fitness in annual species because they reproduce during only one year (Sultan and Bazzaz 1993b). In contrast, perennial species may allocate biomass to organs that capture limiting resources and, consequently, forego reproduction in the short-term to enhance lifetime reproductive output (Bloom et al. 1985). However, the plasticity observed across different levels of organization and the functional significance of this plasticity in perennial species are largely unexplored.

We examined plasticity in leaf- and shoot-level traits in eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.) in response to different nutrient and water availabilities. *Populus deltoides* is a fast-growing tree with indeterminate growth that often occurs in disturbed areas characterized by high, but spatially patchy and temporally fluctuating, resource availability.

Because of their fast growth, high potential for cloning, commercial importance and high sensitivity to various biotic and abiotic factors (including drought and nutrient availability), *Populus* species are frequently studied to examine the influence of various stresses on physiology and growth (e.g., Coleman et al. 1998, Coyle and Coleman 2005, Marron et al. 2006, Monclus et al. 2006). We examined how leaf- and shoot-level traits correlate with plant productivity. Specifically, we tested the hypothesis that *P. deltoides* displays high correlations between: (1) shoot-level growth and leaf-level assimilation traits (e.g., leaf initiation and photosynthetic rates) and productivity under high resource availability; and (2) resource conservation traits (e.g., water-use efficiency and leaf mass per area) and productivity under low resource availability.

Interpreting the functional significance of plasticity is complicated, because several environmental factors can evoke plastic responses from the same suite of plant traits at the same time. For example, changes in nutrient and water availability can simultaneously alter leaf longevity and patterns of belowground biomass allocation (Chapin 1991). Furthermore, the functional significance of a plastic response may depend on the environmental context in which the response occurs (Lechowicz and Blais 1988). For example, Heschel et al. (2004) found that plasticity in a leaf-level physiological trait (water-use efficiency) had a greater influence on fitness in water-limited habitats, whereas plasticity in a plant-level trait (root biomass allocation) was more closely correlated with fitness in moist habitats. Thus, our third objective was to determine the extent to which water and nutrient availability influence the relationships between leaf- and shoot-level traits and productivity.

Materials and methods

Experimental design

We conducted the study in a field nursery at the Institute of Ecosystem Studies in Millbrook, NY (41°51' N, 73°45' W, 130 m a.s.l.) between June and August 1999. Eastern cottonwood individuals were propagated vegetatively from a single clone (ST109, Stoneville, MS) one year before planting as single-leader saplings. Phenotypic variation within plant species (V_P) consists of genetic variation (V_G), environmental variation (V_E) and variation associated with genotype by environment interactions ($V_{G \times E}$; see DeWitt and Scheiner 2004). Therefore, to examine V_E in the absence of potentially confounding sources of V_G and $V_{G \times E}$, we studied a single genotype.

Plants were randomly assigned to one of three fertilization treatments (low nutrient, control or high nutrient) and one of two irrigation treatments (irrigated or non-irrigated) in a completely randomized 3×2 factorial design for nutrient and water availability. Plants in control and high-nutrient treatments were planted in nursery soil, which is rocky and relatively nutrient-poor (Wait 1997). In the field nursery, holes were dug with a post-hole digger and plants were spaced 2 m apart. For

the low-nutrient treatment, holes were filled with a 2:1 (v/v) mixture of sawdust and nursery soil. Although it is possible that adding organic matter to the soil increased its water holding capacity, stomatal conductance (g_s) data suggest that plant water status was similar across fertilization treatments (see Results). Plants in the high-nutrient treatment received 3.5 g N, 3.7 g P and 3.7 g K spread over six applications. Irrigated plants received water by drip irrigation (5–10 l) every 2 to 4 days. In addition, plants in both irrigation treatments received a total of 118.4 mm of precipitation during the study. All plants received full sunlight. Overall, there were 20 irrigated and 16 non-irrigated plants in each of the nutrient treatments. Three plants (of 108) died during the experiment: two in the control + non-irrigated treatment and one in the low nutrient + non-irrigated treatment.

Physiological measurements

Leaves were assigned positions, with the most apical leaf greater than 2 cm in length and with fully unfurled leaf edges designated as Leaf 0. A consecutive number was given to each successive leaf from the apex to the base. To determine the leaf position of full expansion (LPFE; Table 1 lists studied trait abbreviations, descriptions and units), the lengths of Leaves 0–9 were measured over 3 to 4 days from June 23–25, July 7–9, July 26–29 and August 16–18. A leaf was considered fully expanded when its length changed no more than 1 mm over 24 h.

Physiological measurements were conducted on Leaves 1, 3, 5, 7 and 9. Leaves 1, 3, 7 and 9 were measured on each of seven irrigated plants from each fertilization treatment. Leaf 5 (fully expanded) was measured on 17 irrigated and eight non-irrigated plants from each fertilization treatment. Photosynthetic rate (A_{1200}), g_s and transpiration rate were measured at a photosynthetic photon flux of $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$, an

Table 1. Abbreviations and descriptions of measured traits.

