Cowpea Genotypic Variations in N_2 Fixation, Water Use Efficiency ($\delta^{13}C$), and Grain Yield in Response to *Bradyrhizobium* Inoculation in the Field, Measured Using Xylem N Solutes, ^{15}N, and ^{13}C Natural Abundance

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To evaluate cowpea genotypic differences in response to inoculation with *Bradyrhizobium* sp. strains BR 3267 and CB756 in the field, 15 genotypes were assessed for N_2 fixation using ^{15}N natural abundance and assays of xylem N solutes (ureides, nitrates, and amino-N). Carbon isotope discrimination ($\delta^{13}C$) was used as a surrogate for water use efficiency and grain yield determined at maturity. Within each location, the tested cowpeas elicited contrasting responses to inoculation with either *Bradyrhizobium* strains. For example, inoculating Apagbaala and IT90K-277-2 with strain BR 3267 doubled N-fixed and grain yield at Nyankpala when compared to inoculation of the same genotypes with strain CB756, whereas the inoculation of Padituya with strain CB756 increased those same parameters relative to non-inoculation. Similar contrasting results were also obtained at Savelugu and Gbalahi, where bacterial inoculation with each strain resulted in up to a five-fold increase in N-fixed and a four-fold increase in grain yield depending on the cowpea/inoculant combinations used and the planting location. The main effect of genotype showed that the percent N derived from N_2 fixation (%Ndfa) by the test cowpeas ranged between 74.4 and 89.8%, 11.7 and 50.5%, and 71.9 and 90.3% at Nyankpala, Savelugu, and Gbalahi, respectively. The genotypes grown at Savelugu generally exhibited low %Ndfa which was augmented by greater soil N uptake leading to grain yield increases, a finding supported by significant positive correlations when N-fixed and N content were each plotted against grain yield within locations. The inverse relationship between symbiosis and soil N uptake was also supported by negative correlations between xylem nitrate concentration and relative ureide N abundance (RU-N), and between xylem nitrate concentration and %Ndfa measured from ^{15}N natural abundance. Some high yielding genotypes also elicited greater water use

efficiency (δ^{13}C), a trait that could be exploited for increased cowpea production in water limiting environments. This study demonstrates the presence of genotypic differences in cowpea response to inoculation in the field and contributes to the literature regarding the factors influencing legume inoculation response in the tropics.

Keywords: symbiosis, ureides, N-fixed, water use efficiency, δ^{13}C

INTRODUCTION

Grain legumes are highly valued because of their nutritional and health benefits in addition to their contribution to soil fertility through symbiotic relationships with diverse rhizobia in the soil (Foyer et al., 2016). Moreover, their inclusion in cropping systems reduces chemical fertilizer usage and greenhouse gas emissions, both of which contribute to global warming (De Ron et al., 2017). In Africa, cowpea (*Vigna unguiculata* L. Walp.) is widely cultivated for its edible leaves and grain (Ehlers and Hall, 1997; Xiong et al., 2016) which are rich in protein (23.9–34.1%), carbohydrate (64%), and essential S-containing amino acids, such as lysine, which is often lacking in cereals (Iqbal et al., 2006; Hall, 2012; Santos et al., 2012; Ddamulira and Santos, 2015; Shevkani et al., 2015). Furthermore, cowpea has considerable tolerance to drought, a trait that makes it a preferred crop in low rainfall areas and an integral component of low-input farming systems where its N$_2$-fixing activities supply N to subsequent non-legumes planted in rotation (Dakora and Keya, 1997; Peoples et al., 2009). Because of the low C/N ratio of legumes like cowpea, their residues have faster rates of decomposition, which makes them important in conservation agriculture where they also help in C sequestration and improved soil microbial activities (Guopeng et al., 2020).

Despite the nutritional and agronomic importance of cowpea in Africa, average grain yield of the crop is low (160–810 kg.ha^{-1}) across the continent due to poor soil fertility, pests and diseases, and the cultivation of low yielding landraces by farmers (Tittonell and Giller, 2013; De Ron et al., 2017). For example, average cowpea yields of 171, 220, 362, and 417 kg.ha^{-1} have been reported in major producing countries like Niger, Mali, Senegal and Nigeria, respectively (Hall, 2012). Nevertheless, a greater proportion of the world's cowpea comes from Africa, where Nigeria and Niger together produced 67% of the global output in 2019 (FAO, 2021). In Ghana, up to 2,440 kg.ha^{-1} of grain yield is attainable with recommended agronomic practices. Yet, the yield gap is further widened due to much lower yields recorded by most farmers compared to the potential yield of 3,500 kg.ha^{-1} (MoFA, 2010, 2016). Despite the low yields however, cowpea production in Ghana is still more than its consumption, suggesting that the country could increase its net export of the crop by bridging the yield gap through improved agronomic practices and selection for drought tolerance and increased symbiosis in low N soils (MoFA, 2010; Belane and Dakora, 2011; Boddey et al., 2017).

Several studies with different cowpea genotypes in the field have reported up 96% symbiotic dependence, suggesting that the N$_2$-fixing ability of the crop could be tapped for its increased production in the N limited soils that characterize most parts of Africa (Belane and Dakora, 2009, 2010; Belane et al., 2011). When planted in soils that contain large numbers of ineffective rhizobia, however, legumes such as cowpea are often inoculated with superior N$_2$-fixing rhizobial strains to optimize the symbiosis (Ulzen et al., 2016). Nevertheless, inoculation failures often occur when the introduced strain has poor competitive ability for nodule occupancy compared to the already adapted native rhizobial population (Catroux et al., 2001; Herridge et al., 2002). Aside the adverse effects of environmental factors, negative inoculation response in the fields can also result from poor quality of inoculants, suggesting the need to address existing lapses in quality control (Herrmann and Lesueur, 2013). To enhance inoculation success in the field, seed inoculation is often adopted to promote the colonization of nodules by the introduced strains for improved plant growth (Lopes et al., 2021). Moreover, in the formulation of inoculants, the choice of a suitable carrier can enhance rhizobial survival by protecting them against adverse soil factors (Herrmann and Lesueur, 2013). Although previous studies have shown the presence of effective cowpea rhizobia in African soils (Hall, 2004), recent studies have observed inoculation success in Ghana, Mozambique, Tanzania, and Brazil that led to effective nodulation and significant grain yield increases (Martins et al., 2003; Nyoki and Ndakidemi, 2014; Ulzen et al., 2016; Boddey et al., 2017; Kyei-Boahen et al., 2017). However, despite the widespread cultivation of diverse cowpea germplasm in Africa, the few reports regarding the inoculation response of the crop are based on a limited number of genotypes (Ulzen et al., 2016; Boddey et al., 2017; Kyei-Boahen et al., 2017). Furthermore, most breeder-released cowpea varieties, breeding lines, and landraces have not been adequately characterized in in terms of their symbiotic performance, especially in relation to *Bradyrhizobium* inoculation response, an aspect much needed for varietal choice, recommendation, and breeding purposes aimed at improving the cowpea-rhizobia symbiosis. An evaluation of possible cowpea genotypic differences in inoculation-response in the field is therefore required to guide the selection of cowpea/rhizobia combinations that optimize symbiosis for cheaper and sustainable farming systems (Ulzen et al., 2016).

As with legumes in the tribe Phaseoli, cowpea exports its fixed N products in root nodules to the aerial parts as ureides (allantoin and allantoic acid) through the xylem stream (Baral et al., 2016). Therefore, the concentration of ureides relative to other xylem N solutes (i.e., nitrates and amino acids) is often used as a measure of the N sources being assimilated by the legume (Herridge et al., 1990). Furthermore, the ^{15}N natural abundance method has been widely used to quantify N$_2$ fixation by grain legumes, including cowpea (Belane and Dakora, 2010, 2011), soybean (Mapope and Dakora, 2016), and Bambara groundnut (Mohale et al., 2014),

under field conditions. The joint application of such different techniques is expected to yield a more reliable assessment of legume N_2 fixation.

