Mycorrhizal fungi transfer nitrogen from tree to maize in subsistence farmers’ fields

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Abstract

Trees within farmers’ fields can enhance systems’ longer-term productivity e.g., via nutrient amelioration, which is indispensable to attain sustainable agroecosystems. While arbuscular mycorrhizal fungi (AMF) are known to improve plant access to soil nutrients, the potential of AMF to facilitate nutrient transfer from trees to crops is unclear. We used the $^{15}$N (nitrogen) natural abundance technique together with root and AMF exclusion plots to assess if *Faidherbia albida* (faidherbia) trees deliver N to maize via associated AMF in smallholder fields. We show, here, that within one cropping season, maize obtained approximately 35 kg biologically fixed N ha$^{-1}$ from fidherbia and AMF significantly contribute to this transfer of N. One third of tree-derived N in maize leaves was attributed to transfer via AMF and two thirds were explained by tree leaf litter input. Thus, the fidherbia-AMF association can enhance agroecosystem functioning and as such, attain greater sustainability of low-input cropping systems.

Introduction

Food security is at continuous risk in sub-Saharan Africa where the majority of people live from subsistence farming$^1$. Traditional fallow practices have been abandoned due to increasing land pressure and continuous maize cultivation has become the norm$^{2,3}$. This has resulted in soil degradation$^4$ and a negative feedback on food security. Ecologically sound management practices are essential to attain food security. Agroforestry can provide a framework for sustainable farming: trees distributed throughout farmers’ fields can enhance soil fertility via above- and belowground organic matter inputs$^{5-9}$. Nutrient availability of these inputs to surrounding crops depends on indirect and direct transfer mechanisms (e.g., mineralization$^{5,7}$ and transfer via arbuscular mycorrhizal fungi (AMF)$^{10,11}$, respectively).
Arbuscular mycorrhizal fungi, mutualistic root symbionts, provide several benefits to their host plants e.g., enhanced access to nutrients and in return, receive plant carbohydrates\textsuperscript{12}. Mycorrhizal mycelia may connect interspecific plants\textsuperscript{13} and facilitate interplant nutrient transfer\textsuperscript{11,14,15}. Nutrient transfer between agroforestry trees and crops via AMF could improve crop performance\textsuperscript{16,17}. To date, greater yields within agroforestry systems relative to crop monocultures have been ascribed to the positive effect of nitrogen (N)-fixing trees on soil nutrient content and availability\textsuperscript{18-22}. Nitrogen-fixing trees can provide microdose fertilization to farmers’ fields\textsuperscript{22} but the role of AMF in making tree nutrient inputs accessible to crops within agroforestry systems remains unknown. In this study, we quantified the biologically fixed tree-derived N (hereafter referred to as “tree-derived N”) incorporated into crop biomass within a given season. Further, we investigated whether tree-to-crop nutrient transfer via mycorrhizal mycelia occurs in farmers’ fields and to what extent this may affect crop yield. Tree-to-crop nutrient transfer facilitated by AMF could be particularly important in subsistence farming where nutrient inputs are highly restricted and farmers need to capitalize on ecosystem processes to improve food security.

We estimated the total amount of biologically fixed N derived from \textit{Faidherbia albida} (Fabaceae; faidherbia) trees obtained by surrounding maize and revealed the role of AMF in making tree-derived N accessible to maize plants in Malawian farmers’ fields. We used the \textsuperscript{15}N natural abundance technique which enables distinguishing between biologically fixed N derived from faidherbia and N derived from soil, in combination with root and AMF exclusion plots (Fig. 1). Three types of plots were installed around eight faidherbia trees for three types of interaction between faidherbia and maize: 1) fully restricted belowground interactions, limiting tree-derived N inputs to maize to those provided by tree leaf litter only (Litter plot; “litter” hereafter refers to tree leaf litter only), 2) belowground interaction between tree and maize restricted to those facilitated via AMF only (Litter\&AMF plot), and...
3) unrestricted interaction between tree and maize (Litter&AMF&Roots plot). This set-up allowed disentangling the effect of litter, AMF, and tree roots on the amount of tree-derived N in maize leaves and thereby gaining insight into the underlying mechanisms that lead to transfer of N from tree to maize. We focused our study on maize-based agroforestry systems of Malawian smallholders because they exemplify a common agroecosystem that must be managed more sustainably to attain food security.