Variable	Description	Units
<i>Leaf-level traits</i>		
A_{1200}	Photosynthetic rate at $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$	$\mu\text{mol m}^{-2} \text{s}^{-1}$
g_s	Stomatal conductance	$\text{mol m}^{-2} \text{s}^{-1}$
WUE	Water-use efficiency	$\mu\text{mol mol}^{-1}$
ϕ	Apparent quantum yield	$\mu\text{mol } \mu\text{mol}^{-1}$
V_{cmax}	Maximum rate of carboxylation	$\mu\text{mol m}^{-2} \text{s}^{-1}$
R_{day}	Daytime respiration rate	$\mu\text{mol m}^{-2} \text{s}^{-1}$
N_a	Area-based leaf nitrogen concentration	g m^{-2}
N_m	Mass-based leaf nitrogen concentration	mg g^{-1}
PNUE	Photosynthetic nitrogen-use efficiency	$\mu\text{mol mol}^{-1} \text{s}^{-1}$
LMA	Leaf mass per area	g m^{-2}
<i>Shoot-level traits</i>		
RGR	Relative growth rate of apical stem	day^{-1}
D	Stem diameter	mm
LIR	Leaf initiation rate	leaves day^{-1}
LSR	Leaf senescence rate	leaves day^{-1}
LPFE	Leaf position at full expansion	leaf number from leaf 0 ¹

¹ First leaf > 2 cm in length with fully unfurled margins.

ambient CO₂ concentration of 400 $\mu\text{l l}^{-1}$ and a leaf temperature between 26 and 28 °C with a LI-6400 portable photosynthesis system (LI-COR). Water-use efficiency (WUE) was calculated as the ratio of photosynthesis to transpiration.

Light and CO₂ response curves were determined from photosynthetic measurements of Leaf 5 from each of six irrigated and four non-irrigated plants from each fertilization treatment. Light curves were determined by varying irradiance between 50 and 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at an intercellular CO₂ concentration (C_i) of 280 $\mu\text{l l}^{-1}$ and leaf temperature between 26 and 28 °C. We estimated apparent quantum yield (ϕ) and daytime respiration rate (R_{day}) from light curves fit to a rectangular hyperbola as in Causton and Dale (1990). The value of ϕ indicates carbon assimilation efficiency at low irradiance (light-use efficiency). The CO₂ response curves were determined by varying CO₂ concentration between 0 and 800 $\mu\text{l l}^{-1}$ at an irradiance of 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a leaf temperature between 26 and 28 °C. The maximum carboxylation rates (V_{cmax}) were estimated from the CO₂ response curves when $C_i < 200 \mu\text{l l}^{-1}$ following Wullschlegel (1993) and based on the photosynthesis model of Farquhar et al. (1980). We fixed the CO₂ compensation point in the absence of R_{day} at 38.9 $\mu\text{l l}^{-1}$ (Funk et al. 2004). Michaelis-Menten constants for carboxylation (K_c) and oxygenation (K_o) were 310 $\mu\text{l l}^{-1}$ and 155 ml l^{-1} , respectively, and the partial pressure of O₂ was 210 ml l^{-1} following Kirschbaum and Farquhar (1984).

All photosynthetic measurements were made between August 12 and 31 from 0900 to 1500 h. After each gas exchange measurement, leaves were dried at 65 °C for three days, ground and analyzed for leaf N with an elemental analyzer (CE Instruments Flash EA 1112, CE Elantech, Lakewood, NJ). Photosynthetic nitrogen-use efficiency (PNUE) was calculated as the ratio of photosynthesis to leaf N. Leaf N and leaf mass per area (LMA) were measured only for irrigated plants.

Growth and development measurements

One week after planting, we measured initial height, stem diameter and leaf number. Leaf 3 was tagged and its position monitored over time to determine the leaf initiation rate (LIR). The stem diameter (D) at a fixed point (initially Leaf 3) was measured three times. Stem diameter, which is highly correlated with total plant biomass in *Populus* species across nutrient and water treatments (Coyle and Coleman 2005, Monclus et al. 2006), was taken as a measure of productivity.

Stem elongation was monitored from the base of the current-year growth to the widest point of the apical bud. Relative growth rate of the apical stem (RGR) was calculated as: $\text{RGR} = (\ln L_2 - \ln L_1) / (t_2 - t_1)$, where L_1 and L_2 are the lengths of the apical stem at times t_1 and t_2 , respectively. Evaluating relative growth rate rather than absolute height or size allowed us to account for large increases in absolute growth in large plants. During each measurement period, we counted the total number of leaves with unfurled edges, including senescing leaves. Leaf initiation rates for a given measurement period were calculated as the number of new leaves initiated between measurements divided by the number of days in the measurement period. Leaf senescence rate (LSR) was calculated as the total

leaf number minus the number of new leaves initiated.

