

Acacia tree density strongly affects N and P fluxes in savanna

Judith Sitters · Peter J. Edwards ·
Werner Suter · Harry Olde Venterink

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Abstract Studies of nutrient cycling in savanna ecosystems rarely consider how fluxes are affected by local variations in tree density and nutrient redistribution by herbivores. We studied how the density of *Acacia zanzibarica* trees in a humid savanna ecosystem affected the input of nitrogen (N) through N₂-fixation and N and phosphorus (P) outputs through fire and also internal pathways of N and P return through herbivores. We found that N inputs and P outputs both increased with increasing density of N₂-fixing trees, the N effect being due to tree density rather than to differences in the rate of N₂-fixation. However, total N outputs due to fire did not vary with tree density because losses from the herb layer decreased as losses

from the tree layer increased. In contrast, total P outputs did increase with tree density because P losses from the tree layer exceeded those from the herb layer. We suggest that variation in the density of N₂-fixing trees coupled with the effects of fire can cause substantial differences in the local N and P balances in savanna vegetation. To some extent, these differences may be evened out by the tendency for browsing herbivores to transfer nutrients from *Acacia*- to grass-dominated areas. We conclude that encroachment by N₂-fixing trees and shrubs has important consequences for ecosystem properties such as N and P dynamics.

Keywords Fire · Herbivore dung · Nitrogen fixation · Nutrient balances · Phosphorus · Woody encroachment

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J. Sitters (✉) · P. J. Edwards · H. Olde Venterink
Institute of Integrative Biology, ETH Zurich,
Universitätsstrasse 16, 8092 Zurich, Switzerland
e-mail: Judith.Sitters@vub.ac.be

Present Address:

J. Sitters · H. Olde Venterink
Plant Biology and Nature Management, Vrije Universiteit
Brussel, 1050 Brussels, Belgium

W. Suter
Swiss Federal Research Institute WSL,
Zürcherstrasse 111, 8903 Birmensdorf, Switzerland

Introduction

The woody cover of many savanna ecosystems has increased in recent decades. This apparently global phenomenon has been attributed to a combination of factors including climate change and changes in livestock grazing regimes (Scholes and Archer 1997; Bond et al. 2003; Van Auken 2009). Woody encroachment is environmentally important because an increase in tree density may have a large effect upon ecosystem processes such as soil and plant nutrient dynamics (Bond 2008). Indeed, recent studies have found large changes in soil carbon (C), nitrogen

(N) and phosphorus (P) pools and availabilities along tree density gradients, with consequences for plant nutrient stoichiometry and type of limitation (Blaser et al. 2014; Sitters et al. 2013).

Trees can affect the nutrient dynamics of savanna ecosystems by altering the inputs and outputs of particular nutrients. Because N₂-fixing trees are common in many tropical savannas (Schulze et al. 1991), biological N₂-fixation is often the largest source of N in these ecosystems (Cleveland et al. 1999). On the other hand, the largest losses of N are often due to burning (van der Werf et al. 2006; Lioussé et al. 2010), especially in wet savannas (Delon et al. 2012) that produce more biomass than arid savannas to fuel the fires. Hence, sites with a high cover of N₂-fixing trees are likely to show a net gain of N, while treeless savanna sites, especially those with a high grass biomass, may lose N through fire (Kauffman et al. 1994; Geesing et al. 2000; Cech et al. 2010a). So far, studies of nutrient balances in savanna ecosystems been performed in either grassland or woodland sites, and most have been performed in treeless savannas (Sanhueza and Crutzen 1998; Abbadie 2006; López-Hernández et al. 2006), and none has examined the effects of varying tree density. Furthermore, most studies have focused upon N, and much less is known about the P dynamics of savanna ecosystems (but see Cech et al. 2010a).

Another important component of savanna ecosystems are large mammalian herbivores, which by feeding and excreting in different places may bring about a spatial redistribution of nutrients (Augustine et al. 2003; Abbas et al. 2012). For example, herbivores that feed on tree foliage might deposit dung in open areas and hence transport nutrients from woodland to grassland sites. Indeed, it has been observed that browsing herbivores (especially giraffe) often deposit dung in sites with lower tree density (Riginos and Grace 2008; Sitters et al. 2014). However, few studies have investigated the magnitude of this spatial transfer by savanna herbivores and its consequences for local N and P dynamics (but see Augustine et al. 2003; Wolf et al. 2013).

In an earlier paper we showed that soil N and P pools in a humid Tanzanian savanna increased strongly with increasing density of N₂-fixing *Acacia zanzibarica* trees and that N₂-fixation rates of trees and leguminous herbs showed contrasting responses to density (Sitters et al. 2013). Here, we investigate at the site level how tree density affects N inputs due to N₂-

fixation and N and P outputs due to fire. A full list of our working hypotheses is presented in **Box 1**. We also consider the varying importance of plant litter and herbivore dung as pathways for nutrient return along a tree density gradient. We predict increasing returns of N and P in leaf litter and decreasing returns in dung of browsers, at least in areas dominated by giraffe.

Methods

Study area

The study was conducted in Saadani National Park, which is located on the coast of Tanzania (5°43'S, 38°47'E). The northern Mkwaja area (~470 km²) was used as a cattle ranch from 1954 to 2002, and while operational supported up to 13,000 cows. By the 1960's the ranch experienced heavy bush encroachment, especially of the leguminous tree *A. zanzibarica* (S. Moore) Taub. var. *zanzibarica* (Tobler et al. 2003). Much of the southern Saadani area (~210 km²) was used for plantation agriculture during the first half of the twentieth century, and from 1969 was managed as a game reserve. In 2002, the two areas were united to form the Saadani National Park.