Although cowpea is widely reported for its drought tolerance *via* several mechanisms, which include stomatal closure and drought escape (Itani et al., 1992), not much information is documented on the water relations of most cultivated varieties in Ghana and elsewhere. With climate change however, information on drought tolerance is needed to select climate-smart cowpea genotypes to bridge the existing yield gap of the crop (Vadez et al., 2012). Carbon isotope discrimination in C3 plants during photosynthesis is known to correlate with water use efficiency, and therefore allows for the use of the former as a reliable surrogate for assessing plant water use efficiency (Farquhar et al., 1989). Plants subjected to water limiting conditions show higher water use efficiency *via* reduced discrimination against $^{13}CO_2$, leading to higher $\delta^{13}C$ values. Conversely, under adequate water supply, plants exhibit lower $\delta^{13}C$ values due to increased discrimination against $^{13}CO_2$ (Cernusak et al., 2013). Consequently, carbon isotope discrimination has been successfully used to estimate plant water relations in several grain legumes under field conditions (Mohale et al., 2014; Mokgehle et al., 2014; Mapope and Dakora, 2016).

This study evaluated 15 cowpea genotypes, including breeding lines and a local landrace, for genotypic differences in response to two *Bradyrhizobium* inoculants at three locations in the Northern region of Ghana in relation to symbiotic performance and grain yield. The ^{15}N natural abundance technique and colorimetric assays of xylem N solutes were used to assess N_2 fixation while the ^{13}C isotopic technique was used as a surrogate for plant water use efficiency.

MATERIALS AND METHODS

Experimental Site Description

The experiments were conducted at three locations (Nyankpala, Savelugu and Gbalahi) in the Northern region of Ghana. Nyankpala is located on latitude 9.404 and longitude −0.982, while Gbalahi is located on latitude 9.437 and longitude −0.734. Savelugu is located on latitude 9.569 and longitude −0.830. The field at Nyankpala had been fallowed for over 5 years while the Savelugu and Gbalahi locations were respectively planted with okra and maize in the year prior to this experiment. Prior to experimental layout and planting, soil samples were cored from several points in each field, bulked, and sub-sampled for analysis of pH (H_2O), %C (Walkley and Black, 1934), P (Bray and Kurtz, 1945), B, Ca, Cu, Fe, K, Mg, Mn, Na, S (ammonium acetate method), and total N (using N digest).

The Northern Region has a unimodal rainfall of 800–1,200 mm which is distributed between April and October each year (Owusu and Waylen, 2013). The total annual rainfall during the study year (in 2013) was 1081.5 and 1009.7 mm at Nyankpala and Savelugu, respectively. Rainfall data at Gbalahi (857.3 mm) was collected from January to October 2013. The total rainfall from planting until plant sampling (early podding stage of cowpea) was 481 mm at Nyankpala, 471 mm at Savelugu, and

305 mm at Gbalahi. The average weekly distribution of rainfall at the three locations are illustrated in **Supplementary Figure 1**.

Source of Seeds, Experimental Design, and Planting

The 15 cowpea genotypes used in this study included breeder-released varieties (Apagbaala, Asetenapa, Bawutawuta, Marfotuya, Nhyira, Padituya, Songotra, Soronko, and Zayura), breeding lines (SARVx-09-001, SARVx-09-002, SARVx-09-003, and SARVx-09-004, IT90K-277-2), and a local landrace (Omandaw) sourced from CSIR-Savanna Agricultural Research Institute (CSIR-SARI) in Tamale and the Crops Research Institute (CRI) in Kumasi, both in Ghana. The local landrace is adapted to the savanna agroecology of Ghana where it is widely cultivated by rural farmers. The study was a factorial experiment involving the 15 cowpea genotypes together with three levels of inoculation (*Bradyrhizobium* sp. BR 3267, *Bradyrhizobium* sp. CB756 and control/non-inoculation). Thus, there were a total of 45 treatment combinations, which were laid in a randomized complete block design with four replications at each location. Planting was done on July 2, 2013 in Nyankpala, July 10, 2013 in Savelugu, and on July 16, 2013 in Gbalahi. Seeds were sown in plots measuring 2.4 × 2 m at a spacing of 60 cm between rows and 20 cm between plants, with 1 m path between plots and 1.5 m between blocks. Weeding was done using a hand hoe.

Inoculation of Seeds With *Bradyrhizobium* Strains

Seed inoculation was done in the shade using 10 g peat-based inoculant per kg seed which yielded 10^8 rhizobial cells.g^{-1} of inoculant. The seeds were coated with stimulin (a gel sticker) to aid attachment of the inoculant to the seed. To avoid cross contamination, uninoculated/control plots were planted first, followed by inoculated treatments.

Sampling and Processing of Plants

Plants were sampled at early podding at 54 days after planting (DAP) in Nyankpala and Savelugu, and at 55 DAP in Gbalahi. For this, five plants were randomly dug up from each plot in all experiments and separated into shoots, roots, and nodules. The number of nodules per plant was counted and recorded. The shoots and nodules were separately oven-dried (65°C for 48 h) and weighed to determine shoot dry matter and nodule dry matter, respectively. The shoots were then finely ground (0.50 mm sieve) for ^{15}N and ^{13}C isotopic analysis. Shoots of non-legume reference plants were concurrently sampled in each location and similarly processed for ^{15}N isotopic analysis.

$^{15}N/^{14}N$ Isotopic Analysis on Plant Shoots

Weighed ground shoot samples (2.0–2.5 mg) of both legume and reference plants were prepared, and each sample fed into a Carlo Erba NA1500 elemental analyzer (Fisons Instruments SpA, Strada, Rivoltana, Italy) coupled to a Finnigan MAT252 mass spectrometer (Fisons Instrument SpA, Strada, Rivoltana, Italy) *via* conflo II open-split device to measure $^{15}N/^{14}N$ isotopic composition, as described by Mapope and Dakora (2016). The ^{15}N natural abundance of the test plants were expressed as

δ notation and is the per mille deviation of the ^{15}N natural abundance of the sample from that of atmospheric N_2 (0.36637 atom % ^{15}N). An internal standard of *Nasturtium spp.* was included after every five runs. The $\delta^{15}N$ of shoot samples was calculated as (Unkovich et al., 2008):

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

where the $^{15}N/^{14}N_{sample}$ is the abundance ratio of ^{15}N and ^{14}N in the sample and $^{15}N/^{14}N_{atm}$ is the abundance ratio of ^{15}N and ^{14}N in the atmosphere.

Shoot N

The %N of shoot samples were obtained directly from the mass spectrometer, and shoot N content was measured as the product of shoot dry matter and %N (Pausch et al., 1996).

Percent N Derived From Fixation (%Ndfa) and N-Fixed

Shoot %Ndfa of cowpea was calculated as (Shearer and Kohl, 1986):

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

where $\delta^{15}N_{ref}$ is the ^{15}N natural abundance of reference plant, $\delta^{15}N_{leg}$ is the ^{15}N natural abundance of legume plant, and the *B value* is the ^{15}N natural abundance of the shoot of legume completely dependent on N_2 fixation for its N nutrition. The *B value* (−1.95‰) used in this study was earlier determined by inoculating cowpea genotype Padituya with *Bradyrhizobium* sp. TUTVUSA28 which was isolated from cowpea nodules in South Africa (Mohammed et al. unpublished data). The mean $\delta^{15}N$ of all reference plants at each location was determined and used to calculate the %Ndfa of cowpea genotypes per location.

The N-fixed by cowpea was calculated as (Maskey et al., 2001):

$$N-fixed = \%Ndfa \times N\ content\ of\ Kersting's\ groundnut\ shoots$$

The soil N uptake by legume plants was calculated as the shoot N content less N-fixed in shoot.

Shoot $^{13}C/^{12}C$ Isotopic Analysis

The ground shoot samples of cowpea were similarly subjected to $^{13}C/^{12}C$ isotopic analysis, as described for $^{15}N/^{14}N$. The ^{13}C natural abundance ($\delta^{13}C$) was calculated as (Farquhar et al., 1989):

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

where $^{13}C/^{12}C_{sample}$ is the isotopic ratio of the sample and $^{13}C/^{12}C_{standard}$ is the isotopic ratio of Pee Dee Belemnite (PDB), a universally accepted standard from Belemnite Pee Dee limestone formation (Craig, 1957).