Statistical analysis

To compare plasticity among leaf- and shoot-level traits, we generated a plasticity index (PI_V) for each trait (Valladares et al. 2006). The index ranges from zero (no plasticity) to one (maximum plasticity) and is the difference between the minimum and maximum mean value of a trait among treatments divided by the maximum value. To quantify plasticity in response to nutrient availability, plasticity was calculated across the fertilization treatments for each irrigation treatment. To quantify plasticity in response to water availability, plasticity was calculated across the irrigation treatments within each of the nutrient treatments.

Treatment differences for all traits were analyzed by two-way analysis of variance (ANOVA) with fertilization and irrigation as main effects. Pearson product-moment correlation coefficients were generated to evaluate the linear association among traits and D . Because PI_V was calculated from mean values, we had insufficient degrees of freedom to evaluate treatment effects on PI_V for individual traits. Instead, we employed one-way ANOVA to evaluate treatment effects on PI_V of leaf- versus shoot-level traits, with individual traits as replicates. Data that violated the ANOVA assumptions of normality and homogeneity of variance were rank transformed.

Results

Leaf-level responses

Stomatal conductance of non-irrigated plants was 14–35% less than that of irrigated plants (Table 2), which indicates moderate drought stress. For Leaf 5, water stress led to higher WUE and lower g_s , R_{day} and ϕ , whereas fertilization resulted in higher A_{1200} , V_{cmax} and N_m (Table 2). Photosynthetic nitrogen-use efficiency was higher in control and high-nutrient plants than in low-nutrient plants. There were no significant correlations among PNUE, WUE and ϕ ($P > 0.10$). Photosynthetic rate and leaf N concentration were positively correlated on an area ($r = 0.33$, $P = 0.03$) and a mass ($r = 0.86$, $P < 0.0001$) basis and the slope of the relationship was similar among fertilization treatments (data not shown).

Leaves were measured along a leaf developmental sequence from Leaf 1 to 9 to determine changes in photosynthetic capacity. Photosynthetic rate was maximal at Leaf 3 in low-nutrient plants, but was highest in Leaves 5 and 7 in control plants (Figure 1a). Photosynthetic rate in high-nutrient plants did not peak over the developmental sequence examined, but remained high in Leaves 5, 7 and 9. Thus, photosynthetic rate was at a maximum in Leaf 5 (or close to maximum for low-nutrient plants). Patterns of leaf N along the developmental sequence did not mirror patterns of photosynthesis (Figure 1b). Although leaf N in young leaves was higher in high-nutrient plants relative to low-nutrient and control plants, leaf N of older leaves was similar among fertilization treatments.

Shoot-level responses

Fertilization and irrigation influenced shoot-level growth

Table 2. Leaf-level traits measured in irrigated and non-irrigated *Populus deltoides* saplings across three fertilization treatments. Measurements were taken on Leaf 5. Data are means \pm 1 SE. Sample sizes are given in parenthesis. Significant effects of fertilization (F) or irrigation (I) treatments are given if $P < 0.05$. Abbreviation: ns = not significant. See Table 1 for abbreviation definitions.

	Low nutrient		Control		High nutrient		Signif. effects
	Irrigated	Not irrigated	Irrigated	Not irrigated	Irrigated	Not irrigated	
A_{1200}	22.6 \pm 0.9 (17)	24.0 \pm 1.2 (8)	23.8 \pm 0.8 (17)	24.6 \pm 1.8 (8)	24.6 \pm 0.6 (17)	27.5 \pm 1.3 (8)	F
g_s	0.59 \pm 0.06 (17)	0.50 \pm 0.09 (8)	0.73 \pm 0.05 (17)	0.48 \pm 0.08 (8)	0.84 \pm 0.06 (17)	0.50 \pm 0.11 (8)	I
WUE	3.45 \pm 0.20 (17)	4.17 \pm 0.39 (8)	3.29 \pm 0.19 (17)	4.81 \pm 0.86 (8)	3.25 \pm 0.17 (17)	5.90 \pm 1.12 (8)	I
ϕ	0.085 \pm 0.004 (6)	0.084 \pm 0.005 (4)	0.087 \pm 0.004 (6)	0.075 \pm 0.005 (4)	0.088 \pm 0.004 (6)	0.076 \pm 0.005 (4)	I
V_{cmax}	88.1 \pm 5.0 (6)	86.3 \pm 3.8 (4)	85.9 \pm 4.4 (6)	76.7 \pm 8.9 (4)	90.1 \pm 2.5 (6)	102.0 \pm 5.9 (4)	F
R_{day}	3.7 \pm 0.3 (6)	3.1 \pm 0.4 (4)	4.2 \pm 0.3 (6)	3.4 \pm 0.4 (4)	4.7 \pm 0.3 (6)	3.4 \pm 0.4 (4)	I
N_a	2.02 \pm 0.09 (14)		1.81 \pm 0.06 (15)		1.97 \pm 0.04 (15)		ns
N_m	19.3 \pm 1.1 (14)		21.5 \pm 0.8 (15)		25.3 \pm 0.7 (15)		F
PNUE	145.5 \pm 6.9 (14)		172.0 \pm 6.1 (15)		163.0 \pm 4.1 (15)		F
LMA	107.6 \pm 6.0		84.9 \pm 3.5		79.0 \pm 3.1		F

