

LINKS BETWEEN TRANSPIRATION AND PLANT NITROGEN: VARIATION WITH
ATMOSPHERIC CO₂ CONCENTRATION AND NITROGEN AVAILABILITYH. Wayne Polley,¹ Hyrum B. Johnson, Charles R. Tischler, and H. Allen Torbert

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Transpiration is closely linked to plant nitrogen (N) content, indicating that global or other changes that alter plant N accumulation or the relative requirements of plants for water and N will affect transpiration. We studied effects of N availability and atmospheric CO₂ concentration, two components of global biogeochemistry that are changing, on relationships between whole-plant transpiration and N in two perennial C₃ species, *Pseudoroegneria spicata* (a tussock grass) and *Gutierrezia microcephala* (a half-shrub). Two indices of plant N requirement were used: N accretion (N in live and dead tissues) and N loss in litter (N in dead tissues). Transpiration was analyzed as the product of N accretion or loss by plants and the ratio of transpiration to N accretion or loss. The two indices of plant N requirement led to different conclusions as to the effects of N availability on plant use of water relative to N. Transpiration scaled proportionally with N accretion, but transpiration per unit of N loss declined at high N. Carbon dioxide enrichment had little effect on the ratio of transpiration to N accretion and no effect on transpiration per unit of N loss. The two species accumulated similar amounts of N, but the half-shrub used more than twice as much water as the grass. Nitrogen availability and CO₂ concentration influenced whole-plant transpiration more by changing plant N accumulation than by altering the stoichiometry between transpiration and plant N. Species differences in total water use, by contrast, reflected differences in the scaling of transpiration to plant N. A better understanding of species differences in water and N dynamics may thus be required to predict transpiration reliably.

Keywords: global change, nitrogen accretion, nitrogen turnover, resource use efficiency.

Introduction

Transpiration is highly and often linearly correlated with various measures of plant nitrogen (N) (Schulze et al. 1994; Polley et al. 1995). The link between transpiration and N is particularly strong at the leaf level and has been used to estimate water and carbon fluxes of different vegetation types (Schulze et al. 1994). Leaf-level relationships alone, however, may fail to predict responses of transpiration to changes in resources such as N (Vitousek 1994) and atmospheric CO₂ (Trabalka et al. 1986) that alter resource requirements of plants.

At the whole-plant level, the correlation between water use and N can be expressed simply by equating transpiration to the product of plant N content and the ratio of transpiration to plant N (ratio of whole-plant nitrogen use efficiency to plant water use efficiency, or NUE/WUE): Transpiration = Plant N × (NUE/WUE) = Plant N × [(Biomass/Plant N)/(Biomass/Transpiration)] = Plant N × (Transpiration/Plant N). According to this equation, global or other changes that (1) alter plant N acquisition or loss or (2) change the relative amounts of water and N used in production will indirectly alter total transpiration.

Plant resource use efficiency is sensitive to changes in both CO₂ concentration (Polley et al. 1995; Drake et al. 1997) and

N availability (Toft et al. 1989; Aerts and de Caluwe 1994). Carbon dioxide enrichment, for example, may increase WUE and NUE (Drake et al. 1997), but the increase may be more consistent for the former than latter (Polley et al. 1995). The requirement that N be retained at a minimal concentration in all plant tissues imposes an upper bound on NUE that may reduce its response to CO₂ when N is limiting. If WUE increases at high CO₂ but NUE does not, transpiration per unit of plant N will decline (Polley et al. 1995). Indeed, theory predicts that NUE, WUE, and their ratio, transpiration per unit of N, will change to parallel changes in the availabilities and relative costs of acquiring these resources (Bloom et al. 1985). Plant and photosynthetic N use efficiency (rate of net CO₂ uptake per unit of leaf N), for example, often decline as N availability (Shaver and Melillo 1984; Birk and Vitousek 1986; Aerts and de Caluwe 1994) or the availability of N relative to soil phosphorus (Lajtha and Klein 1988; Reich and Schoettle 1988) or water increases (Reich et al. 1989; Lajtha and Whitford 1989). Water use efficiency may vary similarly in response to relative availabilities of water and other plant resources. Photosynthetic water use efficiency (transpiration efficiency) often is lower in well-watered than in droughted plants (Reich et al. 1989) and declines as the availability of water relative to soil N availability increases (Toft et al. 1989).