Results

Foliar \( ^{15}N \) and N concentration of faidherbia ranged from -0.24 to 1.59 % with an average of 0.92 ± 0.24 % and from 3.62 to 5.38 % with an average of 4.44 ± 0.21 %, respectively. Maize foliar \( ^{15}N \) was different between plot types (i.e. type of interaction between tree and maize) \((F_{2,94} = 10.71; \text{Table 1})\). Maize grown in the Litter&AMF plot and Litter&AMF&Roots plot were depleted in \( ^{15}N \) i.e., obtained significantly more faidherbia-derived N relative to maize grown in the Litter plot \((p < 0.001)\). But there was no significant difference in foliar \( ^{15}N \) for maize grown in the Litter&AMF versus the Litter&AMF&Roots plot \((p = 0.977)\). Distance from faidherbia also affected maize foliar \( ^{15}N \) \((F_{4,94} = 5.93)\), with maize grown at 1 m being more depleted in \( ^{15}N \) than at 4 and 5 m from the tree \((p = 0.001\) and \(p < 0.001\), respectively). There was no interaction between the two main factors i.e., plot type and distance from faidherbia on foliar \( ^{15}N \). Similar results were obtained for the effect of plot type and distance from faidherbia on the proportion of tree-derived N in maize (data not shown). Percent foliar N in maize was significantly affected by distance from faidherbia \((F_{4,94} = 5.80; \text{Table 1})\) with reduced percent foliar N in maize grown at 5 m compared to 1 and 2 m \((p < 0.001\) and \(p = 0.004\), respectively) for all three plot types. Foliar N concentration in maize at 4 m was marginally significantly lower than at 1 m \((p = 0.047)\). Nitrogen concentration and \( ^{15}N \) in soil were not affected by plot type \((F_{2,94} = 1.53\) and \(F_{2,94} = 1.34\),
respectively) or distance from faidherbia ($F_{4,94} = 0.91$ and $F_{4,94} = 1.47$, respectively) and were on average $0.16 \pm 0.002$ % and $5.74 \pm 0.04$ %, respectively (Table 1). The proportion of tree-derived N in maize as a result of litter-, AMF-, and root-mediated processes did not differ with distance from faidherbia trees ($F_{4,27} = 1.24$, $F_{4,27} = 0.30$, and $F_{4,25} = 0.90$, respectively; Fig. 2). The effect of roots on tree-derived N in maize was negligible (Fig. 2). Biomass, yield, N content and total tree-derived N in maize leaves on a per plot basis did not significantly differ between plot types ($F_{2,14} = 0.35$, $F_{2,14} = 0.86$, $F_{2,14} = 0.32$, and $F_{2,14} = 2.09$, respectively; Table 2). Total tree-derived N in maize grown within 5 m from faidherbia across all three plot types summed up to approximately $35 \pm 7$ kg N ha$^{-1}$ out of an estimated total N content of $120 \pm 7$ kg N ha$^{-1}$ thereby making up about 30 % of total N in maize.

Discussion

Capitalizing on agroecological processes such as those provided by the faidherbia-AMF association in maize-based agroforestry systems in Malawi is essential to improve agroecosystem functioning on smallholder farms. Our results confirm the importance of faidherbia trees in improving the N budget in farmers’ fields and further highlight that maize obtains tree-derived N not only via faidherbia litter inputs but also through N transfer from faidherbia to maize via mycorrhizal mycelia. Tree-to-maize N transfer via mycorrhizal mycelia accounts for one third of the tree-derived N in maize leaves while two thirds of the tree-derived N in maize leaves come from faidherbia litter inputs (Table 1, Fig. 2). Consequently, AMF substantially contribute to making faidherbia-derived N inputs available to maize.

