PHYSIOLOGICAL ECOLOGY - ORIGINAL PAPER

Spatial variation of the stable nitrogen isotope ratio of woody plants along a topoedaphic gradient in a subtropical savanna

Edith Bai · Thomas W. Boutton · Feng Liu · X. Ben Wu · Steven R. Archer · C. Thomas Hallmark

Abstract Variation in the stable N isotope ratio (δ^{15}N) of plants and soils often reflects the influence of environment on the N cycle. We measured leaf δ^{15}N and N concentration ([N]) on all individuals of *Prosopis glandulosa* (deciduous tree legume), *Condalia hookeri* (evergreen shrub), and *Zanthoxylum fagara* (evergreen shrub) present within a belt transect 308 m long × 12 m wide in a subtropical savanna ecosystem in southern Texas, USA in April and August 2005. Soil texture, gravimetric water content (GWC), total N and δ^{15}N were also measured along the transect. At the landscape scale, leaf δ^{15}N was negatively related to elevation for all the three species along this topoedaphic sequence. Changes in soil δ^{15}N, total N, and GWC appeared to contribute to this spatial pattern of leaf δ^{15}N. In lower portions of the landscape, greater soil N availability and GWC are associated with relatively high rates of both N mineralization and nitrification. Both soil δ^{15}N and leaf [N] were positively correlated with leaf δ^{15}N of non-N_2 fixing plants. Leaf δ^{15}N of *P. glandulosa,* an N_2-fixing legume, did not correlate with leaf [N]; the δ^{15}N of *P. glandulosa*'s leaves were closer to atmospheric N_2 and significantly lower than those of *C. hookeri* and *Z. fagara*. Additionally, at smaller spatial scales, a proximity index (which reflected the density and distance of surrounding *P. glandulosa* trees) was negatively correlated with leaf δ^{15}N of *C. hookeri* and *Z. fagara*, indicating the N_2-fixing *P. glandulosa* may be important to the N nutrition of nearby non-N_2-fixing species. Our results indicate plant ^{15}N natural abundance can reflect the extent of N retention and help us better understand N dynamics and plant-soil interactions at ecosystem and landscape scales.

Keywords Nitrogen isotope discrimination · Nitrogen cycling · Dinitrogen fixation · Spatial scale · Nitrogen availability

Communicated by John Ehleringer.

E. Bai · T. W. Boutton · F. Liu · X. B. Wu
Department of Ecosystem Science and Management,
Texas A&M University, College Station, TX 77843-2138, USA

S. R. Archer
School of Natural Resources,
University of Arizona, Tucson, AZ 85721-0043, USA

C. T. Hallmark
Department of Soil and Crop Sciences, Texas A&M University,
College Station, TX 77843-2474, USA

Present Address:
E. Bai (✉)
Department of Land, Air, and Water Resources,
University of California, Davis, CA 95616, USA
e-mail: ebai@ucdavis.edu

Present Address:
F. Liu
Forest Landscape Ecology Lab,
Department of Forest and Wildlife Ecology,
University of Wisconsin, Madison, WI 53706-1520, USA

Introduction

Variation in natural stable N isotope ratio (δ^{15}N) values of plants and soils reflects N-cycling processes in ecosystems (Hogberg 1997; Robinson 2001; Amundson et al. 2003; Houlton et al. 2006, 2007). At ecosystem to landscape scales, topography appears to be a major influence on soil and plant δ^{15}N due to its impacts on soil moisture availability (Garten and van Miegroet 1994) and soil nutrient availability (Schmidt and Stewart 2003). In topographic depressions or lower-lying landscape positions where soil

moisture tends to be higher, soil $\delta^{15}N$ values are generally higher than in more upland portions of the landscape (Sutherland et al. 1993). This is probably because rates of denitrification and other N-losing transformations are higher (Garten and van Miegroet 1994; Farrell et al. 1996). Similarly, soil nutrients (notably N and P) have been reported to be positively related to soil and leaf $\delta^{15}N$ (Garten and van Miegroet 1994; McKee et al. 2002; Schmidt and Stewart 2003) because higher soil N concentrations result in high rates of microbial N transformations (Booth et al. 2005) that result in preferential ^{14}N losses. Disturbances and land use activities, such as fire (Handley et al. 1999; Cook 2001), grazing (Evans and Belnap 1999; Schulze et al. 1999), forest production (Chang and Handley 2000), and fertilization (McKee et al. 2002; Choi et al. 2003) are also known to influence spatial variation of plant $\delta^{15}N$ at ecosystem and landscape scales.

At the scale of the individual plant, mycorrhizal symbioses (Michelsen et al. 1998; Schmidt and Stewart 2003) and N_2 fixation (Virginia and Delwiche 1982; Schulze et al. 1991) have been found to be related to plant $\delta^{15}N$. Plants associated with mycorrhizal fungi usually have lower $\delta^{15}N$ than those non-mycorrhizal species, presumably because discrimination against ^{15}N occurs during fungal N assimilation (Högberg et al. 1996; Hobbie et al. 2000). N_2 fixers have been reported to have both lower leaf $\delta^{15}N$ and greater leaf N concentration ([N]) compared to non-fixing species because the $\delta^{15}N$ of atmospheric N_2 (0 ‰) can be lower than soil-derived N (Schulze et al. 1991, 1998; Schmidt and Stewart 2003). In addition, the depth(s) in soil from which N is acquired and the form(s) of soil N used (organic N, NH_4^+, NO_3^-) may also affect plant $\delta^{15}N$ (Hogberg 1997).

In the Rio Grande Plains of southern Texas, subtropical woodlands dominated by N_2-fixing tree legumes have become significant components of landscapes that were once almost exclusively dominated by C_4 grasslands (Boutton et al. 1998; Archer et al. 2001). Previous studies have suggested that honey mesquite (*Prosopis glandulosa* Torr. var. *glandulosa*) initiated this vegetation change and served as recruitment foci, facilitating the establishment of other woody species beneath its canopy (Archer et al. 1988; Archer 1995). This dramatic vegetation change has significantly impacted N-cycling processes by increasing: (1) rates of N input to the ecosystem through the fixation process (Zitzer et al. 1996), (2) the quantity and quality of organic matter inputs to the soil (Liao et al. 2006), and (3) rates of N transformations such as ammonification, nitrification, denitrification, and NO flux (Archer et al. 2001; Hibbard et al. 2001; McCulley et al. 2004). We reasoned that these have left an imprint on the pattern of $\delta^{15}N$ in plants and soils in this landscape.

The objectives of this study were to: (1) document multiple-scale spatial variation in leaf $\delta^{15}N$ of three dominant woody species along a topographic gradient in a subtropical savanna: a deciduous tree legume [*P. glandulosa*, (Fabaceae)], and two evergreen shrubs [*Condalia hookeri* M.C. Johnst. (Rhamnaceae) and *Zanthoxylum fagara* (L.) Sarg. (Rutaceae)]; (2) describe seasonal variation in leaf $\delta^{15}N$ of these species; and (3) identify the factors that may be controlling spatial and seasonal variation in leaf $\delta^{15}N$.