Annual rainfall in the area varies considerably among years, ranging from 610 to 1,700 mm between 1957 and 1998, and averaging 900 mm (Tobler et al. 2003). Most rain falls during the wet seasons from March until June and from mid-October until mid-November. Mean annual temperature is 25 °C. Soils in the study area are mainly grey vertisols derived from coral sands, relatively uniform and unstructured up to a depth of more than 1.5 m, but ranging from sand to clay (Cochard and Edwards 2011a).

Due to their differing management histories, the density and diversity of herbivores is much lower in Mkwaja than Saadani (Treydte et al. 2005). Annual precipitation is sufficient to allow for a high percentage of woody cover (Sankaran et al. 2005), and the fact that vegetation is now mainly savanna probably reflects a long history of deliberate burning in the dry months (cf., Bond et al. 2005). We studied tall-grass savanna vegetation invaded to different degrees by *A. zanzibarica* trees. Cech (2008) observed nodulation of the roots of this species and their foliar δ¹⁵N values also suggest N₂-fixation (Online Resource 1). Dominant grass and sedge species in this vegetation

are *Heteropogon contortus*, *Panicum infestum*, *Schoenefeldia transiens*, *Sporobolus pyramidalis* and *Abildgaardia triflora*.

We assessed annual N and P fluxes in 21 sites in Mkwaja and 22 sites in Saadani, selected to provide gradients in both areas in the density of *Acacia* trees (Sitters et al. 2013). The sites were 30 m × 30 m and tree densities ranged from 0 to 2,000 trees ha⁻¹. In Mkwaja we avoided areas where cattle were kept overnight during the ranch time (i.e., the paddocks), as these patches are atypical in their soil nutrients and vegetation composition (Cech et al. 2008). Soils in both the Saadani and Mkwaja area show similar nutrient patterns (total and extractable N and P) with increasing *Acacia* tree density with no significant differences between the areas (Sitters et al. 2013).

N input through symbiotic N₂-fixation by *Acacia* trees and leguminous herbs

In a previous study (Sitters et al. 2013), we determined foliar N concentrations and δ¹⁵N values of individual *A. zanzibarica* trees and leguminous herbs in each of our sites (using the ¹⁵N natural abundance method to estimate % N derived from the atmosphere (*Ndfa*); see Online Resource 1 for further details on the method). Here we take a step further and estimate the total input flux of N₂-fixation by *Acacia* trees and leguminous herbs at the site level through the addition of data on annual biomass production.

At the end of July 2009 we measured diameters of 15 *Acacia* trees per site at a height of 10 cm. Additionally, we visually estimated the degree of branching (ranging from a tree with almost no branching to a tree with ample crown and a high level of branching) and overall foliage cover for each tree using four categories per parameter. We calculated foliage biomass at the site level using allometric equations derived for this species in the same area (Cochard and Edwards 2011a; Table A1 in Online Resource 1). Because *Acacia* trees tend to lose most of their foliage during the dry season (Cochard and Edwards 2011a), we assumed that the net annual production of leaves at the site level corresponded to the foliage biomass after the long wet season (from March to June). In August 2010, we collected, dried and weighed all aboveground biomass of leguminous herbs in a randomly placed 10 m × 10 m plot at each site (Table A3). This biomass was used as a proxy for the annual production of these herbs at the site level.

N and P outputs through fire from herbaceous and *Acacia* tree biomass

To estimate N and P outputs through fire from both herbaceous and *Acacia* tree biomass we first estimated the biomass available as fuel at the site level. For herbaceous biomass, we clipped aboveground biomass per site in one or two 50 × 50 cm squares in July 2009, a month when fires are particularly frequent. The harvested material was dried and weighed, and subsamples were ground for chemical analysis. We determined total N and P concentrations after Kjeldahl digestion using a continuous flow injection analyser (AutoAnalyzer 3 HR, Seal Analytical) (Table B1 in Online Resource 2). We used the measurements made in July 2009 (see *symbiotic N₂-fixation* above) to estimate foliage and branch biomass of *Acacia* trees at the site level up to the burning height of 2 m (personal observation; also see Abbadie 2006). We collected leaves of several *Acacia* trees in both areas, which we dried and ground for N and P analyses (Table B1).

We then visited recently burned sites with different tree densities and visually estimated the proportions of herbaceous and tree biomass damaged by fire at the site level by comparing burned versus unburned herbaceous plots and trees within the same site. For this, we chose 15 burned sites of 30 m × 30 m ranging from 0 to 2,800 trees ha⁻¹ and estimated proportions of burned herbaceous biomass and *Acacia* trees damaged by fire. For further details on these estimations see Online Resource 2.

To determine how much N and P was lost through fire we performed several burning experiments with herbaceous and tree biomass. We obtained the material for these, by clipping herbaceous biomass in six 50 × 50 cm squares and collecting *Acacia* leaves (c. 150 g wet weight each) from several trees at five unburned locations. Subsamples were taken from both herbaceous biomass and tree leaves for nutrient analyses of the pre-burning reference material. The remaining material was weighed before burning, and the ash carefully collected and weighed. We also collected *Acacia* branches that we burned for periods of 1 or 3 min to simulate differing exposures to a moving fire (Galang et al. 2010). Both the pre-burning reference material and the ash samples were analysed for N and P concentrations (Online Resource 2). We calculated total N and P outputs at the site level by combining our estimates on proportions of herbaceous

and *Acacia* tree biomass burned with their N and P losses (Table B1, B2). For further details on the method see Online Resource 2.