Microdose N fertilization by Faidherbia albida

Incorporating N$_2$-fixing trees in agroecosystems can benefit crop yields by providing high-quality above- and belowground organic matter inputs to the soil e.g. in the form of tree litter
input, root exudates, and root turnover\textsuperscript{5-7}. Faidherbia litter input alone can provide 50 to 80 kg N ha\textsuperscript{-1} to the soil under faidherbia trees within a given season\textsuperscript{22}. However, how much of this tree litter-derived N is effectively incorporated into the soil and subsequently incorporated into crop biomass is unclear. The distinct isotopic N signature i.e. $^{15}$N:$^{14}$N ratio of N\textsubscript{2}-fixing faidherbia allows distinguishing between biologically fixed tree-derived N from residual soil N. Thus, the $^{15}$N natural abundance technique allows tracing tree-derived N into maize. Here, we demonstrate that in total, over the course of one season, tree-derived N accounts for 35 kg N ha\textsuperscript{-1} in maize which makes up about 30 % of total N in maize. Therefore, our results confirm the importance of faidherbia trees in improving the N budget of crops in farmers’ fields. The recommended rate of N fertilization in Malawi is 96 kg N ha\textsuperscript{-1} but on average only 18 kg N ha\textsuperscript{-1} are being applied by farmers\textsuperscript{23,24}. Consequently, faidherbia provides more than one third of the recommended fertilization and almost twice the amount that is on the average applied by farmers.

Microdose fertilization has been shown to result in significant yield increase e.g. microdose fertilization of 24 kg N ha\textsuperscript{-1} resulted in a 64 % increase in maize grain yield relative to an unfertilized control\textsuperscript{17}. We found yields within 5 m of faidherbia were 52 % greater compared to yield away from faidherbia. Specifically, maize grain yield was 3.7 ± 0.4 t ha\textsuperscript{-1} under faidherbia compared to 2.5 ± 0.6 t ha\textsuperscript{-1} away (i.e., ~35 m ) from faidherbia. We note that the yield obtained away from faidherbia was based on green cob dry weight while yield under faidherbia was determined at the mature state, after the plants had fully dried, which might have resulted in a slight underestimation of yield away from faidherbia and therefore a slight overestimation of the 52 % increase in yield under relative to away from faidherbia. Nevertheless, our estimates are in line with previous findings\textsuperscript{17}. Furthermore, our results show that foliar N concentration and tree-derived N in maize leaves were greater within the immediate vicinity (1-2 m) of faidherbia compared to further distances (4-5 m).
We conclude that faidherbia trees are effective in providing a microdose N fertilization to maize in subsistence farmers’ fields and have the potential to increase maize yields.

**Arbuscular mycorrhizal fungi mediate tree-to-crop N transfer**

Greater crop yields previously observed around N$_2$-fixing trees within agricultural fields have been mostly ascribed to high quality organic matter inputs to the soil$^{18-22}$. The contribution of AMF in making these inputs available to crops, specifically via interplant nutrient transfer has gained much less attention. There has been some evidence that AMF transfer N from trees to surrounding plants$^{11}$ but verification of this mechanism under field conditions on smallholder farms has not been done. The combined use of the $^{15}$N natural abundance technique and root and AMF exclusion plots allowed us to disentangle the effect of AMF-facilitated transfer of tree-derived N from other belowground transfer mechanisms between maize and trees (i.e., root-to-root contact and direct uptake of tree root exudates by maize roots), and further quantify their respective magnitudes in farmers’ fields. In our study system, AMF-mediated N transfer accounted for 28% of the total tree-derived N in maize leaves within a 5-m radius around faidherbia (Table 2). Tree litter was responsible for most of the tree-derived N in maize and tree roots had a negligible effect on the tree-derived N in maize leaves (Fig. 2, Table 2).

