Symbiotic N nutrition, C assimilation, and plant water use efficiency in Bambara groundnut (*Vigna subterranea* L. Verdc) grown in farmers' fields in South Africa, measured using ^{15}N and ^{13}C natural abundance

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Abstract Bambara groundnut (*Vigna subterranea* L. Verdc) is the second most important indigenous food legume in Africa. The aim of this study was to evaluate plant growth, N_2 fixation, N contribution, C accumulation, and plant water relations of Bambara groundnut grown in 26 farmers' fields in Mpumalanga Province of South Africa. The data revealed marked ($p \leq 0.05$) differences in plant dry matter (DM) yield, N concentration and content, $\delta^{15}\text{N}$, the proportion of N derived from symbiotic fixation (%Ndfa), and actual amounts of N-fixed between and among the 26 farms surveyed. Bambara groundnut plants obtained 33–98 % (mean=72 %) of their N nutrition from symbiotic fixation and contributed 4–200 kg N-fixed ha^{-1} (mean=102 kg N-fixed ha^{-1}). Plant density correlated positively with %N ($r=0.31^{***}$), $\delta^{15}\text{N}$ ($r=0.126^{***}$), and amount of N-fixed ($r=0.15^*$), indicating that the high %Ndfa values obtained for Bambara groundnut in this study and the low symbiotic N yield associated with some farms were due to low plant density rather than poor symbiotic functioning. Bambara groundnut obtained more N from soil (e.g., 173 kg N ha^{-1}) than from symbiosis (e.g., 135 kg N-fixed ha^{-1}) in some fields, implying that the N_2 -fixing efficacy of the microsymbionts nodulating Bambara groundnut was low at some locations in South Africa. The data from this study showed $\delta^{13}\text{C}$ values ranging from -28.01 to -26.20‰ , which indicates differences in plant water use efficiency on the different fields studied. Furthermore, the positive

correlations between $\delta^{13}\text{C}$ and N-fixed ($r=0.15^*$) and between $\delta^{13}\text{C}$ and N content ($r=0.14^*$) suggest a functional relationship between water use efficiency and N_2 fixation, just as the positively significant correlations between $\delta^{15}\text{N}$ and DM yield ($r=0.24^{***}$), N-fixed and DM weight ($r=0.76^{**}$), and N content and DM yield ($r=0.99^*$), as well as N-fixed and C content ($r=0.76^{**}$) also indicate a functional relationship between N_2 fixation and photosynthesis. In the same way, the positive correlation between $\delta^{13}\text{C}$ and DM weight ($r=0.14^*$), or $\delta^{13}\text{C}$ and C content ($r=0.15^*$), also implies a functional link between water use efficiency and plant growth. Thus, an increase in water use efficiency in Bambara groundnut, whenever it occurs, seems to functionally enhance plant growth, symbiotic N_2 fixation, and photosynthetic activity, just as N_2 fixation in nodules also seems to stimulate leaf photosynthesis.

Keywords Reference plants · Plant growth · Plant density · N_2 fixation · %Ndfa · Isotope analysis · $\delta^{15}\text{N}$ · $\delta^{13}\text{C}$ · Soil N uptake · Net N returns

Introduction

Nitrogen and P deficiency limits crop plant growth and grain yield in global agriculture (Vance 2001; Eickhout et al. 2006). Although synthetic fertilizers can improve soil N fertility, and thus increase crop yields, their continuous use can negatively impact on soil health and cause environmental pollution (Mosier and Kroezen 2000; Eickhout et al. 2006). Additionally, chemical fertilizers are expensive and inaccessible to smallholder farmers with limited credit (Bockman 1997). Taken together, these constraints limit the use of chemical inputs by resource-poor farmers in African agriculture. Incorporating N_2 -fixing legumes into cropping systems is therefore a much cheaper and sustainable way of tapping

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atmospheric N₂ for increased crop yields. Recent studies have shown that grain legumes such as cowpea (*Vigna unguiculata* L. Walp.), groundnut (*Arachis hypogaea* L.), and soybean (*Glycine max* L. Merr.) can contribute significant amounts of symbiotic N to traditional cropping systems in Africa (Belane and Dakora 2009; Naab et al. 2009; Pule-Meulenberg and Dakora 2009; Nyemba and Dakora 2010; Pule-Meulenberg et al. 2010). The ¹⁵N natural abundance, in particular, has been found to be one of the few techniques able to provide on-farm estimates of N₂ fixation by legumes (Pule-Meulenberg and Dakora 2009; Nyemba and Dakora 2010).

Bambara groundnut is a food legume that is under-researched and underutilized in Africa. As a result, there are no improved varieties or recommended agronomic practices for this legume in Africa (Ntundu et al. 2006). Although Bambara groundnut is generally regarded as being drought tolerant, there is little information on its water use efficiency under field conditions. Currently, carbon isotope discrimination is recognized as a potential tool for studying water use efficiency in C3 plant species such as Bambara groundnut. Interestingly, Basu et al. (2007) have shown that, in the case of Bambara groundnut, carbon isotope discrimination is under multigene control and could therefore serve as a true indicator of plant water relations in this species.

The $\delta^{13}\text{C}$ of C3 plants such as Bambara groundnut has been reported to reflect the water use efficiency of those species (Farquhar et al. 1989). During photosynthesis, plants discriminate against the heavier ¹³CO₂ in favor of the lighter ¹²CO₂; therefore, ¹³C becomes more abundant (or less negative) in plant tissues relative to ¹²C in the atmosphere. However, during periods of soil water deficit, a partial closure of stomata can occur, leading to an intercellular CO₂ concentration that is just sufficient to saturate photosynthesis. Under those circumstances, less ¹³C discrimination occurs, and less water is also lost. This forms the basis for the use of $\delta^{13}\text{C}$ as a tool for measuring water use efficiency in C3 species such as Bambara groundnut.

As a food crop, Bambara groundnut is cultivated throughout sub-Saharan Africa, stretching from the sahel regions of West Africa, across Central Africa to East Africa, and then down to Southern Africa. The ability of Bambara groundnut to grow in these different agro-ecologies (Jørgensen et al. 2010), with soils differing in pH, mineral nutrients, soil moisture, rhizosphere temperature, and N₂-fixing microsymbionts, is rather intriguing. In the African continent, it is ranked the second most important indigenous food legume after cowpea (Azam-Ali et al. 2001). Nutritionally, the grain of Bambara groundnut is a complete meal with 14–24 % protein, 60 % carbohydrate, and 6–12 % oil (Brough and Azam-Ali 1992; Mahala and Mohammed 2010), components ideal for human health. Furthermore, the spread of its cultivation into new areas such as Malaysia, Indonesia, Thailand, and the Papuan region of Southeast Asia (Ilyas and Sopian 2013; Redjeki et al. 2013; Somta et al. 2013) suggests that Bambara groundnut is a major

food security crop in the world. Yet, little is known about its nodulation, N₂ fixation, microsymbiont biodiversity, plant water relations, and nutritional adaptation to different environments. These alone ought to justify renewed research interest in this species, in addition to the superior dietary value of its grain as food. The aim of this study was to evaluate plant growth, N₂ fixation, water use efficiency, pod yield, and net N returns to soil of Bambara groundnut plants sampled from farmers' fields in Mpumalanga Province of South Africa, using ¹⁵N and ¹³C natural abundance. The report presented here is part of a wider study on Bambara groundnut symbiosis, including its microsymbiont biodiversity in Africa.