N and P returns through deposition of herbivore dung and urine

In a previous study (Sitters et al. 2014), we estimated the annual input of herbivore dung at each site in our study area (also see Online Resource 3 for further details on the method). Using these data we now also estimated urine deposition at each site for each herbivore species, using a method that was developed by Frank et al. (1994) and has been applied in other savanna studies (Augustine et al. 2003; Fornara and Du Toit 2008) (Table C1 in Online Resource 3). To distinguish between flows of N and P originating from grass and from the tree layer, we separately estimated dung/urine deposition by grazing and browsing herbivores.

N and P returns through deposition of *Acacia* tree litter

We placed two litter traps (cotton cloth; 50 × 50 cm²) in nine sites in Mkwaja and ten in Saadani, evenly spaced over the range of tree densities. These litter traps were placed under the canopy of *Acacia* trees, thereby trapping all falling leaves and twigs from these trees. Trees lose most of their leaves during the dry seasons (Cochard and Edwards 2011a), and traps were therefore set out from mid-December 2009 until the end of February 2010 and again from end of July 2010 until beginning of October 2010. During each period the litter was collected on two occasions. We took fish-eye lens photographs directly above the litter traps at the end of the wet season (December 2009) to relate canopy cover to litter deposition rates at the level of the individual tree (Online Resource 4). We also took photographs at the four corners of each site to estimate mean canopy cover per site and hence calculate annual litter deposition rates at the site level. Litter was dried, sieved to remove soil particles, and weighed. Samples were analysed for total N and P concentrations by means of a continuous flow injection analyser (Auto-Analyzer 3 HR, Seal Analytical) after Kjeldahl digestion (Online Resource 4). For further details on the method see Online Resource 4.

Statistical analyses

We used linear, log-linear and quadratic (i.e., 2nd order polynomial) regression models to analyse the effects of *Acacia* tree density on the estimated N and P fluxes, with separate regressions fitted for both areas (see Box 1 for the hypotheses associated with each regression model). We also performed regressions using the pooled data for both areas when no significant difference was detected using area as grouping variable. All regressions were performed using the estimated N and P inputs and outputs at the site level and are also discussed at this level. We used Akaike's information criterion (AIC) for model selection and a model was considered the best model and hence chosen for presentation in the figures when it had the lowest AIC value (Burnham and Anderson 2002) (see Tables 1, 3 for statistical summaries). To analyse the effect of tree density on the relative importance of plant litter and herbivore dung as pathways for nutrient return we used binomial regression models as we had data on proportions. Concerning N input by leguminous herbs, we missed data on biomass production in two sites in the Mkwaja and Saadani area as they were recently burned (Table A3 in Online Resource 1). To predict N input in these sites, we used the linear regression equation for biomass production (Table A4). Prior to the regression analyses the site in Mkwaja with no trees was excluded, as the soil nutrient availabilities in this site were much higher, probably because it was located in a former paddock (values comparable to soil nutrient availabilities from paddocks in Cech et al. 2008). In addition, we used one-way analyses of variance (ANOVA) followed by Tukey–Kramer HSD tests to test for differences in N and P volatilization losses through experimental burning for the different types of reference material (i.e., herbaceous, *Acacia* foliar or branch biomass). All analyses were performed with the open source statistical software R (R Development Core Team 2011).

Results

N input through symbiotic N₂-fixation by *Acacia* trees and leguminous herbs

Annual N input from N₂-fixation by *Acacia* trees increased with tree density from zero in the absence of

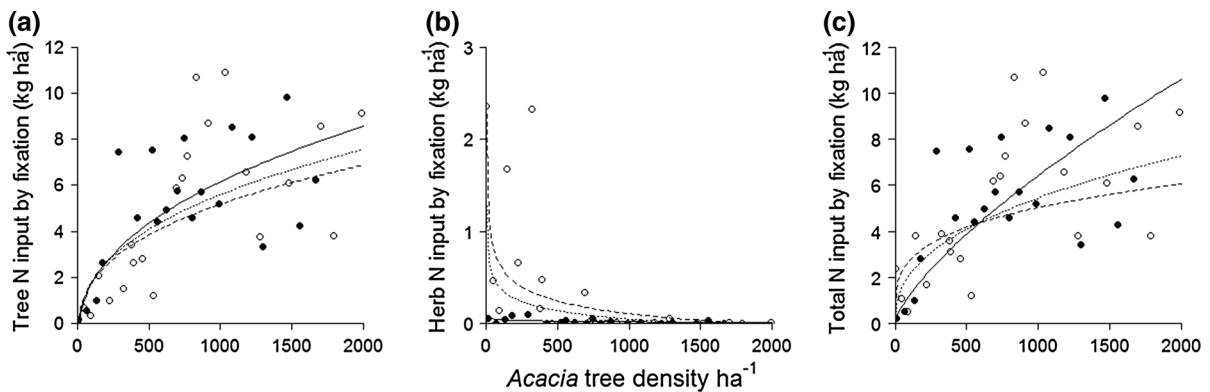


Fig. 1 Estimated annual N input fluxes through symbiotic fixation by *Acacia* trees (a) and leguminous herbs (b) along a density of *Acacia* trees in two savanna areas (solid points and lines Mkwaja area; open points and dashed lines Saadani area). Total annual input (c) was calculated by adding the estimated N

