

Increases of Soil C, N, and P Pools Along an *Acacia* Tree Density Gradient and Their Effects on Trees and Grasses

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ABSTRACT

Nitrogen (N) fixing trees including many species of *Acacia* are an important though variable component of savanna ecosystems. It is known that these trees enrich the soil with carbon (C) and N, but their effect on the combined C:N:P stoichiometry in soil is less well understood. Theory suggests that they might reduce available phosphorus (P), creating a shift from more N-limited conditions in grass-dominated to more P-limited conditions in tree-dominated sites, which in turn could feed back negatively on the trees' capacity to fix N. We studied the effects of *Acacia zanzibarica* tree density upon soil and foliar N:P stoichiometry, and the N₂-fixation rates of trees and leguminous herbs in a humid Tanzanian savanna. Foliar N:P ratios and N₂-fixation rates of trees remained constant across

the density gradient, whereas soil C, N and organic P pools increased. In contrast, the N:P ratio of grasses increased and N₂-fixation rates of leguminous herbs decreased with increasing tree density, indicating a shift towards more P-limited conditions for the understory vegetation. These contrasting responses suggest that trees and grasses have access to different sources of N and P, with trees being able to access P from deeper soil layers and perhaps also utilizing organic forms more efficiently.

Key words: carbon sequestration; legume; nitrogen-fixing trees; nutrient limitation; N:P stoichiometry; plant–soil feedback; phosphorus; savanna; tree–grass interactions; woody encroachment.

INTRODUCTION

Savanna vegetation is composed of both grass and trees layers, with the density of the latter being

influenced by many factors, including precipitation, soil type, fire, grazing, and management (Scholes and Archer 1997; Sankaran and others 2005). In recent decades, a general increase in savanna tree density has been recorded throughout the tropics, which has been attributed to increasing CO₂ concentrations and land-use changes such as increased grazing by livestock (Scholes and Archer 1997; Bond and others 2003; Van Auken 2009). Despite the natural variability in tree density, and the recent increases, studies of how trees influence nutrient cycling in savanna have either considered

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the effects of individual trees or have compared woodland with open grassland (Belsky and others 1993; Hibbard and others 2001; Ludwig and others 2004; Hagos and Smit 2005; Boutton and Liao 2010; Cech and others 2010). However, neither approach adequately reflects the range of effects that trees may have in savanna ecosystems, and in particular upon soil and plant nutrient dynamics (Bond 2008).

Many savanna trees including several species of *Acacia* fix nitrogen (N) symbiotically and thereby add N to the ecosystem (Ludwig and others 2004; Boutton and Liao 2010; Cech and others 2010). To fix N, however, trees require a relatively high supply of soil phosphorus (P) (Binkley and others 2000; Perreijn 2002; Vitousek and others 2002), with the consequence that although the availability of N is usually higher beneath N₂-fixing trees than in open grassland, that of P may be reduced (Hibbard and others 2001; Cech and others 2008; Boutton and Liao 2010). Such a shift in the relative availabilities of N and P could potentially reduce the trees' capacity to fix N and produce biomass (Crews 1993; Perreijn 2002; Binkley and others 2003; Isaac and others 2011), though there is some evidence that trees can access P not available to grasses. This is supported by the fact that the growth of N₂-fixing trees is not always associated with a measurable decline in soil P (Geesing and others 2000; Ludwig and others 2004), and that the cover of leguminous trees is increasing in many savannas (Archer and others 1995; Van Auken 2009). Given that most savanna trees have very extensive root systems, any additional P could be acquired directly, either by lateral roots extending beyond the canopy radius or by roots descending into deeper soil layers (Belsky and others 1989; Scholes and Archer 1997). However, P could also become available indirectly through capillary flow or hydraulic lift (the mechanism by which trees passively redistribute water through their root systems), both of which might promote the transport of dissolved P from deeper soil layers to the drier surface soil (Scholes and Archer 1997; Ludwig and others 2004; McCulley and others 2004). It is not known, however, whether any of these mechanisms is important for savanna trees. Furthermore, even if one or more of these mechanisms were important for trees, the understory vegetation might still be affected by a shift towards more P-limited growth conditions, which—together with asymmetric competition for other resources—could reduce the availability of forage for grazing animals (Scholes and Archer 1997; Riginos and others 2009; Van Auken 2009).

Several studies have shown that N₂-fixing trees tend to increase soil C and N pools (Belsky and

others 1993; Ludwig and others 2004; Hagos and Smit 2005), but much less is known about how trees affect combined soil C:N:P stoichiometry, despite its potential importance for savanna dynamics. We studied these processes in two areas of humid Tanzanian savanna with different management histories, selecting sites that represented gradients from open grassland to dense *Acacia zanzibarica* woodland (a total of 43 sites). The main aim of our study was to determine the effects of *Acacia* tree density upon pools of soil C, N, and P, and availabilities of N and P to trees and understory vegetation. We expected C and N pools to increase with increasing tree density, as has been found in other studies, due to the higher production of organic matter and the accumulation of symbiotically fixed N beneath trees. We were especially interested, therefore, in determining whether the soil P pool also increased along the density gradient and, if so, the probable source of the additional P. We argued that if trees acquire P from surface soil at some distance from the canopy, extractable P pools in these areas would decrease, with this effect increasing with tree density; however, if trees acquire P from deeper soil layers, then we would expect no depletion of soil P pools outside the canopy. We also investigated the foliar N:P ratios of both trees and plants in the herbaceous layer, and N₂-fixation rates of trees and leguminous herbs. If trees are able to acquire sufficient P to meet their needs, then we would expect to find no change in N:P ratios and N₂-fixation rates along the density gradient. However, even if this were the case for trees, we would expect foliar N:P ratios of the understory vegetation to increase, reflecting a shift towards more P-limited conditions for this component of the vegetation.