Material and methods

Site description, plant sample collection, and processing

The selection of fields and plants was done, as described by Nyemba and Dakora (2005). Ten plants were randomly sampled from each farm by walking in a zigzag manner across the length of the field starting from one corner. The plants were dug up using a shovel and separated into nodulated roots and shoots plus pods. In all, 26 farmers' fields were surveyed in six villages (namely, Machipe, Majakaneng, Dikgwale, Malekutu, Phameni, and Skhwahlane) within the Mpumalanga Province of South Africa in 2009. The number of farmers' fields sampled varied from village to village depending on the willingness of farmers to allow plants to be collected from their farms. A paired legume–reference plant sampling procedure was used, as described by Unkovich et al. (1994). The shoots of non-legume species growing near the Bambara groundnut plants in each farm were harvested as reference plants for the estimation of soil N uptake by the legume. All plant materials (i.e., shoots plus pods of Bambara groundnut and shoots of reference plants) were oven dried at 60 °C for 48 h, weighed, and milled to fine powder (0.85 mm sieve) for ¹⁵N and ¹³C isotope analysis.

Soil sampling and analysis

Bulk soil (i.e., soil not associated with plant roots) was sampled from a 0 to 20 cm depth on wide empty spaces in between plants in each farm using an augur. The soil samples were taken to the laboratory, sieved (2 mm), and stored in the fridge (4 °C) prior to analysis for chemical properties.

Measurement of N₂ fixation

¹⁵N/¹⁴N isotopic analysis

Finely ground plant material was weighed into Al tin capsules (1–2 mg/legume sample and 2–3 mg/reference plant) and

analyzed for %N and $^{15}\text{N}/^{14}\text{N}$ ratio using a Carlo Erba NA1500 elemental analyzer (Fisons Instruments SpA, Strada, Rivoltana, Italy) coupled to a Finnigan MAT252 mass spectrometer via ConFlo II open-split device.

The ^{15}N natural abundance is expressed as δ (delta) notation and is the per mille deviation of the ^{15}N natural abundance of the sample from atmospheric (atm) N_2 (0.36637 atom % ^{15}N). The isotopic composition ($\delta^{15}\text{N}$) was measured as described by Unkovich et al. (2008):

$$\delta^{15}\text{N} = \frac{\left(\frac{^{15}\text{N}}{^{14}\text{N}}\right)_{\text{sample}} - \left(\frac{^{15}\text{N}}{^{14}\text{N}}\right)_{\text{atm}}}{\left(\frac{^{15}\text{N}}{^{14}\text{N}}\right)_{\text{atm}}} \times 1000$$

where $^{15}\text{N}/^{14}\text{N}_{\text{sample}}$ is the abundance ratio of ^{15}N and ^{14}N in the Bambara groundnut sample, and $^{15}\text{N}/^{14}\text{N}_{\text{atm}}$ is the abundance ratio of ^{15}N and ^{14}N in the atmosphere.

The combined ^{15}N natural abundance of shoots plus pods was calculated as an average of the ^{15}N natural abundance values of shoots and pods weighted by their respective total N contents as (Robinson et al. 2000):

$$\delta^{15}\text{N}_{\text{whole plant}} = \frac{\sum (\delta^{15}\text{N}_{\text{pod}} \times \text{N}_{\text{pod}} + \delta^{15}\text{N}_{\text{shoot}} \times \text{N}_{\text{shoot}})}{\sum (\text{N}_{\text{pod}} + \text{N}_{\text{shoot}})}$$

Nitrogen content

The N content of each plant organ was determined as the product of %N and organ weight as (Pausch et al. 1996):

$$\text{Organ N (mg plant}^{-1}) = \text{drymatter of organ} \times \% \text{N of organ}$$

Percent N derived from fixation (%Ndfa)

The percent N derived from symbiotic fixation of atmospheric N_2 was estimated using the equation (Shearer and Kohl 1986; Unkovich et al. 2008):

$$\% \text{Ndfa} = \frac{\delta^{15}\text{N}_{\text{ref}} - \delta^{15}\text{N}_{\text{leg}}}{\delta^{15}\text{N}_{\text{ref}} - B} \times 100$$

where the $\delta^{15}\text{N}_{\text{ref}}$ is the ^{15}N natural abundance of reference plants, $\delta^{15}\text{N}_{\text{leg}}$ is the ^{15}N natural abundance of legume, and the B value is the ^{15}N natural abundance of test legume wholly dependent on N_2 fixation for its N nutrition. The B value replaces the value of atmospheric N_2 as it incorporates the isotopic fractionation associated with N_2 fixation (Unkovich

et al. 2008). In this study, the B value used (−1.4‰) was obtained from Nyemba and Dakora (2010).

The amount of N-fixed was calculated as (Maskey et al. 2001):

$$\text{N-fixed} = \text{amount of N in (shoots + pods)} \times \% \text{Ndfa of (shoots + pods)}$$

N-fixed per hectare was estimated as the product of fixed N in shoots + pods per plant and plant density per hectare. The plant density in each field was measured by counting the number of Bambara groundnut plants in a 4-m² quadrant area and using it to estimate plant population per hectare.

$^{13}\text{C}/^{12}\text{C}$ isotopic analysis

To analyze for $^{13}\text{C}/^{12}\text{C}$ ratio, shoot samples of Bambara groundnut (2–3 mg) were weighed into tin capsules and run on a mass spectrometer, as described for $^{15}\text{N}/^{14}\text{N}$ isotopic ratio. The ratio of $^{13}\text{C}/^{12}\text{C}$ in each sample was used to calculate the ^{13}C natural abundance ($\delta^{13}\text{C}$) as (Stout and Rafter 1978):

$$\delta^{13}\text{C} = \left(\frac{\left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_{\text{sample}} - \left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_{\text{standard}}}{\left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_{\text{standard}}} - 1 \right) \times 1000$$

where $^{13}\text{C}/^{12}\text{C}_{\text{sample}}$ is the isotopic ratio of the sample, and $^{13}\text{C}/^{12}\text{C}_{\text{standard}}$ is the isotopic ratio of Belemnite Pee Dee limestone formation (Craig 1957), a universally accepted standard. Shoot C content per plant was calculated as the product of %C and shoot DM weight.

Statistical analysis

Statistical analyses were carried out with the STATISTICA (StatSoft Inc., Tulsa, OK, USA) package. Data were subjected to a normal distribution test, followed by one-way analysis of variance (ANOVA). Where there were significant differences, treatment means were separated using the Duncan multiple range test.

Results

Soil chemical properties

In general, Skhwahlane and Dikgwale sites appeared to have intermediate to high levels of C, N, Ca, Mg, B, and Fe relative to the other sites (Table 1).