inputs through fixation for both *Acacia* trees (a) and leguminous herbs (b). Log-linear regression lines were drawn depending on best fit based on AIC values (see Table 1), and dotted lines represent both areas when observations were pooled

trees to 6 or 8 kg N ha⁻¹ year⁻¹ at a density of 2,000 trees ha⁻¹ for Mkwaja and Saadani respectively (Fig. 1a; Table 1). These increases were caused primarily by increasing leaf production per unit area, as foliar N, $\delta^{15}\text{N}$ and *Ndfa* showed no significant trends with tree density (Sitters et al. 2013) (in accordance with hypothesis A1; Table A4 in Online Resource 1). In contrast, annual N input from leguminous herbs decreased exponentially with increasing tree density (Fig. 1b; Table 1), partly as a consequence of declining biomass production but also because *Ndfa* values were lower at high tree density (Sitters et al. 2013) (in accordance with hypothesis B2; Table A4). Since the N input from leguminous herbs was low compared to that from *Acacia* trees, however, the net effect was a log-linear increase in total N input from symbiotic N₂-fixation with increasing tree density (Fig. 1c; Table 1).

N and P outputs through fire from herbaceous biomass and *Acacia* biomass

In the burning experiments, we found significant differences in mean volatilization losses of N and P from herbaceous and *Acacia* biomass (Table 2). More N than P was lost when foliage was burned (both herbs and trees), whereas the losses of these nutrients from burning branches were comparable.

At the site level, the losses of N from burning herbaceous biomass decreased with tree density (Fig. 2a;

Table 1), while those from tree biomass increased (log-linearly) (in accordance with hypothesis D2; Fig. 2b; Table 1), resulting in a total N output that showed no significant trend with density in either area (Fig. 2c; Table 1). The decrease in N output from herbaceous biomass was only significant in the Mkwaja area (Table 1), partly because N concentrations at this site did not increase with density as they did in Saadani, and therefore did not compensate for the declining proportion of herbaceous biomass burned (in accordance with hypotheses C1 and C2; Table B1 in Online Resource 2).

In general, trends in P output due to burning paralleled those for N (Fig. 2d, e; Table 1), though in the Mkwaja area total P output showed a log-linear increase with tree density (Fig. 2f; Table 1). The decrease in P output through the burning of herbaceous biomass was only significant in the Saadani area (Fig. 2d; Table 1), perhaps because of greater variation in herbaceous biomass and P concentrations in Mkwaja (Fig. B2; Table B1).

Internal pathways of N and P returns from *Acacia* biomass

Annual N and P returns through browser dung and urine deposition (of which ca. 50 % originated from giraffe and ca. 40 % from bushbuck) declined with increasing *Acacia* tree density in the Saadani area, while site-level returns of N and P in *Acacia* litter

Table 1 Statistical summary of several regression models examining the relationship between the main annual N and P input and output fluxes along a density of *Acacia* trees in two savanna areas

	Mkwaja area			Saadani area			Both areas		
	Regression	R ²	AIC	Regression	R ²	AIC	Regression	R ²	AIC
Nitrogen									
Inputs									
N ₂ -fixation by trees	<u>Log-linear</u>	0.71***	17.1	<u>Log-linear</u>	0.64***	31.5	<u>Log-linear</u>	0.66***	45.7
	Quadratic	0.51**	89.0	Quadratic	0.62***	102.6	Quadratic	0.56***	186.9
N ₂ -fixation by herbs	Linear	0.23*	-85.4	Linear	0.29*	46.5	Linear	0.16**	68.3
	<u>Log-linear</u>	0.21*	-87.4	<u>Log-linear</u>	0.51***	10.7	<u>Log-linear</u>	0.34***	6.9
Total input by N ₂ -fixation	<u>Log-linear</u>	0.79***	31.1	<u>Log-linear</u>	0.31**	49.9	<u>Log-linear</u>	0.46***	88.8
	Quadratic	0.51**	89.0	Quadratic	0.51**	103.9	Quadratic	0.50***	186.8
Outputs									
Fire output herb layer	Linear	0.20†	-	Linear	n.s.	-	<u>Linear</u>	0.14*	-
Fire output tree layer	Linear	0.49***	97.5	Linear	n.s.	116.5	Linear	0.24***	213.4
	<u>Log-linear</u>	0.76***	13.6	<u>Log-linear</u>	0.54***	27.5	<u>Log-linear</u>	0.61***	41.7
	Quadratic	0.50**	99.2	Quadratic	0.26†	114.4	Quadratic	0.34***	209.3
Total output by fire	All	n.s.	-	All	n.s.	-	All	n.s.	-
Phosphorus									
Outputs									
Fire output herb layer	<u>Linear</u>	n.s.	-	<u>Linear</u>	0.27*	-	<u>Linear</u>	0.15*	-
Fire output tree layer	Linear	0.51***	-34.5	Linear	n.s.	-32.3	Linear	0.25***	-70.8
	<u>Log-linear</u>	0.53***	-45.3	<u>Log-linear</u>	0.28*	-43.8	<u>Log-linear</u>	0.36***	-90.8
	Quadratic	0.52**	-34.5	Quadratic	0.26†	-32.3	Quadratic	0.35***	-70.8
Total output by fire	Linear	0.32**	-28.8	Linear	n.s.	-	Linear	0.10*	-58.5
	<u>Log-linear</u>	0.30*	-41.0	<u>Log-linear</u>	n.s.	-	<u>Log-linear</u>	0.19**	-90.2
	Quadratic	0.32*	-26.8	Quadratic	n.s.	-	Quadratic	0.19*	-60.8