Collection of Xylem Sap

Xylem sap was collected from the same plants that were sampled and processed for ^{15}N and ^{13}C isotopic analysis. Five plants from each plot were decapitated at the crown level, and root-bleeding xylem sap were collected into 2 ml Eppendorf tubes using clean Pasteur glass pipettes. The xylem samples were stored at −20°C until analysis for N solutes (ureide-N, nitrate-N, and amino-N).

MEASUREMENT OF XYLEM N SOLUTES

Ureide-N in Xylem Sap

The concentration of ureides in xylem sap was colorimetrically determined, as described by Young and Conway (1942) or Unkovich et al. (2008). Varying concentrations of allantoin (0.1, 0.02, 0.04, 0.1, and 0.15 mM) were prepared as standards for estimating ureides in xylem sap, after colorimetric assay (Young and Conway, 1942).

Nitrate-N in Xylem Sap

Nitrate-N in xylem sap was determined using the salicylic acid method (Cataldo et al., 1975), as described by Unkovich et al. (2008). Again, varying concentrations of nitrate (0, 1.25, 2.5, 5, 10, and 15 mM) were used as standards to construct curves for extrapolating nitrate values from xylem sap.

Amino-N Determination

Amino-N in xylem sap was measured using the ninhydrin method (Yemm and Cocking, 1955; Unkovich et al., 2008). As with ureide and nitrate-N determinations, a standard curve was prepared from assaying different concentrations of amino acid (0, 0.1, 0.2, 0.4, and 1 mM).

Relative Ureide Nitrogen (RU-N)

The relative ureide-N in xylem sap or relative ureide abundance was calculated as (Herridge and Peoples, 1990):

$$Relative\ Ureide - N = \left(\frac{4a}{4a + b + c}\right) \times 100$$

Where *a, b, and c* are the molar concentrations of ureides (ureides contain four nitrogen atoms per molecule), nitrate-N, and amino-N, respectively (Herridge et al., 1990).

Determination of Grain Yield

At maturity, 20 plants were harvested from each plot for determination of grain yield. The pods were detached from plants, air-dried to 15% moisture content, and threshed to obtain seeds. The seeds were weighed, and grain yield was expressed per hectare based on plant population.

Statistical Analysis

All data gathered were tested for normality and then subjected to ANOVA using Statistica data analysis software, version 10.0 (Statsoft Inc, 2011). A two-way ANOVA was used to compare the means of genotype and inoculation within each location while a three-way ANOVA was used to compare the performance of genotypes between locations. Where treatment means showed significant differences, Duncan's Multiple Range Test (DMRT)

was used to separate them at $p \leq 0.05$. Correlation and regression analyses were used to assess functional relationship between measured parameters.

RESULTS

Soil Chemical Properties

Pre-planting soil samples from Nyankpala, Savelugu, and Gbalahi recorded pH (H_2O) of 5.9, 6.1, and 6.3, respectively (**Table 1**). The concentrations of various nutrient elements in the soils at the three test locations are presented in **Table 1**. Both soil N and organic carbon contents were much lower in the soils at Savelugu when compared to values recorded at Nyankpala and Gbalahi.

PLANT GROWTH, SYMBIOTIC PARAMETERS, $\delta^{13}C$, AND GRAIN YIELD OF COWPEA

Main Effect of Cowpea Genotype

A two-way ANOVA of data within locations revealed significant effect ($p \leq 0.05$) of genotype on nodule dry matter, shoot biomass, $\delta^{15}N$, %Ndfa, N-fixed, soil N uptake, C/N ratio, $\delta^{13}C$, and grain yield of cowpea in the three test locations (**Tables 2–4**).

From the analysis of main effects in Nyankpala, genotype Asetenapa recorded the highest nodule mass (115.7 mg.plant^{-1}), followed by genotypes Songotra, Nhyira, SARVx-09-001, SARVx-09-003, and SARVx-09-004 which had relatively lower but similar nodule mass ranging between 76.33 and 81.67 mg.plant^{-1} (**Table 2**). The least nodule mass in Nyankpala was recorded in genotypes Apagbaala, Omandaw, and Marfotuya (**Table 2**).

The genotype Asetenapa, which produced the highest nodule mass, also exhibited the highest shoot biomass, followed by Omandaw and SARVx-09-0001 (**Table 2**). The least shoot biomass in Nyankpala was produced by genotypes Nhyira and Zayura and (**Table 2**).

Genotype Soronko recorded the least $\delta^{15}N$ in Nyankpala followed by Asetenapa, Bawutawuta, IT90K-277-2, Marfotuya, Nhyira, Padituya, and Zayura, also with lower $\delta^{15}N$ values when compared to the other genotypes at the location (**Table 2**). The genotype SARVx-09-004, Apagbaala, and Songotra had the highest $\delta^{15}N$ values in Nyankpala. As expected, %Ndfa was greater in the genotypes which had lower $\delta^{15}N$, with values ranging between 85.02% in genotypes Asetenapa and Zayura and 89.78% in Soronko. Due to their greater $\delta^{15}N$ values, genotypes SARVx-09-0004, Apagbaala, and Songotra recorded relatively

lower %Ndfa (74.4, 76.76, and 78.50%, respectively) in Nyankpala (**Table 2**).

The amount of N-fixed was highest in genotype Asetenapa (92.42 kg.ha^{-1}) followed by Padituya (45.26 kg.ha^{-1}) and Omandaw (44.05 kg.ha^{-1}). Despite their greater %Ndfa however, genotypes IT90K-277-2 and Nhyira recorded lower N-fixed due to their reduced shoot biomass, whilst genotype SARVx-09-004 coupled reduced shoot biomass with lower %Ndfa and N-fixed (**Table 2**). The genotype Asetenapa, which recorded the highest N-fixed at Nyankpala, also recorded the highest soil N uptake at the location, whilst Apagbaala, Songotra, and SARVx-09-004 recorded relatively lower N-fixed but greater soil N uptake (**Table 2**). Soil N uptake was least in genotypes Bawutawuta, IT90K-277-2, Nhyira, Soronko, and Zayura, all of which had high %Ndfa. Despite the high %Ndfa by IT90K-277-2 however, the genotype together with Omandaw recorded high C/N ratio at Nyankpala due to their reduced %N (**Table 6**) when compared to other genotypes at the location. The least C/N ratio was recorded in genotype Asetenapa followed by Apagbaala and SARVx-09-004 (**Table 2**).

Further, the genotypes Asetenapa, Nhyira, Omandaw, Soronko, and Zayura recorded higher shoot $\delta^{13}C$ values in Nyankpala than the other cowpea genotypes (**Table 2**). In contrast, genotype Apagbaala, together with Bawutawuta and SARVx-09-002, elicited lower $\delta^{13}C$ values when compared to the other genotypes in Nyankpala (**Table 2**). Despite the observed high %Ndfa by genotypes in Nyankpala, their grain yield was generally low, with the highest values being recorded in genotypes Asetenapa (419.9 kg.ha^{-1}) and Omandaw (411.4 kg.ha^{-1}) which also exhibited greater $\delta^{13}C$ values. Grain yield was, however, lower but similar between and among the remaining cowpea genotypes, with values ranging between 212.4 and 308.7 kg.ha^{-1} (**Table 2**).

In the Savelugu location, there was a significant effect ($p \leq .05$) of cowpea genotype on nodulation, shoot biomass, symbiotic parameters, soil N uptake, $\delta^{13}C$, and grain yield (**Table 3**). Here, the genotypes Asetenapa, IT90K-277-2, and Soronko, together with Padituya and SARVx-09-001, recorded higher nodule mass when compared to the other genotypes. The least nodule mass at Savelugu was recorded in genotypes Apagbaala and Padituya, followed by Bawutawuta and Nhyira (**Table 3**).