Materials and methods

Study area and transect description

Research was conducted at the Texas AgriLife La Copita Research Area (27°40′N, 98°12′W; elevation 80 m) in the eastern Rio Grande Plains of the Tamaulipan Biotic Province. Climate is subtropical with a mean annual temperature of 22.4°C and mean annual precipitation of 680 mm. Landscapes typically grade (1–3% slopes) from sandy loam uplands (Typic Argiustolls) to clay loam and clay lowlands (Pachic Argiustolls). Upland vegetation is savanna parkland consisting of discrete woody clusters (3–10 m diameter; comprising a single *P. glandulosa* tree with up to 15 understory tree/shrub species) and larger groves (10- to >20-m diameter; comprising several woody clusters that have fused together) embedded within a matrix of grassland vegetation. Upland grasslands are dominated by C_4 grasses and C_3 forbs. Clusters and groves are dominated by *P. glandulosa* and *Z. fagara*. *P. glandulosa* and several other leguminous trees and shrubs present in wooded areas are capable of symbiotic N_2 fixation (Zitzer et al. 1996). Drainage woodlands are covered by closed-canopy woodlands similar in composition to upland clusters and groves. Playas are closed-basin depressions (Ustic Epiaquerts and Vertic Argiaquolls) that occur in the lowest portions of drainage woodlands, and vegetation there consists of a continuous grass layer with *P. glandulosa* and *Acacia farnesiana* trees of variable density (Farley 2000). Woody encroachment has occurred in the study area over the past 75–100 years due to the interaction of heavy livestock grazing and reduced fire frequency (Archer 1995). In the past 20 years, the study area has not burned, and grazing intensity has been light and uniform across the entire transect.

A 308-m transect spanning a hillslope gradient from an upland through a drainage woodland and into a playa was established. All five of the major landscape elements at the study site (grasslands, clusters, groves, drainage woodlands, and playa) occurred along the transect. The transect was marked with steel posts at 1-m intervals, and georeferenced by Trimble GPS pathfinder Pro XRS system (Trimble Navigation, Sunnyvale, Calif.) at 5-m intervals. Elevations were determined by surveying.

Plant and soil sampling and analysis

Three soil cores (0–15 cm) were collected at 1-m intervals along the transect in April 2005. The first soil core was used to determine soil particle size distribution [pipette method (Gee and Bauder 1986)], the second to determine soil total N and δ^{15}N (Harris et al. 2001), and the third to determine soil gravimetric water content (GWC) (Hillel 1982).

Three common woody species were sampled: *P. glandulosa*, *C. hookeri*, and *Z. fagara*. *P. glandulosa* is a deep-rooted deciduous tree capable of symbiotic N$_2$ fixation (Zitzer et al. 1996). *C. hookeri* and *Z. fagara* are evergreen shrubs and non-N$_2$ fixing. Bai et al. (2008) presented detailed biological characteristics of these plants. Leaf tissue from every individual of these three species occurring within 6 m on either side of the 308-m transect was sampled in April and August 2005. Exact spatial coordinates of each plant were recorded. For each species, approximately 10 g of fully-expanded, south-facing, sun-lit leaves in the upper canopy were sampled. Leaves were oven-dried for 48 h at 60°C and pulverized.

δ^{15}N and N concentrations of plant tissues and soils were determined using a Carlo Erba EA-1108 (CE Elantech, Lakewood, N.J.) interfaced with a Delta Plus (ThermoFinnigan, San Jose, Calif.) isotope ratio mass spectrometer operating in continuous flow mode. N isotope ratios are presented in notation:

$$\delta = [(R_{SAMPLE} - R_{STD})/R_{STD}] \times 10^3$$

where R_{SAMPLE} is the ^{15}N/^{14}N ratio of the sample and R_{STD} is the ^{15}N/^{14}N ratio of the atmospheric N$_2$ (Mariotti 1983). Precision of duplicate measurements was <0.2‰.

Statistical analyses and modeling

Correlation analyses were performed to evaluate relationships between plant δ^{15}N and environmental variables using Sigma Plot 8.0 (SPSS, 2002). To determine which environmental variables had the greatest influence over spatial variability in plant δ^{15}N values, stepwise multiple regression analyses were conducted for each of the three woody plant species (SPSS version 16., SPSS). Due to our systematic sampling regime and the potential for spatial autocorrelation in our study, a partial Mantel test was used to test for differences in δ^{15}N between species and sample periods (PASSAGE version 1.1; Rosenberg 2001). The partial Mantel test (Mantel 1967) can account for and remove the effect of spatial autocorrelation between variables, and a significant Mantel *r*-statistic indicates that the correlation between the two variables is not related to a common spatial structure (Fortin and Gurevitch 1993).

A proximity index (PI) was originally developed by Gustafson and Parker (1994) to measure the potential influence of surrounding patches in a neighborhood on a focal patch. Ghazoul et al. (1998) used a variation of PI to measure the relative isolation of flowering trees to study the effect of cross-pollination on reproductive success. We used this modified PI to evaluate the influence of *P. glandulosa* trees on leaf δ^{15}N values of associated shrub species (*C. hookeri* and *Z. fagara*) in two sampling seasons:

$$PI = \sum_{i=1}^{n} 1/Z_i$$

where Z_i is the distance in meters from the shrub to each surrounding *P. glandulosa* tree. This index considers both the number of surrounding *P. glandulosa* trees and their distances, weighted in favor of the nearest neighbor. PI is larger when the shrub is surrounded by closer and/or more *P. glandulosa* and decreases as *P. glandulosa* become more distant and/or sparser.

Results

Temperature and rainfall during the study period

Average monthly temperatures during February (17.1°C), March (19.1°C), and April (22.8°C) 2005 were above the 110-year mean at Alice, Texas. Monthly rainfall in both February (64.3 mm) and March (62.2 mm) was approximately 60% above the 110-year mean for those months; however, rainfall during April (2.54 mm) was only 6% of 110-year mean. Average monthly temperatures during May, June, July and August 2005 were 26.2, 29.4, 30.7, and 31.4°C, respectively. These temperatures were all above the 110-year mean for these months. Monthly rainfall during the same period was 41.4 mm (May), 3.3 mm (June), 64.8 mm (July) and 8.6 mm (August), which was either dramatically less than or comparable to the 110-year mean for these months. Hence, conditions prior to the April foliage sampling were comparatively warm and mesic, while those preceding the August foliage sampling were comparatively hot and dry. Detailed temperature and rainfall data for December up to and including August 2005 at this study site have been presented elsewhere (Bai et al. 2008).

Elevation and soil characteristics

The elevation of the transect ranged from 84.5 to 86.5 m (Fig. 1a). Soil clay content was negatively correlated with elevation (Table 1), with values of approximately 100 g kg^{-1} in the uplands, 100–200 g kg^{-1} in the drainage woodlands, and 250–350 g kg^{-1} in the playas (Fig. 1b).