Our interpretation of the link between transpiration and plant N and its sensitivity to global changes may depend on the measure of plant N employed. Nitrogen accretion, the sum of N in live and dead tissues, is an adequate measure of N requirement for annual plants but is not ideal for perennial

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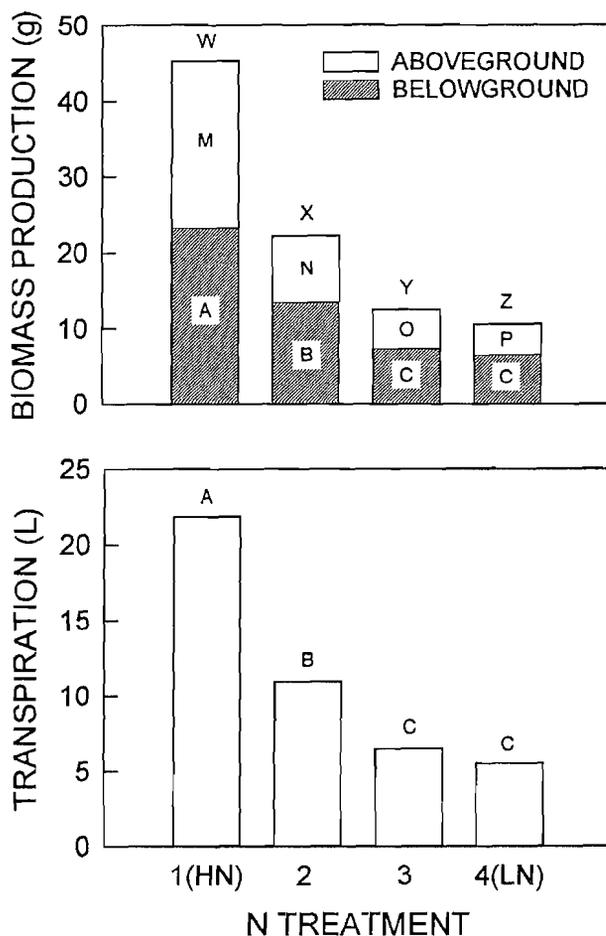


Fig. 1 Biomass production and its division between above- and belowground tissues (upper panel) and transpiration (lower panel) of plants grown from seed for 1 yr at four N treatments: 1 = highest N (HN); 4 = lowest N (LN). Values are averaged across species (*Pseudoroegneria spicata*, *Gutierrezia microcephala*) and CO₂ concentrations (387 and 690 $\mu\text{mol mol}^{-1}$). Column or tissue values that differed significantly ($P < 0.05$) are labeled with different letters ($n = 20$). Arithmetic means are shown, but statistical analyses were performed following logarithmic transformation.

species. Perennial plants lose only a fraction of their N capital each year. Nitrogen that is retained can be used to support further growth, requiring additional transpiration. For perennial plants then, the amount of acquired N that is lost to the plant in litter is a better measure of a plant's N requirement, and thus a preferable index of N for calculating NUE, than either N content or N accretion (Berendse and Aerts 1987).

We investigated effects of atmospheric CO₂ concentration and N availability on the relationship of transpiration to plant N in two perennial species. Two indices of plant N requirement were used: total N accretion, a commonly used measure, and N loss in litter, a measure that may be more appropriate for perennial species. Treatment effects on the scaling of transpiration to plant N were assessed by dividing NUE, calculated with each index of plant N requirement, by WUE. Two questions were of particular interest. (1) Would rising CO₂ concentration and increasing N availability affect the stoichiometry of perennial plants' requirement for water and N by differentially affecting WUE and NUE? (2) Would treatment effects on NUE and transpiration/plant N be sensitive to the index of plant N employed (N accretion, N loss)? Evidence for a positive answer to either question would indicate caution in extrapolating global change effects on transpiration from changes in plant N content alone.

The C₃ species studied, *Pseudoroegneria spicata* (Pursh) A. Löve spp. *spicata* (bluebunch wheatgrass) and *Gutierrezia microcephala* (DC) Gray (threadleaf snakeweed) are abundant on rangelands and differ in growth form. The half-shrub *G. microcephala* is found on grazing lands in northern Mexico and western Texas, New Mexico, Arizona, and Utah of the United States (Pieper and McDaniel 1990). *Pseudoroegneria spicata* is a native tussock grass of the Great Basin region of western North America.