Table 1 Properties (pH and macro- and micronutrients) of bulk soil sampled from 0 to 20 cm in each farmer's field

Location	Farm	pH (KCl)	Ca mg kg ⁻¹	Mg	P	C	N	Mn	B	Fe
Village										
Machipe	1	4.4	10.2	3.12	8	3.5	0.25	7.7	0.03	54.0
	2	5.2	38.8	4.08	5	5.1	0.25	51.7	0.05	65.5
	3	4.5	14.0	4.08	11	3.8	0.23	37.3	0.06	87.1
	4	4.5	14.2	4.2	11	3.8	0.17	40.4	0.06	85.6
	5	4.9	38.2	3.84	6	5.0	0.27	44.8	0.05	54.8
	6	5.7	17.8	5.04	11	4.5	0.36	76.2	0.06	124.5
Majakaneng	7	5.8	18.6	5.28	11	4.4	0.21	76.4	0.06	109.7
	8	4.9	42.6	4.56	6	5.6	0.35	48.6	0.04	59.0
Dikgwale	9	4.9	43.0	4.44	6	5.3	0.35	54.5	0.05	68.3
	10	4.2	11.2	3.0	3	2.3	0.21	62.3	0.02	47.7
Malekutu	11	4.5	11.2	3.0	3	3.0	0.19	25.9	0.03	36.1
	12	4.5	15.8	4.08	5	3.1	0.22	52.9	0.02	41.0
	13	4.4	8.6.0	1.92	4	3.3	0.19	7.8	0.01	39.5
	14	5.2	26.8	3.72	4	4.1	0.19	165.7	0.07	84.6
	15	4.8	11.0	2.4	4	3.2	0.18	34.2	0.03	29.0
	16	4.6	11.4	2.64	4	2.9	0.13	17.1	0.02	30.2
	17	4.4	8.2	1.8	4	3.5	0.17	5.0	0.02	24.5
	18	4.2	9.0	1.8	4	2.5	0.31	11.6	0.03	62.6
Phameni	19	5.1	21.2	4.56	4	4.4	0.19	78.3	0.05	62.6
	20	4.9	22.8	5.16	5	5.3	0.16	18.1	0.04	67.5
	21	5.2	26.4	3.84	4	3.9	0.18	156.3	0.07	81.5
	22	4.5	9.2	2.28	3	3.0	0.21	88.9	0.02	61.8
Skhwahlane	23	7.6	163.4	17.64	136	7.3	0.45	85.1	0.17	176.2
	24	7.6	163.4	18.0	141	7.4	0.33	85.2	0.17	162.5
	25	4.2	8.2	1.56	5	2.5	0.19	120.7	0.03	71.1
	26	4.5	23.8	4.92	5	5.7	0.25	51.3	0.04	89.4

Each value is the average of a duplicate soil analysis

$\delta^{15}\text{N}$ values of reference plants

Several plant species, including grasses, herbaceous weeds, and cereals, were collected from farmers' fields in the six villages (namely, Machipe, Majakaneng, Dikgwale, Malekutu, Phameni, and Skhwahlane) in Mpumalanga Province. Where more than one reference plant was sampled per farm, the average value of the combined $\delta^{15}\text{N}$ values was used to estimate %Ndfa of Bambara groundnut on that farm. A recent study has shown that the combined mean $\delta^{15}\text{N}$ value of herbaceous weeds can be used to estimate a legume's dependence on symbiotic N_2 fixation for its N nutrition (Nyemba and Dakora 2010).

Plant growth

A comparison of Bambara groundnut plant growth between and among the 26 fields surveyed in Mpumalanga revealed marked differences in DM yield (Table 3). Across the board, DM yield was highest at Dikgwale (field 9, 162 g plant⁻¹),

followed by Skhwahlane (field 25, 141.9 g plant⁻¹), Malekutu (field 18, 109.1 g plant⁻¹), Skhwahlane (field 23, 89.2 g plant⁻¹), and Skhwahlane (field 24, 86.5 g plant⁻¹). The lowest DM (6.0 g plant⁻¹) was obtained at Malekutu (field 12).

Dry matter yield of shoots plus pods differed significantly ($p \leq 0.05$) between fields around each village surveyed. For example, at the Machipe site, DM production was highest in field 4 (64 g plant⁻¹) and lowest in field 5 (17 g plant⁻¹), while at Majakaneng, DM yield was 81 g plant⁻¹ in field 6 compared to 20 g plant⁻¹ in field 8 (Table 3). Similarly, Bambara groundnut showed better growth at Dikgwale in field 9 (163 g dry mass plant⁻¹) compared to field 8 (68.6 g plant⁻¹).

Field 10 at Dikgwale similarly showed greater plant growth (163.3 g DM plant⁻¹) than field 9 (68.6 g DM plant⁻¹). Of the 9 farms sampled at Malekutu, farm 12 produced 5.9 g DM plant⁻¹ in contrast to farm 18, which yielded 109.1 g DM plant⁻¹ (Table 3). Significant differences were similarly found in the DM yield of shoots plus pods at Phameni. For example, shoot plus pod biomass production ranged from 7.8 g DM plant⁻¹ (field 22) to 53.4 g plant⁻¹ (field 19) at the Phameni

site, while it ranged from 73 g DM plant⁻¹ (field 26) to 142 g DM plant⁻¹ (farm 25) at Skhwahlane (Table 3).

N concentration and N content

N concentration and content of Bambara groundnut plants sampled across Mpumalanga Province also differed significantly between fields. The N concentration of shoots plus pods ranged from 1.7 to 3.9 % across the 26 farmers' fields studied (Table 3). As a result of those differences in N concentration, N content of shoots plus pods also differed between and among fields. For example, the N content of Bambara groundnut shoots plus pods was much greater at Skhwahlane field 25 (4.4 g plant⁻¹), followed by Dikgwale field 9 (3.9 g plant⁻¹), Malekutu field 18 (3.6 g plant⁻¹), and Skhwahlane field 24 (3.2 g plant⁻¹). In contrast, the lowest DM yield was produced at Malekutu (see fields 12, 14, and 13).

$\delta^{15}\text{N}$ values and %Ndfa

The $\delta^{15}\text{N}$ of Bambara groundnut plants sampled in Mpumalanga Province showed marked differences across the 26 farmers' fields surveyed and varied from -1.77 to +1.92‰ for shoots and -1.29 to +2.61‰ for shoots plus pods (Table 3). The highest $\delta^{15}\text{N}$ value was obtained at Malekutu (field 10), followed by Skhwahlane (fields 26 and 25) and Dikgwale (field 8). In contrast, the lowest $\delta^{15}\text{N}$ values for shoots plus pods were recorded at Malekutu (field 12) and Phameni (field 20). The %Ndfa of Bambara groundnut shoots plus pods also differed markedly between farmers' fields and ranged from 33 % (field 10) to 98 % at Malekutu (field 12). Except for Malekutu (field 10) and Skhwahlane (field 25), where the legume derived below 50 % N (i.e., 33 and 43 %, respectively) from symbiotic fixation, Bambara groundnut was able to obtain between 52 and 98 % of its N nutrition on all the other fields studied (Table 4).

