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Legume and Non-legume Trees Increase Soil Carbon Sequestration in Savanna

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ABSTRACT

Savanna ecosystems are increasingly pressured by climate and land-use changes, especially around populous areas such as the Mt. Kilimanjaro region. Savanna vegetation consists of grassland with isolated trees or tree groups and is therefore characterized by high spatial variation and patchiness of canopy cover and aboveground biomass. Both are major regulators for soil ecological properties and soil-atmospheric trace gas exchange (CO_2, N_2O, CH_4), especially in water-limited environments. Our objectives were to determine spatial trends in soil properties and trace gas fluxes during the dry season and to relate above- and belowground processes and attributes. We selected a Savanna plain with vertic soil properties, south east of Mt. Kilimanjaro. Three trees were chosen from each of the two most dominant species: the legume *Acacia nilotica* and the non-legume *Balanites aegyptiaca*. For each tree, we selected one transect with nine sampling points, up to a distance of 4 times the crown radius from the stem. At each sampling point, we measured carbon (C) and nitrogen (N) content, $\delta^{13}C$ of soil (0–10, 10–30 cm depth) and in plant biomass, soil C and N pools, water content, available nutrients, cation exchange capacity (CEC), temperature, pH, as well as root biomass and greenhouse-gas exchange. Tree species had no effect on soil parameters and gas fluxes under the crown. CEC, C, and N pools decreased up to 50% outside the crown-covered area. Tree leaf litter had a far lower C:N ratio than litter of the C_4 grasses. $\delta^{13}C$ in soil under the crown shifted about 15% in the direction of tree leaf litter $\delta^{13}C$ compared to soil in open area reflecting the tree litter contribution to soil organic matter. The microbial C:N ratio and CO_2 efflux were about 30% higher in the open area and strongly dependent on mineral N availability. This indicates N limitation and low microbial C use efficiency in the soil of open grassland areas. We conclude that the spatial structure of aboveground biomass in savanna ecosystems leads to a spatial redistribution of nutrients and thus C mineralization and sequestration. Therefore, the capability of savanna ecosystems to act as C sinks is both directly and indirectly dependent on the abundance of trees, regardless of their N-fixing status.

Key words: carbon use efficiency; *Balanites aegyptiaca*; *Acacia nilotica*; soil respiration; spatial variability; C:N stoichiometry.

INTRODUCTION

The savanna biome covers nearly 20% of the earth's terrestrial surface (Scholes and Walker 1993). It is a hotspot for biodiversity and wildlife conservation in temperate and tropical regions of America, Asia, Australia, and Africa. Savannas are under strong pressure from climate and land-use changes. They are particularly threatened by desertification, shrub encroachment, and conversion into arable land (Meyer and others 2007; Lambin and others 2003; Goldewijk 2001).

One of the main attributes that define the savanna biome is the co-dominance of trees and grasses (Scholes and Archer 1997). Ecological interactions due to this contrasting vegetation cover have been a major research topic (Huntley and Walker 1982). Most research, however, has focused on species interactions or the impact of disturbances such as fire, grazing, or droughts (Otieno and others 2005; Meyer and others 2009; Schleicher and others 2011). Other approaches estimated carbon (C) and nutrient stocks or fluxes in the ecosystem as a whole (Varella and others 2004; Veenendaal and others 2004; Grace and others 2006; Werner and others 2014; Chen and others 2016).

Several studies combined these approaches and analyzed the spatial effects of the highly heterogeneous vegetation cover on soil ecological properties (Bernhard-Reversat 1982; Belsky and others 1993; Hibbard and others 2001; Ludwig and others 2004; Perakis and Kellogg 2007; Rascher and others 2012; Otieno and others 2015). These studies have shown positive effects of trees on soil fertility, nitrogen (N) availability, understory growth, and C pools compared to open grassland areas. This results in patchy areas of distinctly altered biogeochemical conditions: 'islands of fertility' (Garcia-Moya and McKell 1970). These changes in physical (for example, water budget and temperature), chemical (pH, CEC, and N content), and biological (microbial biomass and composition) soil properties result from a multitude of processes, including altered water balance, shading, and accumulation of biomass in the form of litter. It is often assumed that plant N fixation, whether by the tree itself or by undergrowth species, is a main factor for the increased soil fertility of tree patches (Vitousek and Walker 1989; Sitters and others 2015). Legume trees such as *Acacia* species can resolve N limitation in African savanna grasslands (Ludwig and others 2001). However, the extent of this effect strongly depends on other limiting factors, such as nutrients

and water (Vetaas 1992), and some studies did not show stronger effects of N-fixing tree species on soil parameters compared to other tree species (Bernhard-Reversat 1982; Belsky and others 1989).

