Linkages between soil carbon, soil fertility and nitrogen fixation in *Acacia senegal* plantations of varying age in Sudan

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ABSTRACT

Background: Over the last decades sub-Saharan Africa has experienced severe land degradation and food security challenges linked to loss of soil fertility and soil organic matter (SOM), recurrent drought and increasing population. Although primary production in drylands is strictly limited by water availability, nutrient deficiencies, particularly of nitrogen (N) and phosphorus (P), are also considered limiting factors for plant growth. It is known that SOM (often measured as soil organic carbon (SOC)) is a key indicator of soil fertility, therefore, management practices that increase SOM contents, such as increasing tree cover, can be expected to improve soil fertility. The objectives of this study were to investigate the effect of *Acacia senegal* (*Senegalia senegal*) trees on soil nitrogen, phosphorus and potassium (K) in relation to SOC, the potential of *A. senegal* for N_2 fixation, and to identify possible N and P ecosystem limitations.

Methods: Soil nutrient (total N, P, K and available P and exchangeable K) concentrations and stocks were determined for the 0–10, 10–20, 20–30 and 30–50 cm layers of *A. senegal* plantations of varying age (ranging from 7 to 24-years-old) and adjacent grasslands (reference) at two sites in semi-arid areas of Sudan. At both sites, three plots were established in each grassland and plantation. The potential of *A. senegal* for N_2 fixation in relation to plantations age was assessed using $\delta^{15}N$ isotopic abundances and nutrient limitations assessed using C:N:P stoichiometry.

Results: Soil concentrations of all studied nutrients were relatively low but were significantly and directly correlated to SOC concentrations. SOC and nutrient concentrations were the highest in the topsoil (0–10 cm) and increased with plantations age. Acacia foliage $\delta^{15}N$ values were >6‰ and varied little with plantations age. Soil C:N and C:P ratios did not differ between grassland and plantations and only 0–10 cm layer N:P ratios showed significant differences between grassland and plantations.

Discussion: The results indicated that soil fertility in the Sahel region is strongly related to SOM contents and therefore highlighting the importance of trees in the

landscape. The higher mineral nutrient concentrations in the topsoil of the plantations may be an indication of 'nutrient uplift' by the deeper roots. The high foliar δ^{15}N values indicated that N_2 fixation was not an important contributor to soil N contents in the plantations. The accretion of soil N cannot be explained by deposition but may be related to inputs of excreted N brought into the area annually by grazing and browsing animals. The soil C:N:P stoichiometry indicated that the plantations may be limited by P and the grasslands limited by N.

INTRODUCTION

Over the last decades sub-Saharan Africa has experienced severe land degradation and food security challenges linked to loss of soil fertility and soil organic matter (SOM), recurrent drought and increasing population (*Nkonya et al., 2015*). While soil water availability is the main limitation on primary productivity in drylands, nutrient deficiencies, particularly nitrogen (N), phosphorus (P) and potassium (K), are other important causes (*FAO, 2004*; *Lal, 2004a*). SOM plays an important role in maintaining adequate nutrients and moisture levels (*Tiessen, Cuevas & Chacon, 1994*; *Lal, 2004b*) and soil fertility management practices that increase SOM contents have been adopted in many drylands in order to enhance crop productivity (*FAO, 2004*; *Koohafkan & Stewart, 2008*). The use of a fallow period is a well-known practice in these areas, allowing the soil to restore its SOM content and so recover from years of cultivation (*Sanchez, 1999*). However, the area of land put under fallow and the duration of the fallow period have been reduced as a result of increasing population pressure (*Kaya, Hildebrand & Nair, 2000*; *FAO, 2004*). Other practices aimed at reversing land degradation have focused on the role of trees, particularly N_2-fixing species, in maintaining soil fertility and protecting the soil from wind and water erosion (*FAO, 2001*, *2004*). The deeper roots of trees play an important role in mineral nutrient recycling, enabling mineral nutrients to be taken up from deeper soil layers and making them available to ground vegetation via litterfall—so-called 'nutrient uplift' (*Scholes, 1990*; *Ludwig et al., 2004*).

Sub-Saharan drylands are characterized by woodland savanna with trees and shrubs forming an open canopy with varying proportions of grasses (*Bourlière & Hadley, 1983*; *Torello-Raventosa et al., 2013*). The importance of the facilitative mechanisms (relative to competition) of trees in tree-grass systems has been reported to be greater in drier savanna (*Dohn et al., 2013*; *Moustakas et al., 2013*). The positive effects of trees and shrubs on ground vegetation have been attributed to the effect of shade, improvement in soil moisture conditions, and increased nutrients contents under tree canopies (*Belsky et al., 1993*; *Hagos & Smit, 2005*; *Blaser et al., 2013*). Fire in savanna is typical, although varying in frequency and intensity, and generally results in a loss of C and N from the ecosystem (*Pellegrini et al., 2015*). However, fire may have little effect on soil total N and soil organic carbon (SOC) because of the superficial nature of the fires

(*Coetsee, Bond & February, 2010*; *Coetsee, Jacobs & Govender, 2012*). Savanna ecosystems are also subject to grazing and browsing, the effects of which on ecosystem biogeochemistry and nutrient fluxes are complex and variable, but maybe significant (*Holdo et al., 2007*). In open ecosystems, such as savannas, herbivores may bring in significant quantities of nutrients, particularly N and P, in the form of dung and urine (*Holdo et al., 2007*).

N_2 fixation can increase soil N contents (*Ludwig et al., 2004*; *Blaser et al., 2013*). However, N_2 fixation has a high P requirement (*Vitousek et al., 2002*; *Binkley, Senock & Cromack, 2003*), which is low in dryland soils due to P adsorption either by iron oxide (*Dregne, 1976*) or calcium (*Lajtha & Schlesinger, 1988*). The abundance of stable N isotopes (δ^{15}N) in leaves and, to a lesser extent, soils can be used to assess N_2 fixation and indicate patterns of ecosystem N cycling (*Boddey et al., 2000*; *Aranibar et al., 2004*; *Peri et al., 2012*). Low foliar and soil δ^{15}N values indicate biological N_2 fixation (*Schulze et al., 1999*; *Robinson, 2001*), while the enrichment of soil ^{15}N can be attributed to SOM reprocessing by microorganisms (*Aranibar et al., 2004*; *Swap et al., 2004*).

The biogeochemical cycles of C, N and P are often closely related (*Finzi et al., 2011*) and C:N:P stoichiometry is commonly used to provide an insight into the nature of nutrient limitations in ecosystems (*Jobbágy & Jackson, 2001*; *Bui & Henderson, 2013*). Soil C:N and C:P ratios are useful indicators of the state of SOM decomposition and N and P availability (*Batjes, 1996*; *Tian et al., 2010*), and foliar N:P ratios have been used to assess plant nutrient limitations (*Ludwig et al., 2004*; *Sitters, Edwards & Olde Venterink, 2013*; *Blaser et al., 2014*).

Acacia senegal (L.) Willd. (the new scientific name is *Senegalia senegal* (L.) Britton.) is a highly drought-resistant tree native to Sudan and Sahel zone of Africa (*Obeid & Seif El Din, 1970*). Although the new name has been used in a number of recent publications, we have retained the use of the old name, *A. senegal*, for reasons of consistency with our previous two related articles and with literature in general, and because of the local importance of the old name. *A. senegal* provides a wide variety of benefits, such as fodder for animals, fuelwood, charcoal and gum arabic (*Barbier, 1992*). Gum arabic is an exudate collected from *A. senegal* trees and widely used as an emulsifier in confectionary and beverages, photography, pharmaceutical and other manufacturing industries (*Barbier, 2000*). This tree is also known to be capable of N_2 fixation under different soil types and climatic conditions (*Raddad et al., 2005*; *Gray et al., 2013*). The influence of *A. senegal* on soil physiochemical properties in arid and semi-arid areas of Africa has been documented in a number of studies (*Deans et al., 1999*; *Githae, Gachene & Njoka, 2011*). In Sudan, particular attention has been given to SOC and N contents under *A. senegal* in the north Kordofan region (*Jakubaschk, 2002*; *Olsson & Ardö, 2002*; *Ardö & Olsson, 2004*; *Abaker et al., 2016*) and on the influence of inter-cropping systems with *A. senegal* on soil properties of sandy and clay soils (*Raddad et al., 2006*; *El Tahir et al., 2009*).

The aims of our study were to determine the effects of *A. senegal* plantation age on: (1) soil N (total), P (total and available) and K (total and exchangeable) concentrations, stocks and accretion rates; (2) potential N_2 fixation using foliar δ^{15}N values; and (3) acacia

leaf, ground vegetation N:P ratios and soil C:N:P stoichiometry in order to indicate nutrient limitations, imbalances and cycling in these ecosystems. We hypothesized that soil N, P and K concentrations and stocks would be positively correlated with SOC and increase with planation age, further indicating the benefits of maintaining tree cover in these semi-arid environments. This paper complements two previous papers dealing with effects of *A. senegal* plantation age on SOC stocks (*Abaker et al., 2016*) and on soil moisture and water balance (*Abaker, Berninger & Starr, 2018*). These two studies were carried out at the same sites as in this study.

MATERIALS AND METHODS

Study sites

We conducted our research at two sites in western Sudan: El Demokeya forest reserve (13°16′ N, 30°29′ E, 560 m a.s.l.), an experimental site managed for gum arabic research, and El Hemaira forest (13°19′ N 30°10′ E, 570 m a.s.l.) owned and managed by farmers for gum arabic production (Fig. 1). At both sites there was an area of open grassland which was taken to serve as a control against which the plantations of differing age were compared. Photographs showing the plantations and grasslands at the two sites during the rainy season are given in Supplementary Material S1.