traits, including RGR, LIR, LSR and D (Table 3). Relative growth rate was significantly higher in control and high-nutrient plants than in low-nutrient plants and higher in irrigated

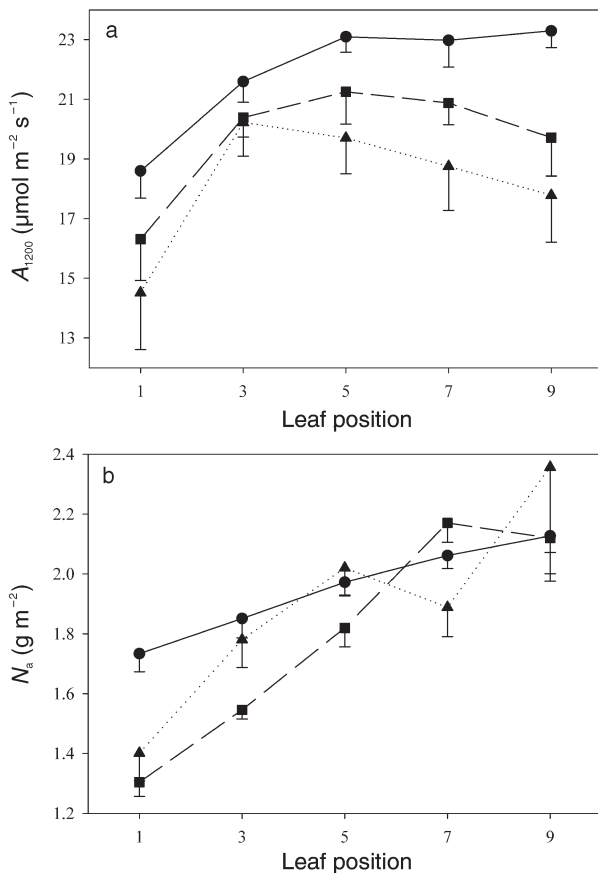


Figure 1. Area-based (a) photosynthetic rate (A_{1200}) and (b) leaf nitrogen concentration (N_a) of *Populus deltoides* leaves. The youngest leaf, with a length > 2 cm and fully unfurled edges, was designated leaf position 0. Thus, leaf maturity increases from Leaf 1 to 9. Fertilization treatments were low nutrient (\blacktriangle ; $n = 14$), control (\blacksquare ; $n = 15$) and high nutrient (\bullet ; $n = 15$). Values are means \pm 1 SE.

plants than in non-irrigated plants. In irrigated plants, RGR increased in the first few weeks following planting and decreased slightly or remained constant throughout the remainder of the growing season (Figure 2a). Throughout most of the season, RGR remained higher in irrigated fertilized and control plants than in irrigated low-nutrient plants. In contrast, there was no effect of fertilization on seasonal patterns of RGR in non-irrigated plants after Week 2. In all non-irrigated treatments, RGR decreased substantially after Week 2 (Figure 2b).

Stem diameter and LIR were higher in fertilized and irrigated plants than in unfertilized and non-irrigated plants (Table 3). Across treatments, LIR decreased over the growing season (Figure 3). As with RGR, the effect of fertilization on LIR was maintained throughout the growing season; however, nutrient treatment differences were apparent in both irrigated and non-irrigated plants. LSR and leaf position at full expansion (LPFE) (integrated across the growing season) were significantly higher in fertilized and irrigated plants than in unfertilized non-irrigated plants (Table 3).

Plasticity index and correlations between traits and productivity

In response to fertilization, shoot-level traits were more plastic (mean $PI_V = 0.48$ and 0.41 for irrigated and non-irrigated plants, respectively) than leaf-level traits (mean $PI_V = 0.15$ for both irrigation treatments; Table 4; $P < 0.01$ for both irrigation treatments). In contrast, mean PI_V values for shoot- and leaf-level traits were similar in response to irrigation ($P > 0.10$ in all nutrient treatments).