The genotypes Asetenapa and Songotra, which had high nodule mass and genotypes Omandaw and SARVx-09-004, recorded greater shoot biomass in Savelugu. However, Padituya had high shoot biomass despite reduced nodule biomass in the Savelugu location (**Table 3**). In contrast, genotypes IT90K-277-2, Soronko, and Zayura exhibited much lower shoot biomass

TABLE 1 | Soil chemical properties of the three test locations before planting.

Location	pH (H_2O)	%		mg.kg^{-1}									
		N	C	B	Ca	Cu	Fe	K	Mg	Mn	Na	P	S
Nyankpala	5.9	0.023	0.40	0.4	250	0.30	42.0	48	55.2	131.4	7	7	2.70
Savelugu	6.1	0.016	0.03	0.1	272	0.20	23.96	29	51.6	66.46	4	5	2.10
Gbalahi	6.3	0.031	0.41	0.8	460	0.78	46.69	58	130.8	65.51	17	7	2.20

TABLE 2 | Nodulation, shoot dry matter (DM), symbiotic parameters, soil N uptake, $\delta^{13}C$, and grain yield of 15 cowpea genotypes inoculated with two *Bradyrhizobium* strains and planted in Nyankpala in 2013.

Treatment	Nodule DM mg.plant^{-1}	Shoot DM g.plant^{-1}	$\delta^{15}N$ ‰	%Ndfa %	N-fixed kg.ha^{-1}	Soil N uptake kg.ha^{-1}	C/N ratio g.g^{-1}	$\delta^{13}C$ ‰	Grain yield kg.ha^{-1}
GENOTYPE									
Apagbaala	36.1 ± 4.43e	8.4 ± 0.88b–e	−0.85 ± 0.08ab	76.7 ± 1.72fg	36.0 ± 3.64b–e	12.1 ± 2.19ab	12.9 ± 0.36c	−29.39 ± 0.07e	301.5 ± 28.6b
Asetenapa	115.7 ± 8.93a	14.4 ± 2.73a	−1.24 ± 0.10efg	85.0 ± 2.12abc	92.4 ± 19.04a	13.9 ± 2.16a	11.1 ± 0.23d	−28.27 ± 0.19a	419.9 ± 54.3a
Bawutawuta	58.5 ± 5.18c	8.2 ± 0.54cde	−1.38 ± 0.07gh	87.9 ± 1.46ab	40.4 ± 2.57b–e	5.6 ± 0.76e	14.1 ± 0.52bc	−29.24 ± 0.14de	283.7 ± 20.3b
IT90K-277-2	64.0 ± 4.99bc	7.6 ± 0.50def	−1.37 ± 0.05gh	87.7 ± 1.06ab	32.6 ± 2.54de	4.7 ± 0.61e	16.6 ± 0.50a	−28.67 ± 0.15abc	281.9 ± 35.6b
Marfotuya	41.0 ± 5.96de	7.6 ± 0.53def	−1.26 ± 0.08efg	85.5 ± 1.59abc	39.7 ± 2.52b–e	6.9 ± 0.98cde	13.0 ± 0.41bc	−28.91 ± 0.21bcd	261.3 ± 21.8b
Nhyira	76.3 ± 8.62b	6.3 ± 0.28f	−1.28 ± 0.11efg	85.9 ± 2.27abc	31.8 ± 1.99e	5.4 ± 0.90e	13.5 ± 0.57bc	−28.39 ± 0.11a	221.8 ± 18.6b
Omandaw	32.8 ± 4.28e	9.9 ± 0.84b	−1.11 ± 0.09cde	82.2 ± 1.87cde	44.1 ± 3.63bc	10.1 ± 1.48bc	14.60 ± 0.76b	−28.37 ± 0.16a	411.4 ± 61.7a
Padituya	66.7 ± 7.11bc	8.7 ± 0.59bcd	−1.38 ± 0.07gh	87.9 ± 1.39ab	45.3 ± 3.15b	6.6 ± 1.26de	13.6 ± 0.43bc	−29.03 ± 0.13cde	262.1 ± 32.0b
Songotra	81.7 ± 9.92b	8.5 ± 0.75bcd	−0.93 ± 0.08abc	78.5 ± 1.79efg	40.2 ± 3.29b–e	11.4 ± 1.62ab	13.4 ± 0.61bc	−28.83 ± 0.09bcd	284.5 ± 23.5b
Soronko	70.8 ± 5.43bc	7.5 ± 0.66def	−1.47 ± 0.07h	89.8 ± 1.45a	40.6 ± 4.21b–e	4.9 ± 0.98e	13.5 ± 0.68bc	−28.27 ± 0.09a	255.9 ± 34.9b
SARVx-09-001	77.5 ± 4.94b	9.4 ± 0.35bc	−1.09 ± 0.07cde	81.7 ± 1.51cde	42.1 ± 2.04bce	9.5 ± 0.96bcd	14.4 ± 0.65bc	−28.88 ± 0.12bcd	273.5 ± 19.4b
SARVx-09-002	68.7 ± 9.25bc	8.2 ± 0.53cde	−1.21 ± 0.11def	84.4 ± 2.22bcd	39.2 ± 2.80b–e	7.8 ± 1.80cde	13.9 ± 0.49bc	−29.09 ± 0.12de	245.4 ± 31.2b
SARVx-09-003	77.5 ± 4.29b	8.2 ± 0.80cde	−1.01 ± 0.07bcd	80.2 ± 1.57def	39.3 ± 3.81b–e	10.0 ± 1.28bc	13.1 ± 0.32bc	−28.88 ± 0.10bcd	216.1 ± 16.4b
SARVx-09-004	78.3 ± 4.01b	7.2 ± 0.76def	−0.74 ± 0.08a	74.4 ± 1.79g	32.9 ± 3.92de	11.5 ± 1.41ab	12.8 ± 0.14c	−28.94 ± 0.09bcd	212.4 ± 22.8b
Zayura	54.8 ± 3.71cd	6.8 ± 0.21ef	−1.24 ± 0.09efg	85.0 ± 1.93abc	34.9 ± 2.12cde	5.9 ± 0.72e	13.6 ± 0.44bc	−28.58 ± 0.15ab	308.7 ± 35.9b
INOCULATION									
Bradyrhizobium sp. BR 3267	71.2 ± 3.54a	7.6 ± 0.26b	−1.12 ± 0.05a	82.5 ± 1.05b	36.4 ± 1.32b	8.0 ± 0.63b	13.6 ± 0.27a	−28.70 ± 0.08a	297.8 ± 18.7a
Bradyrhizobium sp. CB756	68.4 ± 3.63a	8.0 ± 0.25b	−1.23 ± 0.04b	84.8 ± 0.89a	39.2 ± 1.30b	7.4 ± 0.61b	13.9 ± 0.27a	−28.84 ± 0.07a	261.2 ± 13.0a
Un-inoculated	60.5 ± 4.09b	9.8 ± 0.69a	−1.16 ± 0.04ab	83.3 ± 0.84ab	50.7 ± 4.81a	9.8 ± 0.80a	13.4 ± 0.24a	−28.81 ± 0.06a	289.0 ± 15.9a
F STATISTICS									
Genotype (G) **[df = 14]**	14.13***	13.98***	8.74***	8.74***	23.69***	7.81***	5.27***	6.70***	4.02***
Inoculation (I) **[df = 2]**	5.07**	27.67***	3.12***	3.12***	31.98***	7.12***	1.16ns	1.60ns	1.96ns
G x I **[df = 28]**	2.70***	12.86***	2.80***	2.80***	14.88***	4.32***	0.88ns	0.80ns	1.88**

*Plants were sampled at early podding stage of genotypes. Values (mean ± standard error) with dissimilar letters in a column are significantly different at ***$p < 0.001$ and **$p < 0.01$. ns, not significant.*

TABLE 3 | Nodulation, shoot dry matter (DM), symbiotic parameters, soil N uptake, δ^{13}C, and grain yield of 15 cowpea genotypes inoculated with two *Bradyrhizobium* strains and planted in Savelugu in 2013.