Fig. 1 Changes in elevation (m) (**a**), soil particle size distribution (g kg^{-1}) (**b**), soil gravimetric water content (*GWC*) (mg mg^{-1}) (**c**), soil total N (g N kg^{-1}) and stable N isotope ratio ($\delta^{15}N$) (‰) (**d**), and leaf $\delta^{15}N$ (‰) of three woody species (*Prosopis glandulosa*, *Condalia hookeri*, and *Zanthoxylum fagara*) along the transect in April (**e**) and August (**f**) 2005. *Dashed lines* indicate locations of different landscape elements along the transect
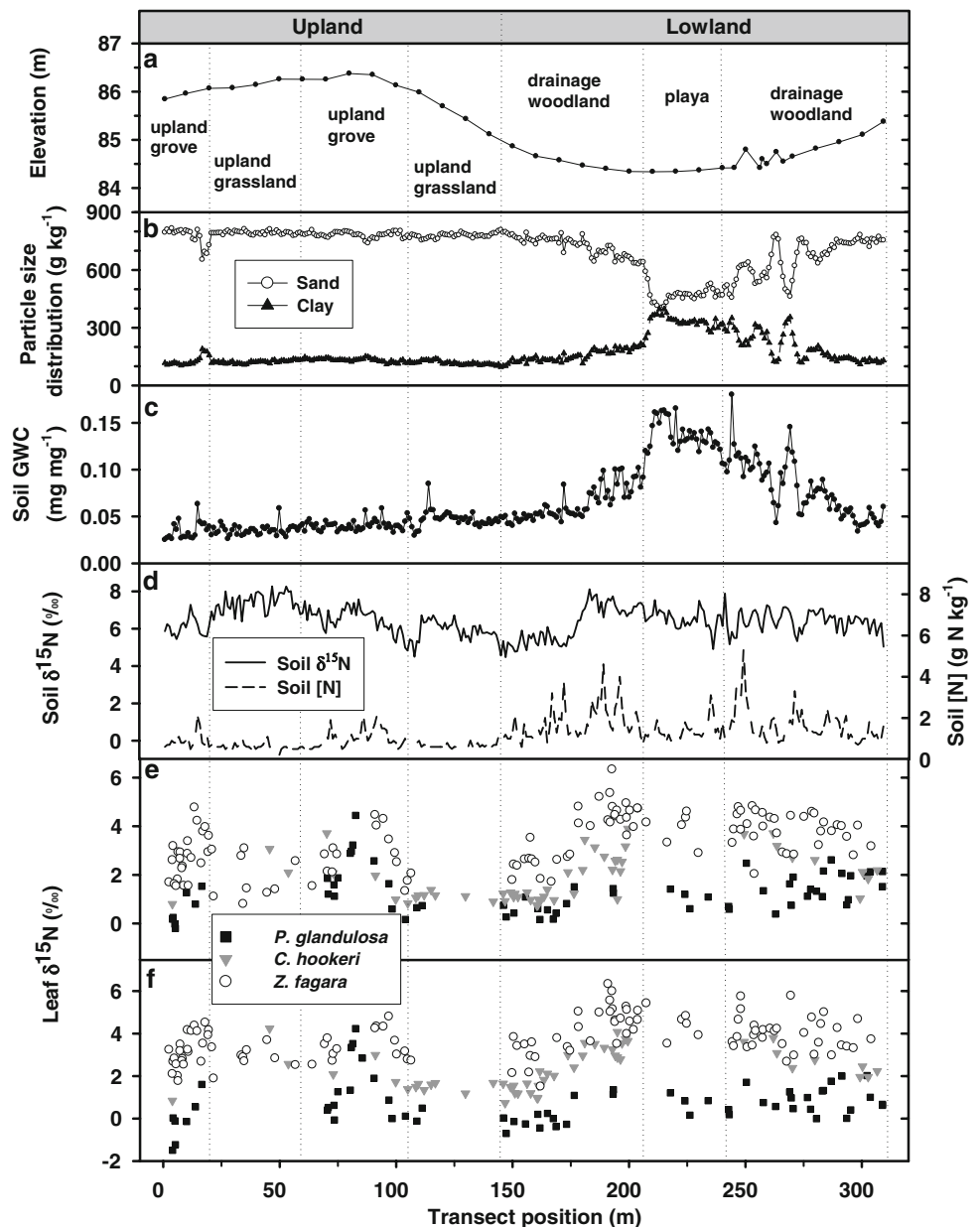
Soil GWC negatively correlated with elevation and positively correlated with soil clay content (Table 1). GWC was generally <0.05 mg mg^{-1} in uplands, but ranged up to 0.15 mg mg^{-1} in the lower-lying drainage woodlands and playas (Fig. 1c).

Soil total N was negatively correlated with elevation along the transect (Table 1), with higher values in the lower-lying drainage woodland and playa (1–5 g N kg^{-1} soil) compared to uplands (0.6–2 g N kg^{-1} soil) (Fig. 1d; $P < 0.01$). In addition, soil total N was higher under all woody vegetation types (1–5 g N kg^{-1} soil) than under grassland (0.6 g N kg^{-1} soil) (Fig. 1d; $P < 0.01$). Soil $\delta^{15}N$ ranged from 4.5 to 8.3‰ but was not strongly related to elevation (Fig. 1d; Table 1). Soil $\delta^{15}N$ was consistently

higher than leaf $\delta^{15}N$ values along the entire transect (Fig. 1d–f).

Plant $\delta^{15}N$ values and their relationships with environmental variables

The Mantel test indicated that leaf $\delta^{15}N$ was significantly different between species ($P < 0.001$; Table 2; Fig. 1e–f). Leaf $\delta^{15}N$ of *P. glandulosa* averaged 1.01‰, which was lower than those for *C. hookeri* (1.96‰) and *Z. fagara* (3.65‰). Sampling season had a significant effect on leaf $\delta^{15}N$ ($P = 0.04$; Table 2). The interaction between species and season also had a significant effect on leaf $\delta^{15}N$ ($P < 0.001$; Table 2). *P. glandulosa*'s foliar $\delta^{15}N$ decreased

Table 1 Pearson product–moment correlation coefficients[a] (r) between leaf stable N isotope ratio ($\delta^{15}N$) values and plant–soil variables along a topoedaphic gradient in a subtropical savanna parkland landscape

	Leaf δ^{15}N (‰)	Leaf [N] (g N kg^{-1})		Soil δ^{15}N (‰)	Soil [N] (g N kg^{-1})	Soil GWC (mg mg^{-1})	Elevation (m)	Soil clay (%)
		April	August					
Leaf δ^{15}N								
Prosopis glandulosa	1.000	0.054	0.186	0.448*	0.196	−0.061	0.220	−0.015
Condalia hookeri	1.000	0.491*	0.649*	0.608*	0.410*	0.500*	−0.342*	0.531*
Zanthoxylum fagara	1.000	0.575*	0.351*	0.451*	0.422*	0.479*	−0.609*	0.371*
Leaf [N]								
P. glandulosa		1.000	1.000	0.307*	0.461*	0.561*	−0.364*	0.531*
C. hookeri		1.000	1.000	0.370*	0.361*	0.478*	−0.420*	0.499*
Z. fagara		1.000	1.000	0.265*	0.387*	0.508*	−0.578*	0.414*
Soil δ^{15}N								
P. glandulosa				1.000	−0.125	0.040	0.165	0.141
C. hookeri				1.000	0.249	0.326*	−0.104	0.492*
Z. fagara				1.000	0.088	0.264*	−0.201*	0.287*
Soil [N]								
P. glandulosa					1.000	0.556*	−0.384*	0.300*
C. hookeri					1.000	0.807*	−0.493*	0.631*
Z. fagara					1.000	0.611*	−0.538*	0.315*
Soil GWC								
P. glandulosa						1.000	−0.735*	0.870*
C. hookeri						1.000	−0.646*	0.829*
Z. fagara						1.000	−0.783*	0.846*
Elevation								
P. glandulosa							1.000	−0.526*
C. hookeri							1.000	−0.452*
Z. fagara							1.000	−0.614*
Soil clay								
P. glandulosa								1.000
C. hookeri								1.000
Z. fagara								1.000

GWC Gravimetric water content

* $P < 0.05$

[a] All correlations are based on data from the April sample period unless otherwise indicated

from 1.30‰ in April to 0.71‰ in August after a period of drought, while leaf δ^{15}N of *C. hookeri* and *Z. fagara* increased during this same time interval (Table 2).