Few studies have measured the broad spectrum of above- and belowground parameters and their interactions to determine the mechanistic effects of tree islands on soil C sequestration. The potential of an ecosystem to sequester C in soil is largely controlled by soil microbial activity and carbon use efficiency (CUE) (Bradford and Crowther 2013). If tree islands alter substrate quality and nutrient supply, this may also change microbial CUE. To date, little is known about how the affected properties interact to control the C and N cycles, especially under water-limited conditions. While savannas are generally considered to be active or potential C sinks (Grace and others 2006), they act as a net source of CO_2 during the dry season (Miranda and others 1997). It remains unclear which factors regulate these C losses and how they are affected by the vegetation. Especially, the spatial distribution of these variables and the connection between above- and belowground processes are important for understanding and predicting ecosystem changes. This is crucial in estimating vulnerability to climate and land-use change.

Our objective was to determine the interrelations and patterns of soil properties and soil greenhouse gas fluxes, depending on the spatial variability and characteristics of the vegetation (that is, legume or non-legume tree). We hypothesize that (1) soil C and nutrient contents increase with the presence of trees through increased litter inputs (independent of tree species); (2) lower litter quality outside the crown area will result in reduced N availability; and (3) C mineralization will increase due to higher microbial N mining outside the crown area.

METHODS

Study Site

The study was conducted in a semi-arid savanna plain of the Lake Chala Game Reserve, close to the Kenyan–Tanzanian border (3°18′39″S, 37°41′8″E). The research area covers about two hectares. It is located at the bottom of the southeastern slope of Mt. Kilimanjaro at an elevation of 950 m a.s.l. Soils of this area were classified as Vertisols and developed on erosion deposits from Mt. Kilimanjaro main peaks and from various parasitic volcanoes along the eastern slope (Kühnel 2015). These soils have high clay (66–79%) and low sand (2%)

content in the upper 40 cm. Bulk density varies from 0.8 to 1.0 g cm^{-3} at 0–10 cm, and from 0.9 to 1.1 g cm^{-3} at 10–30 cm soil depth.

Mean annual temperature and precipitation are 21 °C and 536 mm, respectively (Appelhans and others 2014). Rainfall mainly occurs over a short rainy season around November and a longer rainy season from April to June.

The dominant woody plant species are various acacias (*Acacia nilotica*, *Acacia senegal,* and *Acacia tortilis*) and *Balanites aegyptiaca*. The most abundant grass species are *Heteropogon contortus*, *Eragrostis superba,* and *Bothriochloa insculpta*, which all fix carbon by the C$_4$ pathway.

Field Sampling

Field work was conducted during the dry season in September 2014. We identified the two dominant tree species in our research area: the leguminous *A. nilotica,* and the non-leguminous *B. aegyptiaca*. For both species, we selected three solitary individuals that covered the common range of tree sizes in the region (Table 1). At each tree, one transect was placed in random orientation. Along each transect, we selected nine sampling locations in relation to the respective crown radius r. Locations 50 cm and 0.50 and 0.66 times r distance from the stem represented the area under the canopy. The border zone was defined as 1 × crown radius. The open area outside the crown was sampled at the distances of 1.5, 2.0, 2.5, 3.0, and 4.0 × r.

At each sampling location, collars for greenhouse gas (GHG) chamber measurements were installed (383 cm^2). Before GHG measurements, we measured soil temperature and above-ground grass and herb biomass was collected from inside the collar area. Because of the dry conditions, these samples were assumed to represent dead plant material (that is, undergrowth litter). GHG exchange was measured twice at each transect (9:00 and 12:00 o'clock, two transects per day). Opaque polypropylene chambers (25.2 × 15.2 × 14.7 cm) were fixed gas-tight to the collars and fluxes of CO$_2$, N$_2$O, and CH$_4$ were calculated from concen-

tration changes in the chamber headspace air ($n = 5$ in 60 min). Soil cores were taken from the collar area with a closed soil-core sampler (30 cm × 5 cm Ø) and separated into 0–10 and 10–30 cm depths. Fine roots (<2 mm Ø) with a length ≥10 mm were collected from each soil sample and stored at 4 °C until analysis. In each soil sample, total carbon (C) and nitrogen (N), microbial carbon (MBC) and nitrogen (MBN), water-extractable carbon, density of living and dead roots, gravimetric water content, extractable nutrients (NO$_3^-$, NH$_4^+$, and cations of Al, Mg, K, Mn, Ca, Mg, and Fe), cation exchange capacity (CEC), base saturation (BS), soil pH (in H$_2$O and KCl), and bulk density were measured. Litter traps (70 × 70 cm) were placed under each tree and tree leaf litter was collected for one month.