R²-values, significance levels and AIC values are shown for linear, log-linear and quadratic regression models

Underlined models had lowest AIC values and were therefore used in Figs. 1 and 2

n.s. non-significant trends

Asterisks indicate significance levels, with † (0.05 < P < 0.10), * (P < 0.05), ** (P < 0.01) and *** (P < 0.001)

Table 2 Volatilization losses of N and P through experimental burning of herbaceous and *Acacia* tree biomass to ash

	Herbaceous biomass n = 6	<i>Acacia</i> foliar biomass n = 6	<i>Acacia</i> branch biomass 1 min n = 7	<i>Acacia</i> branch biomass 3 min n = 7
Nitrogen (%)	92.5 ± 1.7 ^a	69.0 ± 6.2 ^a	24.4 ± 8.6 ^a	44.7 ± 6.3 ^a
Phosphorus (%)	25.2 ± 9.2 ^b	20.9 ± 3.9 ^b	16.0 ± 6.3 ^a	47.9 ± 7.7 ^a

Branches were burned either 1 or 3 min

Significant differences in N and P losses for the same type of reference material are indicated by different letters (*t* test, P < 0.05)

showed a log-linear increase with tree density (Fig. 3c, d; Table 3). In the Mkwaja area there was a marginally significant decline in N return through browser dung and urine deposition

(P = 0.06; Fig. 3a, b; Table 3). The relative importance of browser dung deposition was higher at low tree densities (Fig. 3e, f; all binomial regression lines P < 0.05).

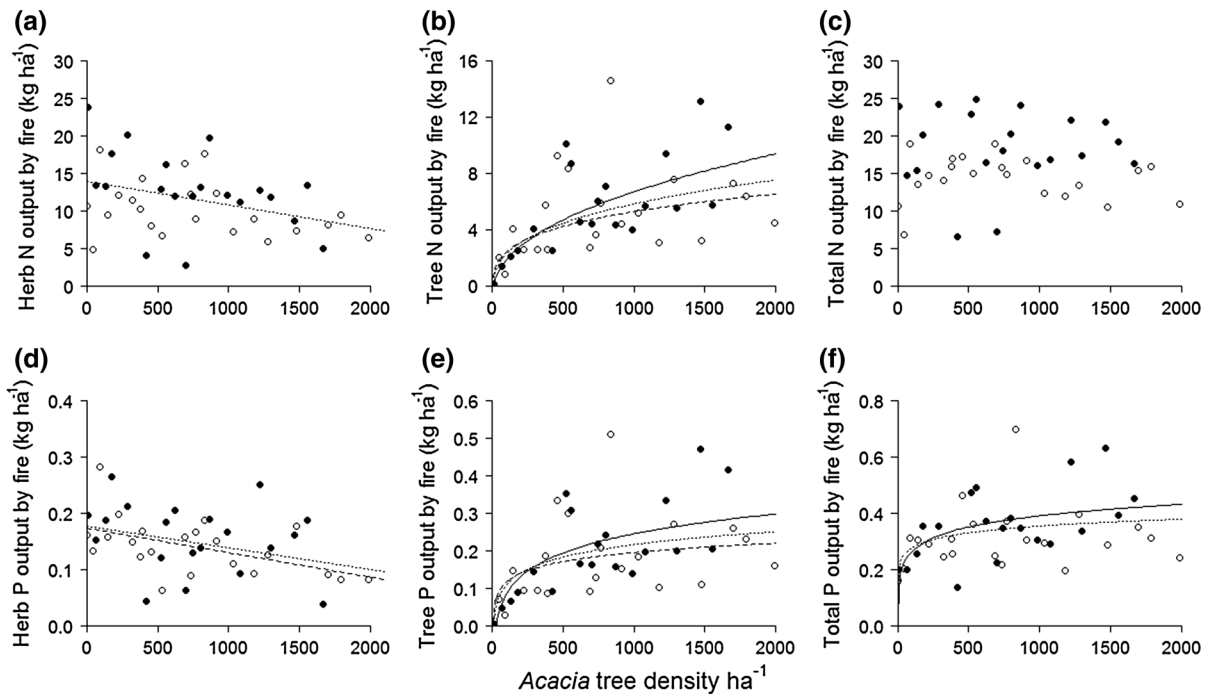


Fig. 2 Estimated N and P output fluxes for a single fire event from herbaceous biomass (**a**, **d**) and *Acacia* tree biomass (**b**, **e**) along a density of *Acacia* trees in two savanna areas (*solid points and lines* Mkwaja area; *open points and dashed lines* Saadani area). Total N and P outputs for a single fire event (**c**, **f**) were calculated by adding the estimated N and P outputs

through fire for both herbaceous biomass and *Acacia* tree biomass (N: **a** and **b**, P: **d** and **e**). Linear or log-linear regression lines were drawn depending on best fit based on AIC values (see Table 1), and *dotted lines* represent both areas when observations were pooled