Amounts of N-fixed

The amounts of N-fixed by Bambara groundnut plants on farmers' fields differed significantly ($p \leq 0.05$) between and among the 26 farms surveyed. As shown in Table 4, symbiotic N contribution by Bambara groundnut across the 26 fields ranged from 4 to 200 kg ha⁻¹, with both minimum and maximum values being recorded at Malekutu fields 12 and 18, respectively. The sites where Bambara groundnut plants made a greater N contribution (116–144 kg N-fixed ha⁻¹) to the N economy of the cropping system included Malekutu (field 16), Skhwahlane (fields 23, 24, and 25), and Machipe (field 4). By contrast, the areas with very low N contribution were Malekutu (field 14) and Phameni (field 21).

Net N returns to soil

The net N returns to soil from Bambara groundnut shoots ranged from 7 to 177 kg N ha⁻¹ (Table 4). Despite the low plant density, the estimated N returns to soil were quite substantial at Skhwahlane (fields 23 and 24), Malekutu (field 16), Machipe (field 4), and Malekutu (field 18) (i.e., 177, 170, 137, 125, and 124 kg N ha⁻¹, respectively; see Table 4), indicating the potential of Bambara groundnut to contribute to the N economy of cropping systems.

Soil mineral N uptake

Soil N uptake differed markedly between and among Bambara groundnut plants sampled from the 26 farmers' fields (Table 4) and ranged from 0.01 kg N ha⁻¹ at Malekutu (field 12) to 173 kg N ha⁻¹ at Skhwahlane (field 25). Other locations where soil N uptake was high included Skhwahlane (116 kg N ha⁻¹ at field 24), Malekutu (89 kg N ha⁻¹ at field 4), and Skhwahlane (63 kg N ha⁻¹ at field 26). Soil N uptake by Bambara groundnut plants was however below 10 kg N ha⁻¹ in nine other farmers' fields (Table 4).

Plant density and its relationship with biomass production and N₂ fixation

There were large differences in Bambara groundnut plant density across the 26 farmers' fields surveyed (Table 3). Relative to other sites, plant density was highest at Malekutu (e.g., fields 13 and 10 with 16 and 12 plants m⁻², respectively). In fact, 15 out of the 26 fields surveyed had very low plant densities, ranging between 3 and 6 plants m⁻² (Table 3).

As shown in Fig. 1, there was evidence to show that low plant density affected DM yield and hence N₂ fixation. Thus, plant biomass increased where legume plant density was high (Fig. 1a). The amount of N-fixed was however also much greater where Bambara groundnut density was higher in the cropping system (Fig. 1b).

Carbon accumulation

C concentration in Bambara groundnut shoots plus pods differed markedly across the 26 farmers' fields studied and ranged from 40 to 49 % (Table 5). Plants at Skhwahlane (field 23) exhibited the highest C concentration (48.9 %), followed by Phameni (46 % in each of fields 20 and 22), Skhwahlane (46 % in field 26), and Majakaneng (46 % in field 6). The lowest C concentration was recorded at Malekutu (40 % at field 10), followed by Machipe (43 % in each of fields 2 and 4), Phameni (43 % in field 19), Dikgwale (43 % in field 9), Majakaneng (43 % in field 7), and Malekutu (43 % in field 18).

As a result, the C content of Bambara groundnut shoots plus pods also differed significantly across all fields and

Fig. 1 Relationship between **a** legume plant density and N-fixed by Bambara groundnut in farmers' fields and **b** legume plant density and dry matter yield by Bambara groundnut in farmers' fields

ranged from 2.6 to 70.9 g plant⁻¹ (Table 5). The highest C content was found at Dikgwale (70.9 g plant⁻¹ in field 9), followed by Skhwahlane (62.5 g plant⁻¹ in field 25) and Malekutu (46.8 g plant⁻¹ in field 18). In contrast, the lowest C content was recorded in plants harvested from Malekutu (2.6 g plant⁻¹ in field 12), Phameni (3.6 g plant⁻¹ in field 21), and Malekutu (4.3 g plant⁻¹ in each of fields 13 and 14).

C/N ratio and photosynthetic fixed N use efficiency

The C/N ratio is a measure of the C and N relationship in plants and is therefore an estimate of photosynthetic N use efficiency.

As with other parameters, there were significant differences in C/N ratios across all 26 fields where Bambara groundnut was sampled. Overall, the C/N ratio ranged from 10.7 g g⁻¹ in field 13 to 26.6 g g⁻¹ in field 12 at Malekutu (Table 5).

The photosynthetic N-fixed use efficiency (measured as shoot C per unit N-fixed in shoots) also varied with the amounts of symbiotic N and C accumulated by field-grown Bambara groundnut plants. As shown in Table 5, the Bambara groundnut material sampled from Malekutu (field 10) showed the highest photosynthetic fixed N use efficiency (154 g C g⁻¹ N-fixed), followed by Malekutu (83 g C g⁻¹ N-fixed in field 12), Majakaneng (58 g C g⁻¹ N-fixed in field 6), and Phameni

(54 g C g⁻¹ N-fixed in field 22). The Bambara groundnut shoots with the lowest photosynthetic N-fixed use efficiency were sampled from Skhwahlane (field 24), Dikgwale (fields 8 and 9), and Skhwahlane (field 23), and they respectively recorded 5.0, 5.9, 6.5, and 6.1 g C g⁻¹ N-fixed (Table 5).

$\delta^{13}\text{C}$ values

A one-way ANOVA of $\delta^{13}\text{C}$ from Bambara groundnut shoots revealed significantly marked variation between and among farmers' fields at the different study sites. The $\delta^{13}\text{C}$ values of Bambara groundnut shoots were much greater (i.e., less negative) at Skhwahlane (fields 23 and 24, -26.20 and -26.68‰, respectively) when compared to the other sites/fields (Table 5). However, the fields that showed much lower, or more negative, $\delta^{13}\text{C}$ values in Bambara groundnut shoots include Phameni (-28.24‰ in field 21), Malekutu (-28.06‰ in field 13), and Dikgwale (-28.01‰ in field 9). Thus, the $\delta^{13}\text{C}$ values of Bambara groundnut shoots ranged from -28.24 to -26.20‰ across the 26 farmers' fields studied (Table 5).

Correlation analysis

Bambara groundnut plant density in farmers' fields was found to correlate positively with N-fixed ($r=0.15^*$), with %N ($r=0.31^{***}$) and with $\delta^{15}\text{N}$ ($r=0.126^{***}$). The $\delta^{13}\text{C}$ of Bambara groundnut shoots plus pods also correlated positively with dry matter ($r=0.14^*$), with C content ($r=0.15^*$), with N content ($r=0.14^*$), and with N-fixed ($r=0.15^*$). Furthermore, N-fixed correlated positively with DM yield ($r=0.76^{**}$) and with C content ($r=0.76^{**}$), just as shoot N content correlated positively with DM yield ($r=0.86^*$), with C content ($r=0.95^{**}$), and with $\delta^{13}\text{C}$ ($r=0.0.14^*$).