Laboratory Analyses

Soil chemical analyses were conducted in the laboratory of the Department of Soil Science of Temperate Ecosystems, University of Göttingen. Carbon and nitrogen contents were determined using a dry combustion automated C:N analyzer (Vario EL, Elementar). We considered total C as equal to organic C because the inorganic C content was negligible at our site (Kühnel and Becker, Unpublished Data). Microbial biomass C (MBC) and microbial biomass N (MBN) were estimated by fumigation extraction (Vance and others 1987) with the correction factors of 0.45 for MBC (Joergensen 1996) and 0.54 for MBN (Joergensen and Mueller 1996). K$_2$SO$_4$-extractable C was taken as extractable organic C (Beck and others 1997). NH$_4^+$ and NO$_3^-$ concentrations in the extracts were measured by continuous flow injection colorimetry (SEAL Analytical AA3, SEAL Analytical GmbH, Norderstedt, Germany). Samples were prepared by salicylate and dichloro isocyanuric acid reaction for NH$_4^+$ and by cadmium reduction with NH$_4$Cl buffer for NO$_3^-$. Availability of major nutrient cations (Al^{3+}, Ca^{2+}, Fe^{2+}, H$^+$, K$^+$, Na$^+$, Mg^{2+}, Mn^{2+}) was determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Spectro Analytical Instru-

Table 1. Tree Characteristics and Transect Orientation

ID	Species	Tree height (m)	DBH (cm)	Crown radius (m)	Transect orientation (°N)
AN1	*Acacia nilotica*	4.9	73.6	3.0	142
AN2	*Acacia nilotica*	4.5	41.8	2.4	304
AN3	*Acacia nilotica*	2.8	32.2	1.9	84
BA1	*Balanites aegyptiaca*	3.0	46.0	1.8	338
BA2	*Balanites aegyptiaca*	2.6	35.0	1.5	302
BA3	*Balanites aegyptiaca*	4.0	50.4	2.2	316

ments) following a preparative extraction in un-buffered salt solution (1.0 mol l^{-1} NH$_4$Cl). Total cation exchange capacity (CEC) and base saturation were calculated as described by Chesworth (2008). Soil pH was measured in H$_2$O as well as in KCl solution.

Dried and ground bulk soil, tree leaf litter, and grass biomass samples were analyzed for ^{13}C natural abundance by isotope ratio mass spectrometry (Delta V Advantage with Conflo III interface, Thermo Electron, Bremen Germany) and a Flash 2000 elemental analyzer (Thermo Fisher Scientific, Cambridge, UK). Delta values (δ^{13}C) are given as the divergence from the standard reference for ^{13}C-to-^{12}C ratio (Vienna-PDB).

Fine root samples were analyzed according to Hertel and Leuschner (2002) with slight modification. Tree roots were separated from herb and grass roots under a stereomicroscope and separated into living and dead roots based on morphological criteria. All root samples were dried for 48 hours at 70°C and weighed.

CO$_2$, CH$_4$ and N$_2$O concentrations from 10-ml vials of chamber headspace were determined at the IMK-IFU (Garmisch-Partenkirchen, Germany), using a gas chromatograph (8610 C, SRI Instruments, Torrance, USA) equipped with an electron capture detector and a flame ionization detector. The calculated flux rates were corrected for pressure and air temperature measured in the field. All flux rates lower than the minimum detection limit (CO$_2$–C: 0.09 mg m^{-2} h^{-1}; CH$_4$–C: 5.76 µg m^{-2} h^{-1}; N$_2$O–N: 0.83 µg m^{-2} h^{-1}), were set to zero.

Data Analysis

Dixon's Q test was used to identify and remove outliers from each factor. We used all data points and applied linear mixed effect model (LME) analysis of variances for nested designs (each tree as a random factor) at significance level $p < 0.05$ to identify differences between the tree species (as a fixed factor). The same method was used to compare areas below and outside the crown, with the addition of soil depth (if available) as a second fixed factor and using Tukey's HSD post hoc adjustment for multiple comparisons. Satterthwaite approximation of degrees of freedom was used to correct for unbalanced replicate number when appropriate.

Variable interactions (GHG fluxes vs. soil parameters and tree characteristics vs. soil parameters) were analyzed by Pearson product moment correlation at p value < 0.05. Statistical analysis was conducted in R 3.3.0 (R Core Team 2013).

RESULTS

Effect of Tree Species and Characteristics

The total N content as well as extractable N fractions in soil under the crown were the same for both tree species and were also unaffected by structural variables such as tree size.

For *A. nilotica* and *B. aegyptiaca*, mean N contents at 0–30 cm soil depth were 0.14 and 0.16%, respectively (Table 2). C content was also unaffected, and therefore the soil C:N ratio was the same under both tree species. The concentration of plant-available NO$_3^-$-N varied from below the detection limit (0.15 mg l^{-1}) to 1.05 mg l^{-1} under *A. nilotica* N and to 0.84 mg l^{-1} under *B. aegyptiaca*. Available NH$_4^+$-N was mainly below the detection limit and reached 0.85 and 0.75 mg l^{-1} for *A. nilotica* and *B. aegyptiaca,* respectively. Microbial C and N contents were the same in soil under both tree species. Tree height and crown radius positively affected water content at 0–10 cm ($p < 0.01$) but did not affect any other measured property at either depth under the crown ($p > 0.05$).