Discussion

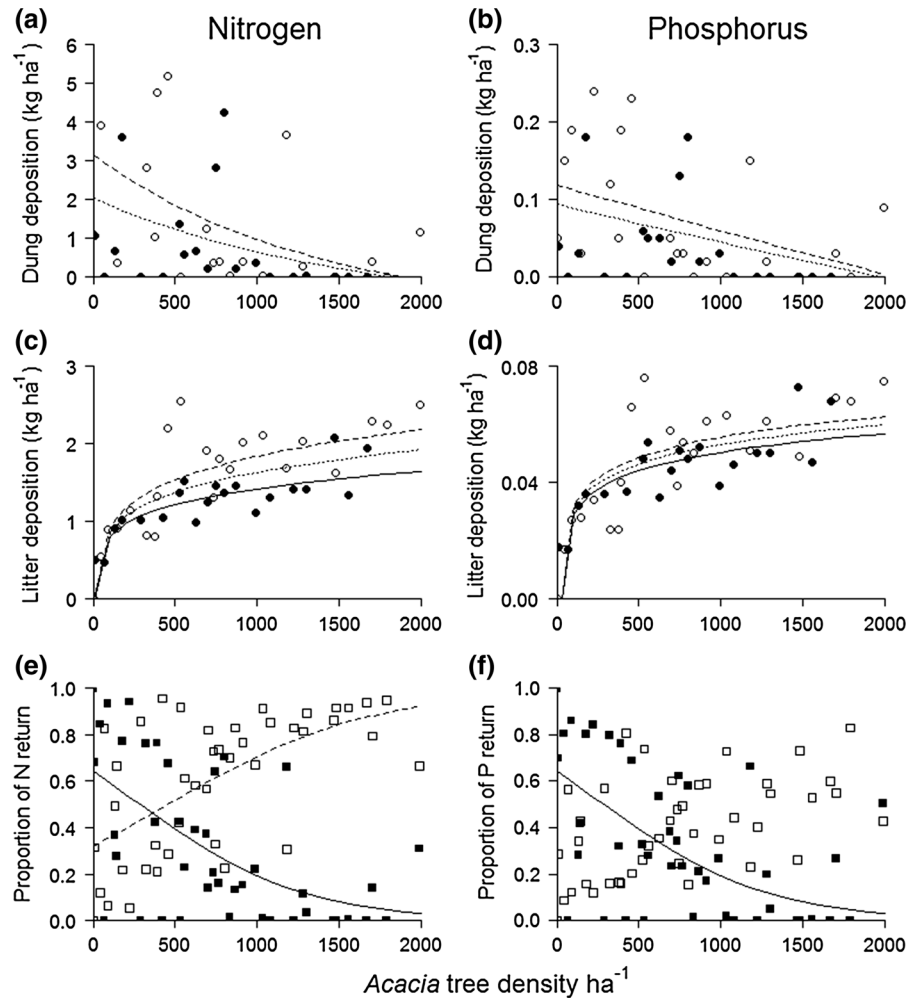
Quantifying N and P input and output fluxes

To our knowledge, this is the first study to quantify nutrient fluxes across a gradient of tree density in a savanna landscape. To achieve this, we had to make a number of simplifying assumptions by deriving flux estimates from sequential calculations using mean values. Although this approach leads to the propagation of errors that cannot be precisely quantified, these are unlikely to invalidate the broad trends in the data. Our results clearly show that N inputs increase with increasing tree density to ca. 7 kg N ha⁻¹ year⁻¹ in the densest woodland sites (Fig. 1a; Online Resource 1). This is slightly lower than values reported for N₂-fixing savanna woodlands (12 kg N ha⁻¹ year⁻¹ at 1,110 trees ha⁻¹ in Australia; Langkamp et al. 1979; 7–30 kg N ha⁻¹ year⁻¹ for West Africa; Robertson and Rosswall 1986, mean of 16.7 kg N ha⁻¹ year⁻¹ for African savannas; Chen et al. 2010), but consistent

with results from a previous study in the same area (3 or 12 kg N ha⁻¹ year⁻¹ at 840 or 2,620 trees ha⁻¹: Cech et al. 2010a). In contrast, leguminous herbs, which were very scarce in our site, contributed very little to the total N input (Fig. 1b; <1 kg N ha⁻¹ year⁻¹). Indeed, Cech et al. (2010b) have attributed this low abundance of N₂-fixing herbs to the superior ability of C₄-grasses in competing for soil N and P.

Many factors influence how much N is fixed by trees in savanna ecosystems, including species (Schulze et al. 1991), water availability (Aranibar et al. 2004; Delon et al. 2012), and availabilities of nutrients such as P and Mb (Vitousek et al. 2002). Although our study area was a relatively wet savanna dominated by *Acacia* trees, which could favour N₂-fixation, the soils were poorer in P than more arid savannas in East Africa (Cech et al. 2008). Even so, the proportion of N derived from the atmosphere by fixation was estimated to be 63 % (Sitters et al. 2013), which lies in the middle of a published range of 40–70 % for several African *Acacia* tree species (Cramer et al. 2007). The

Fig. 3 Annual estimated pathways of N and P returns from *Acacia* tree biomass through browser dung and urine deposition (**a, b**) and *Acacia* tree litter deposition (**c, d**) along a density of *Acacia* trees in two savanna areas (solid points and lines Mkwaja area; open points and dashed lines Saadani area; dotted lines both areas when observations were pooled). The relative importance of browser dung and urine deposition and *Acacia* tree litter to the total returns of N (**e**) and P (**f**) are shown for pooled observations (solid squares and lines browser dung and urine; open squares and dashed lines *Acacia* tree litter). Log-linear regression lines were drawn depending on best fit based on AIC values for **a, b, c** and **d** (see Table 3) while binomial regression lines were drawn for **e** and **f**



apparently lower N inputs could reflect differences in methodology; whereas we used the ¹⁵N abundance method based on biomass production of tree foliage, some authors (e.g. Langkamp et al. 1979) estimated N-inputs using the acetylene reduction method.