The long-term mean annual rainfall and temperature for both sites is 318 mm and 27.3 °C. The soils at both sites are classified as Cambic Arenosols (FAO) (≥90% sand). The topography is very gently sloping eastwards at El Demokeya and flat at El Hemaira and the hydrology similar at the two sites. Water balance modelled runoff from the grasslands was 32 and 95 mm for 2011 and 2012 respectively, zero for the plantations in 2011 and 63 mm in 2012 at both sites (*Abaker, Berninger & Starr, 2018*). Drainage was higher in 2011 than in 2012, and somewhat less at El Hemaira (ranging from 0 to123 mm) than at El Demokeya (ranging from 25 to 128 mm). The vegetation at both sites falls within the low rainfall woodland savanna type (*Ayoub, 1998*; *FAO, 2006*). Main components of the ground vegetation at both sites were grasses such as *Cenchrus biflorus*, *Aristidia pallida* and *Eragrostis tremula*, and some herbs, including *Geigeria alata*, *Justicia kotschyi*, *Trianthema pentandra* and *Acanthus* spp. A complete list of ground vegetation species found at the two sites in given in Supplementary Material S2. Although site-specific information about grazing and frequency of fire at the two sites is unavailable, it is known that there is over-grazing by sheep and browsing by camels, even within the forest reserve at El Demokeya. Additional information about the study sites and sampling have been described in *Abaker et al. (2016).*

Experimental design, sample plots and sampling

The plantations were 15 and 24-years-old (in 2011) at El Demokeya and seven, 15 and 20-years-old at El Hemaira. The same experimental design was used at both sites. Three circular plots (17.8 m radius; 0.1 ha) were established in each plantation of differing age at both sites. Three square plots (50 × 50 m at El Demokeya and 30 × 30 m at El Hemaira) were located in the adjacent grassland, the difference in size being due to the difference in the area of grassland available at the two sites. Square rather than circular
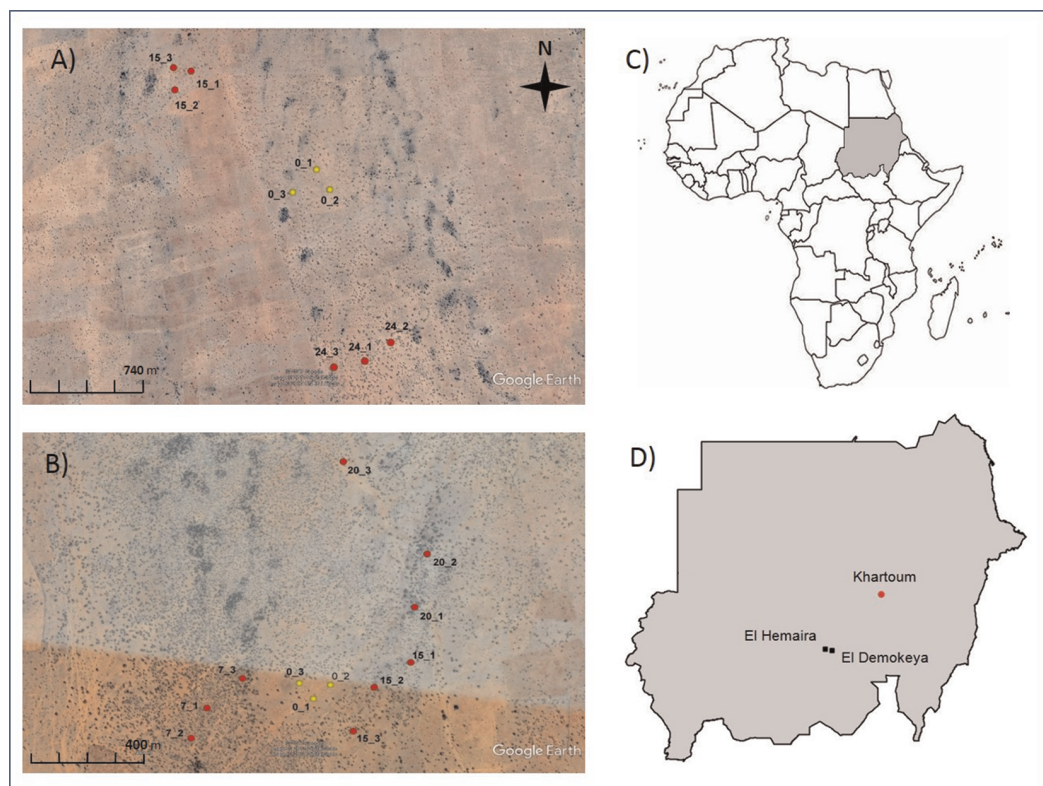
Figure 1 Satellite images of the two study sites El Demokeya (A) and El Hemaira (B) showing location of the plots. Number preceding the underscore refers to plantation age in years (0 = grassland) and number following the underscore refers to plot number. Inset maps showing Sudan's location in Africa (C) and location of study sites in Sudan (D). Image: © 2017 Google, DigitalGlobe and CNES/Airbus.
Full-size ▣ DOI: 10.7717/peerj.5232/fig-1

plots were used for the grasslands as it was easier to delineate in the field and to carry out the sampling. Although constituting a pseudoreplicated experimental design (*Hurlbert, 1984*), which limits the generality of our results concerning patterns about plantation age effects sensu stricto, the design was determined by the spatial layout of the plantations at the study sites.

Acacia leaf samples were taken from three trees (randomly selected) per plot when the foliage was fully developed. Seven randomly selected terminal branches were collected from each of the three trees, air dried, and the leaves excised and composited by tree ($n = 45$).

Ground vegetation samples were collected from one (randomly selected) of the three replicate plots per plantation age and the ground vegetation (a mixture of grasses and herbs) from 14 quadrats (1×1 m^2) harvested. For the grasslands, ground vegetation samples were collected from three quadrats located in one of the grassland plots at each study site. Sampling was carried when the ground vegetation was fully developed. All the plants within each quadrat were manually uprooted, separated into above and belowground parts in the field and placed into separate bags. There were a total of 76 quadrats.

Soil samples were taken from the 0–10, 10–20, 20–30 and 30–50 cm layers of all plantation and grassland plots. For each of the plantation plots, samples were taken from

under the canopy of one (randomly) selected tree at three distances (0.5, 1 and 2.5 m) from the stem. For the grassland plots, samples were taken from the four corners and centre of each plot and composited by layer. For determination of bulk density for the grasslands, a separate sample was collected from the centre of only one of the grassland plots at each site.

Sample pre-treatment and laboratory analysis

The tree-wise composited acacia leaf samples were further composited by plot for chemical and N isotope analyses ($n = 15$). The above and belowground vegetation biomass samples were dried at 60 °C for 48 h and weighed. However, in order to reduce analytical costs, the samples from only five of the 14 quadrats per plantation plot and two of the three quadrats from each of the grassland plots were selected (randomly) for analyses and only the aboveground samples analysed ($n = 29$). The soil samples were air-dried and passed through a two mm sieve and the <2 mm fraction saved for analysis. In the case of the soil samples from the plantations, the samples from the three distances from the stem were combined for total elemental analysis, otherwise the other analyses were carried out on the individual samples.

Contents of C and N in the acacia leaves, vegetation and soil samples were determined directly using an elemental CN analyser (Vario MAX CN; Elementar Analysensysteme GmbH, Germany). Contents of P and K were determined by digesting the samples (300 mg plant material, one g soil) in concentrated HNO_3 acid ($10 + 1$ ml H_2O_2) and microwaving, and measuring elemental concentrations using an ICP-OES spectrometer (Thermo Scientific iCAP 6000 Series, USA). Particle size analysis of the sieved soil samples was performed using a laser diffraction device (Coulter LS230; Coulter Corporation, Miami, FL, USA) and the percentage of clay, silt and sand fractions calculated. The total elemental and particle size analyses were carried out on oven-dried samples (105 °C). Soil available phosphorous (P_{av}) was extracted using 0.5 M sodium bicarbonate solution (pH 8.5) and concentrations determined using the Molybdenum blue spectrophotometer method and exchangeable K (K_{ex}) was extracted with one M ammonium acetate (pH 7.0) and concentrations determined by flame-photometer, both following FAO guidelines (*Dewis & Freitas, 1970*) and were determined from the air-dried samples. Apparent (also known as 'tapped') bulk density was determined using approximately 20 ml of soil placed into a measuring cylinder, tapped 10 times, and the volume and weight of the soil used to calculate the bulk density (*Tan, 2005*). This method is recommended because of the difficulty in taking intact volumetric samples from loose sandy soils with no structure (*Tan, 2005*), as was the case with our sites. The determination of P_{av}, K_{ex} and bulk density was made in the laboratory of the Agricultural Research Corporation, Ministry of Agriculture, Sudan while the total elemental and particle size analyses were carried out in the laboratory of the Department of Forest Sciences, University of Helsinki.

The abundance of stable nitrogen isotope, ^{15}N, was determined from the acacia leaf, ground vegetation aboveground biomass and soil (only for one grassland plot per site) samples. $\delta^{15}N$ values were determined using continuous-flow isotope ratio mass

spectrometry at the Centre for Stable isotopes at IMK-IFU/KIT Garmisch-Partenkirchen (Germany). The precision (standard deviation) of internal standard for stable N isotopic composition was better than 0.2‰. The stable isotopic composition of nitrogen is expressed relative to atmospheric N_2 (international standard for N).