Treatments	Nodule DM mg.plant^{-1}	Shoot DM g.plant^{-1}	δ^{15}N ‰	%Ndfa %	N-fixed kg.ha^{-1}	Soil N uptake kg.ha^{-1}	C/N ratio g.g^{-1}	δ^{13}C ‰	Grain yield kg.ha^{-1}
GENOTYPE									
Apagbaala	14.4 ± 2.00d	11.0 ± 0.71cd	2.63 ± 0.10a	11.7 ± 1.96h	7.6 ± 1.56g	58.6 ± 4.28bc	11.8 ± 0.23c–f	−28.28 ± 0.15a–d	412.5 ± 40.1d
Asetenapa	46.5 ± 7.53a	12.4 ± 0.60ab	1.88 ± 0.27cde	26.3 ± 5.28def	20.4 ± 3.65de	62.2 ± 6.15b	11.1 ± 0.26f	−28.05 ± 0.17ab	896.7 ± 140.0a
Bawutawuta	22.5 ± 2.65cd	10.6 ± 0.82cde	1.98 ± 0.15b–e	24.2 ± 2.95d–g	16.1 ± 2.14ef	50.4 ± 4.31cde	11.9 ± 0.27cde	−28.65 ± 0.17de	650.8 ± 76.4bc
IT90K-277-2	45.1 ± 4.74a	8.4 ± 0.67g	1.20 ± 0.28g	39.3 ± 5.44b	18.5 ± 1.97def	32.8 ± 4.91g	12.8 ± 0.35b	−28.12 ± 0.17abc	401.0 ± 56.3d
Marfotuya	26.8 ± 4.86bc	9.9 ± 0.86def	0.62 ± 0.18h	50.5 ± 3.42a	31.2 ± 1.80a	33.9 ± 5.04fg	12.0 ± 0.15bcd	−28.56 ± 0.11cde	338.2 ± 60.4d
Nhyira	22.0 ± 4.68cd	11.5 ± 0.56bc	1.21 ± 0.10g	39.1 ± 1.94b	27.4 ± 2.03ab	42.8 ± 2.84def	12.4 ± 0.18bc	−28.44 ± 0.16b–e	440.4 ± 52.6d
Omandaw	29.7 ± 5.95bc	12.4 ± 0.68ab	1.06 ± 0.21g	42.0 ± 4.03b	28.6 ± 2.67ab	42.22 ± 5.30d–g	13.5 ± 0.33a	−28.06 ± 0.15ab	646.5 ± 72.0bc
Padituya	13.7 ± 2.60d	12.9 ± 0.65a	1.74 ± 0.18cde	28.8 ± 3.42def	24.4 ± 3.17bcd	59.6 ± 3.86bc	11.5 ± 0.28def	−28.31 ± 0.17a–e	1036.1 ± 288.1a
Songotra	34.6 ± 4.20b	14.2 ± 1.22a	1.69 ± 0.17de	29.8 ± 3.30de	26.9 ± 3.86abc	62.3 ± 5.72b	12.0 ± 0.19bcd	−28.52 ± 0.16cde	506.1 ± 86.1bcd
Soronko	48.2 ± 4.62a	9.2 ± 0.43fg	2.04 ± 0.21bcd	23.2 ± 3.95efg	13.8 ± 2.39f	46.2 ± 3.83de	11.9 ± 0.23cde	−27.95 ± 0.09a	615.5 ± 67.4bc
SARVx-09-001	34.2 ± 4.67b	10.7 ± 0.57cd	1.67 ± 0.21g	39.9 ± 4.01b	28.0 ± 3.26ab	41.1 ± 2.82efg	11.8 ± 0.31c–f	−28.61 ± 0.19de	679.8 ± 122.8b
SARVx-09-002	29.4 ± 5.74bc	11.3 ± 0.69bc	1.28 ± 0.09fg	37.8 ± 1.82bc	27.7 ± 1.95ab	45.9 ± 3.30de	11.8 ± 0.19c–f	−28.54 ± 0.11cde	424.3 ± 63.6d
SARVx-09-003	30.8 ± 7.17bc	10.3 ± 0.88c–f	1.61 ± 0.18ef	31.4 ± 3.39cd	21.2 ± 3.69cde	41.9 ± 3.09d–g	12.4 ± 0.26bc	−28.76 ± 0.16e	484.5 ± 47.1cd
SARVx-09-004	29.1 ± 3.02bc	16.0 ± 1.37a	2.33 ± 0.13ab	17.5 ± 2.50gh	17.0 ± 2.19ef	87.7 ± 8.62a	11.4 ± 0.25def	−28.38 ± 0.09a–e	425.1 ± 44.9d
Zayura	29.7 ± 4.24bc	9.3 ± 0.23efg	2.14 ± 0.13bc	21.2 ± 2.51fg	13.3 ± 1.45f	51.3 ± 3.09cd	11.2 ± 0.23ef	−28.52 ± 0.16cde	405.8 ± 63.0d
INOCULATION									
Bradyrhizobium sp. BR 3267	32.4 ± 3.02a	12.0 ± 0.48a	1.67 ± 0.09a	30.2 ± 1.78a	22.3 ± 1.43a	54.2 ± 2.95a	11.9 ± 0.15a	−28.41 ± 0.07a	524.2 ± 32.5a
Bradyrhizobium sp. CB756	28.2 ± 1.98a	11.0 ± 0.33b	1.68 ± 0.09a	30.1 ± 1.76a	20.8 ± 1.29a	49.1 ± 1.96b	12.0 ± 0.13a	−28.35 ± 0.07a	587.7 ± 48.6a
Un-inoculated	30.8 ± 2.23a	11.01 ± 0.43b	1.56 ± 0.12a	32.3 ± 2.40a	21.3 ± 1.57a	48.5 ± 2.98b	12.1 ± 0.13a	−28.38 ± 0.08a	560.7 ± 67.3a
F STATISTICS									
Genotype (G) **[df = 14]**	10.23***	22.33***	16.76***	16.76***	11.47***	20.88***	5.98***	2.7**	11.91***
Inoculation (I) **[df = 2]**	271.60ns	9.90***	1.17ns	1.17ns	0.76ns	5.34**	0.92ns	0.2ns	1.54ns
G x I **[df = 28]**	8.09***	14.49***	6.01***	6.01***	4.86***	8.90***	0.72ns	1.1ns	14.51***

Plants were sampled at early podding stage of genotypes. Values (mean ± standard error) with dissimilar letters in a column are significantly different at ***p < 0.001 and **p < 0.01. ns, not significant.

TABLE 4 | Nodulation, shoot dry matter (DM), symbiotic parameters, soil N uptake, δ¹³C, and grain yield of 15 cowpea genotypes inoculated with two *Bradyrhizobium* strains and planted in Gbalahi in 2013.