Discussion

Symbiotic N nutrition by Bambara groundnut in farmers' fields

The ^{15}N natural abundance technique was used to evaluate N_2 fixation and N contribution by Bambara groundnut plants grown in farmers' fields in Mpumalanga Province of South Africa. In estimating soil N uptake by legume, many different non-legume plant species were sampled from study sites and analyzed for their $^{15}\text{N}/^{14}\text{N}$ ratios (Table 2). The number of weeds, grasses, and crop species sampled ranged from 2 to 10/field, with mean $\delta^{15}\text{N}$ values ranging from +2.22 to +7.83‰ (Table 2). The difference between combined mean $\delta^{15}\text{N}$ value of reference plants and that of legume was generally above +2.00‰ (Unkovich et al. 1994), indicating the potential for high precision in the use of the $\delta^{15}\text{N}$ natural abundance

Table 2 The $\delta^{15}\text{N}$ values of total identified and unidentified reference plants sampled from the various villages for N_2 fixation in Bambara groundnut

Village	Farm	No. of plants	$\delta^{15}\text{N}$ values (‰)		
			Min	Max	Mean
Machipe	1	4	5.60	8.76	6.94
	2	10	2.95	5.59	4.50
	3	5	4.34	7.27	5.38
	4	6	3.01	5.14	3.24
	5	5	3.59	5.70	4.82
Digwale	6	4	3.44	5.61	4.71
	7	10	2.60	3.66	3.36
Majakaneng	8	4	2.02	6.55	5.34
	9	9	2.27	6.07	5.95
Malekutu	10	3	3.12	5.59	4.72
	11	5	2.22	6.11	4.28
	12	4	5.79	6.92	6.33
	13	3	2.12	5.97	4.03
	14	8	2.29	7.04	4.83
	15	4	3.29	6.11	5.34
	16	3	3.73	5.96	4.41
	17	5	1.80	6.70	4.57
	18	3	5.77	6.58	6.23
Phameni	19	4	0.58	5.38	3.43
	20	3	2.26	5.12	3.98
	21	2	3.09	3.95	3.53
	22	7	3.13	7.34	5.18
Skhwahlane	23	7	4.55	6.05	5.96
	24	9	3.39	5.57	3.49
	25	8	2.94	4.20	3.51
	26	6	3.14	5.65	5.40

technique for measuring N_2 fixation in Bambara groundnut (Nyemba and Dakora 2005).

Due to marked differences in legume plant density across the 26 farmers' fields surveyed, the growth of Bambara groundnut plants (measured as DM yield) also differed between and among experimental fields of each study site (Table 3). In most instances, farms with lower legume density produced greater shoot and pod biomass on an individual plant basis (Table 3). But the number of pods per plant appeared to be genetically controlled, as some farms with fewer Bambara groundnut stands produced low number of pods per plant than those with high density.

As to be expected, N concentration in pods (containing seeds) was higher than shoots (Table 3) due to N translocation from green leaves to the developing ovules. There was however no difference in N concentration of shoots and pods between fields (Table 3). But N content and %N derived from fixation differed markedly between and among farms due to variations in $\delta^{15}\text{N}$ values of shoots and pods (Table 4). The

Table 3 Comparison of plant density, dry matter yield, N content, and N concentration of Bambara groundnut sampled from 26 farmers' fields of Mpumalanga Province, South Africa

Location	Farm	Plant density No m ⁻²	Pod no Per plant	Dry matter		N concentration			N content			
				Pods g plant ⁻¹	Shoots	Pods + shoots	Pods %	Shoots	Pods + shoots	Pods mg plant ⁻¹	Shoots	Pods + shoots
Village	Machipe	1	4±0h	15±0f	2.4±0.0gh	29.7±2.6f	32.1±2.6ij	4.5±0.1b	2.3±0.1cd	108±4g	685±57e	1,102±91e
		2	3±0hi	8±0 g	3.3±0.0g	59.0±5.3c	62.2±5.3g	3.9±0.0c	3.0±0.1b	128±1g	1,798±198bc	2,165±195cd
		3	4±0h	12±0fg	1.6±0.0h	23.8±1.7fg	25.4±1.7j	3.2±0.8cd	3.1±0.1b	51±12h	758±69d	826±132ef
		4	8±0c	52±0d	11.9±0.0ef	54.1±6.7d	66.1±6.7g	5.1±0.3a	2.8±0.1c	609±39de	1,518±206.8c	2,627±303c
	Majakaneng	5	5±0g	5±0 h	1.0±0.0h	16.2±2.2 g	17.2±2.2k	4.7±0.0ab	3.0±0.1b	48±0h	457±65f	654±83f
		6	4±0gh	11±0fg	1.4±0.0h	18.7±2.5 g	20.1±2.5jk	3.7±0.0c	3.1±0.1b	52±0h	580±82ef	678±85f
		7	5±0g	72±0c	16.5±0.0e	64.7±7.6c	81.3±7.6ef	3.3±0.2cd	2.2±0.1d	551±41e	1,385±122c	2,227±203cd
	Dikgwale	8	7±0e	56±0cd	20.8±0.0d	57.4±8.8cd	78.2±8.8f	3.6±0.0c	2.1±0.2d	757±10d	1,176±195cd	2,228±238cd
		9	3±0i	185±0a	77.8±0.0a	84.4±4.6b	162.2±4.6a	2.4±0.0e	2.5±0.1cd	1,869±33a	2,075±117b	3,945±92ab
	Malekutu	10	12±0b	3±0h	0.4±0.0i	15.8±2.0g	16.2±2.0k	2.8±0.0d	3.3±0.1b	3.1±0.0b	11±0i	523±72ef
11		8±0de	38±0e	7.2±0.0fg	17.7±2.4g	24.9±2.4j	3.1±0.3cd	3.1±0.3b	3.1±0.2b	221±25f	542±89ef	782±130ef
Phameni	12	5±0 g	0±0j	0.0±0.0j	5.9±0.5i	6.0±0.5 m	0.0±0.0f	3.4±0.1b	1.7±0.0f	0±0j	195.1±13f	98±6i
	13	16±0a	2±0i	0.4±0.0i	9.2±1.0h	9.6±1.0k	4.7±0.0ab	3.7±0.1ab	4.2±0.0a	19±0hi	346±42f	405±45g
	14	4±0gh	0±0j	0.0±0.0j	9.5±0.9h	9.5±0.9k	0.0±0.0f	3.7±0.1ab	1.9±0.1e	0±0j	360±44f	180±22h
	15	5±0g	52±0d	8.9±0.0f	46.4±9.5e	55.3±9.5h	3.1±0.2cd	2.9±0.1c	3.0±0.1b	278±22f	1,295±222cd	1,617±232d
	16	8±0cd	52±0d	9.0±0.0f	61.2±8.2c	70.2±8.2fg	3.7±0.3c	2.8±0.1c	3.3±0.1ab	334±26f	1,717±232bc	2,274±252cd
	17	6±0f	18±0f	1.7±0.0h	27.2±3.9f	28.9±3.9ij	3.3±0.3cd	3.0±0.1b	3.1±0.2b	54±6h	811±105d	912±133ef
	18	6±0f	117±0b	40.0±0.0bc	69.1±14.0c	109.1±14.0c	3.7±0.4c	2.9±0.1c	3.3±0.2ab	1,470±144b	1,982±406b	3,562±486b
	19	8±0cd	61±0cd	27.2±0.0cd	29.5±4.2f	56.7±4.2h	2.8±0.0d	2.7±0.3c	2.8±0.1c	767±3d	831±159d	1,583±159d
	20	7±0de	14±0f	1.7±0.0h	22.8±4.7fg	24.5±4.7j	3.7±0.0c	2.5±0.1cd	3.1±0.0b	64±0h	569±114ef	766±145ef
	21	3±0hi	4±0h	0.4±0.0i	7.3±1.3hi	7.8±1.3 l	2.5±0.1e	2.8±0.4c	2.7±0.2c	11±0i	211±49f	210±39h
Skhwahlane	22	8±0d	33±0e	16.6±0.0e	22.2±2.2fg	38.8±2.2i	3.3±0.2cd	3.8±0.2a	3.5±0.1ab	544±34e	844±101d	1,363±61de
	23	6±0f	59±0cd	32.0±0.0c	57.2±12.7cd	89.2±12.7d	3.7±0.1c	2.8±0.1c	3.2±0.0b	1,185±21c	1,635±393bc	2,890±413c
	24	8±0cd	67±0c	20.9±5.2d	65.6±2.5c	86.5±5.2d	4.3±0.2b	3.2±0.5b	3.7±0.2ab	853±161d	2,126±326ab	3,243±272bc
	25	7±0de	72±0c	46.6±0.0b	95.2±4.5a	141.9±4.5b	3.5±0.0c	2.6±0.2cd	3.1±0.1b	1,653±13ab	2,527±271a	4,396±238a
F statistics	26	5±0g	52±0d	20.6±0.0d	52.2±5.4d	72.8±5.4fg	3.9±0.2c	3.9±0.1a	3.9±0.1a	802±33d	2,067±251b	2,870±271c
		1,154,2**	1.5**	482.0**	15.2**	37.0**	21.2**	9.8**	16.1**	164.8**	12.5**	30.2**