METHODS

Study Area

The study was conducted in Saadani National Park, located on the Tanzanian coast (5°43'S, 38°47'E). This park, formed in 2002, unites two areas with different management histories. Mkwaja in the north (~470 km²) was used as a cattle ranch between 1954 and 2002; during this period it supported up to 15,000 animals, though from the 1960s onwards its operations were impeded by severe bush encroachment (Tobler and others 2003). Saadani in the south (~210 km²) was used until the 1960s for various types of agriculture including cattle, cashew nuts and sisal, and was subsequently managed as a game reserve (Baldus and others 2001).

The annual rainfall in the area varies widely, from 500 to 1700 mm, with the mean for the 4-year period preceding and during our study being around 580 mm. Most rain falls during two wet seasons—one from March until June and the other from mid-October until mid-November. Mean annual temperature is 25°C. Due to their differing management histories, Saadani supports a much higher density and diversity of wild herbivores than Mkwaja (Treydte and others 2005).

Annual precipitation in the region is sufficient to support woodland (Sankaran and others 2005), and the fact that vegetation is now mainly savanna probably reflects a long history of anthropogenic fire in the dry months (compare Bond and others 2005). We studied tall-grass savanna vegetation invaded to different degrees by the leguminous tree *A. zanzibarica* (S. Moore) Taub. var. *zanzibarica*. This slender tree grows up to 12 m in height and has a strongly monopodial growth form, with a dominant main axis and relatively few, elongated primary branches. The canopy casts little shade, and even dense woodland supports a continuous grass layer. Tree trunks and branches bear large stipular woody spines, some of which have thick, bilobed pseudo-galls at their base, these usually being inhabited by symbiotic crematogastine ants (Cochard and Edwards 2011). The *A. zanzibarica* trees in our study area probably fix N symbiotically, because they form nodules (Odee and others 1995; Cech 2008) and their foliar $\delta^{15}\text{N}$ values are markedly lower than those of non-leguminous trees (see *Acacia* tree variables below). Dominant grass and sedge species in this savanna vegetation are *Heteropogon contortus*, *Panicum infestum*, *Schoenefeldia transiens*, *Sporobolus pyramidalis*, and *Abildgaardia triflora*. Soils in the study area are mainly gray vertisols derived from coral sands. They are relatively uniform and unstructured up to a depth of more than 1.5 m, although they are variable in structure, ranging from sand to clay (Cochard and Edwards 2011).

We studied the effects of *A. zanzibarica* tree density upon soil and vegetation nutrient conditions in both the Mkwaja and Saadani areas. In Mkwaja, the trees probably established during the ranching period in the 1960s, and were subject to various unsuccessful attempts to remove them during the 1970s and 1980s (Cochard and Edwards 2011). We have less information about the history of the Saadani area, but we assume trees were approximately the same age as those in Mkwaja (c. 30–50 years). This is supported by the relatively uniformity in tree size along the gradients. If sites with high tree density had established earlier (that

is, space-for-time substitution) we would expect at least some larger trees at these sites, but this is not the case (Appendix A in Supplemental material).

In a preliminary survey, we drove through the study area and estimated the tree density in randomly selected patches of *A. zanzibarica* woodland. Subsequently, we selected 21 study sites of 30 × 30 m in Mkwaja and 22 sites in Saadani with densities of *A. zanzibarica* ranging from 0 to 2000 trees ha⁻¹. We were careful to choose sites on predominantly sandy soils, and in Mkwaja we avoided the paddocks where cattle were formerly kept overnight, as these areas still have extremely high availabilities of N and P (Cech and others 2010).

Soil Variables

The top 15 cm of soil at each site was sampled in February 2009 using a 4.0 cm diameter corer. At each site we took one soil core each from beneath the canopy of three randomly chosen *Acacia* trees (<1.5 m from the stem), and three from positions outside the canopy (>3 m from the stem of an *Acacia* tree). Before sampling we removed the aboveground litter layer. We only sampled the topsoil layer as nutrients in this layer are highly influenced by the vegetation growing there. Nutrients that are considered most limiting to plant growth in the particular system will be strongly cycled in this layer (Jobbágy and Jackson 2001). Soil bulk density was determined by drying cores to constant weight. We then pooled the cores for each site according to canopy type (that is, beneath canopy or outside canopy), and dried and ground the soil for nutrient analyses. Total C and N concentrations together with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were determined using an elemental analyzer (NCS-2500, Carlo Erba) coupled in continuous flow to an isotope ratio mass spectrometer (Optima, Micro-Mass). Total P concentrations were measured after Kjeldahl digestion by means of a continuous flow injection analyzer (AutoAnalyzer 3 HR, Seal Analytical). Mechlich 1 extraction (0.0125 M H₂SO₄ + 0.05 M HCl) was used to determine extractable soil P as described by Sims (2000). We extracted 5 g soil with 20 ml Mechlich 1 solution and determined P concentrations in the extracts using the ammonium molybdate–ascorbic acid method (Murphy and Riley 1962), measuring absorbance values at 882 nm on a photospectrometer (DR 2800 BIS, Hach Lange). To determine organic soil P concentrations we used the ignition method as described by O'Halloran and Cade-Menun (2008). We ignited 1 g soil at 550°C for 1 h, after which we extracted it with 25 ml