Elevation was not correlated with leaf δ^{15}N for *P. glandulosa* (Table 1). However, there was a significant negative relationship between elevation and leaf δ^{15}N for *C. hookeri* ($r = -0.342$) and *Z. fagara* ($r = -0.609$) (Table 1). Soil GWC showed a significantly positive relationship with leaf δ^{15}N of *C. hookeri* and *Z. fagara* ($r = 0.500$ and 0.479, respectively); however, this relationship was absent for *P. glandulosa* (Table 1). Soil clay content was also positively correlated with leaf δ^{15}N of *C. hookeri* ($r = 0.531$) and *Z. fagara* ($r = 0.371$), but not correlated with leaf δ^{15}N of *P. glandulosa* (Table 1). Soil total N was positively correlated with leaf δ^{15}N of

C. hookeri ($r = 0.422$) and *Z. fagara* ($r = 0.410$). Although the same trend was evident for *P. glandulosa*, the relationship was not significant (Table 1). Soil δ^{15}N was positively related to leaf δ^{15}N for all three species. Leaf [N] was positively correlated with leaf δ^{15}N for *C. hookeri* and *Z. fagara* in both April and August sampling periods (Table 1). However this relationship was not significant for *P. glandulosa*. Leaf [N] was consistently higher in *P. glandulosa* (20–46 g N kg^{-1}) than in the other two non-N$_2$-fixing plants (5–40 g N kg^{-1}).

Stepwise multiple regression was used to develop predictive models for the δ^{15}N values of leaf tissue for the three plant species based on the environmental variables measured in this study (Table 3). Soil δ^{15}N was the only predictor variable common to the models developed for

Table 2 Results of Mantel test for differences in leaf $\delta^{15}N$ (‰) between species and sample periods

Source of variation	Categories	Mean $\delta^{15}N$ (‰)	Mantel test	
			r	P
Species (S)	*P. glandulosa*	1.01	0.42	<0.001
	C. hookeri	1.96		
	Z. fagara	3.65		
Sample period (P)	April	2.22	0.06	0.04
	August	2.25		
S × P	*P. glandulosa*			
	April	1.30	0.03	<0.001
	August	0.71		
	C. hookeri			
	April	1.76	0.04	<0.001
	August	2.16		
	Z. fagara			
	April	3.44	0.02	<0.001
	August	3.86		

each species. Leaf N, soil N, elevation, and GWC were also included in one or more the models. Multiple regression models were able to account for approximately 45% of the variation in plant $\delta^{15}N$ values at this site (Table 3).

Leaf $\delta^{15}N$, rates of nitrification, and concentrations of inorganic N in soil

In an effort to further explain variation in plant $\delta^{15}N$ found in this present study, rates of nitrification and concentrations of soil NH_4^+–N and NO_3^-–N from a previous study (McCulley et al. 2004) were examined. McCulley et al. (2004) found that these parameters were all higher in wooded areas than in grassland, and were higher in drainage woodlands than in upland clusters and groves (data not shown). When mean $\delta^{15}N$ values for each plant species are plotted with respect to patch-specific rates of nitrification and concentrations of soil inorganic N, positive

relationships are obtained (Fig. 2) for both April and August 2005.

Effect of the N-fixer *P. glandulosa* on $\delta^{15}N$ of other woody species

The PI was used to evaluate the potential influence of the N_2-fixer *P. glandulosa* on the $\delta^{15}N$ of the other two woody species. This index accounts for both the number of surrounding *P. glandulosa* trees and their distances, weighted in favor of the nearest neighbor. Therefore, higher PI corresponds to more and/or closer neighbor *P. glandulosa* trees to the sampled species. By iterative solution of the relationship between PI and plant $\delta^{15}N$, we found that *P. glandulosa* trees >12 m from *C. hookeri* or >6 m from *Z. fagara* had no influence on the $\delta^{15}N$ values of those species. Thus, PI was based on a range of 12 m for *C. hookeri*, and 6 m for *Z. fagara*. Results also showed that as PI increased, leaf $\delta^{15}N$ of both *C. hookeri* and *Z. fagara* decreased significantly ($r^2 = 0.21$ and 0.22, respectively) (Fig. 3).

Discussion

Landscape-scale variation in plant $\delta^{15}N$

Topography exerts strong control over soil water and N (Jenny 1980; Schimel et al. 1985; Ruess and Seagle 1994; Turner et al. 1997), and has been reported to influence plant $\delta^{15}N$ at the landscape scale due to its impacts on soil moisture, nutrient availability, and rates of N-cycling processes (Garten 1993; Sutherland et al. 1993; Garten and van Miegroet 1994; Schmidt and Stewart 2003). We found that both soil water and soil total N increased as elevation decreased along a topoedaphic gradient in a subtropical savanna parkland landscape. In addition, elevation was negatively correlated with leaf $\delta^{15}N$ of *C. hookeri* and *Z. fagara*, and both soil GWC and soil total N were positively correlated with leaf $\delta^{15}N$ of these two non-N_2-fixing

Table 3 Stepwise multiple regression equations[a] for predicting leaf $\delta^{15}N$ values of three woody plant species in a subtropical savanna parkland landscape in southern Texas

Plant species	Stepwise regression equation	SE_{EST}	r	Adjusted r^2 (%)
P. glandulosa	$\delta^{15}N = -60.49 + 0.604$ (Soil δ) $+ 0.668$ (Elev) $+ 0.908$ (Soil N)	0.60	0.68	44
C. hookeri	$\delta^{15}N = -2.075 + 0.514$ (Soil δ) $+ 17.252$ (GWC)	0.69	0.69	46
Z. fagara	$\delta^{15}N = -3.145 + 0.099$ (Leaf N) $+ 0.515$ (Soil δ) $+ 0.333$ (Soil N)	0.85	0.68	45

Soil δ $\delta^{15}N$ value of soil total N, *Soil N* soil N concentration (g N kg^{-1} soil), *Elev* elevation along the transect (m), *Leaf N* leaf N concentration (g N kg^{-1} leaf tissue)