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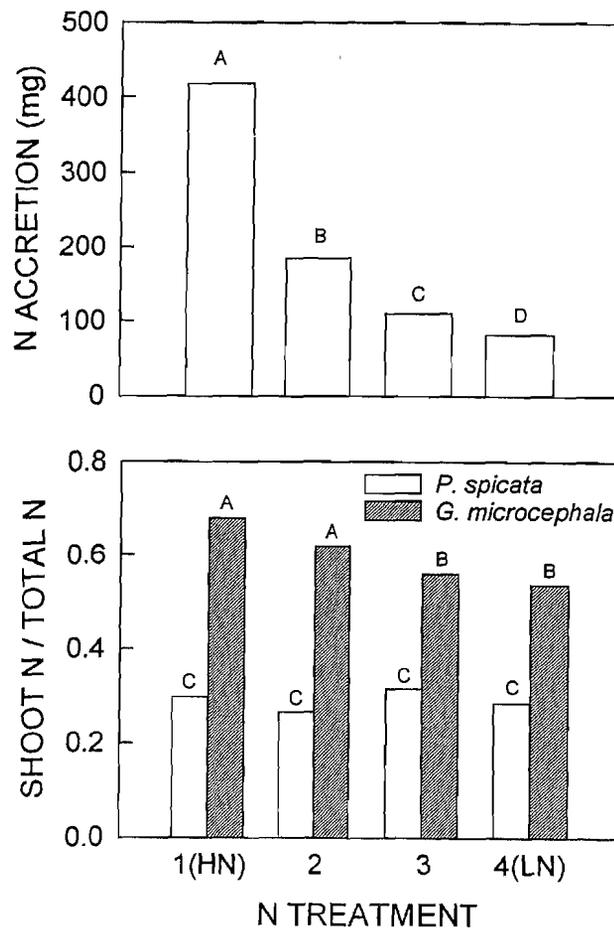


Fig. 2 Total N accretion (upper panel; $n = 20$) and the ratio of shoot N to total N accretion (lower panel; $n = 10$) of plants grown for 1 yr at four N treatments: 1 = highest N (HN); 4 = lowest N (LN). Values for each variable are averaged across remaining treatments (species, *Pseudoroegneria spicata*, *Gutierrezia microcephala*; CO₂ concentrations, 387 and 690 $\mu\text{mol mol}^{-1}$). Values that differed significantly are labeled with different letters. Arithmetic means are shown, but statistical analyses were performed following logarithmic transformation.

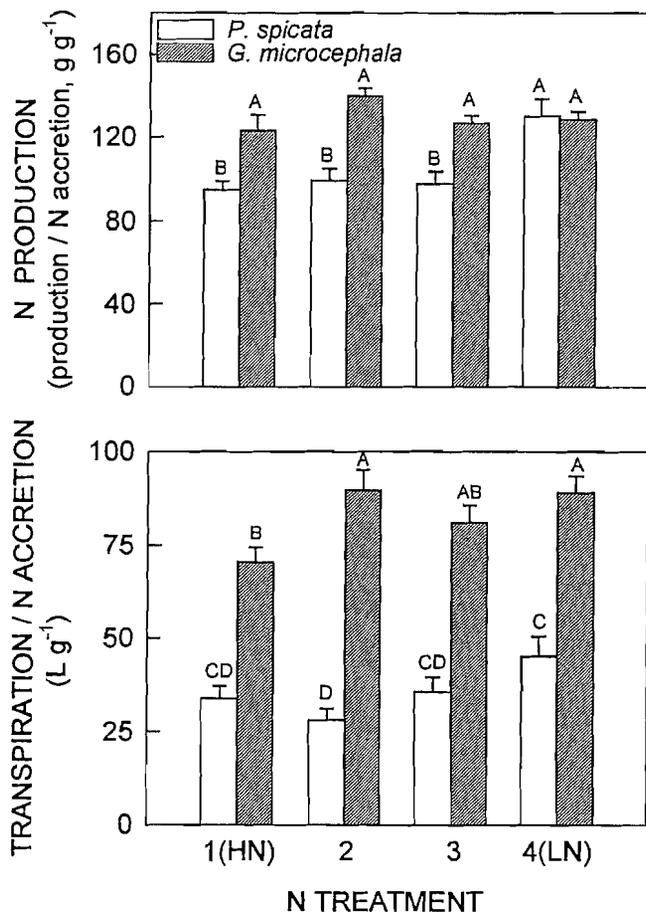


Fig. 3 Nitrogen production (upper panel) and the ratio of transpiration to N accretion (lower panel) of *Pseudoroegneria spicata* and *Gutierrezia microcephala* grown for 1 yr at four N treatments: 1 = highest N (HN); 4 = lowest N (LN). Values are averaged across CO₂ concentrations. Values that differed significantly are labeled with different letters. Error bars indicate 1 SEM.