Values (mean ± SE) with dissimilar letters in a column are significantly different at $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$ *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$

Table 4 Comparison of amount of $\delta^{15}\text{N}$ values, %Ndfa, N-fixed, net N returns, and soil N uptake of Bambara groundnut sampled from 26 farmers' fields of Mpumalanga Province, South Africa

Location	Farm	$\delta^{15}\text{N}$			Ndfa	N-fixed		Net N returns		Soil N uptake
		Pods	Shoots	Pods + shoots	Pods + shoots	Pods + shoots		Shoots		Pods + shoots
		‰			%	g plant ⁻¹	kg ha ⁻¹	g plant ⁻¹	kg ha ⁻¹	kg ha ⁻¹
Village										
Machipe	1	0.05±0.24g	-0.10±0.10e	0.03±0.22g	83±3bc	0.9±0.1d	36±3f	0.7±0.1d	27±2hi	8±2i
	2	0.38±0.19de	0.90±0.26bc	0.43±0.17e	69±3e	0.5±0.1c	48±4e	1.8±0.2bc	58±6f	23±4g
	3	-0.24±0.4li	0.36±0.32c	-0.17±0.39ij	82±6bc	0.7±0.1de	28±5g	0.8±0.1d	30±3 h	6±2j
	4	0.51±0.23d	0.33±0.07c	0.47±0.15e	60±3fg	0.6±0.2c	128±16b	1.5±0.2c	125±17bc	89±13c
	5	-0.61±0.25j	0.41±0.09c	-0.49±0.22k	85±4b	0.6±0.1e	25±3gh	0.5±0.1de	21±3i	5±2j
Majakaneng	6	0.28±0.39e	-0.30±0.29f	0.23±0.34f	73±6d	0.5±0.1e	21±3h	0.6±0.1de	25±5i	8±2i
	7	0.41±0.35de	-0.62±0.07g	0.09±0.25g	69±5e	1.5±0.2c	72±8cd	1.4±0.1c	66±6ef	34±6f
Dikgwale	8	1.59±0.26d	1.11±0.11b	1.37±0.13bc	62±2f	1.4±0.1c	90±9c	1.2±0.2cd	77±13d	55±7d
	9	2.11±0.58ab	-0.58±0.35g	0.82±0.44d	70±6e	2.7±0.2a	75±6cd	2.1±0.1b	57±3f	33±7f
Malekutu	10	2.68±0.13a	-0.47±0.01fg	2.61±0.13a	33±2i	0.2±0.0fg	19±3hef	0.5±0.1de	60±8ef	38±5e
	11	0.74±0.33cd	-1.77±0.42j	-0.04±0.26h	77±4cd	0.6±0.1e	46±9ef	0.5±0.1de	41±7g	13±3h
	12	-1.29±0.40k	0.00±0.00d	-1.29±0.40o	98±7a	0.1±0.0h	4±oj	0.2±0.0f	9±1k	0±0l
	13	0.93±0.22c	-0.60±0.05g	0.85±0.21d	62±4f	0.2±0.0f	38±2f	0.3±0.0e	55±7f	26±5g
	14	-0.14±0.29hi	0.00±0.00d	-0.14±0.29i	79±5c	0.1±0.0h	6±1i	0.4±0.0e	15±2j	2±0k
	15	0.53±0.25d	-0.28±0.00d	0.40±0.20e	70±3e	1.1±0.2d	58±15de	1.3±0.2cd	69±20e	27±7g
	16	0.13±0.13f	-0.98±0.36i	-0.09±0.13hi	80±2c	1.8±0.2bc	144±15b	1.7±0.2bc	137±19b	38±7e
	17	-0.65±0.27j	-0.52±0.006fg	-0.64±0.26l	88±3ab	0.8±0.2bc	48±6e	0.8±0.1d	49±6fg	7±3i
Phameni	18	-0.81±0.21jk	-0.70±0.20h	-0.73±0.09m	90±1ab	3.2±0.4a	200±28a	2.0±0.4b	124±25bc	23±4gh
	19	-0.03±0.33h	-0.47±0.0fg	-0.20±0.19j	76±4cd	1.2±0.1cd	94±7c	0.8±0.2d	66±13e	33±9f
	20	-0.90±0.10jk	-0.30±0.01f	-0.83±0.09n	90±2ab	0.7±0.1de	49±10e	0.6±0.1de	40±8g	5±1j
	21	-0.32±0.29ij	-0.17±0.01e	-0.32±0.27k	77±6cd	0.2±0.0fg	6±1i	0.2±0.0f	7±2k	2±0k
Skhwahlane	22	0.93±0.16jk	-0.39±0.04fg	0.71±0.11m	90±2ab	1.2±0.1cd	92±4c	0.8±0.1d	63±8ef	10±2h
	23	0.94±0.26c	1.55±0.37ab	1.15±0.19c	66±3ef	1.9±0.3b	116±18bc	1.6±0.4c	98±24c	58±7d
	24	0.50±0.26d	1.92±0.17a	0.89±0.26d	54±5g	0.8±0.3bc	143±21b	2.1±0.3b	170±26a	116±14b
	25	1.67±0.25b	0.92±0.41bc	1.38±0.17bc	43±4h	1.9±0.3b	135±18b	2.5±0.3a	177±19a	173±5a
	26	2.51±0.42a	0.18±0.22cd	1.81±0.31b	52±5g	1.5±0.2c	66±8d	2.1±0.3b	93±11cd	63±9d
F statistics		12.1***	14.3***	13.4***	14.3**	21.6**	20.8**	12.5**	11.8***	31.0**

Values (mean ± SE) with dissimilar letters in a column are significantly different at $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$

shoot $\delta^{15}\text{N}$ ranged from -1.77 to +1.92‰ and that of pods from -1.29 to +2.51‰ (Table 4). Calculating the average of $\delta^{15}\text{N}$ in shoots and pods weighted by their total N contents (Robinson et al. 2000) produced mean $\delta^{15}\text{N}$ data ranging from -1.29 to +2.61‰, and it is these mean $\delta^{15}\text{N}$ values of shoots plus pods that were used to estimate %Ndfa. Because of the low $\delta^{15}\text{N}$ values obtained in this study, the Bambara groundnut plants derived between 33 and 98 % of their N nutrition from symbiotic fixation, levels comparable to those obtained for this species in Zambia (Nyemba and Dakora 2010). This clearly indicates that, in South Africa, Bambara groundnut relies heavily on N_2 fixation for meeting its N nutrition.