Shoot-level traits were strongly positively correlated with productivity (D) in low-nutrient, high-nutrient, irrigated and non-irrigated plants (Table 5). Of the shoot-level traits, LSR was uncorrelated with productivity only in nutrient-stressed and water-stressed plants. In contrast, leaf-level traits were generally uncorrelated with productivity. Both A_{1200} and g_s were weakly positively correlated with productivity only in irrigated plants (from all fertilization treatments). Water-use efficiency was not correlated with plant productivity in either ir-

Table 3. Shoot-level traits measured in irrigated and non-irrigated *Populus deltoides* saplings across three fertilization treatments. Data are means \pm 1 SE. Sample sizes are given in parentheses. Values of RGR, LPFE, LIR and LSR were integrated across the growing season. Stem diameter was measured at the end of the season. Significant effects of fertilization (F) or irrigation (I) treatments are given if $P < 0.05$. See Table 1 for abbreviation definitions.

	Low nutrient		Control		High nutrient		Signif. effects
	Irrigated	Not irrigated	Irrigated	Not irrigated	Irrigated	Not irrigated	
RGR	0.023 \pm 0.001 (17)	0.017 \pm 0.001 (14)	0.027 \pm 0.001 (14)	0.019 \pm 0.001 (15)	0.028 \pm 0.001 (24)	0.020 \pm 0.001 (15)	F, I
<i>D</i>	3.4 \pm 0.2 (17)	3.0 \pm 0.1 (15)	4.6 \pm 0.3 (17)	3.9 \pm 1.0 (16)	6.7 \pm 0.4 (31)	4.9 \pm 0.4 (16)	F, I
LIR	0.17 \pm 0.01 (17)	0.14 \pm 0.01 (14)	0.21 \pm 0.01 (14)	0.18 \pm 0.20 (15)	0.28 \pm 0.01 (26)	0.23 \pm 0.01 (16)	F, I
LSR	0.007 \pm 0.003 (17)	0.051 \pm 0.011 (13)	0.013 \pm 0.005 (15)	0.091 \pm 0.015 (13)	0.058 \pm 0.007 (30)	0.146 \pm 0.017 (16)	F, I
LPFE	1.42 \pm 0.12 (16)	1.18 \pm 0.10 (15)	2.05 \pm 0.17 (14)	1.76 \pm 0.13 (16)	2.64 \pm 0.13 (25)	2.27 \pm 0.16 (16)	F, I

rigation treatment. In nutrient-stressed plants, PNUE and LMA were positively and negatively correlated with productivity, respectively. In high-nutrient plants, N_a was negatively correlated with productivity.

Discussion

The adaptive value of individual traits is known to vary across environments (Lechowicz and Blais 1988). Few studies, however, have examined adaptive plasticity in perennial species

(Pigliucci et al. 1997, Pons and Anten 2004, Casper et al. 2005, Valladares et al. 2005), which experience a complex array of spatially and temporally variable resources. We examined how plasticity in leaf- and shoot-level traits relate to plant productivity, and to what extent water and nutrient availability influence the relationships between traits and productivity.

Effects of resource availability on leaf- and shoot-level traits

Populus deltoides responded to differences in water and nutrient availability with higher plasticity in shoot-level traits com-

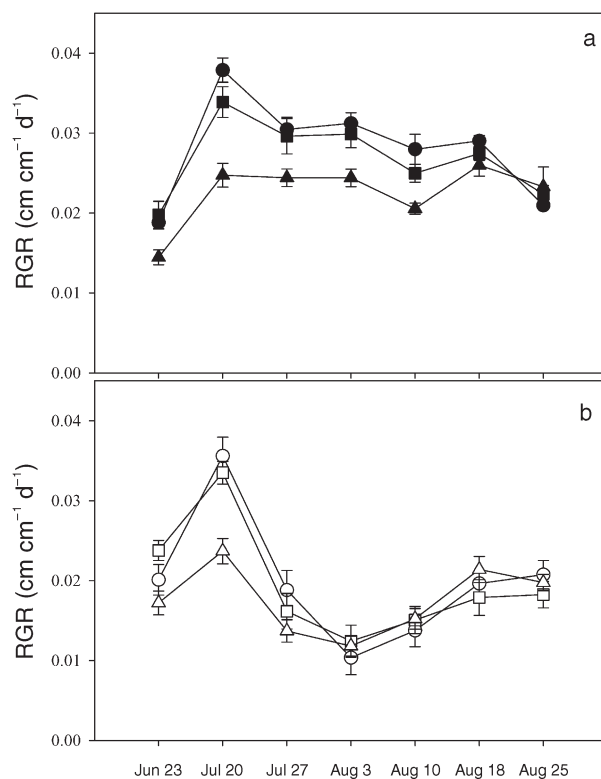


Figure 2. Relative growth rate of the apical stem (RGR) in (a) irrigated and (b) non-irrigated *Populus deltoides* plants across the growing season. Fertilization treatments were low nutrient (\blacktriangle , \triangle ; $n = 14$), control (\blacksquare , \square ; $n = 15$) and high nutrient (\bullet , \circ ; $n = 15$). Values are means \pm 1 SE. Error bars smaller than graph symbols not shown.

