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

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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 N and 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 (N), phosphorus (P) and potassium (K) in relation to SOC, the potential of A. senegal for N\textsubscript{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 in 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\textsubscript{2} fixation in relation to plantations age was assessed using \(\delta^{15}\text{N}\) 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 \(\delta^{15}\text{N}\) 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 \(\delta^{15}\text{N}\) values indicated that N\textsubscript{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.
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Abstract

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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 δ\textsuperscript{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 $\delta^{15}$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.
1. 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\textsubscript{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-Raventos et al., 2013). The importance of the facilitative mechanisms (relative to competition) of trees in
tree-grass systems has been reported to be greater 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, 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 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).

N₂ fixation can increase soil N contents (Ludwig et al., 2004; Blaser et al., 2013). However, N₂ 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 (Drenge, 1976) or calcium (Lajtha & Schlesinger, 1988). The abundance of stable N isotopes (δ¹⁵N) of leaves and, to a lesser extent, soils can be used to assess N₂ fixation and indicate patterns of ecosystem N cycling (Boddey et al., 2000; Aranibar et al., 2004; Peri et al., 2012). Low foliar and soil δ¹⁵N values indicate biological N₂ fixation (Schulze et al., 1991; Robinson, 2001), while the enrichment of soil ¹⁵N 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 ecological benefits, such as fodder for animals, fuelwood, and
charcoal and economic benefits mainly from gum arabic production (Barbier, 1992). Gum arabic
is an exudate collected from *A. senegal* trees and widely used as an emulsifiers 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 and 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 \( \text{N}_2 \) fixation using foliar \( \delta^{15}\text{N} \) 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 \( \text{A. senegal} \) 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.

2. Material and Methods

2.1 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 1.

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
Drainage was higher in 2011 than in 2012, and somewhat less at El Hemaira (ranging from 0 to 123 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 is given in the Supplementary material 2. 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).

### 2.2 Experimental design, sample plots and sampling

The plantations were 15 and 24-years-old (in 2011) at El Demokeya and 7, 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 x 50 m at El Demokeya and 30 x 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 plots were used for the grasslands as it was easier to delineate in the field and to carry out the sampling. Although constituting a pseudo-replication experimental design (Hurlbert 1984), which limits the generality of our results concerning general 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 x 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.

2.3 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 hours 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 2 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, 1 g soil) in concentrated HNO₃ acid (10 ml + 1 ml H₂O₂) 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, 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 1 M ammonium acetate (pH 7.0) and concentrations determined by flame-photometer, both following FAO guidelines (Dewis and 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 (CF-IRMS) 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).

2.4 Calculation of soil stocks and accretion rates

SOC 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:

\[
\text{Stocks} = \text{soil concentration} \times \text{BD} \times \text{T} \times 100
\]

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.
2.5 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 (ANOVA) 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. Armonk, NY, IBM Corp.).

3. 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 ≤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 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 for El Hemaira than El Demokeya. In case of the $P_{av}$ 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 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 3. 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 the $P_{av}$ 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 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 $\delta^{15}N$ values were generally lower than corresponding plantation $\delta^{15}N$ values. Acacia foliar $\delta^{15}N$ 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 $\delta^{15}N$ values increased with
plantation age and decreased with depth at both study sites, but these trends were not significant 
(p >0.05). Plantation soil δ¹⁵N values were significantly correlated to soil C:N ratios, but the 
relationship for grasslands was clearly different (Fig. 4).

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.

4. 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 to be as otherwise 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 forests were established on areas of homogenous abandoned grassland and at various times areas within them planted with *Acacia senegal*.