As most of the soil attributes and GHG fluxes were unaffected by tree characteristics (Table 2), we pooled data from all trees of both species for further comparisons.

Soil Properties and Understory Vegetation

C and N contents, MBC, and CEC at 0–10 cm depth decreased with distance from the tree (Online Appendix Figure 5). Most of the decline occurred over the transition from crown cover to open area, and there were no further changes with greater distance from the tree. In soil below 10 cm, the decrease was less pronounced or completely absent. We therefore used crown and open area as distance classes for LME analysis. Values directly at the interface (1 radius) could be attributed to either of the distance classes and were not considered in further analysis.

In the upper 10 cm of soil, most variables were lower in the open area than under the crown (Figure 1). Carbon and N content as well as MBC decreased by about 25%. The extractable N fraction and MBN decreased by about 41%. The stronger decline of MBN versus MBC resulted in a wider microbial C:N ratio in the open area. Gravimetric soil water content was the same under both cover classes.

Compared to the upper 10 cm of soil, the values of most parameters were lower at 10–30 cm soil depth (Figure 1). CEC decreased with soil depth and was about 7% lower outside the crown area.

Table 2. Effects of Tree Species on Soil Conditions at 0–30 cm Depth, Understory Biomass (BM), and Trace Gas Fluxes Under the Crown

	Acacia nilotica	*Balanites aegyptiaca*	*p* value
C (%)	2.00 ± 0.05	2.09 ± 0.08	0.2985
N (%)	0.14 ± 0.00	0.16 ± 0.01	0.3004
Soil C:N	13.5 ± 0.3	13.1 ± 0.1	0.5200
$\delta^{13}C$ (‰)	-14.6 ± 0.22	-14.6 ± 0.3	0.9334
$\delta^{15}N$ (‰)	5.79 ± 0.09	5.63 ± 0.07	0.2379
MBC (mg kg^{-1})	287 ± 14	320 ± 22	0.4375
MBN (mg kg^{-1})	18.9 ± 1.97	26.41 ± 2.52	0.2300
WOC (mg l^{-1})	5.59 ± 0.30	6.08 ± 0.36	0.5066
NO_3^- (mg l^{-1})	0.31 ± 0.03	0.26 ± 0.03	0.4654
NH_4^+ (mg l^{-1})	0.20 ± 0.03	0.19 ± 0.02	0.4815
CEC (mmol kg^{-1})	252 ± 4	255 ± 5	0.7990
BM (kg m^{-2})	1.16 ± 0.09	1.36 ± 0.08	0.2403
BM C:N	59.0 ± 2.7	67.5 ± 5.1	0.3229
CO_2 (mg m^{-2} h^{-1})	19.3 ± 1.2	20.3 ± 1.1	0.7365
N_2O (μg m^{-2} h^{-1})	-0.50 ± 0.24	0.17 ± 0.15	0.5999
CH_4 (μg m^{-2} h^{-1})	-20.0 ± 1.3	-19.8 ± 0.7	0.8504

Values are arithmetic means ± standard errors. p values are derived from mixed-effect model for nested ANOVA.

This effect was related to K^+ availability, which declined by 50%. The other dominant cations (Ca^{2+}, Mg^{2+}) decreased with soil depth but were unaffected by vegetation cover.

Above- and belowground grass and herb biomass were lower in the open area than those under the crown (Online Appendix Table 4). Living and dead roots in the topsoil (0–30 cm) mainly originated from grass and herb species. The average N content in the grass biomass was 50% lower than that in the tree leaf litter, and the C:N ratio was much wider in grass (40.6 ± 2.1, LME *p* value = 0.0048).

Isotopic Composition

The abundance of ^{13}C in soil under the crown was shifted toward the values of tree litter (Figure 2).

The $\delta^{13}C$ composition of leaf litter from *A. nilotica* and *B. aegyptiaca* varied between -29.4 and $-31.7‰$. Delta values of grass biomass did not differ between the crown and open area, averaging -15.9 and $-15.5‰$, respectively. Due to the incorporation of grass biomass into soil organic matter (SOM), $\delta^{13}C$ values increased by about $1.0‰$ on average. Mean $\delta^{13}C$ values in the top 10 cm were more negative under the crown than in open area (-16.8 and $-14.5‰$, respectively). $\delta^{13}C$ values increased evenly with soil depth under both cover types. Assuming similar ^{13}C fractionation during the incorporation of tree leaf and grass litter into SOM, we estimated the percentage of biomass input by trees: the isotopic composition in soil under the crown was shifted by $2.3‰$ in the direction of tree leaf litter $\delta^{13}C$, which is equivalent to a 15% mass contribution of tree leaf litter to SOM (Figure 2).