The experimental plots were located under the tree crown (average crown diameter of 10 m) and therefore, it was expected that the proportion of tree-derived N obtained by maize as a result of tree litter input was the same within the 5 m around faidherbia (Fig. 2). The contribution of AMF-facilitated N transfer from tree to crops versus uptake via root-to-root contact and direct uptake of tree root exudates by maize roots to the crops’ N budget likely depends on the tree root system architecture and distance from tree. We did not examine the tree root system architecture, but observed no fine tree roots within a radius of 5 m from faidherbia (at a depth of 0 to 50 cm). Even if maize roots usually grow deeper than 50 cm in
the absence of our experimental plots, the lack of fine tree roots within the top 50 cm
suggests that root-to-root contact between faidherbia and maize is typically minimal and
explains why we found no additional increase in tree-derived N obtained by maize grown in
the Litter&AMF&Roots plot relative to the Litter&AMF plot (Table 2). Further, the uniform
litter input and lack of fine tree roots within the area of the excavated plots explain why the
proportion of tree-derived N obtained by maize as a result of litter and tree roots, and
subsequently transfer via mycorrhizal mycelia did not change with distance from faidherbia
(Fig. 2). Given the apparent spatial separation of faidherbia and maize roots, our results
highlight the importance of mycorrhizal mycelia in bridging the space between the rooting
space of faidherbia and maize, for maize to obtain N from faidherbia that would otherwise
not be available.

Despite the contribution of AMF-facilitated N transfer to the proportion of tree-
derived N obtained by maize leaves, maize biomass and yield were not significantly
increased by AMF (Table 2). This is probably linked to the fact that total foliar N content was
not affected by plot type i.e. type of interaction between tree and maize (Table 2). Total tree-
derived N in maize leaves was also not significantly different between plot types but the data
follow the same trend as shown by the proportion of tree-derived N (Table 2). While
interplant N transfer via AMF may not increase the N content in maize, an AMF-facilitated
transfer of N from the tree rooting space to maize likely reduce soil N mining within the
maize rooting space and as such, improves internal nutrient cycling within the
agroecosystem.

In conclusion, this study provides insight into the underlying mechanisms through
which belowground N input from trees is transferred to crops. Mycorrhizal mycelia bridge
the space between the rooting space of trees and crops and as such, increase the amount of
tree-derived N transferred to crops. Sustainability of agroforestry systems could benefit from
adopting management practices that strive to build up and maintain mycorrhizal mycelia and thus, maximize the potential of AMF-mediated interplant nutrient transfer. Especially in low-input cropping systems such as in Malawi, microdose N fertilization provided by faidherbia trees and AMF-mediated tree-to-maize N transfer could enhance sustainability of agroecosystem in the longer-term.

Materials and methods

Site description

This study was conducted in central Malawi, in the lowlands of Dedza district. The fields are distributed within an area of approx. 5 km² around the village of Ndindi in the Golomoti Extension Planning Area. Soil textures range between sandy clay loam and sandy loam. The climate is sub-tropical, humid with a unimodal precipitation pattern. Most rain falls from November to March (average annual precipitation is 884 mm²). Farmers prepare their fields by hand-hoeing (i.e., ~ 15 cm deep). Maize is planted at the onset of the rainy season around December and harvest typically occurs in April/May.

Tree selection

We focused our study on faidherbia (Faidherbia albida, Fabaceae) because it has been highly promoted as an agroforestry species due to its “reverse phenology”. The trees’ foliage is shed with the onset of the rainy season²² resulting in minimized light competition between tree and maize, and in high-quality litter inputs at a time when soil moisture conditions are favorable for rapid mineralization¹⁹,²¹. Eight single-standing faidherbia trees distributed throughout farmers’ maize fields were selected. All trees were well-established trees of a similar size (on average diameter at breast height 53 ± 3.5 cm, height 16 ± 1.0 m, crown radius 5 ± 0.4 m), single-standing (at least 40 m away from the base of any neighboring tree), and with a recent cropping history of maize.
Experimental set-up (year 1)