Recognising the potential limitations imposed by the pseudoreplicated design, the significant dependence of nutrient concentrations on SOC and the significantly higher N and K\textsubscript{ex} stocks in the oldest plantations compared to the grasslands found in this study nevertheless 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\textsubscript{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\textsubscript{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\textsubscript{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\textsuperscript{-2} for 0-30 cm layer and for total N, P\textsubscript{av} and K\textsubscript{ex} values of 118, 2.5 and 29 g m\textsuperscript{-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\textsubscript{2} fixation as acacia species are considered to be N\textsubscript{2} fixing (Ludwig et al., 2004; Raddad et al., 2005; Boutton & Liao, 2010). Although, A. senegal has been reported to be a N\textsubscript{2} fixer (Raddad et al., 2005; Isaac et al., 2011; Githae et al., 2013; Gray et al., 2013) the high δ\textsuperscript{15}N values we observed for acacia leaves (> 6 ‰) would indicate that A. senegal did not fix N\textsubscript{2} or is very limited in our sites. If there had been significant N\textsubscript{2} fixation in the plantations then one would expect foliar δ\textsuperscript{15}N values to be closer to 0 ‰ (Robinson, 2001; Aranibar et al., 2004; Nardoto et al., 2014). Nevertheless, our acacia foliage δ\textsuperscript{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 δ\textsuperscript{15}N values similar to non-legume species and even higher than known N\textsubscript{2}-fixing species in a study carried in the Kalahari Desert. Pate et al. (1998) reported a mean δ\textsuperscript{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 Acacia tortilis savanna woodlands in Kenya, Belsky et al. (1993) concluded that that N\textsubscript{2} fixation was not an important contributor of N to the soil. N\textsubscript{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 with 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 $\delta^{15}$N with plantation age at our sites is 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 $\delta^{15}$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 $\delta^{15}$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\textsubscript{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 \(\delta^{15}N\) 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 \(\delta^{15}N\) with depth observed in both the grasslands and plantations is somewhat unusual (Hobbie & Ouimette, 2009), but it has also been shown during the wet season in an arid, sandy site in the Kalahari (Wang et al., 2013). The variation in soil \(\delta^{15}N\) 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 \(\delta^{15}N\) 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 \(\delta^{15}N\) 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, however, 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 the 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 the increase in grass N:P ratios with tree density indicates a shift towards P-limiting conditions for the ground vegetation.

5. Conclusions

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 $\delta^{15}$N values did not indicate ongoing N$_2$ fixation in the plantations. The soil N accretion rates observed in the plantations was 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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**References**
Abaker, W.E., 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.

Abaker, W.E., Berninger, F., Starr, M. 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.

Aranibar, J.N, Anderson, I.C., Ringrose, S., Macko, S.A., 2003. Importance of nitrogen fixation in soil crusts of Southern African arid ecosystems: acetylene reduction and stable isotope studies. Journal of Arid Environments 54, 345–358.

Aranibar, J.N., Otter, L., Macko, S.A., Feral, C.J. W., Epstein, H.E., Dowty, P.R., Eckardt, F., Shugart, H.H., Swap, R.J., 2004. Nitrogen cycling in the soil-plant system along a precipitation gradient in the Kalahari sands, Global Change Biology 10, 359–373.

Ardö, J., Olsson, L., 2004. Soil carbon sequestration in traditional farming in Sudanese dry lands. Environmental Management 33(1), 318–329.

Austin, A.T., Vitousek, P., 1998. Nutrient dynamics on a precipitation gradient in Hawai’i, Oecologia 113, 519–529.

Ayoub, A.T., 1998. Extent, severity and causative factors of land degradation in the Sudan. Journal of Arid Environments 38, 397–409.

Barbier, E.B., 1992. Rehabilitating gum arabic systems in Sudan: economic and environmental implications. Environmental and Resource Economics 2, 341–358.

Barbier, E.B., 2000. The economic linkages between rural poverty and land degradation: some evidence from Africa. Agriculture Ecosystems and Environment 82, 355-370.
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, 3342–3354. doi:http://dx.doi.org/10.1002/2013WR015197.

Batjes, N.H., 1996. Total carbon and nitrogen in the soils of the world. European Journal of Soil Sciences 47, 151–163.

Belsky, A.J., Mwonga, S.M., Amundson, R.G., Duxbury, J.M., Ali, A.R., 1993. Comparative effects of isolated trees on their undercanopy environments in high- and low-rainfall savannas. Journal of Applied Ecology 30, 143–155.

Binkley, D., Senock, R., Cromack, K., 2003. Phosphorus limitation on nitrogen fixation by Facaltaria seedlings. Forest Ecology and Management 186, 171–176.