Soil Greenhouse Gas Exchange

Measurements of greenhouse gas exchange revealed generally low soil CO_2 emissions, with higher rates in open areas than under the crown (Figure 3). At the same time the savanna ecosystem was a sink for CH_4, with the average flux rates of -20 μg C m^{-2} h^{-1}. N_2O fluxes varied between -4.0 and 2.7 μg N m^{-2} h^{-1} but were not different from zero (*t* test, $p > 0.05$). Both N_2O and CH_4 fluxes were unaffected by vegetation cover.

GHG fluxes under the crown area were uncorrelated to soil properties at 0–10 cm depth (Table 3). Outside the crown, under low-nutrient conditions, there was a positive effect of soil water content on CO_2 efflux. Furthermore, CO_2 production was negatively correlated to NO_3^- availability, indicating a higher substrate turnover under nutrient-limited conditions. Flux rates of CH_4^+ were not related to any of the measured variables. N_2O fluxes in the open area were positively correlated to the C content in the soil (r = 0.55, p < 0.01).

DISCUSSION

Effects of Savanna Trees on Soil C and Nutrient Contents

The presence of trees increased most soil fertility attributes as well as above- and below-ground grass
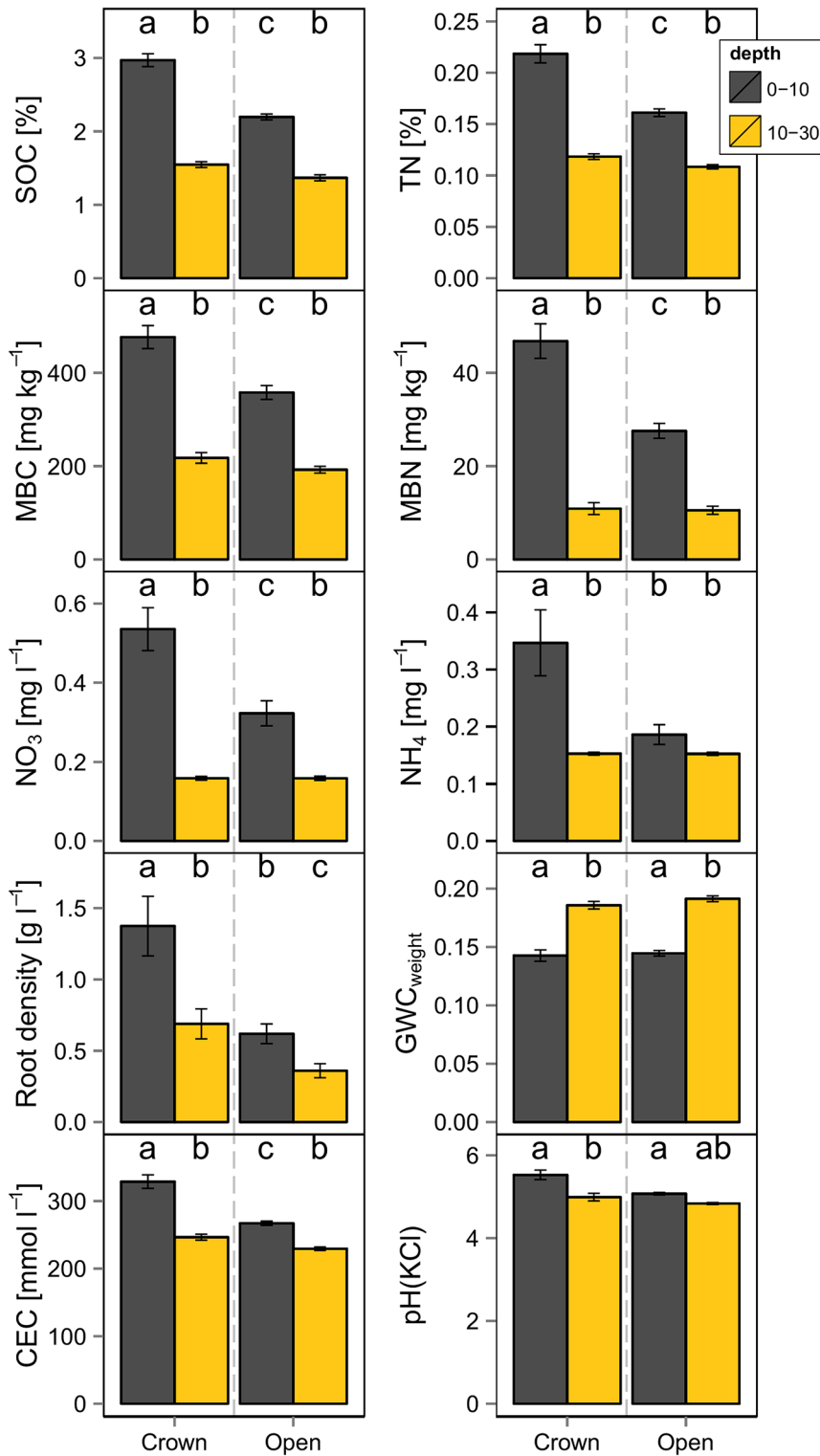
Figure. 1. Soil properties at 0–10 and 10–30 cm depths, under the crown ($n = 18$) and open area ($n = 30$). Standard error of the mean is shown as *error bars* with significance levels (*a–c*) derived from mixed effect model ANOVA for nested designs ($p \leq 0.05$).