Material and Methods

Experimental Design and Plant Culture

Pseudoroegneria spicata and *Gutierrezia microcephala* were grown in air-conditioned glasshouse bays at mean CO₂ concentrations of 387 and 690 $\mu\text{mol mol}^{-1}$. The CO₂ concentration and dewpoint temperature of air in each bay were measured each fourth minute with an infrared gas analyzer (Li-Cor, LI-6262, Lincoln, Nebr.). The CO₂ readings were corrected for atmospheric pressure measured with a pressure indicator (Druck, DPI 260, Fairfield, Conn.). Air temperature was measured in the center of each bay with 25- μm diameter thermocouples and was changed seasonally to approximate temperature outdoors by manually adjusting thermostatic controls. Photosynthetic photon flux density (PPFD) was measured on the glasshouse roof with a silicon photodiode (Li-Cor, LI-190SB, Lincoln, Nebr.) and above plants in each bay with silicon detectors along a 1-m-long sensing surface (Li-Cor, LI-191SA).

Each species was grown in 0.25-m-diameter and 0.60-m-

deep pots in soil mixtures differing in total N content. Plants were grown in 1 : 1, 3 : 1, 7 : 1, and 15 : 1 (v/v) mixtures of washed sand : fine sandy loam soil with total N levels of 6, 2, 1, and <1 $\mu\text{g g}^{-1}$, respectively. These are moderately low to extremely low N levels. Soil pH ranged from 8.3 to 9.0, values typical of soils where *P. spicata* and *G. microcephala* are most abundant. To assure that N would be the most limiting element, 600 mL of Hoagland's solution lacking N were added to each pot before planting (Hoagland and Arnon 1950; modified by replacing CaSO₄ with NaSO₄ and by supplying phosphorus as a 13 : 1 w/w mixture of KH₂PO₄ : K₂HPO₄). An additional 200 mL of the nutrient solution were added to each pot during the fourth and fifth month of plant growth.

Each pot was weighed on a top-loading balance when empty and with a load beam (BLH Electronics, KIS-3, Canton, Mass.) after it was filled with air-dried soil. One soil core (20-mm diameter) was taken from each pot at filling, oven dried at 100°C for 72 h, and weighed. The mass of soil in each pot was calculated using the ratio of the oven-dried mass to air-dried mass of these samples. Before planting, soil was moistened to drip and each pot was reweighed.

Ten pots per soil mixture were seeded with each species during September 1992. Five pots per soil mixture and species were then randomly assigned to each of the two CO₂ treatments. The half-shrub, *G. microcephala*, was replanted during October 1992. The grass, *P. spicata*, was thinned to two plants per pot in November and to one plant per pot in December 1992. One soil core (45-mm diameter) was taken from each pot with grass at the final thinning in December 1992. Holes that resulted from coring were filled with the appropriate soil mixture. Seedlings of *G. microcephala* grew slowly. Because they were small, four seedlings of the half-shrub were retained in each pot at final thinning in January 1993. Soil in all pots was then covered with 1–2 cm of vermiculite to reduce evaporation.

Measurements and Harvest

Pots were weighed weekly from January 1993 through harvest in November/December 1993 to determine water loss to evapotranspiration. Following each weight measurement, water was added to restore soil to ca. 95% of its moisture content at the drip point (ca. 20% volumetric moisture content). Transpiration from planted pots was calculated by subtracting average water loss from two unplanted pots of the appropriate soil mixture at each CO₂ level. To minimize any effect of bay position on plant performance, plants and the appropriate CO₂ treatment were moved monthly among bays until June 1993. Plants from an associated experiment were too large thereafter to move through adjoining doorways.