At the beginning of the growing season of 2016/2017, we excavated three plots (1 x 5 m, 0.5 m deep), around each tree (Fig. 1). Plots started at 0.7 m from the base of the tree and were oriented to 0°, 120°, and 240°. Each plot received a different lining for three types of plots i.e. three types of interaction between tree and maize. The three types of lining were 1) pond liner (AlfaFol PVC pond liner 0.5 mm thick; Oase Living Water) to eliminate all belowground interactions between tree and maize, limiting tree-derived N inputs to maize to those provided by tree leaf litter only (Litter plot), 2) 40 µm mesh (SEFAR Petex 07-40/25) to restrict interactions between tree and maize to those facilitated by AMF only and eliminate intermingling of tree and maize roots (Litter&AMF plot), and 3) no lining for unrestricted interaction between tree and maize, and to control for the potential effect of the disturbance caused by excavation during plot construction (Litter&AMF&Roots plot). Which plot type was placed at 0°, 120°, and 240° around the tree varied for each tree to account for potential differences in microclimate. Prior to excavating the plots, leaf litter that had accumulated on the soil surface was removed. The excavated soil was piled up next to the plot and after the lining had been put in place, the soil was placed back into the plot, moving from the top to the bottom of the pile (roughly placing the soil back to the original depth). After completion of the construction of plots, maize was sown along two lines throughout each plot and within a 10-m radius circle around each tree. Farmers weeded, harvested, and eventually prepared the fields for the next growing season, following their common practices (described above). No measurements were taken in this year to let the system recover from the disturbance caused by the plot installation.

Sample-collection (year 2)

At the beginning of the growing season of 2017/2018, maize was sown into each plot and within a 10-m radius circle around each tree. In each plot, maize was sown along two lines
(0.6 m apart) at every meter, from 1 to 5 m from the base of the tree. Fields were weeded following common practices by the farmers. At the time of harvest (i.e., 17 weeks after sowing), maize leaf samples and paired soil samples (0-15 cm) were collected at every meter, in each plot. Maize leaf samples and soil samples from the same distance of the same plot were combined to composite samples per distance from the tree, resulting in five leaf and five soil samples per plot per tree and a total of 120 leaf and soil samples each. Faidherbia leaf samples were collected to obtain reference δ^{15}N and N concentration values to estimate tree-derived N in maize. Dry, homogenized plant and soil samples were analyzed for δ^{15}N and total N with an elemental analyzer (Vario PyroCube, Elementar) connected to an isotope ratio mass spectrometer (Isoprime 100, Elementar) in continuous flow.

The proportion of tree-derived N (frac_N(tree)) in maize leaves was determined on a per distance basis (i.e. at each distance from the tree) using the following equation:

\[
frac_N(tree) = \frac{\delta^{15}N_{maize} - \delta^{15}N_{soil}}{\delta^{15}N_{tree} - \delta^{15}N_{soil}}
\]  

(1)

where δ^{15}N_{maize} and δ^{15}N_{soil} are the δ^{15}N [%] of maize leaf and soil samples at each distance, respectively and δ^{15}N_{tree} are the δ^{15}N [%] of the corresponding faidherbia tree leaf samples.

We determined AMF-mediated and root-mediated uptake of tree-derived N by maize as follows:

\[
frac_{AMF\_med} = \frac{Litter&AMF}{Litter} - \frac{Litter}{Litter&AMF}
\]  

(2)

\[
frac_{Root\_med} = \frac{Litter&AMF&Roots}{Litter&AMF} - \frac{Litter&AMF}{Litter&AMF}
\]  

(3)

where frac_{AMF\_med} and frac_{Root\_med} are the proportion of tree-derived N in maize leaves obtained via AMF- and root-mediated processes, respectively and frac_{Litter}, frac_{Litter&AMF}, and frac_{Litter&AMF&Roots} are the proportion of faidherbia-derived N found in maize leaves in the Litter plot, Litter&AMF plot, and Litter&AMF&Roots plot, respectively as calculated by equation (1).
For each tree, total biomass and mature cob fresh weight per plot were recorded in the field and homogenized subsamples per tree were oven-dried to determine dry weights. Dry weight of each fraction i.e., leaves, stalks, grain, and cob per tree were determined based on proportional weights obtained for maize previously. Composited maize stalk, grain, and cob subsamples were analyzed for total N and $\delta^{15}$N as described above to determine N content of each fraction (i.e., stalks, grain, and cob) per tree and to estimate total tree-derived N in maize grown within 5 m from faidherbia (see below). The total tree-derived N ($\text{total } N(\text{tree})$ [kg N ha$^{-1}$]) in maize leaves was calculated on a per plot basis as follows:

$$\text{total } N(\text{tree}) = (N_{\text{maize}})(\text{frac } N(\text{tree}))$$  \hspace{1cm} (4)