Blaser, W.J., Sitters, J., Hart, S.P., Edwards, P.J., 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, 1598–1603.

Blaser, W.J., Shanungu, G.K., Edwards, P.J., 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

Boddey, R.M, Peoples, M.B., Palmer, B., Dart, P.J., 2000. Use of the $^{15}$N natural abundance technique to quantify biological nitrogen fixation by woody perennials. Nutrient Cycling in Agroecosystems 57, 235–70.

Bourlière, F., Hadley M., 1983. Present-day savannas: an overview. In: Ecosystems of the World. 13. Tropical Savannas, F. Bourlière. (Ed.), Elsevier, Amsterdam, 17 pp.
Boutton, T.W., Liao, J.D., 2010. Changes in soil nitrogen storage and δ¹⁵N with woody plant encroachment in a subtropical savanna parkland landscape. Journal of Geophysical Research 115:G03019.

Bui, E.N., Henderson, B.L., 2013. C:N:P stoichiometry in Australian soils with respect to vegetation and environmental factors. Plant Soil 373, 553–568.

Coetsee, C., Bond, W.J., February, E.C., 2010. Frequent fire affects soil nitrogen and carbon in an African savanna by changing woody cover. Oecologia 162, 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, 387–402.

Deans, J.D., Diagne, O., Lindley, D.K., Dione, M., Parkinson, J.A., 1999. Nutrient and organic-matter accumulation in Acacia senegal fallows over 18 years. Journal of Arid Environments 59, 499-510.

Deans, J.D., Diagne, O., Nizinski, J., Lindley, D.K., Seek, M., Ingleby, K., Munro, R.C., 2003. Comparative growth, biomass production, nutrient use and soil amelioration by nitrogen fixing-tree species in semi-arid Senegal. Forest Ecology Management. 176, 253-264.

Delon, C., Galy-Lacaux, C., Boone, A., Lioussse, C., Serça, D., Adon, M., Diop, B., Akpo, A., Lavenu, F., Mougim, E., Timouk, F., 2010. Atmospheric nitrogen budget in Sahelian dry savannas. Atmospheric Chemistry and Physics 10, 2691-2708.

Dewis, J. and Freitas, F., 1970. Physical and chemical methods of soil and water analysis. FAO Soils Bulletin 10. FAO, Rome.
Dohn, J., Dembéle, F., Karembé, M., Moustakas, A., Amévor, K.A., Hanan, N.P., 2013. Tree effects on grass growth in savannas: competition, facilitation and the stress-gradient hypothesis. Journal of Ecology 101, 202-209.

Dregne, H.E., 1976. Soils of Arid Regions. Elsevier, Amsterdam. pp. 237.

El Tahir, B.A., Ahmed, D.M., Ardö, J., Gaafar, A.M., Salih, A.A., 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, 499-505.

FAO 2001. Soil carbon sequestration for improved land management. World Soil Resources Reports 96. FAO, Rome.

FAO, 2004. Carbon sequestration in dryland soils. World Soil Resources Reports 102. Rome, Italy.

FAO, 2006. Global forest resources assessment 2005. Estimating forest cover and forest cover change in Sudan. Working paper 109/E. FAO, Rome.

Finzi, A.C., Austin, A.T., Cleland, E.E., Frey, S.D., Houlton, B.Z., Wallenstein, M.D., 2011. Coupled biochemical cycles: responses and feedbacks of coupled biogeochemical cycles to climate change. Examples from terrestrial ecosystems. Frontiers in Ecology and Environment 9, 61–67.

Frank, D.A., Inouye, R.S., Huntly, N., Minshall, G.W., Anderson, J.E., 1994. The biogeochemistry of a north-temperate grassland with native ungulates: nitrogen dynamics in Yellowstone National Park. Biogeochemistry 26: 163–188.

Githae, E.W, Gachene, C. K.K, Njoka, J.T., 2011. Soil physicochemical properties under Acacia senegal varieties in the dryland areas of Kenya. African Journal of Plant Science 5(8), 475-482.
Githae, E.W., Gachene, C.K.K., Njoka, J.T., Omondi, S.F., 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, 327-336.

Gray, A., David, O., 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, 479–496.