Calculation of soil stocks and accretion rates

Soil organic carbon and nutrient stocks (g m^{-2}) were calculated using both the traditional fixed depth method and the minimum equivalent soil mass (ESM) method (*Lee et al., 2009*). The fixed depth stocks were calculated according to the following equation:

$$Stocks = soil\ concentration \times BD \times T \times 100$$

where concentration is in %, BD is soil bulk density (g cm^{-3}) and T the thickness of the soil layer (cm). The ESM stocks for each layer were calculated according the equations given by *Lee et al. (2009)*. This was done in order to eliminate the effect of any alteration in bulk density associated with plantation age. The stocks for the four sampling layers were summed to give the stocks for the 0–50 cm layer. Accretion rates of nutrients in the soil were calculated as the difference between the grassland and the oldest plantation fixed depth stocks divided by the age of the plantation.

Statistical analysis

The effect of plantation age (grassland was taken to be 0-years-old) on SOC, N, P, P_{av}, K and K_{ex} concentrations by layer and stocks (0–50 cm) and on C:N:P ratios by layer were tested for each site separately using one-way analysis of variance followed by Tukey post hoc tests. Differences in acacia leaf N, P and K concentrations, N:P ratios and soil and acacia leaf δ^{15}N abundances between the 7, 15 and 20-year-old plantations at El Hemaria were similarly tested, but for El Demokeya a *t*-test was performed as there were only plantations of two ages.

The dependence of the total soil N, P, P_{av}, K and K_{ex} on SOC was evaluated by fitting linear regressions and the coefficient of determination (R^2). Correlations (Pearson) between SOC contents and total N, P, P_{av}, K and K_{ex} were computed for each soil layer and site separately. All the statistical analyses were performed using SPSS software (IBM SPSS Statistics for Windows, Version 22.0; IBM Corp., Armonk, NY, USA).

RESULTS

All nutrient concentrations were generally higher in the plantations than in the grasslands, increased with plantations age and decreased with depth (Fig. 2). Concentrations of SOC, N, total P and K_{ex} in the top (0–10 cm) layer were significantly ($p \leq 0.05$) higher in the oldest plantations at both sites compared to the grassland plots. Soil concentrations of total N, P, P_{av} and K_{ex} also significantly depended on SOC concentrations (Fig. 3). The strongest dependence was for N ($R^2 = 0.90$) and the weakest was for total K ($R^2 = 0.11$). The correlations between SOC and N concentrations were significant for all layers at both of the sites (Table 1). The correlations between SOC and total P
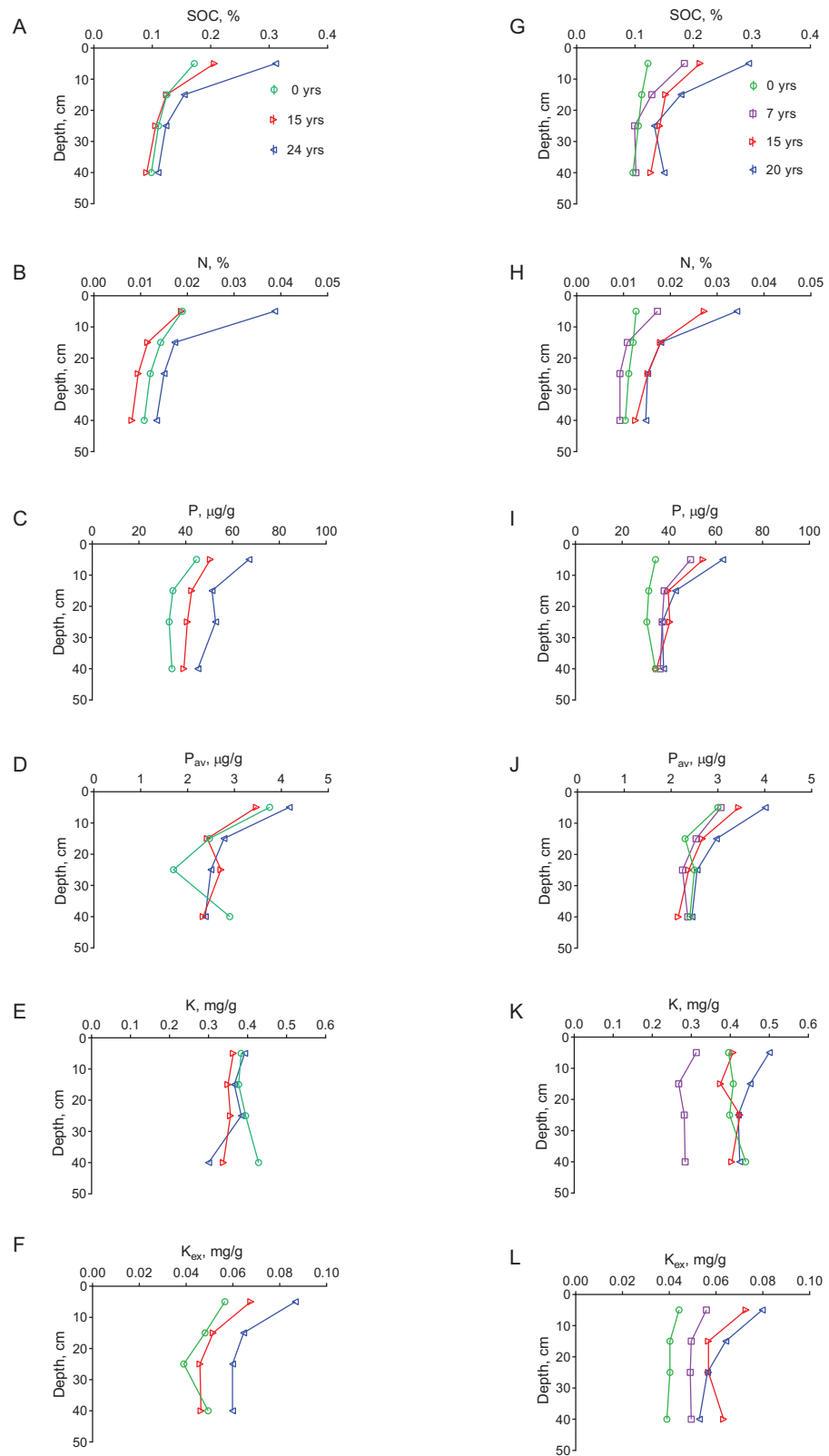
Figure 2 Soil SOC, N, total P, available P, total K and exchangeable K mean ($n = 3$) concentrations plotted against depth for grassland and plantations by age for El Demokeya (A–F) and El Hemaira (G–L) sites. SOC data from *Abaker et al. (2016)*. Full-size ⬜ DOI: 10.7717/peerj.5232/fig-2
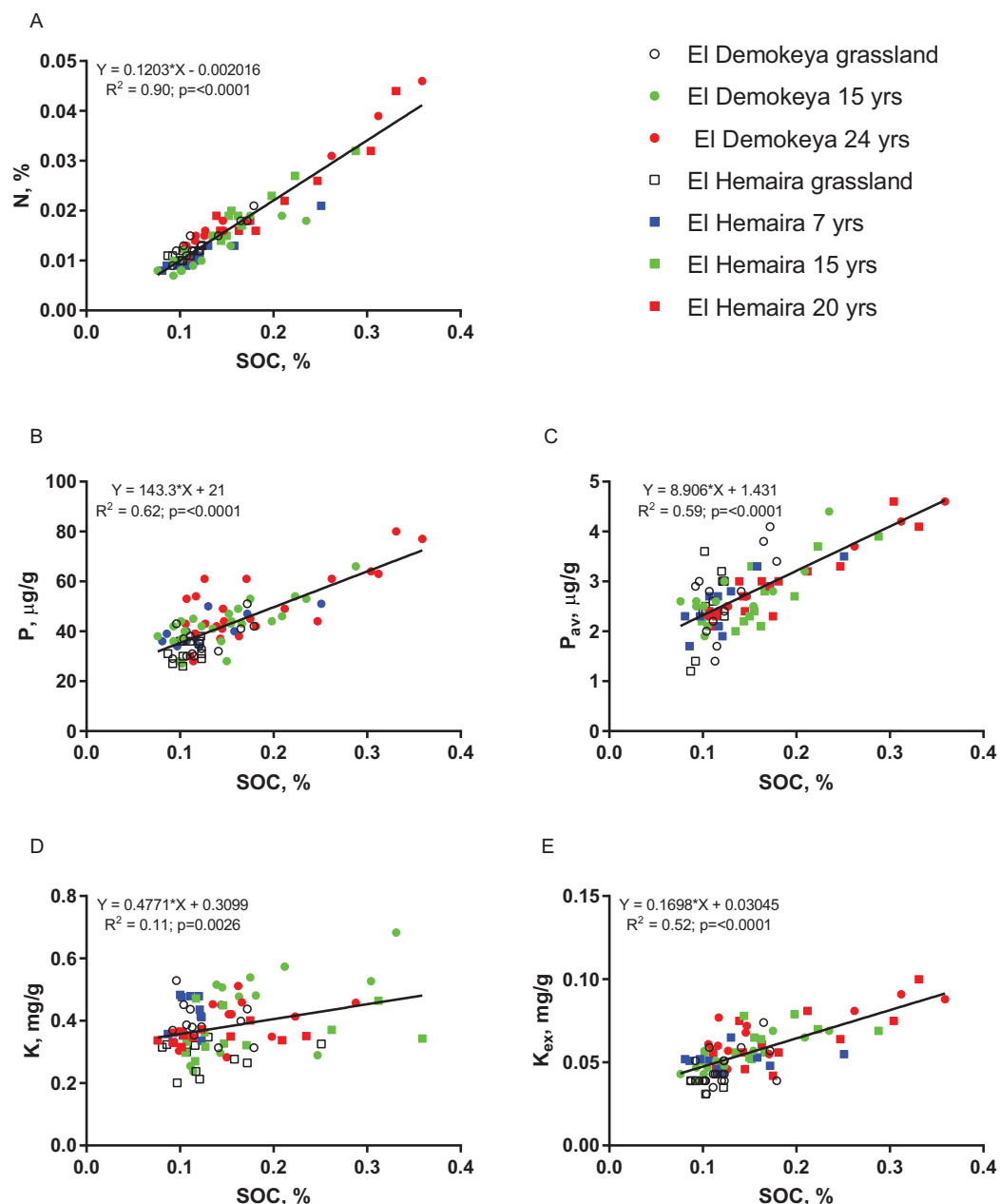
Figure 3 Dependence of soil N (A), total P (B), available P (C), total K (D) and exchangeable K (E) on SOC concentrations for grassland and plantations by age across all soil layers and for the two study sites. Full-size ☒ DOI: 10.7717/peerj.5232/fig-3

concentrations were significant for all layers at El Hemaira but in the case of El Demokeya the correlation was significant only for the top layer. The correlations between SOC and total K concentrations were stronger at El Hemaira than at El Demokeya. In the case of P_{av} and K_{ex}, significant correlations with SOC were associated with the upper layers.