0.5 M H₂SO₄. We also extracted 1 g unignited soil of the same sample. After extraction, we determined P concentrations in both ignited and unignited extracts as described above. The measured P concentrations estimated inorganic P, whereas the difference between the ignited and unignited samples was taken as the organic P concentration of the sample.

To calculate the soil nutrient pools beneath and outside the *Acacia* tree canopy for each site, we multiplied values of soil bulk density with nutrient concentrations from these two canopy types. We had no bulk density data for two sites in Mkwaja, and for these we used the mean value of the other Mkwaja sites (bulk density showed no trend over the tree density gradient).

Soil N and P release rates were measured by adsorption of inorganic N and P to ion-exchange resin bags. Each resin bag (25 cm²) of nylon (60 µm mesh size, Sefar Nitex 03-60/35, Sefar AG) contained 2 g mixed-bed ion-exchange resins (Amberlite IRN 150, H⁺- and OH⁻-forms, Sigma Aldrich). Resins were conditioned by shaking them for 2 h in 2 M KCl solution. Five resin bags were placed at each site in a straight line with a distance of 2 m between bags. We made a slanting incision in the soil (45°) with a knife and carefully inserted the bag at 7.5 cm depth, after which the slides were closed. Bags were left in the soil for periods of 64–67 days in Mkwaja, and 69–75 days in Saadani, the differences in incubation time being randomly distributed over the tree density gradients. After incubation, resin bags were collected, dried, and gently cleaned by brushing. For extraction, resin bags were shaken for 2 h in 50 ml 1 M KCl solution. The extract was then analyzed colorimetrically for PO₄³⁻, NO₃⁻ and NH₄⁺ (AutoAnalyzer 3 HR, Seal Analytical). We then calculated the mean N and P release rates based upon the two to five bags per site that were recovered.

Acacia Tree Variables

We used the ¹⁵N natural abundance method to estimate rates of symbiotic N₂-fixation by *Acacia* trees, following the procedure as described in Cech and others (2010). At each site we collected leaves of several *Acacia* trees from various heights in the canopy in February 2009 and 2010. These were then dried, ground to a powder, and analyzed for C, N, P, and δ¹⁵N, using the same methods as for the soil samples.

We calculated % N derived from the atmosphere (Nd_{fa}) following Amarger and others (1979):

$$\text{Nd}_{\text{fa}} = \frac{\delta^{15}\text{N}_{\text{ref}} - \delta^{15}\text{N}_{\text{leg}}}{\delta^{15}\text{N}_{\text{ref}} - B}$$

where δ¹⁵N_{leg} is the ¹⁵N abundance in the N₂-fixing legume species, δ¹⁵N_{ref} is the ¹⁵N abundance in the non-fixing reference species growing on the same substrate, and *B* is the ¹⁵N abundance of a legume that obtains all its N from N₂-fixation. For the reference tree species we chose *Terminalia spinosa*, the most abundant non-fixing species in our area (though not present at all sites); we collected leaves from eight sites in Mkwaja and seven sites in Saadani. Foliar δ¹⁵N values of *Acacia* and *Terminalia* were significantly different (pairwise *t* test, *t* = 12.40, *df* = 14, *P* < 0.001), which is the essential basis for determining N₂-fixation by the natural abundance method (Boddey and others 2001). As there was no trend in the reference values of δ¹⁵N over either gradient, we used the mean value of 2.14 ± 0.33‰ to calculate Nd_{fa} for the *Acacia* trees. The parameter *B* was set to the lowest detected δ¹⁵N value (−3.35‰ in *Acacia*) following Hansen and Vinther (2001).

Understory Vegetation Variables

To estimate the biomass and production of herbaceous vegetation (predominantly grasses), we clipped the aboveground biomass in one or two squares of 50 × 50 cm placed randomly in each site; this was done on two occasions, once in the middle of the dry season (August 2009) and once at the end of the wet season (January 2010). We divided the material into three fractions—live grass, dead grass, and herbs—which were separately dried and weighed. Before clipping we estimated grass cover and average height in each square and determined the relationship between these variables and the grass biomass measured (Appendix B in Supplemental material). We used these relationships to estimate biomass at the site level, where grass cover and height were recorded in 20 randomly selected plots of 3 × 3 m. Grass production was calculated by subtracting the total biomass in August 2009 from the biomass in January 2010. Samples of live grass were ground and analyzed for total N and P concentrations by means of a continuous flow injection analyzer (AutoAnalyzer 3 HR, Seal Analytical) after Kjeldahl digestion. From the grass samples collected in 2010 we only analyzed samples from 16 sites.