Perhaps as a result of factors such as low plant density (see Fig. 1) and the efficacy of the microsymbionts nodulating

Bambara groundnut (Dakora and Keya 1997; Pule-Meulenberg et al. 2010), N contribution by the species varied hugely between and among fields and ranged from 4 to 200 kg ha⁻¹ (mean=102 kg N-fixed ha⁻¹; Table 4). This variation in symbiotic performance was perhaps not unexpected as the bacterial symbionts isolated from root nodules of Bambara groundnut plants displayed a similarly wide range in symbiotic efficacy (data not shown). The locations where Bambara groundnut made significant N contribution to the cropping system included Skhwahlane fields 23, 24, and 25, which recorded 116, 143, and 135 kg N-fixed ha⁻¹, respectively (Table 4). Incidentally, those fields with greater N contribution either had relatively high pH (e.g., pH 7.6 in fields 23 and 24), greater soil concentrations of Ca, P, and

Fe needed for symbiosis (e.g., fields 23 and 24), or high levels of Mn (e.g., field 25). The endogenous soil factors mentioned here could account for the increased N contribution made by Bambara groundnut plants at those sites (Table 4). However, the differences in symbiotic performance by Bambara groundnut could also be due to its bacterial partner as legumes growing in nutrient-poor, low pH soils in Africa have been found to form root nodules with both α - and β -proteobacteria (Hassen et al. 2012; Kanu and Dakora 2012; Angus et al. 2013; Howieson et al. 2013). It is important to note that even though Bambara groundnut plants derived as high as 98 % of their N nutrition from symbiotic fixation at Malekutu (field 12), the actual amount of N-fixed was very low, only 4 kg ha⁻¹ (Table 4). This small amount of N contributed by plants with 98 % Ndfa value was likely due to low plant density (Table 3) rather than poor symbiotic functioning (Table 4) as positive correlations were found between plant density and N-fixed ($r=0.15^*$), plant density and %N ($r=0.31^{***}$), as well as plant density and $\delta^{15}\text{N}$ ($r=0.126^{***}$). While the N contribution made by Bambara groundnut on some fields could be considered high (e.g., Skhwahlane fields 23, 24, and 25), the actual amounts of N-fixed were nevertheless underestimated by the N content in roots and nodules, which were not harvested.

Similar studies done by Nyemba and Dakora (2010) in farmers' fields found that N contribution by Bambara groundnut also varied with location and was 21, 39, and 68 kg N ha⁻¹ in Southern, Central, and Northern Zambia, respectively. Compared to the data from this study, N contribution by Bambara groundnut is much greater in South Africa relative to Zambia. It was also interesting to note that, at some sites in South Africa, Bambara groundnut obtained more N from soil than symbiosis (e.g., 173 kg N from soil vs. 135 kg N ha⁻¹ from symbiosis on farm 25 at Skhwahlane and 116 kg N from soil vs. 143 kg N ha⁻¹ from symbiosis on farm 24 at Skhwahlane; see Table 4). Although these high levels of N uptake might have, to some extent, inhibited nodule functioning in Bambara groundnut (Streeter 1988; Ayisi et al. 2000), earlier studies have shown that some landraces of the species are tolerant of mineral N in the rhizosphere (Dakora et al. 1992; Dakora 1998). Whatever the case, even under farmer conditions, the net N returns by Bambara groundnut to the cropping system was quite significant, ranging from 7 to 177 kg N ha⁻¹ (Table 4). The mean net N returns of 92 kg N ha⁻¹ from Bambara groundnut grown in farmers' fields indicate the potential of this species for use as a biofertilizer in cropping systems of resource-poor African farmers.

Carbon assimilation and photosynthetic fixed N use efficiency in Bambara groundnut

Carbon accumulation in plant species (whether measured as concentration or content) is directly linked to photosynthetic activity (Belane and Dakora 2011). In this study, C

concentration in Bambara groundnut ranged from 40 to 49 % (Table 5), levels comparable to those reported for symbiotic legumes (Sprent et al. 1996). As to be expected, Bambara groundnut plants that exhibited greater C content (Table 5), always accumulated higher DM (Tables 3), a finding consistent with the report that photosynthetic C accumulation accounts for a substantial amount of plant total biomass (Taiz and Zeiger 2002).

The level of photosynthetic C accumulation in plants is regulated by N nutrition. The C/N ratio is therefore a good measure of the N status in plants and indicates the C-to-N relationship in tissues. Because bacteroids in root nodules of symbiotic legumes reduce atmospheric N₂ to NH₃, N₂-fixing species tend to have C/N values less than 24 g g⁻¹ and non-legumes more than 24 g g⁻¹ (Hobbie et al. 1998). Except for two fields where C/N values were above 24 g g⁻¹, all the other fields exhibited C/N ratios below 24 g g⁻¹ (Table 5), which are consistent with those of symbiotic legumes (Hobbie et al. 1998). In all instances, the C/N value increased (Table 5) with decreasing $\delta^{15}\text{N}$ values (Table 4), suggesting that photosynthetic C supply was increased by higher levels of N₂ fixation in Bambara groundnut.

Furthermore, Bambara groundnut plants sampled from Malekutu (field 10) showed the highest photosynthetic fixed N use efficiency (154 g C g⁻¹ N-fixed), followed by Malekutu field 12 (83 g C g⁻¹ N-fixed), Majakaneng field 6 (58 g C g⁻¹ N-fixed), and Phameni field 22 (54 g C g⁻¹ N-fixed). By contrast, Bambara groundnut shoots from Skhwahlane field 24, Dikgwale fields 8 and 9, and Skhwahlane field 23 displayed the lowest photosynthetic fixed N use efficiency (5.0, 5.9, 6.5, and 6.1 g C g⁻¹ N-fixed, respectively; see Table 5). These findings seem to support the view that photosynthetic activity in Bambara groundnut was stimulated by symbiotic N nutrition, as sites producing low C/N-fixed ratios (Table 5) yielded high symbiotic N (Table 4; also see Belane and Dakora 2011).