Aboveground litter was collected once during the growing season (February–March 1993). Plants were destructively harvested at the end of the growing season, on November 2–3 (grass) and December 1–2, 1993 (shrub). Some leaves were green at harvest, but most aboveground material apparently had undergone normal senescence. Aboveground structures and the large roots retained on a 12.7-mm mesh sieve were separated into live and dead components. Included with live roots were tap and lateral roots of the half-shrub. Crowns were separated from live roots of the grass. A mixed sample

Table 1
Parameters Related to Biomass Production and Turnover, Total Transpiration, and N Dynamics of Plants Grown from Seed for 1 yr at Four N Treatments

Parameter	Nitrogen treatment			
	1	2	3	4
Production/shoot N (g g^{-1})	295.6 ^b	309.2 ^{ab}	268.4 ^b	376.2 ^a
[N] of aboveground litter	1.05 ^a	0.88 ^b	0.94 ^{ab}	0.84 ^b
Turnover aboveground (litter fraction)	0.68 ^a	0.59 ^b	0.56 ^b	0.56 ^b
Fractional turnover of N (litter N/N accretion)	0.45 ^a	0.34 ^b	0.33 ^b	0.29 ^b
NUE _p [$\text{g (biomass) g (N loss)}^{-1} \text{ yr}^{-1}$]	296.3 ^c	425.3 ^{ab}	359.3 ^b	501.4 ^a
Transpiration/N loss (L g^{-1})	127.9 ^b	208.6 ^a	187.1 ^{ab}	240.7 ^a

Note. 1 = highest N, 4 = lowest N. NUE_p (plant N-use efficiency) is biomass production divided by N loss in litter during the year. Values are averaged across species (*Pseudoroegneria spicata*, *Gutierrezia microcephala*) and CO₂ concentrations (387 and 690 $\mu\text{mol mol}^{-1}$). Values within a row that differed significantly ($P < 0.05$) are labeled with different letters ($n = 20$). Arithmetic means are shown, but statistical analyses of most parameters were performed following logarithmic transformation.

of sieved soil from each pot (ca. 3 kg) was collected. Small roots in these samples were hand separated and sorted into live and dead categories based on color. All tissues were dried to constant mass at 60°C and weighed. Soil samples from which roots were removed were oven dried (100°C) and weighed. The mass of small roots that passed through the 12.7-mm sieve was calculated by multiplying root biomass per dry mass of the soil sample by the total dry mass of soil in each pot. The derived mass of small roots was added to that of the large roots retained on the sieve to obtain total root biomass per pot.

Nitrogen Accretion and Use Efficiency

Nitrogen concentration was measured on plant material collected before and at harvest. Dry mass and N mass weights of live and dead tissues were summed to calculate biomass production and N accretion per pot.

Nitrogen use efficiency was calculated using each of two indices of plant N requirement: N accretion and N loss in litter. Plant nitrogen use efficiency (NUE_p) was calculated as biomass production divided by the amount of acquired N lost in aboveground and belowground litter. Nitrogen production was biomass production divided by N accretion.

Water Use Efficiency

Plant WUE was calculated for the period between the final thinning and harvest of each species: the ratio of total biomass production to transpiration during the period. Biomass of *P. spicata* that remained after thinning was estimated from measurements on plants that were removed. Aboveground biomass of the grass in each pot was taken as the product of aboveground biomass per tiller of the plant removed (including biomass of crowns) and tiller number of the remaining plant. Belowground biomass was calculated by multiplying root biomass per tiller at thinning (estimated from soil cores) by tiller number of the remaining plant. Seedlings of *G. microcephala* were small at the final thinning ($\bar{X} = 30 \text{ mg plant}^{-1}$), so biomass produced during the period that transpiration was measured was assumed equal to that present at harvest plus litter collected during the growing season. The ratio of plant tran-

spiration to plant N was calculated in two ways: (1) transpiration/N accretion (N production/WUE) and (2) transpiration/N loss (NUE_p/WUE).

Statistical Analyses

Data were analyzed with a three-way ANOVA that included CO₂ concentration, species, N treatment, and appropriate interactions (Sokal and Rohlf 1981). Student-Newman-Keuls multiple-range test was used to determine significant differences among three or more means. Data were transformed logarithmically before analysis when required to satisfy assumptions of ANOVA. Standard errors for transformed data were omitted in presentation of results using the original scale of measurement. Variable means are presented for individual treatments (N, species, or CO₂) only when statistical interactions with other treatments were not significant ($P > 0.05$).

Environmental Conditions

Mean daytime temperature in glasshouse bays declined from 30°C in September 1992 to 15°C in December 1992, then increased to a maximum of 27°C in late July 1993, before declining to 20°C at harvest in November–December 1993. Mean vapor pressure deficit of air during daylight followed a similar temporal trend, declining from 1.5 kPa in September 1993 to 0.3 kPa in December 1992, before increasing linearly to 1.4 kPa in August–September 1993. The daily integral of PPF inside bays averaged 55% of that measured above the glasshouse, although instantaneous PPF inside the glasshouse approached 90% of that measured outdoors at midday. Standard deviation of CO₂ concentration was calculated daily. The average of these values was 9.4 $\mu\text{mol mol}^{-1}$ at the high CO₂ concentration and 22.2 $\mu\text{mol mol}^{-1}$ in the low CO₂ bay, where concentration was not directly controlled.