Hagos, M.G., Smit, G.N., 2005. Soil enrichment by *Acacia mellifera* subsp. detinens on nutrient poor sandy soil in a semi-arid southern African savanna. Journal of Arid Environment 61, 47–59.

Hedin, L.O., Brookshire, E.N.J., Menge, D.N.L., Barron, A.R., 2009. The nitrogen paradox in tropical forest ecosystems. Annual Review of Ecology, Evolution and Systematics 40, 613–635.

Hurlbert, S.H., 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54, 187-211.

Hobbie, E.A., Ouimette, A.P., 2009. Controls of nitrogen isotope patterns in soil profiles. Biogeochemistry 95, 355–371.

Holdo, R.M., Holt, R.D., Coughenour, M.B., Ritchie, M.E., 2007. Plant productivity and soil nitrogen as a function of grazing, migration and fire in an African savanna. Journal of Ecology 95, 115–128.

Ilstedt, U., Bargués Tobella, A., Bazié, H.R., 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, 21930.

Isaac, M.E, Harmand, J.M., Lesueur, D., Lelon, J., 2011. Tree age and soil phosphorus conditions influence N₂-fixation rates and soil N dynamics in natural populations of Acacia senegal. Forest Ecology Management 261, 582–588.

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

Jobbágy, E.G, Jackson, R.B., 2001. The distribution of soil nutrients with depth: global patterns and the imprint of plants. Biogeochemistry 53, 51–77.

Kaya, B., Hildebrand, P.E., Nair, P.K., 2000. Modeling changes in farming systems with the adoption of improved fallows in southern Mali. Agricultural Systems 66, 51-68.

Khanna, P.K., 1998 Nutrient cycling under mixed-species tree systems in Southeast Asia. Agroforest Systems 38, 99–120.

Koohafkan, P., Stewart, B.A., 2008. Water and Cereals in Drylands. FAO, Malta, Italy.

Lajtha, K., Schlesinger, W.H., 1988. The biogeochemistry of phosphorus cycling and phosphorus availability along a desert soil chronosequence. Ecology 69, 24–39.

Lal, R., 2004a. Carbon Sequestration in Dryland Ecosystems. Environmental Management 33(4), 528–544.

Lal, R., 2004b. Soil carbon sequestration to mitigate climate change. Geoderma 123, 1–22.
Lee, J., Hopmans, J.W., Rolston, D.E., Baer, S.G., Six, J., 2009. Determining soil carbon stock changes: Simple bulk density corrections fail. Agriculture, Ecosystems and Environment 134, 251-256.

Ludwig, F., de Kroon, H., Berendse, F., Prins, H.H.T., 2004. The influence of savanna trees on nutrient, water and light availability and the understorey vegetation. Plant Ecology 170, 93–105.

Moustakas, A., Kunin, W.E., Cameron, T.C., Sankaran, M., 2013. Facilitation or competition? Tree effects on grass biomass across a precipitation gradient. PLoS ONE, 8(2), e57025.

Mubarak, A.R., Abdalla, M.H. 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.

Nardoto, G.B., Quesada, C.A., Patiño, S., Saiz, G., Baker, T.R., Schwarz, M., Schrodt, F., Feldpausch, T.R., Domingues, T.F., Marimon, B.S., Marimon Junior, B.H., Vieira, I.C.G., Silveira, M., Bird, M.I., Phillips, O.L., Lloyd, J., Martinelli, L.A., 2014. Basin-wide variations in Amazon forest nitrogen-cycling characteristics as inferred from plant and soil $^{15}\text{N}:^{14}\text{N}$ measurements. Plant Ecology and Diversity 7, 173–187.

Nkonya, E., Johnson, T., Kwon; H.Y., Kato, E., 2015. Economics of Land Degradation in Sub-Saharan Africa. In Economics of land degradation and improvement – a global assessment for sustainable development, Nkonya, E., Mirzabaev, A., von Braun, J (eds). International Food Policy Research Institute, Washington, DC, USA, Center for Development Research, University of Bonn: Germany, Ch. 9; 215–259.
Nygren, P., Fernández, M.P., Harmand, J-M., Leblanc, H.A., 2012. Symbiotic dinitrogen fixation by trees: an underestimated resource in agroforestry systems? Nutrient Cycling in Agroecosystems 94, 123–160.