Nutrient stocks in the soil of the plantations were generally greater than those in the grassland and increased with plantation age (Table 2). As the fixed depth SOC stock

Table 1 Pearson correlations between SOC and N, total P, available P, total K, exchangeable K concentrations by soil layer across all plots separately for El Demokeya ($n = 9$) and El Hemaira ($n = 12$) sites.

Site	Layer, cm	N	P	P_{av}	K	K_{ex}
El Demokeya	0–10	**0.942**	**0.915**	0.634	0.006	**0.749**
	10–20	**0.675**	0.600	**0.817**	−0.323	0.637
	20–30	**0.652**	0.442	0.144	−0.064	0.366
	30–50	**0.729**	0.182	−0.302	−0.307	**0.757**
El Hemaira	0–10	**0.950**	**0.869**	**0.848**	0.566	**0.762**
	10–20	**0.827**	**0.699**	**0.657**	0.558	**0.862**
	20–30	**0.906**	**0.732**	0.434	**0.732**	0.529
	30–50	**0.936**	**0.663**	0.170	**0.576**	0.365

Note:
Significant ($\alpha = 0.05$) correlations are given in bold.

Table 2 Soil stocks (g m^{-2}; 0–50 cm layer) of SOC, N, total P, available P, total K and exchangeable K for grassland and plantations (under canopy) by age for the two study sites.

Site	Age	SOC*	N	P	P_{av}	K	K_{ex}
El Demokeya	0**	950(51)[a]	105 (11)[a]	28 (3.1)[a]	2.1 (0.1)[a]	315 (33)[a]	38.0 (8.0)[a]
	15	1024(143)[ab]	93 (10)[a]	35 (1.5)[ab]	2.2 (0.2)[a]	291 (5)[a]	43.0 (4.5)[a]
	24	1260(122)[b]	153 (15)[b]	41 (7.6)[b]	2.2 (0.1)[a]	273 (28)[a]	51.5 (2.1)[b]
El Hemaira	0**	867(59)[a]	92 (1)[a]	27 (2.0)[a]	2.1 (0.6)[a]	339 (43)[a]	33.0 (0.6)[a]
	7	982(190)[ab]	89 (13)[a]	32 (1.2)[a]	2.0 (0.3)[a]	230 (40)[a]	40.6 (3.1)[ab]
	15	1216(138)[ab]	136 (27)[ab]	33 (6.4)[a]	2.0 (0.2)[a]	323 (60)[a]	50.1 (2.0)[b]
	20	1422(240)[b]	151 (32)[b]	34 (6.4)[a]	2.3 (0.3)[a]	349 (119)[a]	48.1 (9.6)[b]

Notes:
Values are mean values ($n = 3$) followed by standard deviation (in parentheses). Within each site, mean values sharing the same superscript letters (a, ab, b) are not significantly different from each other (Tukey's HSD, $\alpha < 0.05$).
* SOC values from *Abaker et al. (2016)*.
** Grassland.

values showed better relationships with SOC concentrations and with plantation age than did ESM SOC stock values, only the fixed depth stock SOC and nutrient values are presented in Table 2 and handled further. However, the ESM SOC and nutrient stock values are presented in Supplementary Material S3. At El Demokeya SOC, N, total P and K_{ex} stocks were significantly higher in the oldest plantation than those in the grassland, but not P_{av} and total K stocks. At El Hemaira SOC, N and K_{ex} stocks were also significantly higher in the oldest plantation than in the grassland. K_{ex} stocks in the 15-year-old plantation were also significantly higher than in the grassland. Assuming that the significant difference between grassland and the oldest plantation SOC, N and total P stocks represents the addition of these elements brought about by the effect of the plantation, the average under canopy accretion rates of SOC and N at El Demokeya would be respectively 12.9 and 2.0 g m^{-2} yr^{-1}. At El Hemaira, the corresponding SOC and N accretion rates would be 27.8 and 3.0 g m^{-2} yr^{-1}. The total P accretion rate at El Demokeya

Table 3 Soil C, N and P stoichiometric ratios for the grassland and plantations by age and layer (cm). Values are plot age mean values ($n = 3$).

Site	Age (years)	C:N				N:P				C:P			
		0–10	10–20	20–30	30–50	0–10	10–20	20–30	30–50	0–10	10–20	20–30	30–50
El Demokeya	0*	9.1	8.8	9.2	9.2	4.3[a]	4.2	3.7	3.2	38.9	36.7	34.0	29.7
	15	11.1	10.9	11.2	11.2	3.7[a]	2.7	2.4	2.1	41.0	29.2	26.1	23.1
	24	8.1	8.9	8.2	8.2	5.7[b]	3.4	2.9	3.0	46.4	30.4	23.9	24.8
El Hemaira	0*	9.6	9.2	9.5	9.3	3.8[a]	3.9	3.7	3.1	36.2	35.6	35.1	28.2
	7	10.5	11.9	10.7	11.1	3.5[a]	2.9	2.5	2.5	37.4	34.1	26.8	28.0
	15	8.7	8.7	9.5	10.3	5.0[ab]	4.6	3.7	3.6	43.4	40.4	35.4	36.8
	20	8.8	10.0	9.0	10.3	5.5[b]	4.2	4.1	3.9	48.1	41.5	36.3	39.6

Notes:
Values within each site and soil layer sharing the same superscript letter (a, ab, b) or having no letter are not significantly different from each other (Tukey's HSD, $\alpha < 0.05$).
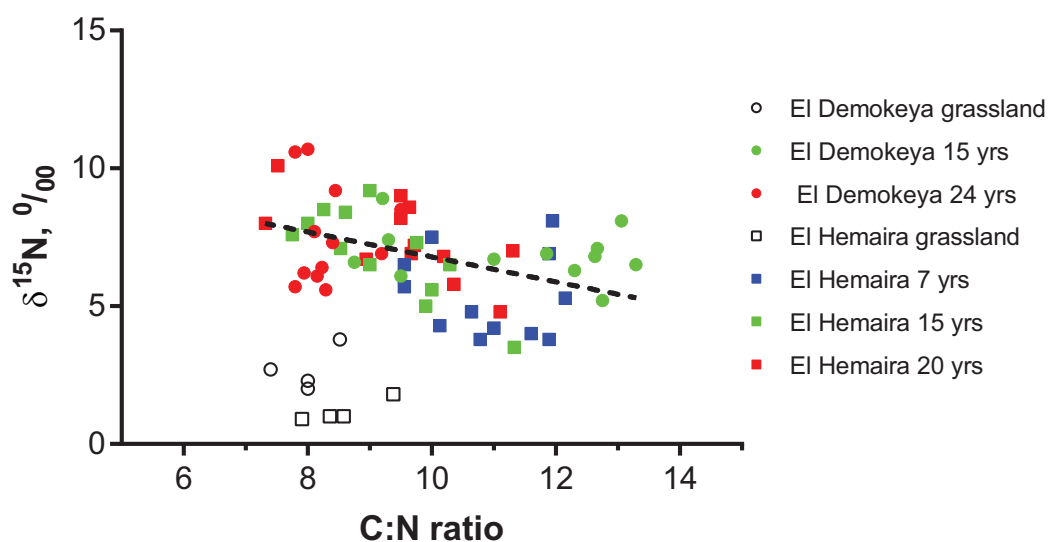* Grassland.

Figure 4 Relationship between soil δ^{15}N (‰) and soil C:N ratios for grasslands and plantations by age for the two study sites across all plots and layers. Dashed line is the linear regression fitted to the plantation data only ($Y = -0.452^*X + 11.31$, $R^2 = 0.1926$, $p = 0.0005$).
Full-size ◲ DOI: 10.7717/peerj.5232/fig-4

would be 0.5 g m^{-2} yr^{-1} (as the difference in total P stocks between the grassland and oldest plantation at El Hemaira was not significant, the accretion rate is considered zero).

Grassland ground vegetation and soil δ^{15}N values were generally lower than corresponding plantation δ^{15}N values. Acacia foliar δ^{15}N values were higher than ground vegetation values, but neither showed a difference related to plantation age (Table 3). The number of ground vegetation samples was too small to allow for significance testing. Soil δ^{15}N values increased with plantation age and decreased with depth at both study sites, but these trends were not significant ($p > 0.05$). Plantation soil δ^{15}N values were significantly correlated to soil C:N ratios, but the relationship for grasslands was clearly different (Fig. 4).

Table 4 N, P and K concentrations (mg g^{-1}) and N:P ratios in acacia leaves ($n = 3$), aboveground vegetation in the grassland ($n = 2$) and plantations ($n = 5$) at each of the two study sites.