biomass through higher litter inputs and quality. Tree species (and therefore N-fixing capability) had no effect on soil C, N, or soil greenhouse gas fluxes under the crown.

The most apparent effect of trees was an increase in C and N contents, microbial biomass (C and N),

understory biomass, and soil nutrient content. This is a common phenomenon for savanna ecosystems (Scholes and Archer 1997). It becomes more distinct with tree age and can remain for several years after tree dieback (Ludwig and others 2004). Nonetheless, the underlying mechanisms of this effect are under debate.

The N-fixing capability of *Acacia* species is often seen as one of the main mechanisms for subsequent C and nutrient accumulation under the trees (Yelenik and others 2004). In contrast to this interpretation, we found no effect on a large set of
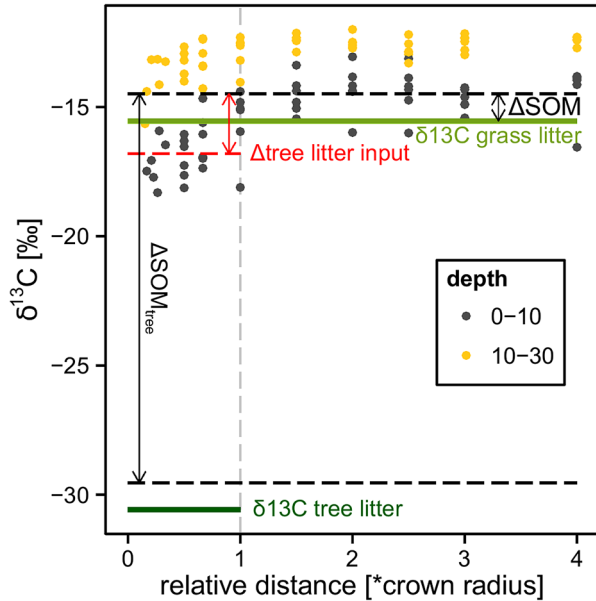

Figure. 2. $\delta^{13}C$ at 0–10 and 10–30 cm soil depths with the increasing distance from the stem. *Solid lines* indicate the average $\delta^{13}C$ composition of tree and grass litter. *Dashed lines* show the mean $\delta^{13}C$ values in 0–10 cm soil under the crown (*black*) and outside the crown (*red*) and potential value at 100% tree litter contribution. *Black arrows* indicate ^{13}C fractionation through grass litter incorporation in soil organic matter (ΔSOM) and the difference to tree litter incorporation (ΔSOM_{tree}). Contribution of tree litter to SOM ($\Delta tree$ litter input) is calculated as the percentage of ΔSOM_{tree}.

soil properties of leguminous versus non-leguminous tree species (Table 2). Particularly, N content and availability as well as N_2O fluxes were the same under the crown of either species. Bernhard-Reversat (1982) attributed a similar finding (comparing *B. aegyptiaca* and *A. senegal*) to N-fixation by an altered species composition in the herb layer under the tree, rather than by the tree itself. In our case, tree root densities in 0–30 cm soil depth were low and had nearly no visible nodules. Grass and herb roots showed no nodulation under or outside the crown. Even though nodulation potential increases with soil depth and maxima can occur more than 4 m below ground (Virginia and others 1986), we would expect at least a sporadic occurrence in the topsoil. Rhizobial nodulation depends on environmental conditions and decreases in dry soil. At the end of the dry season, topsoil horizons are dry and symbiotic N fixation is shifted to lower horizons (Vetaas 1992). Although this may still play a direct role for plant and tree nutrition, the N turnover rates and N availability in the most microbially active soil horizons are independent of N-fixing effects.