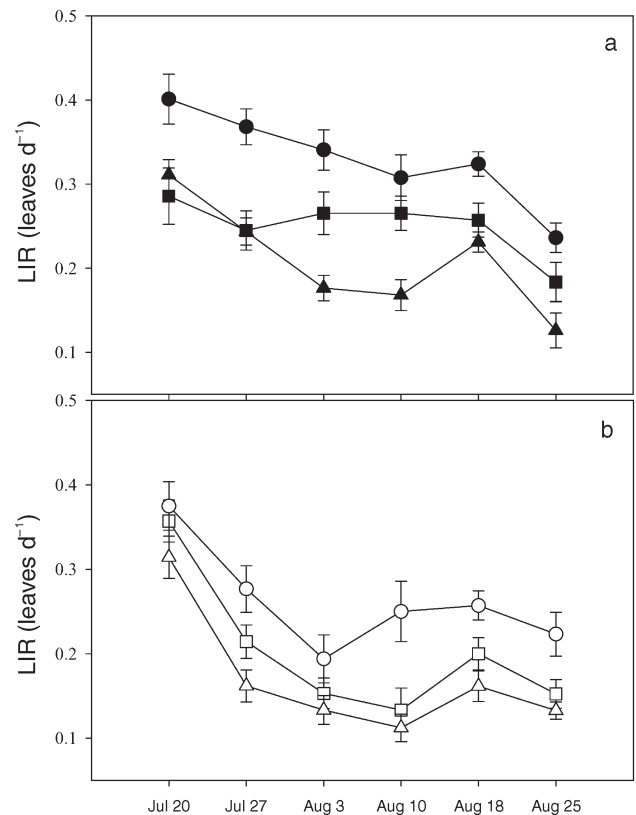


Figure 3. Leaf initiation rates (LIR) in (a) irrigated and (b) non-irrigated *Populus deltoides* plants across the growing season. Fertilization treatments were low nutrient (\blacktriangle , \triangle ; $n = 14$), control (\blacksquare , \square ; $n = 15$) and high nutrient (\bullet , \circ ; $n = 15$). Values are means \pm 1 SE.

Table 4. Plasticity indices (PI_V) for leaf- and shoot-level traits in response to variation in nutrient and water availability. Plasticity indices range from zero to one. To quantify plasticity in response to nutrient availability, PI_V was calculated using maximum and minimum values from all three fertilization treatments within each irrigation treatments (Fertilization). To quantify plasticity in response to water availability, PI_V was calculated for maximum and minimum values in both irrigation treatments within each of the nutrient treatments (Irrigation). See Table 1 for abbreviation definitions.

	Fertilization		Irrigation		
	Irrigated	Not irrigated	Low nutrient	Control	High nutrient
<i>Leaf-level traits</i>					
A_{1200}	0.08	0.13	0.06	0.03	0.11
g_s	0.30	0.04	0.15	0.34	0.40
WUE	0.06	0.29	0.17	0.32	0.45
ϕ	0.03	0.11	0.01	0.14	0.14
V_{cmax}	0.05	0.25	0.02	0.11	0.12
R_{day}	0.21	0.09	0.16	0.19	0.28
N_a	0.10				
N_m	0.24				
PNUE	0.15				
LMA	0.27				
Mean	0.15	0.15	0.10	0.19	0.25
<i>Shoot-level traits</i>					
RGR	0.18	0.15	0.26	0.30	0.29
D	0.49	0.39	0.12	0.15	0.27
LIR	0.39	0.39	0.18	0.14	0.18
LSR	0.88	0.65	0.86	0.86	0.60
LPFE	0.46	0.48	0.17	0.14	0.14
Mean	0.48	0.41	0.32	0.32	0.30

pared with leaf-level traits. However, nutrient and water availability differentially affected the degree of plasticity in both leaf- and shoot-level traits. Within the leaf-level responses, the irrigation treatment led to high plasticity in WUE and g_s in high-nutrient plants only. Among shoot-level traits, RGR was more variable in response to water availability, whereas D , LIR and LPFE were more responsive to nutrient availability. Thus, the degree of plasticity was dependent on the resource examined as well as resource interactions.

As a consequence of indeterminate growth, *P. deltooides* can respond rapidly to environmental signals at the shoot level. We found that patterns of RGR and LIR were strongly influenced by nutrient and water availability across the growing season. Few studies have examined the functional significance of dynamic plasticity (Pigliucci et al. 1997, Bell and Sultan 1999, Weinig 2000) or its prevalence across different groups of species. In contrast to *P. deltooides*, perennial species with semi-determinate growth strategies (e.g., *Quercus*, *Pinus*) may have limited shoot-level plasticity (Linder and Rook 1984) and, instead, may display a high degree of dynamic leaf-level plasticity.