Fires are common in savanna ecosystems and can result in large N and P losses at sites with a high biomass of dry grass (Laclau et al. 2002; Abbadie 2006; Cech et al. 2010a). We estimated how much N and P were lost through a single fire, considering both the herbaceous layer and trees. As far as we know, trees have not previously been included in such estimates, despite the fact that they may be severely affected by fire (see also Cochard and Edwards 2011b). Indeed, we found significant losses of N and P from the tree layer due to burning, which increased along the tree density gradients to c. 8 kg N ha⁻¹ and to c. 0.3 kg P ha⁻¹ in the

densest woodland sites (data pooled over both areas; Fig. 2b, e; Online Resource 2). In contrast, the estimated nutrient outputs from burning of the herbaceous layer declined along the tree density gradients from c. 14 to 8 kg N ha⁻¹ and from c. 0.2 to 0.1 kg P ha⁻¹ (data pooled over both areas; Fig. 2a, d; Online Resource 2). The lower N outputs from trees compared to herbs probably resulted from a higher volatilization of N from the herbaceous biomass, which burned more completely (Table 2). The losses from the herbaceous biomass are at the lower end of the ranges reported for other savanna ecosystems (9–24 kg N ha⁻¹ and 0.1–0.4 kg P ha⁻¹; Laclau et al. 2002; Abbadie 2006; Cech et al. 2010a); this is probably because previous studies assumed that all herbaceous biomass burned to ash, whereas we measured a lower proportion that was completely consumed by fire (see Online Resource 2). This might

Table 3 Statistical summary of several regression models examining the relationship between the main internal pathways of N and P returns from *Acacia* tree biomass along a density of *Acacia* trees in two savanna areas

	Mkwaja area			Saadani area			Both areas		
	Regression	R ²	AIC	Regression	R ²	AIC	Regression	R ²	AIC
Nitrogen									
Browser dung and urine	Linear	n.s.	–	Linear	0.16†	131.7	Linear	0.12*	227.5
	<u>Log-linear</u>	0.14†	–	<u>Log-linear</u>	0.26*	55.1	<u>Log-linear</u>	0.19**	92.3
<i>Acacia</i> tree litter	Linear	0.64***	3.7	Linear	0.55***	33.3	Linear	0.52***	47.8
	<u>Log-linear</u>	0.74***	–33.5	<u>Log-linear</u>	0.79***	–17.6	<u>Log-linear</u>	0.71***	–40.7
Phosphorus									
Browser dung and urine	Linear	n.s.	–	Linear	0.20*	–48.3	Linear	0.14*	–103.3
	<u>Log-linear</u>	n.s.	–	<u>Log-linear</u>	0.20*	–52.8	<u>Log-linear</u>	0.14*	–111.2
<i>Acacia</i> tree litter	Linear	0.64***	–130.5	Linear	0.56***	–121.2	Linear	0.58***	–251.3
	<u>Log-linear</u>	0.68***	–134.0	<u>Log-linear</u>	0.66***	–129.0	<u>Log-linear</u>	0.65***	–263.1

R²-values, significance levels and AIC values are shown for linear and log-linear regression models

Underlined models had lowest AIC values and were therefore used in Fig. 3

n.s. non-significant trends

Asterisks indicate significance levels, with † (0.05 < P < 0.10), * (P < 0.05), ** (P < 0.01) and *** (P < 0.001)

be an attribute of our wetter climate as it is likely that a larger proportion of herbaceous biomass burns to ash when climate conditions are drier (van der Werf et al. 2008).

To extrapolate from our estimates to a complete year requires information on fire frequency, which varies widely even within a site. In our study area, as in many other national parks, fire is used as a management tool in the early dry season to attract wildlife and prevent uncontrollable fires in the late dry season (Eriksen 2007). With a typical fire frequency of once or twice every 3 years (Cech et al. 2010a), the annual N output would be somewhere between 6 and 11 kg ha^{–1} year^{–1}, these values being little affected by tree density (see Fig. 2c for single fire event). In contrast, the annual P output flux increases with tree density, ranging from 0.04 to 0.09 kg ha^{–1} year^{–1} assuming one fire every 3 years, or from 0.13 to 0.25 kg ha^{–1} year^{–1} assuming two fires every 3 years (see Fig. 2f for single fire event).

Possible implications for savanna N and P balances

We were unable to calculate complete N and P balances for the ecosystem because no information was available for some fluxes, including inputs due to dry and wet deposition (but see Online Resource 5) and

outputs through denitrification. However, our data suggest that the N balance in this savanna is probably neutral in areas with intermediate tree densities (ca. 1,100–1,400 trees ha^{–1}), where the N gains through N₂-fixation are similar to the losses through fire (see Online Resource 6 for rough nutrient balance estimates), while sites with a low tree density probably lose N. Contrasting conclusions apply for P, for which the balance may become negative with increasing tree density (Online Resource 6). If so, the long-term nutrient balance of the ecosystem would be maintained through a mosaic cycle, in which the vegetation at any location alternates between open grassland and woodland (also discussed by Skarpe 1992 and Wiegand et al. 2006). Cycles like these have been attributed to water availability, fire and activities of herbivores (especially elephants) (Skarpe 1992; Wiegand et al. 2006; Cochard and Edwards 2011b). Indeed, the finding that our *Acacia* woodlands do not represent a successional stage towards forest, but that they eventually break up and revert to grassland (Cochard and Edwards 2011b), supports the concept of such a cyclical process. And over periods shorter than the full cycle, spatial redistributions might occur that to some extent balance out local variation in nutrient input and output fluxes. For N, this would mean the redistribution of N fixed by trees from *Acacia* woodland areas to open grasslands.