Treatments	Nodule DM mg.plant^{-1}	Shoot DM g.plant^{-1}	$\delta^{15}N$ ‰	%Ndfa %	N–fixed kg.ha^{-1}	Soil N uptake kg.ha^{-1}	C/N ratio g.g^{-1}	$\delta^{13}C$ ‰	Grain yield kg.ha^{-1}
GENOTYPE									
Apagbaala	53.6 ± 6.64fg	13.2 ± 1.67a	−0.38 ± 0.23cde	78.1 ± 3.23def	49.5 ± 6.43a–d	14.4 ± 3.54b	15.3 ± 0.52a–d	−29.54 ± 0.05ab	280.5 ± 38.90b–e
Asetenapa	56.9 ± 4.53fg	8.5 ± 0.58f	−0.46 ± 0.20def	79.3 ± 2.72cde	37.4 ± 2.15gh	10.9 ± 2.14bc	14.1 ± 0.52cd	−29.32 ± 0.11a	139.2 ± 10.53g
Bawutawuta	85.4 ± 5.71cd	11.2 ± 1.05bcd	0.07 ± 0.38a	71.9 ± 5.33h	43.2 ± 2.79d–g	21.6 ± 6.25a	13.7 ± 0.46d	−29.54 ± 0.16ab	213.9 ± 21.69d–g
IT90K-277-2	98.2 ± 8.62b	13.2 ± 1.60a	−0.55 ± 0.23fg	80.5 ± 3.15bc	50.7 ± 5.23abc	13.7 ± 3.03b	15.9 ± 0.60ab	−29.58 ± 0.07b	200.9 ± 22.96efg
Marfotuya	64.5 ± 6.38ef	9.9 ± 0.90def	−0.20 ± 0.12b	75.6 ± 1.73g	40.5 ± 3.83fgh	13.8 ± 2.02b	14.4 ± 0.64bcd	−29.73 ± 0.10b	211.1 ± 17.93d–g
Nhyira	65.6 ± 6.69ef	8.6 ± 1.09f	−1.12 ± 0.10h	88.4 ± 1.39a	36.6 ± 4.98gh	4.3 ± 0.43e	16.9 ± 0.87a	−29.52 ± 0.07ab	181.2 ± 16.18fg
Omandaw	74.7 ± 4.88de	9.8 ± 1.01ef	−0.26 ± 0.22bc	76.5 ± 3.03fg	36.1 ± 3.08gh	12.8 ± 2.35bc	15.5 ± 0.43abc	−29.69 ± 0.04b	256.5 ± 32.86b–f
Padituya	50.9 ± 5.70g	9.7 ± 1.42ef	−0.20 ± 0.08b	75.7 ± 1.08g	39.5 ± 5.68fgh	13.7 ± 2.62b	14.8 ± 0.50bcd	−29.51 ± 0.09ab	314.2 ± 32.30b
Songotra	70.2 ± 6.04e	11.4 ± 0.45bc	−0.48 ± 0.14ef	79.5 ± 1.92cd	48.1 ± 3.01b–e	12.0 ± 1.00bc	15.0 ± 0.52bcd	−29.60 ± 0.08b	440.3 ± 46.89a
Soronko	117.8 ± 4.64a	9.4 ± 0.33ef	−1.26 ± 0.08h	90.3 ± 1.16a	46.7 ± 2.48c–f	5.0 ± 0.69e	14.8 ± 0.65bcd	−29.65 ± 0.05b	198.8 ± 25.40efg
SARVx-09-001	94.4 ± 10.27bc	12.3 ± 0.85ab	−1.14 ± 0.08h	88.7 ± 1.11a	55.6 ± 4.03a	7.4 ± 1.19de	15.5 ± 0.74abc	−29.52 ± 0.09ab	285.8 ± 28.89bcd
SARVx-09-002	84.5 ± 15.05cd	8.8 ± 0.41f	−0.15 ± 0.35b	74.9 ± 4.91g	35.3 ± 2.84h	12.4 ± 2.74bc	14.5 ± 0.78bcd	−29.57 ± 0.04b	324.9 ± 33.17b
SARVx-09-003	85.1 ± 8.26cd	9.1 ± 0.63f	−0.43 ± 0.06def	78.8 ± 0.87cde	37.2 ± 2.66gh	10.0 ± 0.85cd	15.3 ± 0.58a–d	−29.56 ± 0.09b	226.2 ± 28.64c–f
SARVx-09-004	57.1 ± 446g	10.5 ± 0.76cde	−0.31 ± 0.18bcd	77.2 ± 2.53efg	42.2 ± 3.17efg	12.0 ± 1.12bc	15.1 ± 0.59bcd	−29.49 ± 0.11ab	232.1 ± 24.56c–f
Zayura	87.7 ± 4.92bc	11.4 ± 1.53bc	−0.68 ± 0.14g	82.3 ± 1.89b	54.9 ± 6.82ab	13.0 ± 2.26bc	13.6 ± 0.54d	−29.72 ± 0.11b	302.9 ± 63.92bc
INOCULATION									
Bradyrhizobium sp. BR 3267	69.1 ± 3.92b	11.4 ± 0.49a	−0.29 ± 0.12a	76.9 ± 1.73c	46.9 ± 2.08a	15.5 ± 1.65a	14.2 ± 0.27c	−29.54 ± 0.05a	263.7 ± 17.20a
Bradyrhizobium sp. CB756	78.9 ± 4.48a	10.1 ± 0.53b	−0.57 ± 0.09b	80.7 ± 1.20b	40.3 ± 1.89c	10.3 ± 1.00b	15.8 ± 0.29a	−29.59 ± 0.04a	248.4 ± 15.40a
Un-inoculated	81.2 ± 3.30a	9.9 ± 0.44b	−0.65 ± 0.07c	82.0 ± 0.96a	43.2 ± 2.00b	9.5 ± 0.69b	14.9 ± 0.24b	−29.58 ± 0.04a	249.6 ± 17.51a
F STATISTICS									
Genotypes (G) **[df = 14]**	21.70***	12.26***	55.23***	55.20**	9.26***	15.57***	2.65**	2.0*	8.57***
Inoculation (I) **[df = 2]**	12.31***	14.96***	66.68**	66.7**	10.21***	47.42***	12.49***	1.0ns	0.56ns
G x I **[df = 28]**	13.45***	25.48***	73.18**	73.20**	13.93***	26.86***	2.16**	4.0***	4.75***

Plants were sampled at early podding stage of genotypes. Values (mean ± standard error) with dissimilar letters in a column are significantly different at ***p < 0.001, **p < 0.01, and *p < 0.05. ns, not significant.

in Savelugu despite their relatively high nodule biomass at the location (**Table 3**).

Further, shoot $\delta^{15}N$ values were positive for all cowpea genotypes planted in Savelugu (**Table 3**). Here, Marfotuya recorded the least $\delta^{15}N$ value ($+0.62$‰), with genotypes, such as IT90K-277-2, Nhyira, Omandaw, SARVx-09-001, and SARVx-09-002, also exhibiting much lower $\delta^{15}N$ values ($+1.06$ to $+1.67$‰) when compared to other genotypes at the location (**Table 3**). As a result, %Ndfa was highest in Marfotuya (50.5%), followed by the other genotypes which had lower $\delta^{15}N$ values in that order, with values ranging from 37.8 to 42.0% (**Table 4**). Because of their high $\delta^{15}N$ values, %Ndfa was much lower in genotypes Apagbaala (11.7%), SARVx-09-004 (17.5%), Soronko (23.2%), and Zayura (21.2%) (**Table 3**).

The highest N-fixed was recorded in genotype Marfotuya ($31.18 \, kg.ha^{-1}$) followed by Nhyira, Omandaw, SARVx-09-001, SARVx-09-002, and then Songotra with values ranging between 26.91 and $28.60 \, kg.ha^{-1}$ (**Table 3**). Genotype Apagbaala, together with Zayura, SARVx-09-004, Soronko, and Bawutawuta, recorded markedly lower N-fixed values (7.58–$17.02 \, kg.ha^{-1}$) (**Table 3**). Soil N uptake was highest in genotype SARVx-09-004 due to its higher $\delta^{15}N$ and lower %Ndfa values. Other genotypes with high soil N uptake in Savelugu include Asetenapa and Songotra. However, soil N uptake was lower in genotypes Bawutawuta, IT90K-277-2, and SARVx-09-001 which all recorded relatively high %Ndfa in Savelugu (**Table 3**). Shoot C/N ratio was highest in genotype Omandaw ($13.5 \, g.g^{-1}$) followed by IT90K-277-2 ($12.8 \, g.g^{-1}$), and least in genotypes Asetenapa and Zayura. Other genotypes with low C/N ratio include Apagbaala, Padituya, SARVx-09-001, SARVx-09-002, and SARVx-09-004 with values ranging between 11.2 and $11.8 \, g.g^{-1}$ (**Table 3**).

At Savelugu, the cowpea genotype Soronko recorded the highest water use efficiency measured as shoot $\delta^{13}C$ (-27.95‰), followed by Asetenapa and Omandaw all with high $\delta^{13}C$ at Nyankpala (**Table 3**). The least $\delta^{13}C$ was recorded in genotype SARVx-09-004 (-28.38‰) followed by Bawutawuta (28.65‰) and SARVx-09-001 (28.61‰). The highest grain yield in Savelugu ($1036.1 \, kg.ha^{-1}$) was recorded in genotype Padituya followed by Asetenapa ($896.7 \, kg.ha^{-1}$), Omandaw ($646.5 \, kg.ha^{-1}$), and Soronko ($615.5 \, kg.ha^{-1}$) which all recorded high $\delta^{13}C$ at the location (**Table 3**). Cowpea genotype SARVx-09-004 also recorded relatively high grain yield ($679.8 \, kg.ha^{-1}$) despite much lower $\delta^{13}C$ value (**Table 3**). The least grain yield at Savelugu was recorded in genotypes Apagbaala, IT90K-277-2, Marfotuya, Nhyira, SARVx-09-002, SARVx-09-004, and Zayura with values between 338.4 and $440.4 \, kg.ha^{-1}$ (**Table 3**).