Higher A_{1200} in fertilized plants corresponded with higher N_m , V_{cmax} and lower LMA, responses typically observed in response to fertilization (Linder and Rook 1984, Evans 1989, Coleman et al. 1998, Rosati et al. 1999, Wilson et al. 2000). In contrast to observations in other nutrient-stressed trees (Reich and Schoettle 1988, Reich et al. 1989), PNUE was lower in low-nutrient plants relative to control and high-nutrient plants.

A positive relationship between PNUE and leaf N, as observed here, can occur if low-nutrient plants differentially allocate resources to structural or defensive compounds rather than to photosynthetic components that maximize carbon assimilation (Sage and Pearcy 1987). The high LMA observed in low-nutrient plants is consistent with this hypothesis.

We found similar A_{1200} for irrigated and non-irrigated plants. Although we did not measure leaf water potential, the 14–35% difference in g_s between irrigation treatments suggests that non-irrigated plants were only moderately stressed at the time of measurement. Furthermore, the similar V_{cmax} observed in irrigated and non-irrigated plants indicates that the biochemical capacity for photosynthesis was unaffected by the drought treatment. Similar A_{1200} and low g_s in non-irrigated plants resulted in high WUE, with the greatest difference between irrigated and non-irrigated plants occurring in the high-nutrient treatment. Greater WUE in fertilized plants likely results from increased biochemical capacity for photosynthesis in these plants (e.g., Morgan 1984, Reich et al. 1989).

Correlations among traits and productivity

In examining the relationships between leaf- and shoot-level traits and productivity, we expected positive correlations between productivity and most shoot-level growth traits, A_{1200} and leaf N in fertilized and irrigated plants. In addition, we expected positive correlations between productivity and traits associated with resource conservation in nutrient- and water-

Table 5. Correlations of leaf- and shoot-level traits with productivity (stem diameter; D). The fertilization comparison is among irrigated low- and high-nutrient plants. The irrigation comparison is among irrigated and non-irrigated plants in all nutrient treatments. Significant correlations are shown in bold type. The number of plants is shown in parentheses. Leaf nitrogen concentration and LMA were not measured in non-irrigated plants. See Table 1 for abbreviation definitions.

	Fertilization		Irrigation	
	Low	High	Irrigated	Not irrigated
<i>Leaf-level traits</i>				
A_{1200}	-0.02 (14)	0.00 (15)	0.33 (44)	0.32 (24)
g_s	0.18 (14)	0.08 (15)	0.38 (44)	0.28 (24)
WUE	-0.25 (14)	0.32 (15)	0.11 (44)	-0.03 (24)
ϕ	0.10 (6)	0.16 (5)	0.14 (17)	0.48 (12)
V_{cmax}	0.22 (6)	0.43 (6)	0.16 (17)	0.40 (12)
R_{day}	0.24 (6)	-0.05 (5)	-0.36 (17)	-0.59 (12)
N_a	-0.35 (14)	-0.61 (15)		
N_m	0.47 (14)	0.02 (15)		
PNUE	0.63 (14)	0.36 (15)		
LMA	-0.66 (14)	-0.45 (15)		
<i>Shoot-level traits</i>				
D	0.60 (17)	0.79 (26)	0.75 (57)	0.75 (45)
LIR	0.85 (17)	0.94 (26)	0.95 (57)	0.85 (43)
LSR	0.37 (17)	0.62 (31)	0.72 (65)	0.15 (45)
LPFE	0.78 (16)	0.87 (25)	0.81 (55)	0.88 (45)

stressed plants (Chapin 1980, Bloom et al. 1985, Lambers and Poorter 1992).

In accordance with our expectations, shoot-level growth traits were strongly linked to productivity across fertilization and irrigation treatments. Only the correlation between LSR and productivity was influenced by resource availability. Although LSR was positively correlated with productivity in both fertilized and irrigated plants, there was no correlation between LSR and productivity in either low-nutrient or non-irrigated plants. In both low-nutrient and non-irrigated plants, the lack of correlation may reflect a trade-off between short-term productivity and resource conservation. Low-nutrient plants displayed low LSR, which increases leaf lifespan and maximizes carbon assimilation per unit nutrient over the lifespan of the leaf, a pattern that is consistent with the resource conservation strategy employed by plants growing in resource-poor environments (Chapin 1980). In contrast, water-limited plants had higher LSR, which likely minimized water loss by reducing whole-plant transpiration (Orians and Solbrig 1977).

In agreement with our expectations, PNUE was positively associated with productivity in low-nutrient plants, indicating that this trait is adaptive under low-nutrient conditions. Low-nutrient plants also had higher LMA relative to control and high-nutrient plants, reflecting greater allocation of carbon to structural compounds in leaves which should increase leaf lifespan and, ultimately, maximize carbon assimilation per unit nutrient over the lifespan of the leaf (Chapin 1980). We observed a negative correlation between LMA and productiv-

ity in low-nutrient plants that may reflect a trade-off between short-term productivity, as measured in this study, and resource conservation under low-nutrient conditions.