Site	Age (years)	Sample	N	P	K	N:P
El Demokeya	0*	Grd. veg.	12.1	2.7	23.5	4.6
	15	Acacia leaves	40.0 (1.8)	0.7 (0.05)	4.6 (0.1)	59.3
		Grd. veg.	11.6 (1.6)	2.3 (0.6)	19.9 (3.5)	5.3
	24	Acacia leaves	39.4 (1.7)	0.7 (0.04)	4.2 (0.6)	59.2
		Grd. veg.	13.0 (1.6)	1.8 (0.3)	16.6 (5.8)	7.6
El Hemaira	0*	Grd. veg.	11.8	0.9	17.1	13.5
	7	Acacia leaves	38.4 (2.9)	0.5 (0.0)	5.2 (1.3)	70.3
		Grd. veg.	16.1 (4.5)	1.5 (0.3)	16.6 (8.0)	11.1
	15	Acacia leaves	41.4 (2.1)	0.6 (0.0)	3.5 (0.5)	73.7
		Grd. veg.	11.6 (3.7)	1.8 (0.6)	20.3 (4.6)	6.9
	20	Acacia leaves	40.0 (5.3)	0.9 (0.4)	4.4 (0.8)	52.6
		Grd. veg.	19.2 (5.3)	3.9 (0.7)	30.7 (8.3)	4.9

Note:
Values are means followed by standard deviation (in parentheses).
* Grassland.

Table 5 δ^{15}N values (‰) for acacia leaves ($n = 3$), aboveground vegetation ($n = 2$ for grassland, and $n = 5$ for plantations) and soil ($n = 3$) by plantation age at the two study sites.

Site	Age (years)	Acacia leaves	Ground veg.	Soil layer (cm)			
				0–10	10–20	20–30	30–50
El Demokeya	0*	–	2.9	3.8	2.7	2.3	2.0
	15	6.5 (1.5)	3.2 (1.1)	7.9 (1.1)	6.9 (0.2)	6.8 (0.6)	5.9 (0.7)
	24	7.0 (1.5)	3.8 (1.5)	10.2 (0.9)	7.7 (0.8)	6.4 (0.8)	6.0 (0.4)
El Hemaira	0*	–	5.8	1.8	1.0	1.0	0.9
	7	8.8 (1.2)	7.9 (2.0)	7.4 (0.8)	5.4 (1.3)	4.5 (1.1)	4.3 (0.5)
	15	8.9 (0.5)	5.5 (1.7)	8.7 (0.4)	7.1 (1.3)	6.5 (1.2)	5.5 (1.7)
	20	8.0 (1.4)	6.7 (1.2)	9.1 (0.9)	7.4 (1.0)	6.9 (1.1)	6.2 (1.3)

Notes:
Values are mean values followed by standard deviation (in parentheses). Soil grassland value is for a single composite sample from one plot.
* Grassland.

Soil C:N:P ratios did not significantly differ with depth and the N:P ratios only showed significant differences between plantation age for the 0–10 cm layer (Table 4). The 0–10 cm soil layer C:N and C:P ratios did not show significant differences with age at either of the sites. At El Demokeya, the 0–10 m soil layer N:P ratio in the 24-year-old plantation was significantly ($p < 0.05$) higher than those in the grassland and 15-year-old plantation. At El Hemaira, the 0–10 cm soil layer N:P ratio in 20-year-old plantation was significantly greater ($p < 0.05$) than those in the grassland and 7-year-old plantation. Acacia leaf nutrient concentrations and N:P ratios did not show significant differences related to plantations age at either of the sites (Table 5). There were too few ground vegetation samples for statistical testing of nutrient concentrations and ratios.

DISCUSSION

In this study we aimed to determine whether the previously reported increase in SOC contents with plantation age at the two sites (*Abaker et al., 2016*) would also result in higher nutrient (N, P and K) concentrations and stocks, which would further support the importance of maintaining or increasing tree cover in the region. In an earlier paper, we showed that the increases in SOC with plantation age at the two sites resulted in increased available water capacities which then had an effect on the water balance of the plantations (*Abaker, Berninger & Starr, 2018*). Because of the pseudoreplicated design of our study, general patterns about plantation age effects may not be strictly inferred. However, given the inevitable within site variation in site conditions, the climate, soil type and topography were uniform across each site and the replicate three plots for each treatment (grassland and plantation age) were located so as otherwise to be as similar and comparable as possible. Unfortunately, documented information about land-use prior to the establishment of the plantations at the two sites was not available. However, from discussions with local staff, the *A. senegal* plantations were established on areas of homogenous abandoned grassland.

Recognising the potential limitations imposed by the pseudoreplicated design, the significant dependence of nutrient concentrations on SOC and the significantly higher N and K_{ex} stocks in the oldest plantations compared to the grasslands support our initial hypothesis that soil N, P and K are linked to SOC and are in agreement with results reported from other studies. For example, in *A. tortilis* savanna woodlands in northern Tanzania *Ludwig et al. (2004)* found increases in SOM, N, P and P_{av} concentrations with tree growth stage (grassland, under small and large trees), and *Deans et al. (1999)* working with *A. senegal* in Senegal found that N and K_{ex}, but not P concentrations, increased with plantation age. In both these studies, the soil refers to the surface layer (0–10 cm). This layer had the highest SOC contents and would therefore be expected to be the most affected by the plantations. Furthermore, in the study by *Deans et al. (1999)*, soil concentrations of N, P and K_{ex} were all significantly correlated to loss-on-ignition contents, i.e. SOC contents. *El Tahir et al. (2009)* working at El Demokeya site, reported a SOC stock value of 738 g m^{-2} for 0–30 cm layer and for total N, P_{av} and K_{ex} values of 118, 2.5 and 29 g m^{-2}, respectively. We were unable to take into account the effect of fire and grazing on soil SOC and N stocks at our study sites. However, fire has generally been found not to result in a loss of soil total N and SOC because of the superficial nature of the fires (*Coetsee, Bond & February, 2010*; *Coetsee, Jacobs & Govender, 2012*). The effect of grazing at our study sites is discussed below in relation to soil N stocks.

Compared to the grasslands, the higher N, P_{av} and K_{ex} concentrations observed in the upper soil layer of the plantations indicates a significant effect of acacia trees on ecosystem nutrient cycling, at least at our study sites. The higher concentrations in the surface layer was particularly obvious in the older plantations and can be explained by 'nutrient uplift' by the deeper roots of the acacia trees (*Scholes, 1990*; *Ludwig et al., 2004*). *Mubarak, Abdalla & Nortcliff (2012)* also concluded that tree litter input is a significant source of P and K in southern Kordofan soils and the presence of trees has been

shown to contribute to the general maintenance of soil fertility in the Sahel (*Wezel, Rajot & Herbrig, 2000*; *Schlecht et al., 2006*).

The higher N concentrations in the surface soils of the plantations may be thought to be due to N_2 fixation as acacia species are considered to be N_2 fixing (*Ludwig et al., 2004*; *Raddad et al., 2005*; *Boutton & Liao, 2010*). Although, *A. senegal* has been reported to be a N_2 fixer (*Raddad et al., 2005*; *Isaac et al., 2011*; *Githae et al., 2013*; *Gray et al., 2013*) the high $\delta^{15}N$ values we observed for acacia leaves (>6‰) would indicate that *A. senegal* did not fix N_2 or is very limited in our sites. If there had been significant N_2 fixation in the plantations then one would expect foliar $\delta^{15}N$ values to be closer to 0‰ (*Robinson, 2001*; *Aranibar et al., 2004*; *Nardoto et al., 2014*). Nevertheless, our acacia foliage $\delta^{15}N$ values are in agreement with the findings of other studies conducted in arid environments. For example, *Aranibar et al. (2004)* observed that Acacia leaves had $\delta^{15}N$ values similar to non-legume species and even higher than known N_2-fixing species in a study carried in the Kalahari Desert. *Pate et al. (1998)* reported a mean $\delta^{15}N$ value of 9.10‰ for Acacia species in arid Australia, which was identical to those of non-fixing woody species, suggesting little or absence of N fixation. In a study carried out in *A. tortilis* savanna woodlands in Kenya, *Belsky et al. (1993)* concluded that that N_2 fixation was not an important contributor of N to the soil. N_2 fixation by legume trees in drylands has been show to vary considerably, even within the same species (*Nygren et al., 2012*). For example, N_2 fixation by *A. senegal* growing on clay soil in Sudan was shown to vary from 29 to 48 kg N ha^{-1} (*Raddad et al., 2005*).

Our soil N accretion rates in the plantations appear high but are comparable to those reported by *Blaser et al. (2014)* of 1.3–2.0 g N m^{-2} yr^{-1} (for 0–10 cm layer) in Zambian savanna. However, the vegetation at their site was dominated by the N_2-fixing shrub *Dichrostachys cinerea*. As deposition loads of N in the Sahel are about 0.3–0.7 g N m^{-2} yr^{-1} (*Delon et al., 2010*), our high N accretion rates cannot be explained by deposition. The paradox between the accumulation of soil N in the absence of N_2 fixation and sufficient N deposition in humid tropical forests has been identified in several studies (see *Hedin et al., 2009*) and has been explained by heterotrophic N_2 fixation by free-living bacteria decomposing litter and SOM (*Vitousek & Hobbie, 2000*) or by canopy epiphytic N_2 fixation (*Hedin et al., 2009*). However, the rates of such N_2 fixation are low and could not explain our high soil N accretion rates. A possible source of our observed high soil N accretion rates could be from grazing animal excretion. The two study sites are not fenced and seasonal pastoral and nomadic grazing (mainly sheep) and browsing (camels), although varying, takes place throughout the study area (*Poussart, Ardö & Olsson, 2004*). Bigger trees (older plantations) may be expected to provide increased shading and ground vegetation for grazing and browsing. Animals entering the plantations may therefore have added N to the soil in the form of animal excretion derived from grazing outside and in excess of grazing removals from inside the study sites. Studies on elk and bison in north-temperate grassland indicate that herbivore excretion can add significant amounts of N to the soil (*Frank et al., 1994*). However, data on land-use history and animal herbivory at the two sites is not available and therefore this animal excretion N explanation is only speculative.