Results

Nitrogen Treatments

Biomass production, N accretion, and transpiration each increased by a factor of 4–5 from the lowest to highest N

Table 2
Parameters Related to Biomass Production and Turnover, Total Transpiration, and N Dynamics of Plants Grown from Seed for 1 yr at Two CO₂ Concentrations

Parameter	CO ₂ concentration ($\mu\text{mol mol}^{-1}$)	
	387	690
WUE (g L^{-1})	2.09	2.81**
Transpiration (L pot^{-1})	12.75	9.47**
N accretion (g)	0.21	0.18*
N accretion/root biomass (mg g^{-1})	17.2	13.6**
N production [g (biomass) g (N accretion) ⁻¹]	110.0	125.5**
Transpiration/N accretion (L g^{-1})	63.2	55.5*
Tissue turnover (litter fraction)	0.38	0.30**
Fractional turnover of N (litter N/N accretion)	0.39	0.31**
NUE _p [g (biomass) g (N loss) ⁻¹ yr ⁻¹]	320.3	474.4**
Transpiration/N loss (L g^{-1})	184.2	199.7

Note. NUE_p is biomass production divided by N loss in litter during the year. Values are averaged across species (*Pseudoroegneria spicata*, *Gutierrezia microcephala*) and four N treatments. Arithmetic means are shown, but statistical analyses of most parameters were performed following logarithmic transformation; $n = 40$.

* $P < 0.05$.

** $P < 0.001$.

treatment (figs. 1, 2). Each incremental increase in N availability elicited similar proportional increases in biomass production, N accretion, and transpiration. As a result, N availability did not consistently affect plant WUE ($\bar{X} = 2.45 \text{ g L}^{-1}$), N production (biomass/N accretion), or the ratio of N and water use efficiencies' transpiration per unit of N accretion (fig. 3). Production per unit of N invested in shoots (leaves + aboveground stems) declined with increasing N availability (table 1), but in *Gutierrezia microcephala*, the proportion of plant N in shoots also increased at high N (fig. 2).

The N concentration in aboveground litter and turnover of aboveground tissues (litter fraction) both increased with increasing N (table 1). The fraction of accumulated N that was lost to litter (litter N/total N accretion) increased as a result. Both NUE_p (production/N loss) and transpiration per unit of N lost in senescent tissues (ratio of NUE_p to WUE) declined at high N availability because of the increase in fractional turnover of N.

CO₂ Concentration

Elevating CO₂ concentration increased plant WUE by 34% by reducing total transpiration by 26% (table 2). Effects of CO₂ were statistically independent of species or N treatment. Total biomass production was not significantly affected by CO₂ in either *Pseudoroegneria spicata* or *G. microcephala* (fig. 4).

Nitrogen accretion declined at high CO₂ (table 2). Belowground biomass did not differ significantly between CO₂ treatments (not shown), so this decline reflected lower N accretion per unit of belowground biomass at the elevated than ambient CO₂ concentration (table 2). Nitrogen production, the ratio

of biomass production to total N accretion, increased slightly at high CO₂. The increase in N production was proportionally smaller than that in WUE, so transpiration per unit of plant N accretion declined slightly (12%) with CO₂ enrichment.

Plants grown at elevated CO₂ lost a smaller fraction of biomass and of accumulated N to litter, largely because turnover aboveground was lower (table 2). As a result, NUE_p rose 48% at elevated CO₂. The proportional increase in NUE_p was similar to that in WUE, so atmospheric CO₂ concentration did not significantly affect transpiration per unit of N lost in litter.