Contrary to our expectations, measures of leaf quality were negatively correlated (N_a) and uncorrelated (N_m) with productivity, respectively, in fertilized plants. These results support the idea that investing in growth and leaf production at the expense of enhancing leaf quality is adaptive when resources are abundant. These results corroborate previous studies showing that fertilization of forest tree species results in small changes in leaf N, but large changes in relative growth rate (Brix 1981, Walters and Reich 1989, Wendler and Millard 1996, Coleman et al. 1998). However, support for this result from studies of well-watered *P. deltooides* \times *P. nigra* L. clones is mixed (Marion et al. 2005, Monclus et al. 2005), indicating that investment in growth versus leaf quality may be influenced by other environmental factors, such as water availability (Monclus et al. 2006).

Maximum photosynthetic rate and g_s were positively correlated with productivity in irrigated plants. Water-use efficiency displayed high plasticity and was higher in non-irrigated than in irrigated plants, but WUE was not positively associated with productivity in non-irrigated plants, a result that has been observed in *P. deltooides* \times *P. nigra* clones (Monclus et al. 2006). Although most traits associated with resource conservation (e.g., LSR, LMA and WUE) were uncorrelated with short-term productivity, these traits may result in higher productivity over timescales longer than a single growing season.

Conclusions and perspectives

Our results suggest that plasticity in shoot- rather than leaf-level traits of *P. deltooides* will have a greater impact on productivity in response to altered resource availability. This conclusion is similar to that reported for *P. deltooides* \times *P. nigra* clones under irrigated and non-irrigated conditions (Monclus et al. 2006); however, it differs from results obtained for an annual plant, where leaf-level traits more strongly influenced fitness in water-limited habitats, whereas plant-level traits were more influential in wet habitats (Heschel et al. 2004). Our finding that shoot-level growth traits more strongly influenced productivity was not an artifact of constrained responses of leaf-level traits to resource availability (i.e., low plasticity). Several leaf- and shoot-level traits showed some correlation with productivity despite having low plasticity (e.g., A_{1200} , N_a and RGR). Thus, our data demonstrate that small changes in plant traits can have large influences on plant fitness.

There were several limitations associated with our study design. The use of one genotype was necessary in order to collect extensive physiological and developmental data on multiple individuals from six treatments across the growing season, while removing confounding variation due to V_G and $V_{G \times E}$. The *P. deltooides* clone ST109 has been used in numerous ecological studies (e.g., Gregg et al. 2003, Funk et al. 2004) and is likely typical in its response to environmental heterogeneity. Nevertheless, our results may be unrepresentative of the species as a whole. Also, because it is usually impossible to test

the contribution of particular traits to fitness or productivity while holding all other aspects of the phenotype constant (Sultan and Bazzaz 1993a, Ackerly et al. 2000, but see Dudley and Schmitt 1996), the relationships among traits and productivity should be interpreted with caution.

Although our results support the idea that trait plasticity and its functional significance are resource dependent, several questions remain for future research. First, the magnitude of plasticity and the adaptive nature of plant traits will be strongly influenced by the severity of stress. Our study was limited to one water-limitation regime and two nutrient-stress regimes. Future work should examine the adaptive plasticity of various traits under carefully controlled stress conditions or across stress gradients to examine the influence of stress severity on plasticity. In particular, controlling for water stress by assessing soil water content or plant water potential is crucial for gauging the severity of water stress. Second, a comprehensive understanding of how the adaptive value of individual traits varies across environments should incorporate V_G , V_E and $V_{G \times E}$. Quantifying $V_{G \times E}$ will be particularly useful in deciphering intra-specific variation in environmental response. Drought studies on multiple genotypes of *P. deltoides* × *P. nigra* provide an example of this approach (Monclus et al. 2006).

Third, although we focused on aboveground traits, it is possible that plasticity in these traits is linked to belowground processes (e.g., trade-off between leaf-level and root response to water stress, Ibrahim et al. 1998). Belowground traits are inherently difficult to measure. However, incorporating information on root traits will be crucial to understanding how plants respond to environmental factors, particularly nutrient and water availability. Last, although many studies have examined the effects of resource availability on growth and physiology, few studies have explicitly examined the influence of resource availability on relationships among growth and physiology traits and fitness or productivity. Thus, there is a need to examine the functional significance of leaf- and shoot-level plasticity in response to resource availability in other species to identify generalizations across taxa, life forms and environments. In particular, the functional significance of leaf- versus plant-level traits observed here for water and nutrient availability is likely to be different in response to variation in light availability. Because light is intercepted and harvested by leaves, leaf-level traits are likely to have large influences on aboveground carbon gain, growth rate and reproductive output in light-limited habitats (Montgomery 2004, Pons and Anten 2004, but see Sims et al. 1994 and Poorter 1999).

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