In February 2009, we collected leaves of the most abundant leguminous and non-leguminous herbs or small shrubs (mainly re-sprouting leguminous

Dichrostachys cinerea) at each site. We collected leaves from 12 sites in the Mkwaja area and 20 sites in the Saadani area (one to three species per site). Leaves were dried, ground, and their foliar $\delta^{15}\text{N}$ values determined as for *Acacia*. The mean $\delta^{15}\text{N}$ values between the leguminous and non-leguminous herb species per site were significantly different (pairwise t test, $t = 7.14$, $P < 0.001$) (Appendix C in Supplemental material). With these mean $\delta^{15}\text{N}$ values, we calculated mean Ndfa for the leguminous herbs per site with parameter B set to the lowest detected $\delta^{15}\text{N}$ value (-3.09‰ in *Rhynchosia* sp.).

Statistical Analyses

We used linear regressions to analyze the effects of *Acacia* tree density on the measured soil, tree, and grass variables. No significant differences were detected using area as grouping variable, and we therefore repeated the analyses using the pooled data for both areas. If necessary, data were log-transformed to fulfill assumptions of normality and homogeneity of variance. Prior to the regression analyses the site in Mkwaja with no trees was removed, as the soil nutrient availabilities in this site were much higher, probably because it was located in a former paddock (compared to soil nutrient availabilities from paddocks in Cech and others 2010). We furthermore had no soil samples for another site in Mkwaja because it had recently burned and we had collected too little soil beneath *Acacia* tree canopies for organic and extractable P measurements in another site. Concerning the understory grass layer, we excluded three recently burned sites in the Mkwaja area in August 2009 because foliar nutrient concentrations were considerably higher in the fresh regrowth. Paired t tests were used to test for differences in soil bulk density, nutrient concentrations and ratios beneath and outside *Acacia* tree canopies per site. All analyses were performed with the open source R (R Development Core Team 2011).

RESULTS

Soil C, N, and P Along the Tree Density Gradient

As predicted, total C and N pools and N release rates in the topsoil increased with increasing tree density, though the increases in C and N pools were

only significant outside the canopy (Figure 1A, B, G; Table 1). In addition, the soil organic P pool (top 15 cm) also increased significantly, from about 6 g P m^{-2} in sites with no trees to 16 g P m^{-2} at sites with 2000 trees ha^{-1} (values derived from the linear regression; Figure 1D; Table 1). However, there was no change in the total P pool (Figure 1C; Table 1), with the consequence that the organic fraction of total P increased from 30% in sites with no trees to 70% at a tree density of 2000 trees ha^{-1} . Neither the extractable P pool—accounting for about 9% of total P—nor P release rates were related to tree density (Figure 1E, F; Table 1). Total soil N:P ratios also showed no trends, though the ratio of N:P release rates (N:P adsorption to resin) increased significantly with tree density (Figure 1J, K; Table 1). Concentrations of C, N and organic P were significantly higher in soil samples taken from beneath the *Acacia* canopy than in paired samples taken outside the canopy, whereas extractable P tended to be lower (Table 2).

Soil $\delta^{13}\text{C}$ decreased significantly with increasing tree density from approximately -16 to -20‰ (indicating more C derived from C_3 plants), whereas soil $\delta^{15}\text{N}$ remained constant, both beneath and outside the canopy (Figure 1H, I; Table 1). Mean values of soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were significantly lower in samples from beneath the canopy (Table 2).

Nutrient Concentrations in Trees and Understory Grasses Along the Tree Density Gradient

Tree foliar N, P, and N:P ratios showed no trends with increasing tree density (Figure 2A–C), but leaf N:P ratios in understory grasses increased from about 11 to about 17 (Figure 2F; dry season: $P = 0.03$, $R^2 = 0.12$; wet season: $P < 0.001$, $R^2 = 0.68$), due to increasing N (Figure 2D; dry season: trend at $P = 0.09$; wet season: $P = 0.02$, $R^2 = 0.34$) but constant P concentrations (Figure 2E). Grass biomass and productivity remained rather constant along the tree density gradient (Appendix D in Supplemental material).

Foliar $\delta^{15}\text{N}$ values and Ndfa of the *Acacia* trees did not vary significantly along the tree density gradient (Figure 3A, B). However, we did find significant trends for leguminous herbs; values of $\delta^{15}\text{N}$ increased with tree density ($P = 0.046$, $R^2 = 0.13$) whereas those of Ndfa decreased ($P = 0.02$, $R^2 = 0.16$) (Figure 3C, D).