where $N_{\text{maize}}$ is the total N content in maize leaves [kg N ha$^{-1}$], and $\text{frac } N(\text{tree})$ the proportion of tree-derived N in maize leaves as calculated by equation (1). To obtain total tree-derived N in maize on a per tree basis, we estimated total tree-derived N per fraction and subsequently, determined the sum of total tree-derived N per fraction of all fractions (leaves, stalks, grain, and cobs) combined. Hence, we adjusted equation (4) to have $N_{\text{maize}}$ and $\text{frac } N(\text{tree})$ represent the total N content and the proportion of tree-derived N in maize stalk, grain, or cob to determine total tree-derived N ($\text{total } N(\text{tree})$) in maize stalk, grain, or cob, respectively for each tree. The proportion of tree-derived N ($\text{frac } N(\text{tree})$) in maize stalk, grain, and cob on a per tree basis were calculated using equation (1) with $\delta^{15}$N$_{\text{maize}}$ as the $\delta^{15}$N [%] of maize stalks, grain, or cob per tree, respectively, $\delta^{15}$N$_{\text{soil}}$ the average $\delta^{15}$N [%] of soil determined per tree, and $\delta^{15}$N$_{\text{tree}}$ the $\delta^{15}$N [%] of the corresponding faidherbia tree leaf samples.

**Yield comparison under versus away from *Faidherbia albida***

For yield (i.e. dry weight of grain) comparison between under and away from faidherbia, we used the data obtained here from within 5 m of faidherbia i.e. under faidherbia with the yield
(based on green cob dry weight) obtained in a previous study (cropping season 2015/2016) at approximately 35 m from faidherbia.

**Statistical analyses**

All statistical analyses were performed in the software environment R Studio (Version 1.1.383 – © 2009-2017 RStudio, Inc.). The effect of plot type i.e. type of interaction and distance from the tree on $\delta^{15}$N in maize leaves and soil, foliar and soil N concentration, and $\text{frac}_{\text{N(tree)}}$, and the effect of plot type on maize biomass and yield, N content and tree-derived N in maize leaves were analyzed using linear mixed-effects models with individual tree included in the model as a random effect variable. Similarly, the impact of distance from faidherbia on the proportion of tree-derived N determined in maize leaves as a result of litter-, AMF-, and root-mediated processes was assessed with linear mixed-effects models. The Cook’s distance measure was used to detect outliers. We obtained no significant interaction of plot type and distance from faidherbia but in case the linear mixed-effects model revealed significant main effects, we applied post hoc pairwise means comparisons using Tukey’s test to calculate least-squares means (function ‘lsmeans’ in package ‘lsmeans’).

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**Author contributions**

J.D., J.S., W.J.B., and H.A.G. designed the experiment. J.D. and J.S. collected the data. J.D. performed data analyses with input from J.S. The manuscript was written by J.D. with input from J.S., W.J.B., and H.A.G.

**Competing interests**

The authors declare no competing interests.

**Figure legends**

**Fig. 1 Low-input maize cropping system in central Malawi and the experimental set-up.**

Top: *Faidherbia albida* trees distributed throughout smallholder farmers’ maize fields. Bottom: Root and AMF exclusion plots together with the use of the $^{15}$N natural abundance technique allowed disentangling the effect of AMF-facilitated transfer of tree-derived N from other belowground transfer mechanisms between maize and trees.

**Fig. 2 Tree-derived N in leaves of maize surrounding *Faidherbia albida* acquired via above- and belowground processes.** The proportion of tree-derived N determined in maize leaves as a result of litter input, transfer via AMF, or direct root-to-root contact between tree and maize (litter-, AMF-, and root-mediated, respectively; see Methods Eq. 2 & 3) with distance from *F. albida*. 
Table 1. Delta$^{15}$N of maize leaf and paired soil samples [%] and foliar N concentration of maize with distance from *Faidherbia albida*.