The higher soil C and N contents are limited to the area under the crown and to the upper 10 cm of soil (Figure 1). This indicates a spatially limited source, such as the amount and quality of plant litter or throughfall water, as the main reason for increased C and N under the trees (Perakis and Kellogg 2007). Overall inputs from grass litter under and outside the crown did not differ in $\delta^{13}C$. A few grass biomass samples under the crown area, however, showed a lower $\delta^{13}C$ value, which implies the co-occurrence of C_3 herbs or grasses with the dominant C_4 grass species (Cerling and others
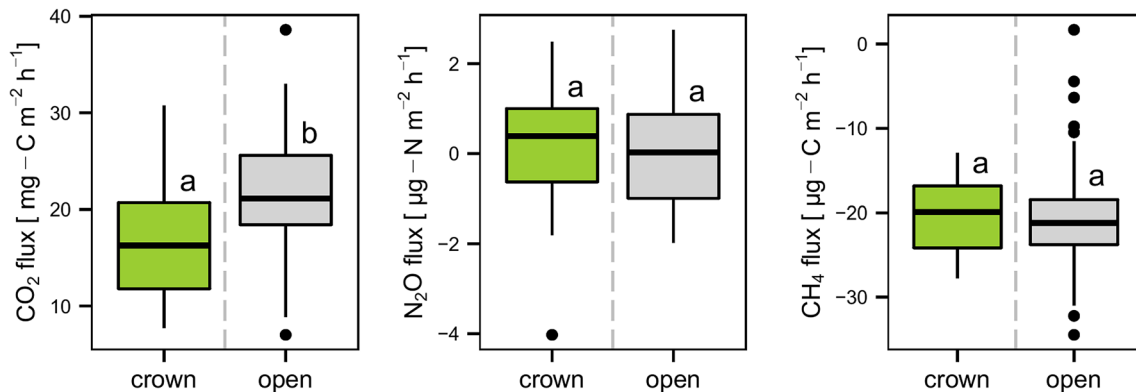

Figure. 3. Comparisons of soil greenhouse gas emissions under tree crowns and in open savanna area. Medians, interquartile range (IQR), and extreme values (>1.5 × IQR deviation) are displayed as *bold lines, boxes with whiskers,* and *dots,* respectively. Significance levels derived from mixed effect model ANOVA for nested designs are shown as letters *a, b* ($p \leq 0.05$).

1997). This agrees with previous findings that the species composition in savanna herb grass layers changes with varying tree cover (Belsky 1994; Ludwig and others 2004). Grass biomass, however, can only partially explain elevated soil C and nutrient contents. Soil $\delta^{13}C$ values under the crown were shifted toward the signal of tree leaf litter, suggesting that tree leaf litterfall contributes about 15% of SOM (Figure 2) and is a major driver maintaining higher SOM levels under the crown. This interpretation is supported by the fact that CEC (that is, available nutrient cations: K^+, Na^+, and Ca^{2+}) showed a redistribution as well (Figure 1) and supports the theory that savanna trees act as nutrient pumps (Scholes 1990). Nutrients from the weathering zone are transported through the tree and return to the soil as litterfall, leachate, root litter, or exudate. This explains the increase in cation availability in topsoil, followed by a decrease with soil depth. Similar to Ludwig and others (2004), there was no obvious lateral pump effect and the absence of tree roots in the topsoil layers indicates a preferential vertical nutrient and water flow from deeper soil layers. Additionally, the accumulation of decomposition-resistant woody debris from trees and roots adds to higher C contents under crowns.

Interactions Between Variables

We expected the strongest effects of trees on soil microbial activity during the dry season because canopy shading and hydraulic lift are especially important under water-limited conditions (Horton and Hart 1998; Raz-Yaseef and others 2010). However, we found no differences in water contents under and outside the tree crowns at the end of the dry season (Figure 1). Water content was below or just around the permanent wilting point (Kühnel 2015), and all activities (that is, biomass production, GHG fluxes) were reduced due to water limitation. This allowed us to look at other parameter effects on GHG exchange without the overriding effect of water content. While soil water content was constant under and outside the crown, CO_2 efflux was higher in the open area (Figure 3). This efflux trend was negatively related to fine root density. Because these variables are usually positively correlated under dry conditions (Ceccon and others 2011), we rule out a large contribution of rhizomicrobial and root respiration or an effect of water content. Instead, the higher CO_2 efflux outside the crown can be attributed to increased microbial respiration by decomposition of SOM and litter. CO_2 production under low-N conditions (that is, outside the crown) is inversely related to NO_3^- availability (Table 3). Because NO_3^- addition is known to reduce microbial C mineralization (Burton and others 2004), this relationship might indicate N limitation. We found a stronger decrease in MBN than in MBC outside the tree crown, widening the microbial C:N ratio. These wide microbial C:N ratios are directly related to the C:N ratio of available substrate (Nicolardot and others 2001) and reflect a low carbon use efficiency (Sinsabaugh and others 2013; Blagodatskaya and others 2014). New available substrate for microbial turnover (i.e., litterfall from trees and grasses) differs in C:N ratio: leaf litter from C_4 grasses has a wider C:N ratio than litter from trees and C_3 grasses. This requires microorganisms to dispose of the C surplus via increased respiration to achieve their optimum C:N stoichiometry (Chen and others 2007; Spohn 2015).