At Gbalahi, there was a marked effect of genotype on nodule mass, shoot biomass, symbiotic parameters, soil N uptake, $\delta^{13}C$, and grain yield of cowpea (**Table 4**). The genotype Soronko recorded the highest nodule mass followed by IT90K-277-2, SARVx-09-001, and Zayura, also, with high nodule dry matter (**Table 4**). The least nodule mass was recorded in genotypes SARVx-09-004 and Padituya. Other genotypes with lower nodule mass at Gbalahi include Apagbaala, Asetenapa, Marfotuya, and Nhyira (**Table 4**).

The genotypes Apagbaala and IT90K-277-2 recorded the highest shoot biomass at Gbalahi followed by SARVx-09-001 and Zayura also with high shoot biomass. The least shoot biomass was recorded in genotypes Asetenapa, Nhyira, SARVx-09-002, SARVx-09-003, and then followed by Omandaw and Padituya (**Table 4**).

Genotypes Nhyira, Soronko, and SARVx-09-001 recorded the lowest $\delta^{15}N$ values followed by Zayura and IT90K-277-2 also with lower $\delta^{15}N$ (**Table 4**). The highest $\delta^{15}N$ value was recorded in genotype Bawutawuta followed by Marfotuya, Padituya, and SARVx-09-002 which also exhibited high $\delta^{15}N$ values (**Table 4**). As a result, %Ndfa was highest in genotypes Nhyira (88.4%), Soronko (90.3%), and SARVx-09-001 (88.7%), followed by Zayura (82.3%) and IT90K-277-2 (80.5%). Genotype Bawutawuta recorded the least %Ndfa in Gbalahi, followed by Marfotuya, Padituya, and SARVx-09-002, all of which had high $\delta^{15}N$ values (**Table 4**). The amount of N-fixed was highest in genotype SARVx-09-001, followed by Zayura and IT90K-277-2, while the least N-fixed was recorded in genotype SARVx-09-002 followed by Omandaw, Nhyira, Padituya, Marfotuya, and SARVx-09-003 also with low N-fixed values (**Table 4**). Genotype Bawutawuta, together with Apagbaala, IT90K-277-2, Marfotuya, and Padituya recorded greater soil N uptake while Nhyira, Soronko, and SARVx-09-001 recorded lower soil N uptake. Shoot C/N ratio ranged between 13.56 and $16.89 \, g.g^{-1}$ in Gbalahi, with genotypes Nhyira, IT90K-277-2, and Omandaw recording higher C/N ratio in the location while Bawutawuta and Zayura recorded the least values (**Table 4**).

Shoot $\delta^{13}C$ ranged from -29.73‰ in genotype Marfotuya to -29.32‰ in Asetenapa at Gbalahi (**Table 4**). The highest grain yield ($440.3 \, kg.ha^{-1}$) in Gbalahi was recorded in genotype Songotra followed by SARVx-09-002 ($324.9 \, kg.ha^{-1}$) and Padituya ($314.2 \, kg.ha^{-1}$), while Asetenapa recorded the least grain yield at the location ($139.2 \, kg.ha^{-1}$). Other genotypes with lower grain yield at Gbalahi include Nhyira, Soronko, Bawutawuta, and Marfotuya (**Table 4**).

Main Effect of Inoculation

The main effect of inoculation was significant ($p \leq .05$) for nodule dry matter, shoot dry matter, $\delta^{15}N$, %Ndfa, N-fixed, and soil N uptake at Nyankpala (**Table 2**). Although inoculation with either *Bradyrhizobium* strains increased dry nodule mass over non-inoculation at Nyankpala, shoot biomass was lowered when compared to uninoculated plots (**Table 2**). Together with non-inoculation, bacterial inoculation with strain CB756 decreased overall shoot $\delta^{15}N$, resulting in greater %Ndfa when compared to inoculation with strain BR 3267 (**Table 2**). As observed with shoot biomass, non-inoculation increased overall N-fixed and soil N uptake in Nyankpala when compared to plots inoculated with either *Bradyrhizobium* strains (**Table 2**). However, overall shoot C/N ratio, $\delta^{13}C$, and grain yield were unaltered by bradyrhizobial inoculation at Nyankpala (**Table 2**).

At Savelugu, the overall plant nodulation and other symbiotic parameters, along with $\delta^{13}C$ and grain yield of cowpea, were unaltered by bradyrhizobial inoculation (**Table 3**). However, the main effect of inoculation was significant for shoot biomass and

soil N uptake in Savelugu (**Table 3**). Overall, seed inoculation with *Bradyrhizobium* sp. strain BR 3267 increased shoot biomass and soil N uptake when compared to non-inoculation or inoculation with strain CB756 (**Table 3**).

At Gbalahi, the main effect of inoculation was significant for nodule mass, shoot biomass, all symbiotic parameters, and soil N uptake (**Table 4**). Here, bacterial inoculation with strain BR 3267 increased shoot biomass despite reduced nodule mass compared to non-inoculation or inoculation with strain CB756. However, δ^{15}N was least with non-inoculation followed by inoculation with strain CB756. As a result, %Ndfa was highest with non-inoculation, followed by inoculation with strain CB756, and least with strain BR 3267 inoculation (**Table 4**). Bacterial inoculation with strain BR 3267 increased soil N uptake and decreased C/N ratio compared to other treatments. Here, overall δ^{13}C and grain yield were unaltered by inoculation (**Table 4**).

Genotype × Inoculation Interaction Effect

There was a marked effect of genotype × inoculation interaction on nodule dry matter, shoot biomass, δ^{15}N, N-fixed, soil N uptake, N content, and grain yield across the three test locations (**Tables 2–4**). However, results are described for shoot biomass, N-fixed, N content, and grain yield for cowpea genotypes that showed consistent patterns of interactions for those parameters within the test locations (**Figures 1–3**).

In Nyankpala, inoculating genotype Apagbaala with *Bradyrhizobium* sp. strain BR 3267 doubled N-fixed and slightly increased the parameter in IT90K-277-2 when compared to inoculation with strain CB756 (**Figures 1A–D**). On the other hand, bacterial inoculation of Bawutawuta, Padituya, and SARVx-09-003 with strain CB756 significantly increased shoot biomass, resulting in a 32–200% increase in the amounts of N-fixed and shoot N accumulation when compared to inoculation with strain BR 3267 (**Figure 1**). Consequently, grain yield was increased by 1.3- to 2-fold in those cowpea/inoculant strain combinations in Nyankpala (**Figure 1**). In contrast, non-inoculation of Apagbaala, Asetenapa, Padituya, and SARVx-09-003 in Nyankpala each increased N-fixed by 1.5- to 3.4-fold (36.5–55.0 kg.ha^{-1}), and also resulted in greater shoot N accumulation. As a result, grain yield was increased by 120–194% in those control plants when compared to inoculation with strain CB756 in the case of Apagbaala and Asetenapa, and relative to strain BR 3267 in the case of Padituya and SARVx-09-003 (**Figure 1**). Furthermore, although non-inoculation of genotypes Songotra and Soronko resulted in greater shoot biomass, N-fixed, and N accumulation when compared to inoculation with either strain, the corresponding increases in grain yield were not significant (**Figure 1**). Similarly, bacterial inoculation of Soronko with strain BR 3267 only increased grain yield slightly despite significant increases in shoot biomass, N-fixed, and N accumulation (**Figure 1**).