[a] Variables were only included in the model if they accounted for a statistically significant ($P < 0.05$) proportion of the residual variance
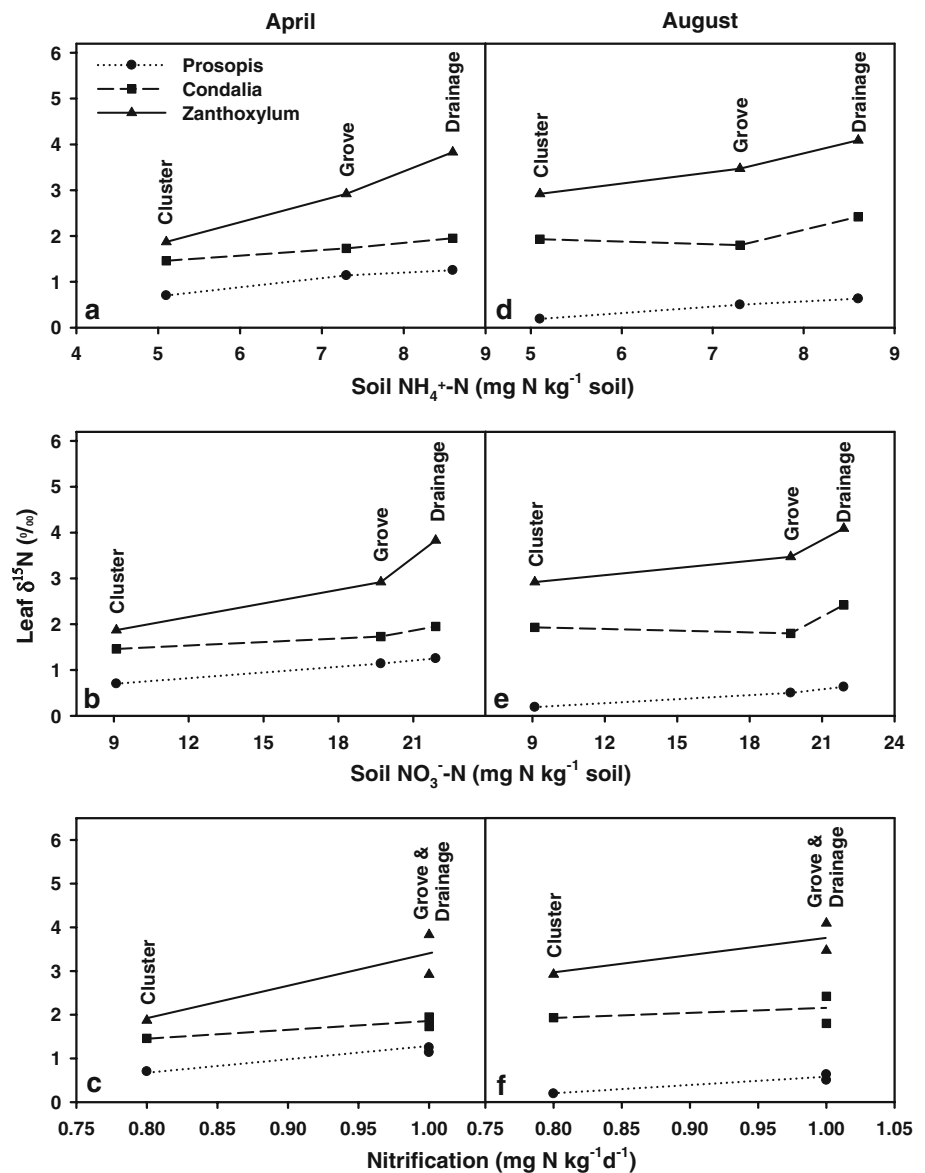
species. Although soil water availability has been shown to be negatively correlated with leaf δ^{15}N at continental and global scales (Austin and Sala 1999; Handley et al. 1999), positive correlations between soil water availability and leaf δ^{15}N have been found in mesic sites at the landscape scale (Garten and van Miegroet 1994; Farrell et al. 1996; Dijkstra et al. 2003). Because soil water and total N are major determinants of decomposition and N-transformation rates (Matson and Vitousek 1981; Booth et al. 2005), sites with higher soil water content may have more microbial activity and higher N-transformation rates, resulting in greater potential for losses of ^{15}N-depleted N via denitrification or leaching, and therefore higher residual δ^{15}N in plants and soils (Garten and van Miegroet 1994). Several other studies have documented a positive relationship between soil N concentrations and leaf δ^{15}N. Garten (1993)

found higher leaf δ^{15}N was associated with greater soil N availability, higher net nitrification, and mineralization potential in valley bottoms of an eastern deciduous forest in Tennessee. Garten and van Miegroet (1994) found leaf δ^{15}N was positively correlated with soil N availability, net mineralization and net nitrification rate in evergreens, maples and ferns. Sah and Brumme (2003) found soil N-poor sites were associated with more negative soil and leaf δ^{15}N in a pine forest in Nepal. Pardo et al. (2006) found a significantly positive relationship between leaf δ^{15}N of many forest tree species and nitrification rates. They also found site-averaged leaf δ^{15}N was strongly related to nitrification, mineralization rates and forest floor δ^{15}N of many sites in northeastern USA.

In our study area, McCulley et al. (2004) found that potential nitrification rates and soil pool sizes of NH_4^+–N

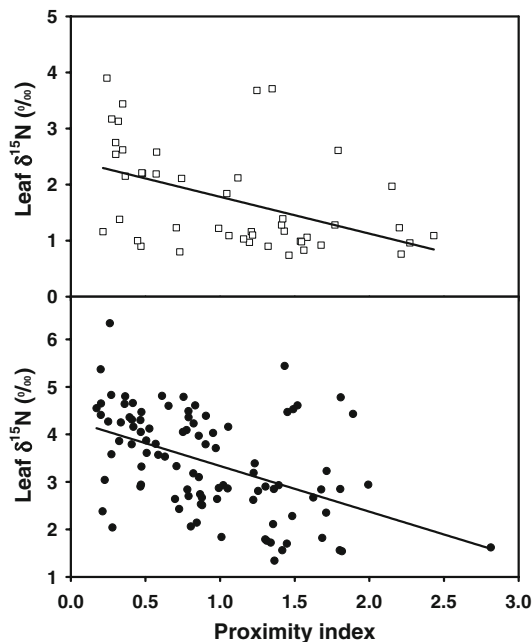**Fig. 3** Leaf δ^{15}N (‰) of *C. hookeri* (*upper panel*; $y = 2.44 - 0.66x$; $r^2 = 0.21$; $P = 0.001$) and *Z. fagara* (*lower panel*; $y = 4.30 - 0.96x$; $r^2 = 0.22$; $P < 0.001$) along a proximity index (PI) gradient. PI accounts for both the number of surrounding *P. glandulosa* trees and their distances, weighted in favor of the nearest neighbor

and NO_3^-–N increased in the sequence clusters < groves < drainage woodlands. The processes of nitrification and denitrification both result in losses of N as N_2O and NO (Firestone and Davidson 1989), and NH_4^+ and NO_3 represent forms of N that could potentially be lost via gaseous and leaching pathways. Thus, we infer that the potential for N losses and the δ^{15}N of residual soil N would also increase in the sequence clusters < groves < drainage woodlands. Indeed, when we plot leaf δ^{15}N against potential nitrification and soil NH_4^+ and NO_3^- concentrations measured by McCulley et al. (2004), it is evident that δ^{15}N of all three plant species increases as these proxies for N-loss potential also increase (Fig. 2). Thus, our results appear to be consistent with prior assessments of landscape-scale variation in plant δ^{15}N in relation to N cycle characteristics.