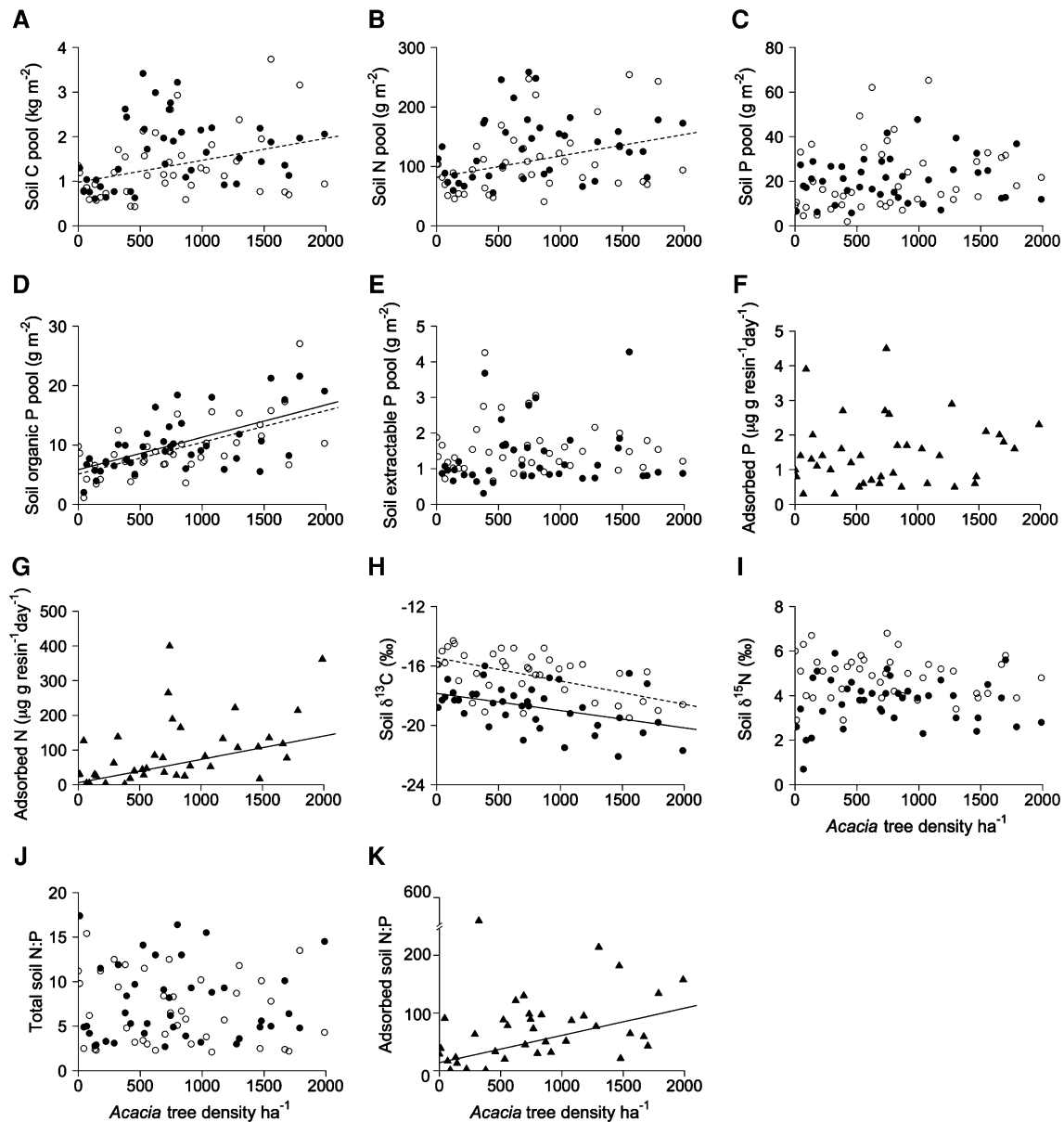


Figure 1. Soil C, N, and P pools and fluxes in the top 15 cm soil beneath and outside *Acacia* tree canopies along a density of *Acacia* trees (solid points and lines: beneath canopy; open points and dashed lines: outside canopy). Soil C (A), N (B), total P (C), organic P (D), and extractable P (E) were measured in these two canopy types together with soil δ¹³C (H) and soil δ¹⁵N (I). Total soil N:P ratios (J) were determined using the total soil pools of these elements. Adsorption rates of inorganic N (G) and inorganic P (F) to ion-exchange resins were measured and adsorbed soil N:P (K) was determined using these rates; note the use of a different symbol as resin bags were randomly placed in each site and not specifically beneath or outside *Acacia* tree canopies. Linear regression lines were drawn where significant (see Table 1 for significance levels and R² values).

DISCUSSION

Soil C and N Pools Along the Tree Density Gradient

Increasing density of *A. zanzibarica* was associated with larger soil C and N pools, as has been shown for individual trees in other *Acacia* savannas (Belsky

and others 1993; Ludwig and others 2004; Hagos and Smit 2005) (Figure 1A, B, G; Table 1). There is little doubt that these increases in C and N pools were caused by the *Acacia* trees, rather than that the abundance of trees was caused by differences in soil nutrients. First, the topsoil beneath the tree canopies was similarly enriched in total C and N in

Table 1. Statistical Results of Linear Regressions Between *Acacia* Tree Density and Soil Variables Beneath and Outside *Acacia* Tree Canopies