It has been shown that N-fixing trees accumulate large amount of N-rich litterfall during the first years of establishment, however once N availability has built up in the soil, N fixation may be ceased or inhibited (*Khanna, 1998*; *Boddey et al., 2000*; *Hedin et al., 2009*) and the older trees/plantations become more dependent on litterfall and N recycling (*Deans et al., 2003*). The relatively high and increasing trend in soil δ^{15}N with plantation age at our sites may be an indication of greater microbiological processing of SOM and a more open N cycle (ammonia volatilization and denitrification during the wet season) resulting in an enrichment of ^{15}N (*Aranibar et al., 2004*; *Swap et al., 2004*; *Hobbie & Ouimette, 2009*). The negative relationship observed between soil δ^{15}N and soil C:N ratios in the plantations is consistent with the notion that low soil C:N ratios in arid environments promote greater N gaseous losses (*Austin & Vitousek, 1998*; *Aranibar et al., 2004*; *Saiz et al., 2016*). The vegetation present at a given site exerts a large influence on SOM dynamics not only because of the quantity and quality of organic matter returning to the soil (*Saiz et al., 2015*), but also because of its impact on soil hydrological conditions (*Abaker, Berninger & Starr, 2018*). In this regard, trees growing on coarse-textured soils in semi-arid regions may promote the maintenance of soil water conditions suitable for the activities of SOM decomposers through the interception and funnelling of rainfall by their canopies and the reduction in soil water evaporation by shading (*Bargués Tobella et al., 2014*; *Ilstedt et al., 2016*). Two recent works have shown potentially faster SOM decomposition rates at locations dominated by trees compared to those dominated by grass vegetation in mixed C_3/C_4 systems occurring on coarse-textured soils (*Saiz et al., 2015*; *2016*). These vegetation-related factors may be responsible for the higher SOC and nutrient contents observed in our acacia plantations. The higher soil δ^{15}N values observed with plantation age is further evidence of SOM decomposition processes being comparatively more dynamic under the direct influence of trees.

Cyanobacteria associated with the formation of cryptogamic soil crusts have been shown to be a significant pathway to fix atmospheric N_2 in arid environments, but their development diminishes with vegetation cover (*Aranibar et al., 2004*; *Wang et al., 2013*). Therefore, N fixation by cyanobacterial soil crusts (which may be expected to be more strongly developed in the grasslands) may explain the low soil δ^{15}N values observed in grassland sites. However, we have no information on the presence and development of such cyanobacterial soil crusts at our sites, but in any case annual N fixation rates associated with cyanobacterial soil crusts are very low (*Aranibar et al., 2003*).

The decreasing rather than increasing trend in soil δ^{15}N with depth observed in both the grasslands and plantations is somewhat unusual (*Hobbie & Ouimette, 2009*), but it has also been observed in an arid, sandy site in the Kalahari (*Wang et al., 2013*). The variation in soil δ^{15}N values with depth are the result of multiple interacting factors, which include N inputs by plant and cryptogamic crusts, vertical transport processes (i.e. leaching, fungal immobilization and bioturbation), soil moisture conditions, and isotopically fractionating processes (e.g. ammonia volatilization and denitrification) (*Hobbie & Ouimette, 2009*; *Wang et al., 2013*; *Saiz et al., 2016*). However, as the N contents in our soils are very low resulting in a low analytical signal for ^{15}N, our soil δ^{15}N results should be interpreted with caution.

Soil C:N ratios often decrease with soil depth as a result of the SOM being older and more decomposed and therefore relatively enriched in N compared to SOC (*Batjes, 1996*; *Tian et al., 2010*). However, there was no consistent trend in C:N ratios with depth in either the grasslands or the plantations at our sites, which may be explained by gaseous losses of N as indicated by the soil δ^{15}N values and discussed above. The significantly lower soil (0–10 cm) N:P ratios in the grasslands than in the oldest plantations at our sites would indicate N limitation in the grasslands. The N:P ratios of the ground vegetation were on the lower side of values presented for savanna grasses by *Ludwig et al. (2004)* and *Sitters, Edwards & Olde Venterink (2013)*. *Ludwig et al. (2004)* considered low grass N:P ratios from open grasslands to indicate N limitation and higher values for grasses sampled from under the canopy of trees to indicate P-limiting conditions for the grasses. *Sitters, Edwards & Olde Venterink (2013)* similarly concluded that their observed increase in grass N:P ratios with tree density indicated a shift towards P-limiting conditions for the ground vegetation.

CONCLUSION

The concentrations of all studied nutrients were relatively low but directly and significantly correlated to SOC, were highest in the topsoil and increased with plantation age at our sites. Although these results are specific to our study sites, we consider these results support our hypothesis that soil N, P and K contents in the Sahel region are strongly controlled by SOM (SOC) contents. Although *A. senegal* is known to be capable of N_2 fixation and may have occurred when the trees were young, current foliar δ^{15}N values did not indicate ongoing N_2 fixation in the plantations. The soil N accretion rates observed in the plantations were unlikely to be due to N deposition but may be related to inputs of excreted N brought into the area annually by grazing and browsing animals. The relatively high surface soil N contents in the plantations at our sites were considered to be the result of litterfall and recycling. The higher total and plant available contents of P and K in the soil surface of the plantations may be an indication of 'nutrient uplift' by the deeper roots of the acacia trees. Soil N:P ratios indicated N limitation in the grasslands and a trend towards P-limitation in the plantations. Our results support the notion that an increase in SOM (SOC) contents related to the retention and preferably planting of trees in the Sahel region would not only increase carbon sequestration, but also significantly improve soil fertility.

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Frank Berninger is an Academic Editor for PeerJ.

Author Contributions
- Wafa E. Abaker conceived and designed the experiments, performed the experiments, analysed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Frank Berninger conceived and designed the experiments, authored or reviewed drafts of the paper, approved the final draft.
- Gustavo Saiz contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
- Jukka Pumpanen authored or reviewed drafts of the paper, approved the final draft.
- Mike Starr conceived and designed the experiments, analysed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.

Data Availability
The following information was supplied regarding data availability:
   The raw data are provided in a Supplemental File.

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REFERENCES
Abaker WE, Berninger F, Saiz G, Braojos V, Starr M. 2016. Contribution of *Acacia senegal* to biomass and soil carbon in plantations of varying age in Sudan. *Forest Ecology and Management* **368**:71–80 DOI 10.1016/j.foreco.2016.03.003.

Abaker WE, Berninger F, Starr M. 2018. Changes in soil hydraulic properties, soil moisture and water balance in *Acacia senegal* plantations of varying age in Sudan. *Journal of Arid Environments* **150**:42–53 DOI 10.1016/j.jaridenv.2017.12.004.

Aranibar JN, Anderson IC, Ringrose S, Macko SA. 2003. Importance of nitrogen fixation in soil crusts of Southern African arid ecosystems: acetylene reduction and stable isotope studies. *Journal of Arid Environments* **54(2)**:345–358 DOI 10.1006/jare.2002.1094.

Aranibar JN, Otter L, Macko SA, Feral CJW, Epstein HE, Dowty PR, Eckardt F, Shugart HH, Swap RJ. 2004. Nitrogen cycling in the soil-plant system along a precipitation gradient in the Kalahari sands. *Global Change Biology* **10(3)**:359–373 DOI 10.1111/j.1365-2486.2003.00698.x.

PeerJ

Ardö J, Olsson L. 2004. Soil carbon sequestration in traditional farming in Sudanese dry lands. *Environmental Management* **33(S1)**:S318–S329 DOI 10.1007/s00267-003-9141-2.

Austin AT, Vitousek PM. 1998. Nutrient dynamics on a precipitation gradient in Hawai'i. *Oecologia* **113(4)**:519–529 DOI 10.1007/s004420050405.

Ayoub AT. 1998. Extent, severity and causative factors of land degradation in the Sudan. *Journal of Arid Environments* **38(3)**:397–409 DOI 10.1006/jare.1997.0346.

Barbier EB. 1992. Rehabilitating gum arabic systems in Sudan: economic and environmental implications. *Environmental and Resource Economics* **2(4)**:341–358 DOI 10.1007/bf00304966.

Barbier EB. 2000. The economic linkages between rural poverty and land degradation: some evidence from Africa. *Agriculture, Ecosystems & Environment* **82(1–3)**:355–370 DOI 10.1016/s0167-8809(00)00237-1.

Bargués Tobella A, Reese H, Almaw A, Bayala J, Malmer A, Laudon H, Ilstedt U. 2014. The effect of trees on preferential flow and soil infiltrability in an agroforestry parkland in semiarid Burkina Faso. *Water Resources Research* **50(4)**:3342–3354 DOI 10.1002/2013WR015197.

Batjes NH. 1996. Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science* **47(2)**:151–163 DOI 10.1111/j.1365-2389.1996.tb01386.x.

Belsky AJ, Mwonga SM, Amundson RG, Duxbury JM, Ali AR. 1993. Comparative effects of isolated trees on their undercanopy environments in high-and low-rainfall savannas. *Journal of Applied Ecology* **30(1)**:143–155 DOI 10.2307/2404278.

Binkley D, Senock R, Cromack K. 2003. Phosphorus limitation on nitrogen fixation by Facaltaria seedlings. *Forest Ecology and Management* **186(1–3)**:171–176 DOI 10.1016/s0378-1127(03)00240-8.