Species

Biomass production was 56% higher in pots with *G. microcephala* than *P. spicata* at the current CO₂ concentration (fig. 4). Biomass was higher in the half-shrub at elevated CO₂, but species differences were not significant. Across N treatments and CO₂ concentrations, transpiration from pots with *G. microcephala* was almost three times that from pots with

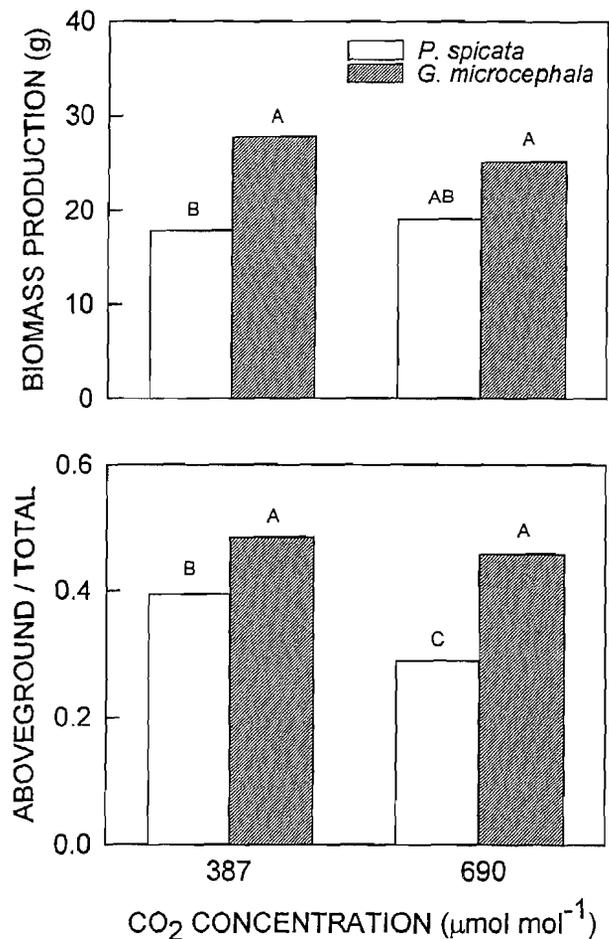


Fig. 4 Total biomass production (upper panel) and the ratio of aboveground to total production (lower panel) of *Pseudoroegneria spicata* and *Gutierrezia microcephala* grown for 1 yr at two CO₂ concentrations ($n = 20$). Values are averaged across four N treatments. Values that differed significantly are labeled with different letters. Arithmetic means are shown, but statistical analyses were performed following logarithmic transformation.

Table 3
Parameters Related to Biomass Turnover, Total Transpiration, and N Dynamics of 1-yr-old Plants of *Pseudoroegneria spicata* and *Gutierrezia microcephala*

Parameter	Species	
	<i>Pseudoroegneria spicata</i>	<i>Gutierrezia microcephala</i>
Transpiration (L pot ⁻¹)	5.76	16.45**
WUE (g L ⁻¹)	3.29	1.61**
N accretion (g)	0.18	0.21
Turnover belowground (litter fraction)	0.21	0.10**
Turnover aboveground (litter fraction)	0.59	0.61
Fractional turnover of N (litter N/N accretion)	0.33	0.37*
NUE _p [g (biomass) g (N loss) ⁻¹ yr ⁻¹]	403.3	391.4
Transpiration/N loss (L g ⁻¹)	134.3	249.6**

Note. NUE_p is biomass production divided by N loss in litter during the year. Values are averaged across four N treatments and two CO₂ concentrations. Arithmetic means are shown, but statistical analyses of most parameters were performed following logarithmic transformation; *n* = 40.

* *P* < 0.05.

** *P* < 0.001.

the grass (table 3). Consequently, WUE of the half-shrub was only one-half that of the grass. Nitrogen accretion was higher (14%) in *G. microcephala* than *P. spicata*. At all but the lowest N treatment, N production was also higher in the half-shrub than grass (fig. 3). The half-shrub *G. microcephala* transpired more than twice as much water per unit of N accretion as *P. spicata*, a reflection of both higher N production and lower WUE.

Species did not differ in the fraction of total biomass that was lost to litter, but fractional turnover of N was 11% higher in *G. microcephala* than *P. spicata* (table 3). The half-shrub invested a much higher proportion of production aboveground (fig. 4) where turnover was a factor of 3 or more times that in belowground tissues (table 3). The NUE_p did not differ significantly between species. The shrub used almost twice as much water per unit of N loss to litter as *P. spicata*, however, because of its low WUE.