Do browsing herbivores spatially redistribute nutrients?

Our results show that the relative importance of litter and dung deposition from browsing herbivores as pathways for nutrient return vary with tree density (Fig. 3). For example, the return of N and P through excreta was highest where tree densities were low, especially in the Saadani area where herbivores were more common (Treydte et al. 2005). Since browsing animals, mainly giraffe, were observed to feed across the range of tree densities, we conclude that they probably bring about some redistribution of nutrients from *Acacia*- to grass-dominated areas (Fig. 3a, b). However, quantifying these fluxes would require data on rates of browsing along the tree density gradients, which would be practically difficult to obtain. Similar spatial transfers have been demonstrated in other studies including both domesticated and wild herbivores (Abbas et al. 2012; Augustine et al. 2003; Edwards and Hollis 1982; Jewell et al. 2007).

Herbivory also has implications for the availability of nutrients, which are usually released more rapidly from dung than from plant litter (Bakker et al. 2004). Since browser dung is not only rich in N but releases this nutrient rather rapidly (Sitters et al. 2014) the deposition of this dung in N-deficient grassland areas may be ecologically important. In the case of grazing herbivores, we observed no spatial patterns in either grazing or excretion, suggesting that these herbivores played a minimal role in redistributing nutrients within our study area (see Online Resource 7 for more details). However, in savannas used for cattle, large quantities of nutrients may be removed from grazing areas and concentrated in places where cattle are kept at night (Augustine et al. 2003).

Implications for savanna management

Our study illustrates that encroachment by leguminous shrubs, which has been reported for savanna ecosystems throughout the world (Scholes and Archer 1997; Bond et al. 2003; Van Auken 2009), affects not only vegetation composition and forage availability, but also ecosystem properties such as N and P dynamics. This has important implications for management and especially for the use of prescribed burning to manage the balance between trees and grasses (Sankaran et al. 2005; Bond 2008). The effects of such management

upon the nutrient balance, especially in nutrient-poor systems, needs to be taken into account to avoid a long-term decline in the nutrient capital of an area. We conclude that in ecosystems such as the humid savanna in the Saadani National Park, no site should be burned more frequently than once every 3 years.

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Box 1. Hypothesized influence of *Acacia* tree density on nutrient fluxes

We have multiple working hypotheses for explaining how an increase in *Acacia* tree density might influence the main N and P input and output fluxes at the site level. Our predictions are based on results from previous research and are set out below.

N input flux through N₂-fixation by trees

The input of N by an individual tree depends on its biomass and N₂-fixation rate (see Sitters et al. 2013 and the methods section of this paper). Biomass production of an individual *Acacia* tree might decrease at higher densities (Smith and Goodman 1986, Pearson and Vitousek 2001, Cochard and Edwards 2011a) likely due to increased intraspecific competition. However, the proportion of N derived from N₂-fixation per tree was independent of tree density in our study area (Sitters et al. 2013). We therefore predict the relationship between N input and tree density to be:

A1: positive log-linear (a saturated response), if trees do reduce their individual biomass beyond a certain tree density but not so strongly that site level inputs are reduced.

A2: quadratic, if trees do reduce their individual biomass beyond a certain tree density so strongly that site level inputs are also reduced.

N input flux through N₂-fixation by leguminous herbs

The proportion of N derived from N₂-fixation by leguminous herbs decreased with increasing tree density (Sitters et al. 2013), which could additionally limit their productivity. We therefore predict the relationship between N input and tree density to be:

B1: negative linear, if trees only reduce N₂-fixation rates of leguminous herbs with increasing tree density, but not their biomass.

B2: negative exponential (log-linear), if trees reduce both N₂-fixation rates and biomass of leguminous herbs with increasing tree density.

N and P output fluxes by fire for the herb layer

Grass biomass at the site level was not related to tree density in our study area (Sitters et al. 2013); hence the amount of herbaceous fuel available is equal in each site. However, trees might act as a buffer against fire at high tree densities and reduce fire probability and intensity (Jeltsch et al. 2000). Additionally, N and P concentrations of the herbaceous biomass might change with increasing tree density. We, for example, observed an increase in herbaceous N concentrations with increasing tree density in our study area (Sitters et al. 2013). We therefore predict the relationship between N and P outputs and tree density to be:

C1: negative linear, if a decrease in the proportion of herbaceous biomass burned with increasing tree density is not compensated by an increase in herbaceous N or P concentrations.

C2: constant, if an increase in herbaceous N or P concentrations compensates for the decrease in proportion of biomass burned.

N and P output fluxes by fire for the tree layer

As trees might act as a buffer against fire at high densities, the proportion of tree biomass burned at the site level might reduce with increasing tree density.

Additionally we did not observe a change in N and P concentrations of tree foliage with increasing tree density (Sitters et al. 2013). We therefore predict the relationship between N and P outputs and tree density to be:

D1: positive linear, if the proportion of tree biomass burned is not significantly reduced.

D2: positive log-linear (a saturated response), if the proportion of tree biomass burned is reduced but not significantly so to compensate for the increase in site level tree biomass.

D3: quadratic, if the proportion of tree biomass burned is significantly reduced to compensate for the increase in site level tree biomass.

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