Olsson, L., Ardö, J., 2002. Soil carbon sequestration in degraded semiarid agro-ecosystems - perils and potentials. Ambio 31, 471–477.

Pate, J.S., Unkovich, M.J., Erskine, P.D., Stewart G.R., 1998. Australian mulga ecosystems –$^{13}$C and $^{15}$N natural abundances of biota components and their ecophysiological significance. Plant, Cell and Environment 21, 1231–1242.

Pellegrini, A.F.A., Hedin, L.O., Staver, A.C., Govender, N., 2015. Fire alters ecosystem carbon and nutrients but not plant nutrient stoichiometry or composition in tropical savanna. Ecology 96, 1275–1285.

Peri, P.L., Ladd, B., Pepper, D.A., Bonser, S.P., Laffan, S.W., Amelung, W., 2012. Carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) stable isotope composition in plant and soil in Southern Patagonia's native forests. Global Change Biology 18, 311–321.

Poussart, J.N., Ardö, J., Olsson, L., 2004. Effects of data uncertainties on estimated soil organic carbon in the Sudan. Environmental Management 33(1), 405–415.

Obeid, M., Seif El Din, A., 1970. Ecological studies of vegetation of Sudan.I. Acacia-senegal (L.) Willd. And its natural regeneration. Journal of Applied Ecology 7, 507-518.

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, 261–269.
Raddad, E.Y., Luukkanen, O., Sali, A.A., Kaarakka, V., El Fadl, M.A., 2006. Productivity and nutrient cycling in young *A. senegal* farming systems on Vertisol in the Blue Nile region. Sudan. Agroforestry Systems. 68, 193–207.

Robinson, D., 2001. $\delta^{15}$N as an integrator of the nitrogen cycle, Trends in Ecology and Evolution 16(3), 153–162.

Saiz, G., Bird, M.I., Wurster, C.M., Quesada, C.A., Ascough, P.L., Domingues, T.F., Schrodt, F., Schwarz, M., Feldpausch, T.R., Veenendaal, E.M., 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, 5041-5059.

Saiz, G., Wandera, F.M., Pelster, D.E., Ngetich, W., Okalebo, J.R., Rufino, M.C., 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.

Sanchez, P.A., 1999. Improved fallow comes of age in the tropics. Agroforestry Systems 47, 3–12.

Schlecht, E., Buerkert, M., 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, 109-136.

Scholes, R.J., 1990. The influence of soil fertility on the ecology of African savannas. Journal of Biogeography 17, 415-419.
Schulze, E.D., Farquhar, G.D., Miller, J.M., Schulze, W., Walker, B., Williams, R.J., 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, P.J., 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, 347–357.

Swap, R.J., Aranibar, J.N., Dowty, P.R., Gilhooly, W.P., Macko, S.A., 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, 350–358. doi: 10.1046/j.1529-8817.2003.00702.x

Tan, K.H., 2005. Soil Sampling, Preparation, and Analysis. Second Edition. Boca Raton, Florida, USA: Taylor and Francis, pp. 680.

Tian, H., Chen, G., Zhang, C., Melillo, J.M., Hall, C.A., 2010. Pattern and variation of C: N: P ratios in China’s soils: a synthesis of observational data. Biogeochemistry 98(1–3), 139–151.

Tiessen, H., Cuevas, E., Chacon, P., 1994. The role of soil organic matter in sustaining soil fertility. Nature 371, 783–785.

Torello-Raventosa, M., Feldpausch, T.R., Veenendaal, E., Schrodt, F., Saiz, G., Domingues, T.R., Djagbletey, G., Ford, A., Kemp, J., Marimon, B.S., Marimon Junior, B.H., Lenza, E., Ratter, J.A., Maracahipes, L., Sasaki, D., Sonk’e, B., Zapfack, L., Taedoumg, H., Villarroel, D., Schwarz, M., Quesada, C.A., Ishida, F.Y., Nardoto, G.B., Affum-Baffoe, K., Arroyo, L., Bowman, D.M.J.S., Compaore, H., Davies, K., Diallo, A., Fyllas, N.M., Gilpin, M., Hien, F., Johnson, M., Killeen, T.J., Metcalfe, D., Miranda, H.S., Steininger, M., Thomson, J., Sykora, K., Mougir, E., Hiernaux, P., Bird, M.I., Grace, J., Lewis, S.L., Phillips, O.L., and
Lloyd, J., 2013. On the delineation of tropical vegetation types with an emphasis on forest/savanna transitions. Plant Ecology and Diversity 6, 101-137.