Discussion

Relationships between whole-plant transpiration and two indices of plant N requirement (N accretion, N loss in litter) were studied to address two primary questions. Is the scaling of transpiration to plant N sensitive to resource (N, CO₂) availability? Is transpiration per unit of N in perennial species sensitive to the index of plant N employed? Schulze et al. (1994) found the close link between stomatal conductance and leaf N a powerful basis for scaling transpiration from the leaf to canopy level. Leaf-level relationships alone, however, are not adequate for predicting responses of transpiration to changes in N or other resources that alter plant allocation, tissue turnover rates, or the relative requirements of plants for different

resources. Analyses that combine information on resource availabilities with the ratios of plant resource use efficiencies provide a simple alternative to more physiologically based models for predicting transpiration and plants effects on processes such as N cycling (Aber et al. 1991).

Scaling of Transpiration to Plant N

A major conclusion of this study is that CO₂ concentration and N availability had little effect on the scaling of water use to N accretion. Elevating CO₂ concentration by ca. 80% reduced transpiration by a mean 26% but reduced use of water relative to N by only 12%. Nitrogen treatments that produced fourfold differences in biomass during the initial year of growth did not significantly affect N production, WUE, or their ratio, transpiration per unit of N accretion. Consequently, N availability and CO₂ concentration influenced whole-plant transpiration more by changing plant N accretion than by altering the stoichiometry between transpiration and plant N.

Others have found that N production and WUE may be sensitive to changes in N availability, as predicted by theory (Patterson et al. 1997). Production per unit of plant N typically declines as N availability increases (Aerts 1989; Aerts and de Caluwe 1994), whereas WUE may increase at high N. Tanner and Sinclair (1983) concluded from a review of early experiments on plant water requirements that WUE became sensitive to variation in plant nutrition when nutrient deficiencies were severe enough to reduce yield to about half that obtained with adequate fertilizer.

Lower transpiration at high CO₂ largely reflected an accompanying decline in N acquisition and N loss. Belowground production did not differ significantly between CO₂ concentrations, so the decrease in N accretion resulted from lower

accumulation per unit of belowground biomass at the elevated than ambient concentration. Whether this decline in root-specific accretion resulted from physiological or morphological changes in plants or a shift in soil N dynamics is not clear. Total N uptake by the C₄ grass *Bouteloua gracilis* (blue grama) and soil solution NO₃⁻ levels both were lower at elevated than ambient CO₂ (Morgan et al. 1994), indicating that CO₂ enrichment can reduce N mineralization and availability to plants. By contrast, Hunt et al. (1996) reported that the N content of perennial organs of two grasses increased 11% at elevated CO₂, despite a decline in the N concentration of senescent shoots. The direction of CO₂ effects on N cycling may depend on plant species. Whether plant N pools and N uptake increased, decreased, or did not change at elevated CO₂ was species specific (Hungate et al. 1996).

Species differences in total transpiration, unlike those between CO₂ or N treatments, reflected differences in the ratio of water use to plant N. Nitrogen accretion and N turnover differed by <15% in *G. microcephala* and *P. spicata*, so differences in WUE largely accounted for the twofold differences in transpiration/N between species. The extent to which these differences might be expressed on dry rangelands obviously depends on whether species variation in WUE persists as water becomes limiting. These results indicate, however, that transpiration in N-limited systems could be much more sensitive to species composition than to direct effects of atmospheric CO₂ concentration.

Relationship of Transpiration to Two Indices of N Requirement

The two indices of plant N requirement (N accretion, N loss) used here led to different conclusions as to effects of N availability on the stoichiometry of plant requirements for water and N. Nitrogen production, WUE, and the ratio of transpiration to N accretion did not vary with N treatments that produced fourfold differences in biomass. By contrast, NUE_p

and transpiration per unit of N in litter declined at high N as plants lost a larger fraction of accumulated N at high compared with low N. Increased turnover of aboveground tissues and a higher N concentration in aboveground litter at high compared with low N contributed to the increase in N turnover, similar to trends measured in some (Aerts 1989), but not all, species (Aerts and de Caluwe 1994). It is important to note that although the pattern of N turnover measured here is relevant to the N dynamics of establishing plants, it may not be representative of turnover in vegetation that has reached equilibrium with respect to its N content. Unanswered by this experiment, therefore, is the question of whether the increase in N turnover that led to lower transpiration per unit of N loss will persist when N acquisition comes into balance with N losses. Transpiration at high N could be lower than predicted from the relationship between N accretion and water use if measured patterns in N turnover persist.

Results from this study thus indicate that a better understanding of factors controlling N turnover is critical to long-term predictions of atmospheric and other changes on water and N use by perennial plants. The stoichiometry between transpiration and plant N varied much more between species than with resource availability, indicating that information on species or growth form differences in water and N requirements will be required to predict transpiration in a changing world.

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