$\delta^{13}\text{C}$ and its relationship with N and C nutrition in nodulated Bambara groundnut

The $\delta^{13}\text{C}$ of C3 plants is a known measure of water use efficiency in those species (Farquhar et al. 1989; Basu et al. 2003). In this study, the variation in $\delta^{13}\text{C}$ of Bambara groundnut plants ranged from -28.24 to -26.20‰ for the 26 fields (Table 5), thus indicating differences in water use efficiency between and among farmers' fields. Interestingly, Bambara groundnut from Skhwahlane (fields 23, 24, and 25), which showed relatively greater $\delta^{13}\text{C}$ values (-26.20, -26.68, and -27.57‰, respectively, see Table 5) made the highest N contribution to the cropping system (Table 4), while Phameni (field 21), which recorded the lowest $\delta^{13}\text{C}$ (Table 5), made the least N contribution (Table 4). This can be interpreted to mean that there is a functional relationship between plant water use efficiency and symbiotic N₂ fixation, and that

Table 5 Comparison of C concentration and content, photosynthetic N use efficiency, and $\delta^{13}\text{C}$ on Bambara groundnut plants sampled from 26 farmers' fields of Mpumalanga Province, South Africa

Location	Farm	Carbon accumulation		PNUE		δ ¹³ C
		C concentration %	C content g plant ^{−1}	C/N-fixed g·g ^{−1}	C/N	
Village						
Machipe	1	44.4±0.3cd	14.2±1.2ef	14.5±0.5fg	13.0±0.3e	−26.94±0.14b
	2	42.8±0.4de	26.7±2.4d	17.1±3.0bc	12.4±0.2ef	−27.19±0.17bc
	3	45.2±1.2c	11.4±0.7f	18.2±4.0e	17.1±3.0bc	−27.32±0.18bc
	4	42.8±0.2de	28.3±2.9d	15.8±1.1f	11.1±0.5f	−27.32±0.16bc
Majakaneng	5	44.9±0.4c	7.7±1.0g	13.0±0.8fg	11.8±0.2ef	−27.79±0.11de
	6	45.8±0.4bc	9.3±1.2fg	57.7±8.3c	13.7±0.4de	−27.34±0.10bc
	7	43.2±0.6de	35.2±3.4c	21.8±3.2e	15.9±0.8c	−26.79±0.12ab
Dikgwale	8	43.6±0.4d	34.1±3.8c	6.5±0.7i	15.3±0.6cd	−27.47±0.16c
	9	43.2±0.65d	70.9±2.0a	5.9±2.6i	18.0±0.6b	−28.01±0.27f
Malekutu	10	40.2±0.7e	6.5±0.8g	153.7±30.5a	13.1±0.2e	−27.67±0.14d
	11	44.4±0.9cd	11.1±1.0f	7.9±2.3h	15.0±1.3cd	−27.70±0.12d
	12	44.6±0.5cd	2.6±0.2j	83.4±12.0b	26.6±0.8a	−27.87±0.15e
	13	45.0±0.5c	4.3±0.4h	19.1±2.2e	10.7±0.2f	−28.06±0.15f
	14	45.0±0.5c	4.3±0.5h	34.8±9.5d	24.2±0.9a	−27.92±0.23e
	15	44.3±0.5cd	24.3±4.1d	14.1±6.3fg	15.0±0.6cd	−27.81±0.21e
	16	45.0±0.6c	31.5±3.6cd	10.8±1.0g	14.0±0.7d	−27.78±0.26d
	17	44.5±0.6cd	12.8±1.7f	28.0±4.5de	14.4±0.6d	−26.89±0.21ab
	18	43.3±0.9d	46.8±5.4b	19.5±7.6e	13.5±1.0de	−27.88±0.11e
Phameni	19	43.0±1.1de	24.6±2.4d	16.8±3.7ef	15.8±0.8c	−27.10±0.70b
	20	45.9±0.4b	11.3±2.2f	41.0±7.3cd	14.7±0.3cd	−27.91±0.17e
	21	45.6±0.2bc	3.6±0.6hi	46.8±10.7cd	17.3±1.5bc	−28.24±0.26f
	22	46.1±0.4b	17.9±1.0e	53.8±32.0c	13.1±0.3e	−27.57±0.00c
Skhwahlane	23	48.9±0.3a	43.6±6.2bc	6.1±1.0i	15.1±0.2cd	−26.20±0.33a
	24	43.7±0.7d	37.8±2.4c	5.0±1.3j	11.9±1.0ef	−26.68±0.13ab
	25	43.9±0.4cd	62.3±2.2a	15.4±8.4f	14.2±0.4d	−27.57±0.26c
	26	45.9±0.7b	33.4±2.6a	18.0±3.6e	11.8±0.4ef	−27.95±0.38ef
F statistics		6.5***	36.3**	10.5**	17.0**	3.8***

Values (mean ± SE) with dissimilar letters in a column are significantly different at $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$

PNUE photosynthetic N use efficiency

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$

improved water use in nodulated legumes supports nodule functioning. This argument is reinforced by the finding that $\delta^{13}\text{C}$ correlated positively with N content ($r=0.14^*$), just as $\delta^{13}\text{C}$ also correlated with amount of N-fixed ($r=0.15^*$). Furthermore, there was a positive correlation between $\delta^{13}\text{C}$ and DM yield ($r=0.14^*$), $\delta^{13}\text{C}$ and C content ($r=0.15^*$), $\delta^{15}\text{N}$ and DM yield ($r=0.24^{***}$), N content and DM yield ($r=0.99^{**}$), amount of N-fixed and DM yield ($r=0.76^{**}$), as well as N-fixed and C content ($r=0.76^{**}$). Taken together, these results suggest the existence of a functional relationship between symbiotic N nutrition and plant water use efficiency, between N_2 fixation and photosynthesis, and between water use efficiency and plant growth in Bambara groundnut.

C3 plant species increase their $\delta^{13}\text{C}$ (or water use efficiency) with drought events (Vandoorne et al. 2012), in the same way that they increase their $\delta^{13}\text{C}$ values when experiencing soil water deficit (Naumburg et al. 2003; Hartman and Danin 2010; Wang et al. 2010). It is therefore likely that the high $\delta^{13}\text{C}$ values or greater water use efficiency of Bambara groundnut plants harvested from fields around Skhwahlane and other locations were due to reduced soil moisture from low or uneven rainfall at those study sites. Unfortunately, no rainfall data could be obtained from farmers' fields to help explain these variations in shoot $\delta^{13}\text{C}$.

In conclusion, we have used the ^{15}N and ^{13}C natural abundance to assess symbiotic functioning and plant water

relations in Bambara groundnut grown in farmers' fields. The data revealed about 33 to 98 % dependency on symbiotic N_2 fixation for the species' N nutrition, an N contribution of 4 to 200 kg N-fixed ha^{-1} to the cropping system, and net N returns of 7 to 177 kg fixed N ha^{-1} . The sometimes small symbiotic N yield per hectare obtained in this study for Bambara groundnut was due to low plant density rather than poor symbiotic functioning in farmers' fields. With proper management of plant density, this legume has the potential to become a significant food security crop and a biofertilizer in cropping systems of resource-poor farmers in Africa. Furthermore, it is important to screen and identify Bambara groundnut landraces with superior symbiosis and improved water use efficiency for increased food security in a climate change scenario.

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