Small-scale variation in plant δ^{15}N: influence of the N_2-fixer *P. glandulosa* on neighboring plants

In our study area, *P. glandulosa* develops active root nodules capable of symbiotic N_2 fixation (Zitzer et al. 1996). In the present study, *P. glandulosa* had consistently lower leaf δ^{15}N (Fig. 1; Table 2) and higher leaf [N] than the non-N_2-fixing species *C. hookeri* and *Z. fagara*, both consistent with N_2-fixing plants in many ecosystems around the world (Virginia and Jarrell 1983; McKey 1994; Salas et al. 2001; Dijkstra et al. 2003; Schmidt and Stewart 2003; Temperton et al. 2007).

By fixing atmospheric N_2 with a δ^{15}N $\approx$ 0‰, *P. glandulosa* could influence the availability and δ^{15}N of soil N near its canopy in a manner that should be reflected in the δ^{15}N of adjacent non-N_2-fixing plants. In fact, our calculated PI was negatively correlated with leaf δ^{15}N for both *C. hookeri* ($r^2 = 0.21$) and *Z. fagara* ($r^2 = 0.22$) (Fig. 3); plants that were near one or more *P. glandulosa* trees generally had lower δ^{15}N than those located farther away. These data indicate that soil N derived from N_2-fixing *P. glandulosa* trees likely makes an important contribution to the N nutrition of associated non-N_2-fixing plant species. By iterative solution of PI, we found that *P. glandulosa* influenced δ^{15}N values of *C. hookeri* located within 12 m, and *Z. fagara* located within 6 m. The average canopy area of *C. hookeri* is approximately 4 times greater than that of *Z. fagara* (Northup et al. 2005); if the lateral extent of their root systems matches that of their canopies, this may explain the larger sphere of influence of *P. glandulosa* on *C. hookeri*. Few studies have examined the potential influences of N_2-fixing plants on adjacent non-N_2-fixing plants in natural ecosystems. Handley et al. (1994) found that leaf δ^{15}N of non-N_2-fixing plants were not related to distance from putative N_2-fixing *Acacia* plants. Belsky et al. (1989) also found that N_2-fixing *Acacia tortilis* did not influence adjacent plants differently than non-N_2-fixing baobab trees in Kenya. However, Temperton et al. (2007) found significantly lower δ^{15}N values in phytometer species growing with legumes in Germany.

Previous studies at this site have shown that open grassland was once dominant across the entire landscape, and that woody plants have only recently encroached into this former grassland during the past 100 years (Archer et al. 1988, 2001; Boutton et al. 1998). Woody encroachment into grassland is initiated by *P. glandulosa*, which then appears to facilitate the establishment of other woody species beneath its canopy. The exact mechanisms by which *P. glandulosa* facilitates woody patch development are not clear, but may include enhanced soil moisture via hydraulic lift (Zou et al. 2005) and accelerated rates of N-cycling processes (Hibbard et al. 2001; McCulley et al. 2004). Our results support the notion that N_2 fixation by *P. glandulosa* is important to the N economy of non-N_2-fixing woody plants, thus representing an important mechanism by which *P. glandulosa* facilitates the formation of woody patches in grasslands in this region.

Individual plant scale variation in δ^{15}N

Leaf δ^{15}N was lower than that of soils (Fig. 1e–f), consistent with numerous other studies at landscape (Garten 1993; Miller and Bowman 2002; Koba et al. 2003), regional (Houlton et al. 2007) and global (Amundson et al.

) scales. The uptake of ^{15}N-depleted inorganic N (Houlton et al. 2007), fractionations via mutualistic relationships with mycorrhizae (Hobbie et al. 2000), and fractionations via internal translocation (Hogberg 1997) have been interpreted as the causes. Leaf δ^{15}N of all the three species had positive relationships with soil δ^{15}N (Table 1). Handley et al. (1999) found a similar correlation globally, but the mechanism underlying this correlation remained unexplained. Although bulk soil δ^{15}N does not represent the sources of plant δ^{15}N because different plants may use different portions of bulk soil N, the positive relationship between plant and soil δ^{15}N suggested that these plants may use the dominant and most available forms of N in soil. Detailed analysis of the δ^{15}N of the various forms of soil N is needed to obtain a complete knowledge of this possibility (e.g., Houlton et al. 2007).

N$_2$-fixing *P. glandulosa* had leaf δ^{15}N values that were significantly lower than those of the two non-N$_2$-fixing shrub species (Fig. 1e–f; Table 2), consistent with other studies comparing N$_2$-fixing versus non-N$_2$-fixing plants (Virginia and Delwiche 1982; Schulze et al. 1991). Apparently this is because *P. glandulosa* obtains at least some of its tissue N from atmospheric N$_2$ which has lower δ^{15}N than that of soil N sources. Nonetheless, stepwise multiple regression revealed that soil δ^{15}N was an important determinant of leaf δ^{15}N values for all three plant species. Therefore, the δ^{15}N of N source is very important in determining plant δ^{15}N at an individual plant scale.

Seasonal variation in plant δ^{15}N

Leaf δ^{15}N of *P. glandulosa* was lower in August than in April; however, those of *C. hookeri* and *Z. fagara* were higher in August than April (Table 2). The difference in temporal responses between *P. glandulosa* and the other two species may be related to the fact that *P. glandulosa* is a N$_2$-fixing plant and therefore has access to a source of N that is unavailable to non-N$_2$-fixing species. We currently lack direct information that can explain these temporal differences. However, in previous studies, seasonal variation in leaf δ^{15}N has been attributed to changes in the rate of the ecosystem N cycle (Schulze et al. 1994; Peñuelas et al. 2000; Filella and Penuelas 2003) and to internal plant N mobilization (Garten 1993; Handley and Scrimgeour 1997; Chang and Handley 2000). For example, Filella and Penuelas (2003) reported leaf δ^{15}N was higher in spring than summer in Mediterranean woody shrubs in north-east Spain. They suggested that spring rains and increasing temperatures accelerated N-cycle processes, resulting in a higher rate of N cycle and higher leaf δ^{15}N at that time. Chang and Handley (2000) found foliar δ^{15}N was lower in August than in May for western red cedar trees and shrub salal on Vancouver Island, British Columbia. They

suggested that higher leaf δ^{15}N values in spring were strongly influenced by the mobilization of internally stored N, while lower leaf δ^{15}N values in summer were the result of uptake of ^{15}N-depleted soil N. Basipetal translocation of N in deciduous plants prior to autumn leaf fall may cause higher leaf δ^{15}N in older leaves (Garten 1993; Chang and Handley 2000; Bustamante et al. 2004).