Soil variables	Beneath canopy			Outside canopy		
	<i>n</i>	<i>R</i> ²	Direction	<i>n</i>	<i>R</i> ²	Direction
Bulk density (g cm ⁻³)	40	n.s.		41	n.s.	
Total C (kg m ⁻²)	40	n.s.		41	0.12*	+
Total N (g m ⁻²)	40	n.s.		41	0.13*	+
Total P (g m ⁻²)	40	n.s.		41	n.s.	
Organic P (g m ⁻²)	39	0.36***	+	41	0.39***	+
Extractable P (g m ⁻²)	39	n.s.		41	n.s.	
δ ¹³ C (‰)	40	0.18**	-	41	0.31***	-
δ ¹⁵ N (‰)	40	n.s.		41	n.s.	
Total N:P	40	n.s.		41	n.s.	
			Random location			
			<i>n</i>	<i>R</i> ²		Direction
Adsorbed N (μg g resin ⁻¹ day ⁻¹)			38	0.29***		+
Adsorbed P (μg g resin ⁻¹ day ⁻¹)			39	n.s.		
Adsorbed N:P			37	0.18**		+

*For adsorbed N, P and N:P ratios regressions were not performed separately per canopy type as resin bags were randomly placed in each site. Asterisks indicate significant regressions over Acacia tree density, with * (P < 0.05), ** (P < 0.01) and *** (P < 0.001). n.s. indicates non-significant trends.*

Table 2. Nutrient Concentrations and Ratios in Topsoil Beneath and Outside *Acacia* Tree Canopies

	Beneath canopy		Outside canopy
Bulk density (g cm ⁻³)	1.40 ± 0.03	***	1.48 ± 0.04
Total C (mg g ⁻¹)	12.0 ± 0.9	**	9.3 ± 0.8
Total N (mg g ⁻¹)	0.96 ± 0.07	***	0.75 ± 0.06
Total P (mg g ⁻¹)	0.15 ± 0.01		0.14 ± 0.01
Organic P (μg g ⁻¹)	74 ± 6	**	63 ± 5
Extractable P (μg g ⁻¹)	9.7 ± 1.1	†	11.5 ± 1.3
δ ¹³ C (‰)	-18.7 ± 0.2	***	-16.6 ± 0.2
δ ¹⁵ N (‰)	3.7 ± 0.2	***	4.9 ± 0.1
Total N:P	7.3 ± 0.8		7.5 ± 0.7

*For each soil variable, paired t-tests were performed with variables paired per site. Asterisks indicate significant differences between canopy types, with † (0.05 < P < 0.10), * (P < 0.05), ** (P < 0.01) and *** (P < 0.001).*

all sites (and along both gradients), which is what we would expect given that all sites were probably of similar age (studies have shown that nutrient enrichment increases with time; Ludwig and others 2004; Boutton and Liao 2010; Blaser and others, unpublished manuscript) (Figure 1A, B; Tables 1; 2). On the other hand, we did find a significant positive relationship between soil C and N and tree density outside the canopy, reflecting the fact that the distance to the nearest tree declines as density increases. Second, in addition to the general increase in C along the density gradient, the soil δ¹³C values indicate an increasing proportion of soil C originating from the trees (Figure 1H) (Rao and

others 1994; Boutton and others 1998). Binkley (2005) has suggested that N enrichment by N₂-fixing trees may cause changes in the soil microbial community that lead to slower turnover of organic matter; this mechanism could account for the accumulation of C (and therefore of organic P) along the tree density gradient.

There is also one puzzling feature of the data: although the foliar δ¹⁵N data indicate that the *A. zanzibarica* trees in our sites were fixing N (Odee and others 1995; Cech 2008; Figure 3A, B), we recorded no decrease in soil δ¹⁵N with increasing tree density, although this might have been expected (Figure 1I; Boutton and Liao 2010).