Vitousek, P.M., Hobbie, S., 2000. Heterotrophic nitrogen fixation in decomposing litter: patterns and regulation. Ecology 81 (9), 2366–2376.

Vitousek, P.M., Cassman, K., Cleveland, C., Crews, T., Field, C.B., Grimm, N.B., Howarth, R.W., Marino, R., Martinelli, L., Rastetter, E.B., Sprent, J.I., 2002. Towards an ecological understanding of biological nitrogen fixation. Biogeochemistry 57, 1–45.

Wezel, A., Rajot, J.L., 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, 383–398.

Wang, L., Okin, G.S., D’Odorico, P., Caylor, K.K., Macko, S.A. 2013. Ecosystem-scale spatial heterogeneity of stable isotopes of soil nitrogen in African savannas. Landscape Ecology 28(4), 685-698.
Figure 1

Satellite images (Image: ©2017 Google, DigitalGlobe and CNES/Airbus) 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).
Figure 2 (on next page)

Soil SOC, N, total P, available P, total K and extractable 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).
Figure 3 (on next page)

Dependence of soil N (A), total P (B), available P (C), total K (D) and extractable K (E) on SOC concentrations all soil layers for grassland and plantations by age across and for the two study sites.
Figure 4 (on next page)

Relationship between soil δ¹⁵N (‰) and soil C:N ratios for grasslands and plantations by age for the two study sites across all plots and depths.

Dotted line is the linear regression fitted to the plantation data only \( Y = -0.452X + 11.31, \quad R^2 = 0.1926, \quad p = 0.0005 \).
Table 1 (on next page)

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

Significant (α=0.05) correlations are given in bold.
| Site      | Layer, cm | N     | P     | $P_{av}$ | K     | $K_{ss}$ |
|-----------|-----------|-------|-------|----------|-------|----------|
| 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    |
Table 2 (on next page)

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

Values are mean values (n=3) followed by standard deviation (in parentheses). Within each site, mean values sharing the same superscript are not significantly different from each other (Tukey's HSD, \(\alpha<0.05\)).

* SOC values from Abaker et al. (2016) ** grassland
| Site       | Age | SOC     | N   | P    | $P_{av}$ | K   | $K_{av}$ |
|------------|-----|---------|-----|------|----------|-----|----------|
| 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)$^{ab}$ | 153 (15)$^b$ | 41 (7.6)$^b$ | 2.2 (0.1)$^b$ | 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)$^b$ |
Table 3 (on next page)

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).

Values within each site and soil layer sharing the same superscript letter or having no letter are not significantly different from each other (Tukey's HSD, α<0.05).

* grassland
| Site          | Age (yrs) | C:N 0-10 | 10-20 | 20-30 | 30-50 | N:P 0-10 | 10-20 | 20-30 | 30-50 | C:P 0-10 | 10-20 | 20-30 | 30-50 |
|--------------|-----------|----------|-------|-------|-------|----------|-------|-------|-------|----------|-------|-------|-------|
| El Demokeya  | 0         | 9.1      | 8.8   | 9.2   | 9.2   | 4.3      | 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      | 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      | 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      | 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      | 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      | 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      | 4.2   | 4.1   | 3.9   | 48.1     | 41.5  | 36.3  | 39.6  |
Table 4 (on next page)

Mean (& standard deviation) N, P and K concentrations (mg g⁻¹) and N:P ratio in acacia leaves (n=3), above ground vegetation in the grassland (n=2) and plantations (n=5) at each of the two study sites.

* grassland
| Site         | Age (yrs) | 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 |
Table 5 (on next page)

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.

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

* grassland
| Site        | Age (yrs) | 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) |