Conclusion

At the landscape scale, spatial patterns of leaf δ^{15}N of non-N$_2$-fixing plants are controlled by topography which influences soil water availability and the concentration and δ^{15}N of soil total N in this subtropical savanna parkland ecosystem. Greater soil N content and soil water availability are usually associated with higher rates of N transformations (e.g., mineralization, nitrification, and denitrification) which result in N losses from the ecosystem and ^{15}N enrichment of the residual N. Thus, plants in lower-lying portions of the landscape have higher leaf δ^{15}N than those in upland areas. In general, the N$_2$-fixing plant *P. glandulosa* had leaf δ^{15}N values that were closer to values for atmospheric N$_2$ and lower than leaf values for the two non-N$_2$-fixing shrub species. At smaller scales, spatial patterns of leaf δ^{15}N from non-N$_2$-fixing plants appear to be influenced by proximity to the N$_2$-fixing *P. glandulosa*, suggesting that encroachment of N$_2$-fixing *P. glandulosa* may influence the N cycle and facilitate N acquisition by other woody plants. Our results indicate that topoedaphic characteristics that influence rates of N-cycling processes are important determinants of plant δ^{15}N values at the landscape scale, and that N fixed by *P. glandulosa* may affect the N economy of neighboring plants and facilitate the encroachment of other woody species into this subtropical savanna parkland landscape.

References

Amundson R, Austin AT, Schuur EAG, Yoo K, Matzek V, Kendall C, Uebersax A, Brenner D, Baisden WT (2003) Global patterns of the isotopic composition of soil and plant nitrogen. Global Biogeochem Cycles 17:1031

Archer S (1995) Tree-grass dynamics in a *Prosopis*-thorn scrub savanna parkland: reconstructing the past and predicting the future. Ecoscience 2:83–89

Archer S, Scifres C, Bassham CR, Maggio R (1988) Autogenic succession in a subtropical savanna: conversion of grassland to thorn woodland. Ecol Monogr 58:111–127

Archer S, Boutton TW, Hibbard KA (2001) Trees in grasslands: biogeochemical consequences of woody plant expansion. In: Schulze E-D, Harrison SP, Heimann M, Holland EA, Lloyd J, Prentice IC, Schimel D (eds) Global biogeochemical cycles in the climate system. Academic Press, San Diego, pp 115–138

Austin AT, Sala OE (1999) Foliar δ^{15}N is negatively correlated with rainfall along the IGBP transect in Australia. Aust J Plant Physiol 26:293–295

Bai E, Boutton TW, Liu F, Wu XB, Archer SR (2008) Variation in woody plant δ^{13}C along a topoedaphic gradient in a subtropical savanna parkland. Oecologia 156:479–489

Belsky AJ, Amundson RG, Duxbury JM, Riha SJ, Ali AR, Mwonga SM (1989) The effects of trees on their physical, chemical and biological environments in a semi-arid savanna in Kenya. J Appl Ecol 26:1005–1024

Booth MS, Stark JM, Rastetter E (2005) Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. Ecol Monogr 75:139–157

Boutton TW, Archer SR, Midwood AJ, Zitzer SF, Bol R (1998) δ^{13}C values of soil organic carbon and their use in documenting vegetation change in a subtropical savanna ecosystem. Geoderma 82:5–41

Bustamante MMC, Martinelli LA, Silva DA, Camargo PB, Klink CA, Domingues TF, Santos RV (2004) ^{15}N natural abundance in woody plants and soils of central Brazilian savannas (cerrado). Ecol Appl 14:200–213

Chang SX, Handley LL (2000) Site history affects soil and plant ^{15}N natural abundances in forests of northern Vancouver Island, British Columbia. Funct Ecol 14:273–280

Choi W, Ro H, Hobbie EA (2003) Patterns of natural ^{15}N in soils and plants from chemically and organically fertilized uplands. Soil Biol Biochem 35:1493–1500

Cook GD (2001) Effects of frequent fires and grazing on stable nitrogen isotope ratios of vegetation in northern Australia. Austral Ecol 26:630–636

Dijkstra P, Williamson C, Menyailo O, Doucett R, Koch G, Hungate BA (2003) Nitrogen stable isotope composition of leaves and roots of plants growing in a forest and a meadow. Isot Environ Health S 39:29–39

Evans RD, Belnap J (1999) Longterm consequences of disturbance on nitrogen dynamics in an arid grassland ecosystem. Ecology 80:150–160

Farley AL (2000) Vegetation, soils and surface hydrology of playa landforms in the Rio Grande Plains, Texas. MS thesis, Department of Rangeland Ecology and Management, Texas A&M University, College Station, TX

Farrell RE, Sandercock PJ, Pennock DD, van Kessel C (1996) Landscape-scale variations in leached nitrate: relationship to denitrification and natural N-15 abundance. Soil Sci Soc Am J 60:1410–1415

Filella I, Penuelas J (2003) Partitioning of water and nitrogen in co-occurring mediterranean woody shrub species of different evolutionary history. Oecologia 137:51–61

Firestone MK, Davidson EA (1989) Microbiological basis of NO and N_2O production and consumption in soil. In: Andreae MO, Schimel DS (eds) Exchange of trace gases between terrestrial ecosystems and the atmosphere. Wiley, New York, pp 7–21

Fortin MJ, Gurevitch J (1993) Mantel tests: spatial structure in field experiments. In: Scheiner SM, Gurevitch J (eds) Design and analysis of ecological experiments. Chapman and Hall, New York, pp 342–359

Garten CT (1993) Variation in foliar ^{15}N abundance and the availability of soil nitrogen on walker branch watershed. Ecology 74:2098–2113

Garten CT, van Miegroet H (1994) Relationships between soil nitrogen dynamics and natural ^{15}N abundance in plant foliage from Great Smoky Mountains National Park. Can J For Res 24:1636–1645

Gee GW, Bauder JW (1986) Particle-size analysis. In: Klute A (ed) Methods of soil analysis. Part I. Physical and mineralogical methods. American Society of Agronomy, Soil Science Society of America, Madison, pp 383–411

Ghazoul J, Liston KA, Boyle TJB (1998) Disturbance-induced density-dependent seed set in Shorea siamensis (dipterocarpaceae), a tropical forest tree. J Ecol 86:462–473

Gustafson EJ, Parker GR (1994) Using an index of habitat patch proximity for landscape design. Landsc Urban Plan 29:117–130

Handley LL, Scrimgeour CM (1997) Terrestrial plant ecology and ^{15}N natural abundance: the present limits to interpretation for uncultivated systems with original data from a scottish old field. Adv Ecol Res 27:133–212

Handley LL, Odee D, Scrimgeour CM (1994) δ^{15}N and δ^{13}C patterns in savanna vegetation: dependence on water availability and disturbance. Funct Ecol 8:306–314

Handley LL, Austin AT, Robinson D, Scrimgeour CM, Raven JA, Heaton THE, Schmidt S, Stewart GR (1999) The ^{15}N natural abundance (δ^{15}N) of ecosystem samples reflects measures of water availability. Aust J Plant Physiol 26:185–199

Harris D, Horwáth WR, van Kessel C (2001) Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Sci Soc Am J 65:1853–1856

Hibbard KA, Archer S, Schimel DS, Valentine DW (2001) Biogeochemical changes accompanying woody plant encroachment in a subtropical savanna. Ecology 82:1999–2011

Hillel D (1982) Introduction to soil physics. Academic Press, New York

Hobbie EA, Macko SA, Williams M (2000) Correlations between foliar δ^{15}N and nitrogen concentrations may indicate plant-mycorrhizal interactions. Oecologia 122:273–283