Blaser WJ, Shanungu GK, Edwards PJ, Olde Venterink H. 2014. Woody encroachment reduces nutrient limitation and promotes soil carbon sequestration. *Ecology and Evolution* **4(8)**:1423–1438 DOI 10.1002/ece3.1024.

Blaser WJ, Sitters J, Hart SP, Edwards PJ, Olde Venterink H. 2013. Facilitative or competitive effects of woody plants on understorey vegetation depend on N fixation, canopy shape and rainfall. *Journal of Ecology* **101(6)**:1598–1603 DOI 10.1111/1365-2745.12142.

Boddey RM, Peoples MB, Palmer B, Dart PJ. 2000. Use of the ^{15}N natural abundance technique to quantify biological nitrogen fixation by woody perennials. *Nutrient Cycling in Agroecosystems* **57**:235–270.

Bourlière F, Hadley M. 1983. Present-day savannas: an overview. In: Bourlière F, ed. *Ecosystems of the World. 13. Tropical Savannas*. Amsterdam: Elsevier, 17.

Boutton TW, Liao JD. 2010. Changes in soil nitrogen storage and δ^{15}N with woody plant encroachment in a subtropical savanna parkland landscape. *Journal of Geophysical Research* **115(G3)**:G03019 DOI 10.1029/2009jg001184.

Bui EN, Henderson BL. 2013. C:N:P stoichiometry in Australian soils with respect to vegetation and environmental factors. *Plant and Soil* **373(1–2)**:553–568 DOI 10.1007/s11104-013-1823-9.

Coetsee C, Bond WJ, February EC. 2010. Frequent fire affects soil nitrogen and carbon in an African savanna by changing woody cover. *Oecologia* **162(4)**:1027–1034 DOI 10.1007/s00442-009-1490-y.

Coetsee C, Jacobs S, Govender N. 2012. An overview of nitrogen cycling in a semiarid savanna: some implications for management and conservation in a large African park. *Environmental Management* **49(2)**:387–402 DOI 10.1007/s00267-011-9779-0.

Deans JD, Diagne O, Lindley DK, Dione M, Parkinson JA. 1999. Nutrient and organic-matter accumulation in *Acacia senegal* fallows over 18 years. *Journal of Arid Environments* **59**:499–510.

Deans JD, Diagne O, Nizinski J, Lindley DK, Seck M, Ingleby K, Munro RC. 2003. Comparative growth, biomass production, nutrient use and soil amelioration by nitrogen fixing-tree species in semi-arid Senegal. *Forest Ecology and Management* **176(1–3)**:253–264 DOI 10.1016/s0378-1127(02)00296-7.

Delon C, Galy-Lacaux C, Boone A, Liousse C, Serça D, Adon M, Diop B, Akpo A, Lavenu F, Mougin E, Timouk F. 2010. Atmospheric nitrogen budget in Sahelian dry savannas. *Atmospheric Chemistry and Physics* **10(6)**:2691–2708 DOI 10.5194/acp-10-2691-2010.

Dewis J, Freitas F. 1970. *Physical and Chemical Methods of Soil and Water Analysis. FAO Soils Bulletin 10.* Rome: FAO.

Dohn J, Dembélé F, Karembé M, Moustakas A, Amévor KA, Hanan NP. 2013. Tree effects on grass growth in savannas: competition, facilitation and the stress-gradient hypothesis. *Journal of Ecology* **101(1)**:202–209 DOI 10.1111/1365-2745.12010.

Dregne HE. 1976. *Soils of Arid Regions.* Amsterdam: Elsevier, 237.

El Tahir BA, Ahmed DM, Ardö J, Gaafar AM, Salih AA. 2009. Changes in soil properties following conversion of *Acacia senegal* plantation to other land management systems in North Kordofan State, Sudan. *Journal of Arid Environments* **73(4–5)**:499–505 DOI 10.1016/j.jaridenv.2008.11.007.

Food and Agriculture Organization of the United Nations (FAO). 2001. *Soil carbon sequestration for improved land management. World Soil Resources Reports 96.* Rome: FAO. *Available at http://www.fao.org/3/a-bl001e.pdf.*

Food and Agriculture Organization of the United Nations (FAO). 2004. *Carbon sequestration in dryland soils. World Soil Resources Reports 102.* Rome: FAO. *Available at http://www.fao.org/3/a-Y5738e.pdf.*

Food and Agriculture Organization of the United Nations (FAO). 2006. Global forest resources assessment 2005. *Estimating Forest Cover and Forest Cover Change in Sudan.* Working paper 109/E. Rome: FAO. *Available at http://www.fao.org/docrep/008/a0400e/a0400e00.htm.*

Finzi AC, Austin AT, Cleland EE, Frey SD, Houlton BZ, Wallenstein MD. 2011. Responses and feedbacks of coupled biogeochemical cycles to climate change: examples from terrestrial ecosystems. *Frontiers in Ecology and the Environment* **9(1)**:61–67 DOI 10.1890/100001.

Frank DA, Inouye RS, Huntly N, Minshall GW, Anderson JE. 1994. The biogeochemistry of a north-temperate grassland with native ungulates: nitrogen dynamics in Yellowstone National Park. *Biogeochemistry* **26(3)**:163–188 DOI 10.1007/bf00002905.

Githae EW, Gachene CKK, Njoka JT. 2011. Soil physicochemical properties under *Acacia senegal* varieties in the dryland areas of Kenya. *African Journal of Plant Science* **5(8)**:475–482.

Githae EW, Gachene CKK, Njoka JT, Omondi SF. 2013. Nitrogen fixation by natural populations of *Acacia senegal* in the drylands of Kenya using ^{15}N natural abundance. *Arid Land Research and Management* **27(4)**:327–336 DOI 10.1080/15324982.2013.784377.

Gray A, Odee D, Cavers S, Wilson J, Telford A, Grant F, Diouf M, Ochieng J, Grant H, Stott A. 2013. Does geographic origin dictate ecological strategies in *Acacia Senegal* (L.) Willd.? Evidence from carbon and nitrogen stable isotopes. *Plant and Soil* **369(1–2)**:479–496 DOI 10.1007/s11104-013-1593-4.

Hagos MG, Smit GN. 2005. Soil enrichment by *Acacia mellifera* subsp. detinens on nutrient poor sandy soil in a semi-arid southern African savanna. *Journal of Arid Environments* **61(1)**:47–59 DOI 10.1016/j.jaridenv.2004.08.003.

Hedin LO, Brookshire ENJ, Menge DNL, Barron AR. 2009. The nitrogen paradox in tropical forest ecosystems. *Annual Review of Ecology Evolution and Systematics* **40(1)**:613–635 DOI 10.1146/annurev.ecolsys.37.091305.110246.

Hobbie EA, Ouimette AP. 2009. Controls of nitrogen isotope patterns in soil profiles. *Biogeochemistry* **95(2–3)**:355–371 DOI 10.1007/s10533-009-9328-6.

Holdo RM, Holt RD, Coughenour MB, Ritchie ME. 2007. Plant productivity and soil nitrogen as a function of grazing, migration and fire in an African savanna. *Journal of Ecology* **95(1)**:115–128 DOI 10.1111/j.1365-2745.2006.01192.x.

Hurlbert SH. 1984. Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* **54(2)**:187–211 DOI 10.2307/1942661.

Ilstedt U, Bargués Tobella A, Bazié HR, Bayala J, Verbeeten E, Nyberg G, Sanou J, Benegas L, Murdiyarso D, Laudon H, Sheil D, Malmer A. 2016. Intermediate tree cover can maximize groundwater recharge in the seasonally dry tropics. *Scientific Reports* **6(1)**:21930 DOI 10.1038/srep21930.

Isaac ME, Harmand JM, Lesueur D, Lelon J. 2011. Tree age and soil phosphorus conditions influence N_2-fixation rates and soil N dynamics in natural populations of *Acacia senegal*. *Forest Ecology and Management* **261(3)**:582–588 DOI 10.1016/j.foreco.2010.11.011.

Jakubaschk C. 2002. *Acacia senegal*, soil organic carbon and nitrogen contents: a study in North Kordofan, Sudan. M.Sc. thesis. Lund: Department of Physical Geography and Ecosystems Analysis, Lund University.

Jobbágy EG, Jackson RB. 2001. The distribution of soil nutrients with depth: global patterns and the imprint of plants. *Biogeochemistry* **53(1)**:51–77 DOI 10.1023/a:1010760720215.

Kaya B, Hildebrand PE, Nair PKR. 2000. Modeling changes in farming systems with the adoption of improved fallows in southern Mali. *Agricultural Systems* **66(1)**:51–68 DOI 10.1016/s0308-521x(00)00036-6.

Khanna PK. 1998. Nutrient cycling under mixed-species tree systems in Southeast Asia. *Agroforest Systems* **38**:99–120.

Koohafkan P, Stewart BA. 2008. *Water and Cereals in Drylands*. Malta: FAO.

Lajtha K, Schlesinger WH. 1988. The biogeochemistry of phosphorus cycling and phosphorus availability along a desert soil chronosequence. *Ecology* **69(1)**:24–39 DOI 10.2307/1943157.

Lal R. 2004a. Carbon sequestration in dryland ecosystems. *Environmental Management* **33(4)**:528–544 DOI 10.1007/s00267-003-9110-9.

Lal R. 2004b. Soil carbon sequestration to mitigate climate change. *Geoderma* **123(1–2)**:1–22 DOI 10.1016/j.geoderma.2004.01.032.

Lee J, Hopmans JW, Rolston DE, Baer SG, Six J. 2009. Determining soil carbon stock changes: simple bulk density corrections fail. *Agriculture, Ecosystems & Environment* **134(3–4)**:251–256 DOI 10.1016/j.agee.2009.07.006.