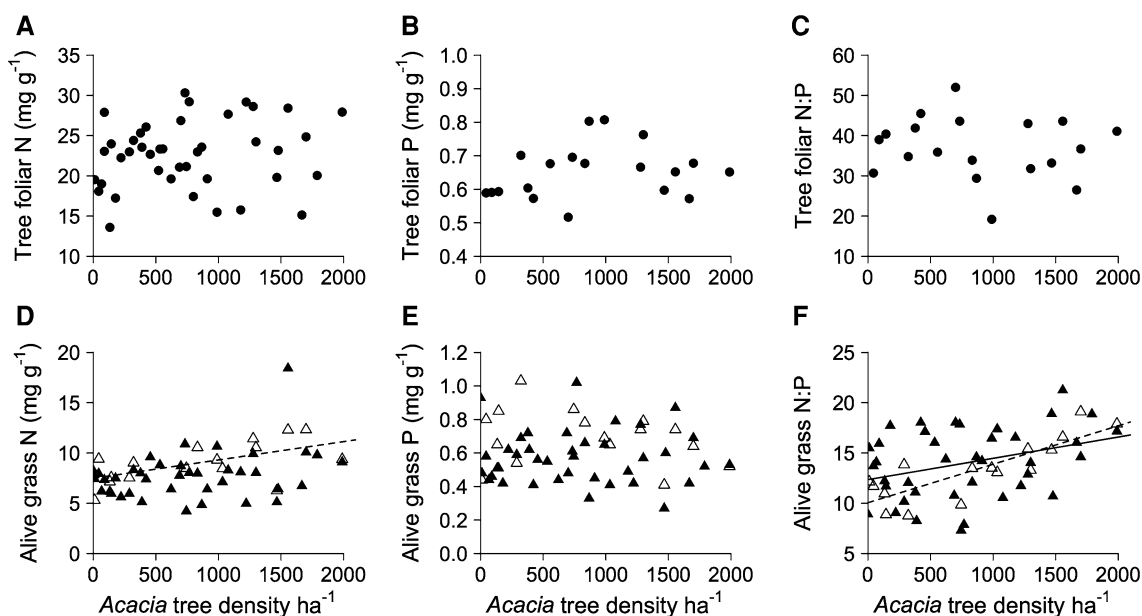


Figure 2. *Acacia* tree and understory grass foliar N (**A**, **D**) and P concentrations (**B**, **E**) together with their N:P ratios (**C**, **F**), along a density of *Acacia* trees. For the understory grasses *solid triangles* and *lines* represent measurements in the middle of the dry season (August 2009) and *open triangles* and *dashed lines* represent measurements at the end of the wet season (January 2010). Linear regression lines were drawn where significant (see text for significance levels and R^2 values).

Possibly, other ^{15}N enrichment processes balanced this effect; for example, ^{15}N enrichment of the N pool could occur through N pumping from deeper soil layers or through transformation processes as litter decomposition (Hobbie and Quimette 2009; Boutton and Liao 2010).

Soil P Pools and C:N:P Stoichiometry Along the Tree Density Gradient

An important finding was the strong increase in the pool of organic P along the tree density gradient (Figure 1D; Table 1). This is in contrast to previous work with *Acacia* spp., which has not revealed any increase in P (in any form) with increasing tree density (up to c. 2000 trees ha^{-1} ; Riginos and others 2009) or age (from 6 to 20 years; Pearson and Vitousek 2001). However, a recent study of encroachment by the leguminous shrub *Dichrostachys cinerea* in Zambia showed increases in total soil P and soil extractable P with increasing shrub density (0–100% canopy cover) and age (from 5 to 35 years; Blaser and others, unpublished manuscript).

We found no evidence that the understory vegetation profited from the increasing pool of organic P along the density gradient. Indeed, the N:P ratio in grass biomass (Figure 2F) closely followed that of soil N:P release rates, which is a better reflection of N:P availabilities than the ratio of total N:P pools

(Figure 1J, K). Following the N:P criterion for savanna vegetation of Cech and others (2008), this would suggest that the herbaceous understory layer was either co-limited by N + P or P-limited, with P-limitation becoming increasingly important as tree density increased. This result is consistent with field fertilization experiments in the same savanna, which show frequent N + P co-limitation as well as a shift induced by *Acacia* trees towards P-limitation (Cech and others 2008). Furthermore, foliar $\delta^{15}\text{N}$ in leguminous herbs increased, and their Ndfa (although of marginal importance compared to the trees) decreased with increasing tree density, which could have been a consequence of increasing shortage of P for N_2 -fixation (Figure 3C, D).

In contrast to the grasses, *A. zanzibarica* showed no sign of being P-limited at high tree densities. Indeed, the fact that trees maintained stable foliar N:P, $\delta^{15}\text{N}$, and Ndfa along the density gradient (Figures 2C; 3A, B) indicates that they were able to obtain the P needed for an increasing biomass by utilizing some source or sources not available to grasses. The root system of a *A. zanzibarica* tree includes both superficial roots radiating out at least 10 m from the trunk, and a tap root and sinkers that extend down at least 4 m, and probably much more (Cochard 2004). One exclusive source of P for the trees, therefore, is likely to be the deeper soil