Hogberg P (1997) Tansley review no. 95: ^{15}N natural abundance in soil-plant systems. New Phytol 137:179–203

Högberg P, Hogbom L, Schinkel H, Hogberg M, Johannisson C, Wallmark H (1996) ^{15}N abundance of surface soils, roots and mycorrhizas in profiles of European forest soils. Oecologia 108:207–214

Houlton BZ, Sigman DM, Hedin LO (2006) Isotopic evidence for large gaseous nitrogen losses from tropical rainforests. Proc Nat Acad Sci USA 103:8745–8750

Houlton BZ, Sigman DM, Schuur EA, Hedin LO (2007) A climate-driven switch in plant nitrogen acquisition within tropical forest communities. Proc Nalt Acad Sci USA 104:8902–8906

Jenny H (1980) The soil resource: origin and behavior. Springer, New York

Koba K, Hirobe M, Koyama L, Kohzu A, Tokuchi N, Nadelhoffer KJ, Wada E, Takeda H (2003) Natural ^{15}N abundance of plants and soil N in coniferous temperate forest. Ecosystems 6:457–469

Liao JD, Boutton TW, Jastrow JD (2006) Organic matter turnover in soil physical fractions following woody plant invasion of grassland: evidence from natural ^{13}C and ^{15}N. Soil Biol Biochem 38:3197–3210

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220

Mariotti A (1983) Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements. Nature 303:685–687

Matson PA, Vitousek PM (1981) Nitrogen mineralization and nitrification potentials following clearcutting in the Hoosier national forest, Indiana. Forest Sci 27:781–791

McCulley RL, Archer SR, Boutton TW, Hons FM, Zuberer DA (2004) Soil respiration and nutrient cycling in wooded communities developing in grassland. Ecology 85:2804–2817

McKee KL, Feller IC, Popp M, Wanek W (2002) Mangrove isotopic (δ^{15}N and δ^{13}C) fractionation across a nitrogen vs. phosphorus limitation gradient. Ecology 83:1065–1075

McKey D (1994) Legumes and nitrogen: the evolutionary ecology of a nitrogen-demanding lifestyle. In: Sprent JI, McKey D (eds) Advances in legume systematics. 5. The nitrogen factor. Royal Botanic Gardens, Kew, pp 211–228

Michelsen A, Quarmby C, Sleep D, Jonasson S (1998) Vascular plant ^{15}N natural abundance in heath and forest tundra ecosystems is closely correlated with presence and type of mycorrhizal fungi in roots. Oecologia 115:406–418

Miller AE, Bowman WD (2002) Variation in nitrogen-15 natural abundance and nitrogen uptake traits among co-occurring alpine species: do species partition by nitrogen form? Oecologia 130:609–616

Northup BK, Zitzer SF, Archer SR, McMurtry CR, Boutton TW (2005) Above-ground biomass and carbon and nitrogen content of woody species in a subtropical thornscrub parkland. J Arid Environ 62:23–43

Pardo LH, Templer PH, Goodale CL, Duke S, Groffman PM, Adams MB, Boeckx P, Boggs J, Campbell J, Colman B, Compton J, Emmett B, Gundersen P, Kjonaas J, Lovett G, Mack M, Magill A, Mbila M, Mitchell MJ, McGee G, McNulty S, Nadelhoffer K, Ollinger S, Ross D, Rueth H, Rustad L, Schaberg P, Schiff S, Schleppi P, Spoelstra J, Wessel W (2006) Regional assessment of N saturation using foliar and root δ^{15}N. Biogeochemistry 80:157–194

Peñuelas J, Filella I, Lloret F, Piñol J, Siscart D (2000) Effects of a severe drought on water and nitrogen use by *Quercus ilex* and *Phyllyrea latifolia*. Biol Plantarum 43:47–53

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18:293–320

Robinson D (2001) δ^{15}N as an integrator of the nitrogen cycle. Trends Ecol Evol 16:153–162

Rosenberg MS (2001) PASSAGE. Pattern analysis, spatial statistics, and geographic exegesis. version 1.1. Department of Biology, Arizona State University, Tempe

Ruess RW, Seagle SW (1994) Landscape patterns in soil microbial processes in the Serengeti National Park, Tanzania. Ecology 75:892–904

Sah SP, Brumme R (2003) Altitudinal gradients of natural abundance of stable isotopes of nitrogen and carbon in the needle and soil of a pine forest of Nepal. J For Sci 49:19–26

Salas E, Nygren P, Domenach AM, Berninger F, Ramirez C (2001) Estimating biological N$_2$ fixation by a tropical legume tree using the non-nodulating phenophase as the reference in the ^{15}N natural abundance method. Soil Biol Biochem 33:1859–1868

Schimel D, Stillwell MA, Woodmansee RG (1985) Biogeochemistry of C, N, and P in a soil catena of the shortgrass steppe. Ecology 66:276–282

Schmidt S, Stewart GR (2003) δ^{15}N values of tropical savanna and monsoon forest species reflect root specialisations and soil nitrogen status. Oecologia 134:569–577

Schulze ED, Gebauer G, Ziegler H, Lange OL (1991) Estimates of nitrogen fixation by trees on an aridity gradient in Namibia. Oecologia 88:451–455

Schulze ED, Chapin FS, Gebauer G (1994) Nitrogen nutrition and isotope differences among life forms at the northern treeline of Alaska. Oecologia 100:406–412

Schulze ED, Williams J, Farquhar GD, Schulze W, Langridge J, Miller JM, Walker BH (1998) Carbon and nitrogen isotope discrimination and nitrogen nutrition of trees along a rainfall gradient in northern Australia. Aust J Plant Physiol 25:413–425

Schulze ED, Farquhar GD, Miller JM, Schulze W, Walker BH, Williams RJ (1999) Interpretation of increased foliar δ^{15}N in woody species along a rainfall gradient in northern Australia. Aust J Plant Physiol 26:296–298

Sutherland RA, van Kessel C, Farrell RE, Pennock DJ (1993) Landscape-scale variations in plant and soil nitrogen-15 natural abundance. Soil Sci Soc Am J 57:169–178

Temperton VM, Mwangi PN, Scherer-Lorenzen M, Schmid B, Buchmann N (2007) Positive interactions between nitrogen-fixing legumes and four different neighbouring species in a biodiversity experiment. Oecologia 151:190–205

Turner CL, Blair JM, Schartz RJ, Neel JC (1997) Soil N and plant responses to fire, topography, and supplemental N in tallgrass prairie. Ecology 78:1832–1843

Virginia RA, Delwiche CC (1982) Natural ^{15}N abundance of presumed N$_2$-fixing and non-N$_2$-fixing plants from selected ecosystems. Oecologia 54:317–325

Virginia RA, Jarrell WM (1983) Soil properties in a mesquite-dominated Sonoran Desert ecosystem. Soil Sci Soc Am J 47:138–144

Zitzer SF, Archer SR, Boutton TW (1996) Spatial variability in the potential for symbiotic N$_2$ fixation by woody plants in a subtropical savanna ecosystem. J Appl Ecol 33:1125–1136

Zou C, Barnes PW, Archer S, McMurtry CR (2005) Soil moisture redistribution as a mechanism of facilitation in savanna tree-shrub clusters. Oecologia 145:32–40