Ludwig F, de Kroon H, Berendse F, Prins HHT. 2004. The influence of savanna trees on nutrient, water and light availability and the understorey vegetation. *Plant Ecology* **170(1)**:93–105 DOI 10.1023/b:vege.0000019023.29636.92.

Moustakas A, Kunin WE, Cameron TC, Sankaran M. 2013. Facilitation or competition? Tree effects on grass biomass across a precipitation gradient. *PLOS ONE* **8(2)**:e57025 DOI 10.1371/journal.pone.0057025.

Mubarak AR, Abdalla MH, Nortcliff S. 2012. Millet (*Pennisetum typhoides*) yield and selected soil attributes as influenced by some tree types of the semi-arid tropics of Sudan. *Journal of Arid Environments* **77**:96–102 DOI 10.1016/j.jaridenv.2011.10.008.

Nardoto GB, Quesada CA, Patiño S, Saiz G, Baker TR, Schwarz M, Schrodt F, Feldpausch TR, Domingues TF, Marimon BS, Marimon Junior BH, Vieira ICG, Silveira M, Bird MI, Phillips OL, Lloyd J, Martinelli LA. 2014. Basin-wide variations in Amazon forest

nitrogen-cycling characteristics as inferred from plant and soil ^{15}N:^{14}N measurements. *Plant Ecology & Diversity* **7(1–2)**:173–187 DOI 10.1080/17550874.2013.807524.

Nkonya E, Johnson T, Kwon HY, Kato E. 2015. Economics of Land Degradation in Sub-Saharan Africa. In: Nkonya E, Mirzabaev A, von Braun J, eds. *Economics of Land Degradation and Improvement—A Global Assessment for Sustainable Development.* Washington, D.C.: International Food Policy Research Institute. Center for Development Research, University of Bonn: Germany, Ch. 9, 215–259.

Nygren P, Fernández MP, Harmand J-M, Leblanc HA. 2012. Symbiotic dinitrogen fixation by trees: an underestimated resource in agroforestry systems? *Nutrient Cycling in Agroecosystems* **94(2–3)**:123–160 DOI 10.1007/s10705-012-9542-9.

Obeid M, Seif El Din A. 1970. Ecological studies of the vegetation of the Sudan. I. *Acacia senegal* (L.) Willd. and its natural regeneration. *Journal of Applied Ecology* **7(3)**:507–518 DOI 10.2307/2401975.

Olsson L, Ardö J. 2002. Soil carbon sequestration in degraded semiarid agro-ecosystems-perils and potentials. *AMBIO: A Journal of the Human Environment* **31(6)**:471–477 DOI 10.1639/0044-7447(2002)031[0471:scsids]2.0.co;2.

Pate JS, Unkovich MJ, Erskine PD, Stewart GR. 1998. Australian mulga ecosystems–^{13}C and ^{15}N natural abundances of biota components and their ecophysiological significance. *Plant, Cell and Environment* **21(12)**:1231–1242 DOI 10.1046/j.1365-3040.1998.00359.x.

Pellegrini AFA, Hedin LO, Staver AC, Govender N. 2015. Fire alters ecosystem carbon and nutrients but not plant nutrient stoichiometry or composition in tropical savanna. *Ecology* **96(5)**:1275–1285 DOI 10.1890/14-1158.1.

Peri PL, Ladd B, Pepper DA, Bonser SP, Laffan SW, Amelung W. 2012. Carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope composition in plant and soil in Southern Patagonia's native forests. *Global Change Biology* **18(1)**:311–321 DOI 10.1111/j.1365-2486.2011.02494.x.

Poussart JN, Ardö J, Olsson L. 2004. Effects of data uncertainties on estimated soil organic carbon in the Sudan. *Environmental Management* **33(S1)**:S405–S415 DOI 10.1007/s00267-003-9148-8.

Raddad EY, Luukkanen O, Salih AA, Kaarakka V, Elfadl MA. 2006. Productivity and nutrient cycling in young *A. senegal* farming systems on Vertisol in the Blue Nile region, Sudan. *Agroforestry Systems* **68(3)**:193–207 DOI 10.1007/s10457-006-9009-6.

Raddad EAY, Salih AA, El Fadl MA, Kaarakka V, Luukkanen O. 2005. Symbiotic nitrogen fixation in eight *Acacia senegal* provenances in dryland clays of the Blue Nile Sudan estimated by the ^{15}N natural abundance method. *Plant and Soil* **275(1–2)**:261–269 DOI 10.1007/s11104-005-2152-4.

Robinson D. 2001. δ^{15}N as an integrator of the nitrogen cycle. *Trends in Ecology & Evolution* **16(3)**:153–162 DOI 10.1016/s0169-5347(00)02098-x.

Saiz G, Bird MI, Wurster CM, Quesada CA, Ascough PL, Domingues TF, Schrodt F, Schwarz M, Feldpausch TR, Veenendaal EM, Djagbletey G, Jacobsen G, Hien F, Compaore H, Diallo A, Lloyd J. 2015. The influence of C_3 and C_4 vegetation on soil organic matter dynamics in contrasting semi-natural tropical ecosystems. *Biogeosciences* **12(16)**:5041–5059 DOI 10.5194/bg-12-5041-2015.

Saiz G, Wandera FM, Pelster DE, Ngetich W, Okalebo JR, Rufino MC, Butterbach-Bahl K. 2016. Long-term assessment of soil and water conservation measures (Fanya-juu terraces) on soil organic matter in South Eastern Kenya. *Geoderma* **274**:1–9 DOI 10.1016/j.geoderma.2016.03.022.

Sanchez PA. 1999. Improved fallow comes of age in the tropics. *Agroforestry Systems* **47**:3–12.

Schlecht E, Buerkert A, Tielkes E, Bationo A. 2006. A critical analysis of challenges and opportunities for soil fertility restoration in Sudano-Sahelian West Africa. *Nutrient Cycling in Agroecosystems* **76(2–3)**:109–136 DOI 10.1007/s10705-005-1670-z.

Scholes RJ. 1990. The influence of soil fertility on the ecology of Southern African dry savannas. *Journal of Biogeography* **17(4/5)**:415–419 DOI 10.2307/2845371.

Schulze ED, Farquhar GD, Miller JM, Schulze W, Walker B, Williams RJ. 1999. Interpretation of increased foliar d15N in woody species along a rainfall gradient in northern Australia. *Australian Journal of Plant Physiology* **26**:296–298.

Sitters J, Edwards PJ, Olde Venterink H. 2013. Increases of soil C, N, and P pools along an Acacia tree density gradient and their effects on trees and grasses. *Ecosystems* **16(2)**:347–357 DOI 10.1007/s10021-012-9621-4.

Swap RJ, Aranibar JN, Dowty PR, Gilhooly WP, Macko SA. 2004. Natural abundance of ^{13}C and ^{15}N in C_3 and C_4 vegetation of southern Africa: patterns and implications. *Global Change Biology* **10(3)**:350–358 DOI 10.1111/j.1365-2486.2003.00702.x.

Tan KH. 2005. *Soil Sampling, Preparation, and Analysis.* Second edition. Boca Raton: Taylor and Francis, 680.

Tian H, Chen G, Zhang C, Melillo JM, Hall CAS. 2010. Pattern and variation of C:N:P ratios in China's soils: a synthesis of observational data. *Biogeochemistry* **98(1–3)**:139–151 DOI 10.1007/s10533-009-9382-0.

Tiessen H, Cuevas E, Chacon P. 1994. The role of soil organic matter in sustaining soil fertility. *Nature* **371(6500)**:783–785 DOI 10.1038/371783a0.

Torello-Raventosa M, Feldpausch TR, Veenendaal E, Schrodt F, Saiz G, Domingues TF, Djagbletey G, Ford A, Kemp J, Marimon BS, Marimon Junior BH, Lenza E, Ratter JA, Maracahipes L, Sasaki D, Sonk B, Zapfack L, Taedoumg H, Villarroel D, Schwarz M, Quesada CA, Ishida FY, Nardoto GB, Affum-Baffoe K, Arroyo L, Bowman DMJS, Compaore H, Davies K, Diallo A, Fyllas NM, Gilpin M, Hien F, Johnson M, Killeen TJ, Metcalfe D, Miranda HS, Steininger M, Thomson J, Sykora K, Mougin E, Hiernaux P, Bird MI, Grace J, Lewis SL, Phillips OL, Lloyd J. 2013. On the delineation of tropical vegetation types with an emphasis on forest/savanna transitions. *Plant Ecology & Diversity* **6(1)**:101–137 DOI 10.1080/17550874.2012.762812.

Vitousek PM, Cassman K, Cleveland C, Crews T, Field CB, Grimm NB, Howarth RW, Marino R, Martinelli L, Rastetter EB, Sprent JI. 2002. Towards an ecological understanding of biological nitrogen fixation. *Biogeography* **57/58**:1–45.

Vitousek PM, Hobbie S. 2000. Heterotrophic nitrogen fixation in decomposing litter: patterns and regulation. *Ecology* **81(9)**:2366–2376 DOI 10.2307/177460.

Wang L, Okin GS, D'Odorico P, Caylor KK, Macko SA. 2013. Ecosystem-scale spatial heterogeneity of stable isotopes of soil nitrogen in African savannas. *Landscape Ecology* **28(4)**:685–698.

Wezel A, Rajot JL, Herbrig C. 2000. Influence of shrubs on soil characteristics and their function in Sahelian agro-ecosystems in semi-arid Niger. *Journal of Arid Environments* **44(4)**:383–398 DOI 10.1006/jare.1999.0609.