| Plot type | Distance from tree [m] | 1      | 2      | 3      | 4      | 5      |
|-----------|------------------------|--------|--------|--------|--------|--------|
| Litter    | $\delta^{15}$N$_{\text{maize}}$ | 3.54 ± 0.41 | 4.14 ± 0.60 | 4.03 ± 0.45 | 4.47 ± 0.53 | 4.65 ± 0.53 |
|           | $\delta^{15}$N$_{\text{soil}}$ | 5.65 ± 0.16 | 5.75 ± 0.16 | 5.76 ± 0.17 | 5.81 ± 0.12 | 5.75 ± 0.13 |
|           | % N$_{\text{maize}}$     | 1.73 ± 0.11 | 1.72 ± 0.15 | 1.63 ± 0.09 | 1.60 ± 0.11 | 1.58 ± 0.09 |
| Litter &  | $\delta^{15}$N$_{\text{maize}}$ | 2.47 ± 0.37 | 3.02 ± 0.22 | 3.41 ± 0.37 | 3.61 ± 0.35 | 3.74 ± 0.52 |
| AMF       | $\delta^{15}$N$_{\text{soil}}$ | 5.74 ± 0.16 | 5.71 ± 0.16 | 5.84 ± 0.12 | 5.81 ± 0.16 | 5.77 ± 0.13 |
|           | % N$_{\text{maize}}$     | 2.05 ± 0.12 | 1.80 ± 0.09 | 1.77 ± 0.11 | 1.49 ± 0.08 | 1.45 ± 0.12 |
| Litter &  | $\delta^{15}$N$_{\text{maize}}$ | 2.40 ± 0.34 | 2.96 ± 0.32 | 2.83 ± 0.26 | 3.93 ± 0.36 | 3.72 ± 0.42 |
| AMF &     | $\delta^{15}$N$_{\text{soil}}$ | 5.54 ± 0.17 | 5.65 ± 0.15 | 5.60 ± 0.16 | 5.81 ± 0.18 | 5.85 ± 0.16 |
| Roots     | % N$_{\text{maize}}$     | 1.79 ± 0.11 | 1.93 ± 0.09 | 1.78 ± 0.08 | 1.79 ± 0.12 | 1.50 ± 0.14 |

Plot type refers to the type of interaction between tree and maize. Litter plot: pond liner to eliminate any belowground contact between tree and maize; Litter&AMF plot: 40 µm mesh to allow contact between tree and maize via mycorrhizal mycelia but restrict root-to-root contact between tree and maize; Litter&AMF&Roots plot: no lining to control for the potential effect of the disturbance caused by excavation during plot construction. Plots were 1 m wide x 5 m long x 0.5 m deep and directly around the base of *F. albida*. Values represent means ± SE (n = 8). For statistical results refer to Results.
Table 2. Maize biomass, yield, and N content and total biologically fixed tree-derived N in maize leaves within a 5-m radius around *Faidherbia albida*.

| Plot type         | Biomass [t ha\(^{-1}\)] | Yield [t ha\(^{-1}\)] | N content in leaves [kg ha\(^{-1}\)] | Tree-derived N in leaves [kg ha\(^{-1}\)] |
|-------------------|---------------------------|------------------------|--------------------------------------|------------------------------------------|
| Litter only       | 4.0 ± 0.3                 | 4.1 ± 0.3              | 18.2 ± 1.3                           | 6.3 ± 1.8                                |
| Litter&AMF        | 3.7 ± 0.6                 | 4.3 ± 0.4              | 17.3 ± 2.4                           | 8.8 ± 1.5                                |
| Litter&AMF&Roots  | 3.7 ± 0.5                 | 3.7 ± 0.4              | 18.5 ± 2.5                           | 8.9 ± 1.8                                |

Plot type refers to the type of belowground interaction between tree and maize. Litter plot: pond liner to eliminate any belowground contact between tree and maize; Litter&AMF plot: 40 µm mesh to allow contact between tree and maize via mycorrhizal mycelia but restrict root-to-root contact between tree and maize; Litter&AMF&Roots plot: no lining to control for the potential effect of the disturbance caused by excavation during plot construction. Plots were 1 m wide x 5 m long x 0.5 m deep and directly around the base of *F. albida*. None of the variables were significantly affected by ‘plot type’ i.e., type of interaction between tree and maize. Values represent means ± SE (n = 8).