Implications and Relevance

We chose two widely different species and individual trees that cover the whole range of tree sizes in the study area to increase representativeness. All

Table 3. Pearson's Correlations Coefficients Between Gas Fluxes and Selected Soil Properties at 0–10 cm Under the Crown ($n = 18$) and Under Open Area

	C	N	MBC	MBN	NO_3^{-a}	$NH_4^{+ a}$	Living roots	Water content	T_{soil}	CEC	pH (H2O)
Crown area											
CO_2	−0.20	−0.13	−0.22	−0.12	−0.14	−0.40	0.15	0.16	−0.29	−0.52*	−0.11
N_2O	−0.05	−0.04	0.09	−0.01	−0.06	−0.04	−0.16	0.29	0.27	0.22	0.01
CH_4	−0.01	−0.07	−0.16	0.01	−0.37	0.06	0.29	−0.25	−0.25	−0.13	−0.17
Open area											
CO_2	−0.25	−0.08	0.23	−0.02	−0.55*	−0.31	0.25	0.37*	−0.15	−0.27	−0.20
N_2O	0.55**	0.30	0.10	0.29	0.26	−0.11	0.12	0.07	0.29	0.27	−0.04
CH_4	0.18	0.05	−0.10	0.07	0.28	0.01	−0.09	0.01	0.22	0.26	−0.05

^{a}*Values below detection limit were excluded ($n = 30$). Significance levels of $p < 0.05$ and $p < 0.01$ are indicated as * and ** respectively.*
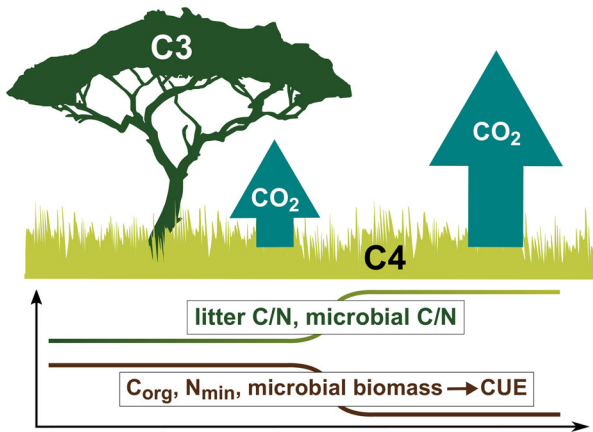
Figure. 4. Effect of savanna trees on soil C and nutrient pools and related changes in soil respiration under dry conditions. The wide C:N ratio of C_4 plant litter reduces N availability (Nmin) and microbial biomass (MBC). Soil microbial C:N ratios and respiration increase due to low carbon use efficiency (CUE).

measured properties were in a typical range for soil characteristics, but the values were highly variable between trees and for each tree. Nonetheless, despite water limitation and the overall reduced biological activity (represented by low soil respiration rates), tree cover effects on soil respiration were evident. Apparently, there is strong competition for nutrients within the microbial community in savanna soils, even under strongly water-limited conditions.

Tree effects on soil properties were independent of tree height, DBH, and crown radius—all characteristics directly linked with tree age (Diallo and others 2013). Therefore, trees can affect the surrounding soil independent of their age. This indicates that soil C pools and fluxes react more rapidly to increased tree cover than to vertical tree growth. Tree cover is expected to change in natural savanna ecosystems of Africa due to improved wildlife management and climate change: On the one hand, the already decreased abundance of megaherbivores and prevention of wildfires will increase tree cover percentage (Staver and others 2011). On the other hand, the predicted irregularity of precipitation and increased air temperatures (IPCC 2013) might lower tree cover. This in turn would decrease soil fertility and directly increase CO_2 losses during the dry season because of lower carbon use efficiency (Figure 4). The potential of savanna ecosystems to act as a C sink, as proposed by Grace and others (2006), is very variable and directly depends on how the vegetation structure affects N availability.

CONCLUSIONS

The occurrence of trees (C_3) in a C_4 grassland increased soil fertility through higher litter inputs and quality in the local area under the crown. In soil deeper than 10 cm, the increase was less pronounced or disappeared completely. This effect is the result of active vertical transport by the trees (nutrient pumping) and a passive accumulation of C and N from litterfall over time. Tree species, whether leguminous or non-leguminous, had the same effects on soil properties. We conclude that soil C pools and fluxes are directly related to the spatial abundance of trees and react more rapidly to increased tree cover than to vertical tree growth.

In the open area and against the background of low N availability, the wider C:N ratio of C_4 grasses compared to C_3 tree litter inputs reduced the carbon use efficiency of soil microbes (Figure 4). This increased microbial respiration and the CO_2 efflux from soil. Therefore, savanna trees affect soil C storage through two processes, (1) actively by increasing biomass inputs and (2) passively by hampering output mechanisms.

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