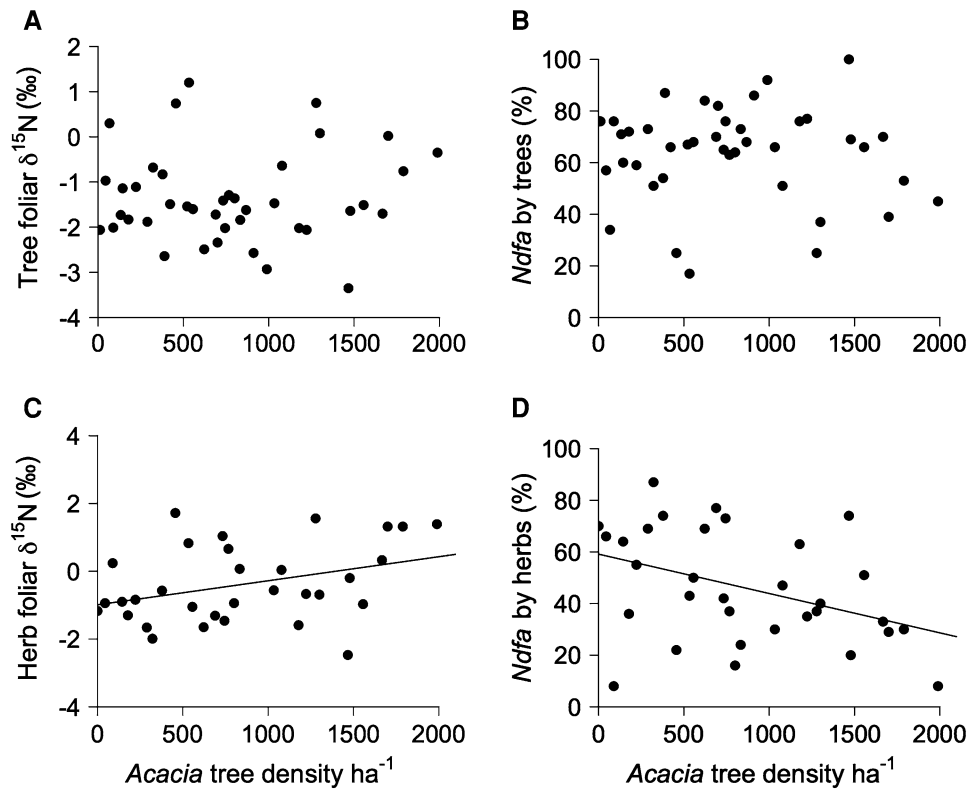


Figure 3. *Acacia* tree and leguminous herb foliar $\delta^{15}\text{N}$ (**A**, **C**) and % N derived from atmosphere (Ndfa) (**B**, **D**) along a density of *Acacia* trees. Linear regression lines were drawn where significant (see text for significance levels and R^2 values).

layers. This would be consistent with our finding that extractable P pools were not depleted in the topsoil beyond the canopy (Figure 1E; Tables 1, 2), and that organic P increased both beneath and outside the canopy (Figure 1D; Table 1). Studies conducted in a variety of ecosystems have shown that deep-rooting plants can acquire a substantial proportion of their nutrients, and specifically P, from deeper soil layers (Marsh and others 2000; Jackson and others 2002; McCulley and others 2004; Göransson and others 2007). Indeed, when woody species invade previously nutrient-poor grasslands this process may cause significant P enrichment of topsoil, as for *Crataegus monogyna* invading calcareous grasslands in Britain (Grubb and Key 1975). In the case of savanna trees, however, the increase in P may be related not only to the deep root-systems of trees, but also to their high transpiration rates; by drying out the surface soil (Scholes and Archer 1997; Oliveira and others 2005; Cochard and Edwards 2011), trees may promote capillary movement of water from deeper soil, and thereby the upward movement of dissolved nutrients.

A second P source that trees may utilize more effectively than grasses is the soil organic fraction. Studies in other ecosystems have shown that N_2 -fixing plants maintain a higher root phosphatase activity than grasses, thereby facilitating the uptake

of organic P forms (Houlton and others 2008). And for other species of *Acacia* mycorrhizal fungi are also important in enabling trees to utilize organic P (for example, Duponnois and others 2005).

These two potential sources of P for *A. zanzibarica* trees are not mutually exclusive, though further research is needed to confirm that they are, indeed, important. Taking up P from deeper soil layers may be the way that trees increase the total pool of nutrient that is actively cycled. More generally, this is also one mechanism by which trees accumulate nutrients beneath their canopy, producing the 'island of fertility' phenomenon that is known from other savanna ecosystems (Scholes and Archer 1997; Ludwig and others 2004). However, trees may also benefit by being more efficient than grasses in utilizing the organic P that is returned to the environment in dying roots and leaf litter. Indeed, our results show that the P supply for grasses is impaired as the organic fraction increases, whereas that of *A. zanzibarica* remains the same or even improves.

The relatively favorable P-status across the density gradient, at least for trees, is no doubt related to the fact that the soils in our study area were relatively young and derived from P-containing coral (Cochard and Edwards 2011). In many tropical regions, the P supply decreases as it becomes immobilized in occluded forms and is lost through

erosion and leaching (Walker and Syers 1976; Crews and others 1995). For example, along a substrate-age gradient in Hawaii where sites ranged from 300 years to more than 4 million years old, soil P was significantly reduced in the older sites, which resulted in a negative feedback upon N₂-fixation rates by *Acacia* trees (Pearson and Vitousek 2002). Further studies are needed to understand the time-scales over which such feedbacks become effective, and how they might be influenced by soil and environmental conditions.

Conclusions and Implications

Various authors have suggested that trees will become increasingly abundant in savanna ecosystems in the future through changes in land use and environmental conditions (Scholes and Archer 1997; Bond and others 2003; Van Auken 2009). This study shows that a negative feedback through soil P depletion on N₂-fixation and density of leguminous trees in savanna does not prevent an open savanna from developing into dense woodland (c. 2000 trees ha⁻¹), at least during the first 50 years of encroachment. Trees in our study area were able to acquire sufficient P for growth, even at high densities, probably because they exploited P originating from deeper soil layers. Furthermore, considerable amounts of C, N, and P were sequestered in the soil during woody encroachment. Because the main constraint on C accumulation is thought to be N-limitation (Hungate and others 2003), the development of *Acacia* woodland in humid savannas offers a potentially important sink for C.

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