At Savelugu, although N_2 fixation was generally low in plants, bacterial inoculation of Asetenapa with either strain BR 3267 or CB756 resulted in a 3- to 5-fold increase in N-fixed, and, respectively, increased grain yield by 2.6-fold (932 kg.ha^{-1}) and 4-fold (1403 kg.ha^{-1}) (**Figure 2**). On the other hand, inoculating genotype Bawutawuta with strain CB756 in Savelugu increased

shoot biomass, N-fixed (by 143%), shoot N accumulation, and, consequently, grain yield by 175% (**Figure 2**). Furthermore, bacterial inoculation of genotypes Nhyira, Omandaw, and Songotra with strain BR 3267 increased N-fixed by 126–157% and grain yield by up to 2-fold when compared to non-inoculation in the case of Omandaw and Songotra, and relative to strain CB756 in the case of Nhyira (**Figure 2**). Although inoculating IT90K-277-2 with *Bradyrhizobium* sp. CB756 resulted in lower N-fixed, the shoot biomass and N accumulation were greater due to higher soil N uptake, which resulted in up to two-fold increase in grain yield when compared to non-inoculation or inoculation with strain BR 3267. Here, non-inoculation increased N-fixed in genotype Nhyira (30.6 kg.ha^{-1}) and doubled it in Padituya when compared to inoculation with strains CB756 and BR 3267, respectively. Consequently, grain yield was also increased by 61% in uninoculated plants of Nhyira (440.7 kg.ha^{-1}) relative to plants inoculated with strain CB756, and up to 5 to 7-fold in Padituya (2318.7 kg.ha^{-1}) relative to inoculation with either strain (**Figure 2**).

In Gbalahi, the genotype x inoculation interaction was significant for several parameters, including shoot biomass, N-fixed, N content, and grain yield (**Table 5**). Here, up to a 2.5-fold increase in N-fixed (84.5 kg.ha^{-1}) and 3.6-fold increase in grain yield (573.7 kg.ha^{-1}) was obtained by inoculating IT90K-277-2, SARVx-09-004 and Zayura with *Bradyrhizobium* sp. BR 3267, and Apagbaala with strain CB756. Consequently, grain yield was increased by 57–149% in those cowpea-inoculant strain combinations (**Figure 3**). On the other hand, non-inoculation increased N-fixed by 2.8-fold and 78% in genotypes Apagbaala (64.7 kg.ha^{-1}) and SARVx-09-004 (51.8 kg.ha^{-1}) relative to inoculation of those genotypes with Bradyrhizobium sp. BR 3267 or Bradyrhizobium sp. CB756, respectively in Gbalahi. Moreover, non-inoculation also resulted in a 2-fold and 97% grain yield increase in genotypes Apagbaala and SARVx-09-004 relative to inoculation of those genotypes with *Bradyrhizobium* sp. strain BR 3267 or CB756, respectively (**Figure 3**).

Main Effects of Genotype and Cropping System on N Solutes in Xylem Sap

Within each location, there was a significant effect of genotype on the concentration of ureides, nitrate, and amino acids in the xylem sap of the test cowpeas (**Table 5**).

At Nyankpala, genotypes Omandaw and Asetenapa had greater concentration of ureides in xylem sap while Nhyira, Soronko, and Zayura recorded the least concentrations of ureides (**Table 5**). Other cowpea genotypes with high concentration of ureides in xylem sap in Nyankpala include Bawutawuta, IT90K-277-2, and SARVx-09-001 (**Table 5**). The genotypes Apagbaala, Songotra, SARVx-09-003, and Zayura coupled lower ureides in xylem sap with greater concentrations of nitrate. However, the concentrations of ureides and nitrates were both high in the sap of genotype Omandaw (**Table 5**). Moreover, the concentration of amino acids was greater in genotypes Apagbaala, Asetenapa, Omandaw, and SARVx-09-004 but least in Bawutawuta, Nhyira, and Padituya (**Table 5**). With high %Ndfa values from ^{15}N
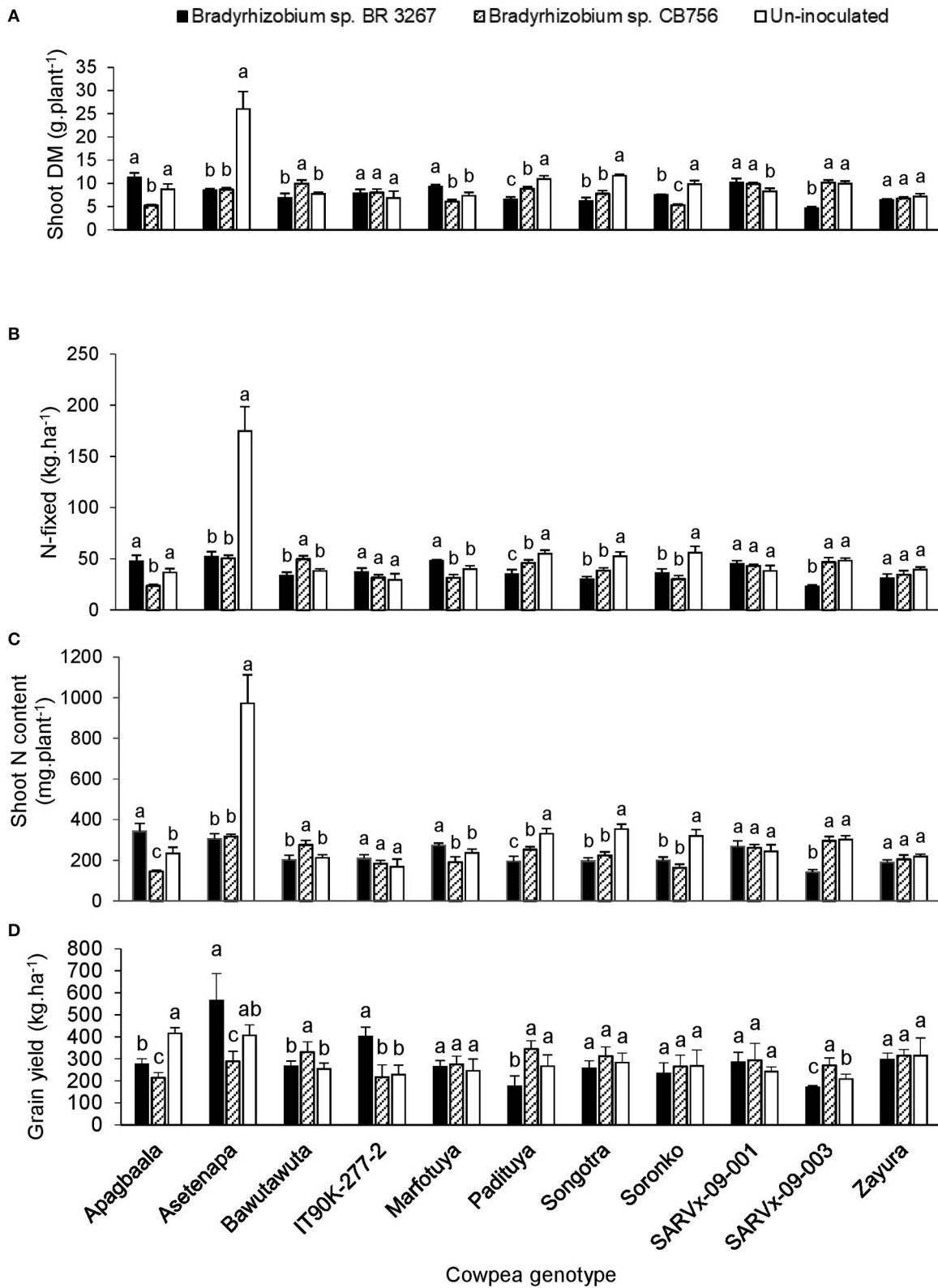
FIGURE 1 | The interactive effect of genotype x inoculation on **(A)** shoot dry matter, **(B)** N-fixed, **(C)** N content, and **(D)** grain yield of 11 cowpea genotypes planted in Nyankpala in 2013. For each genotype, bars with dissimilar letters are significantly different at $p \leq 0.05$. Error bars represent standard errors.
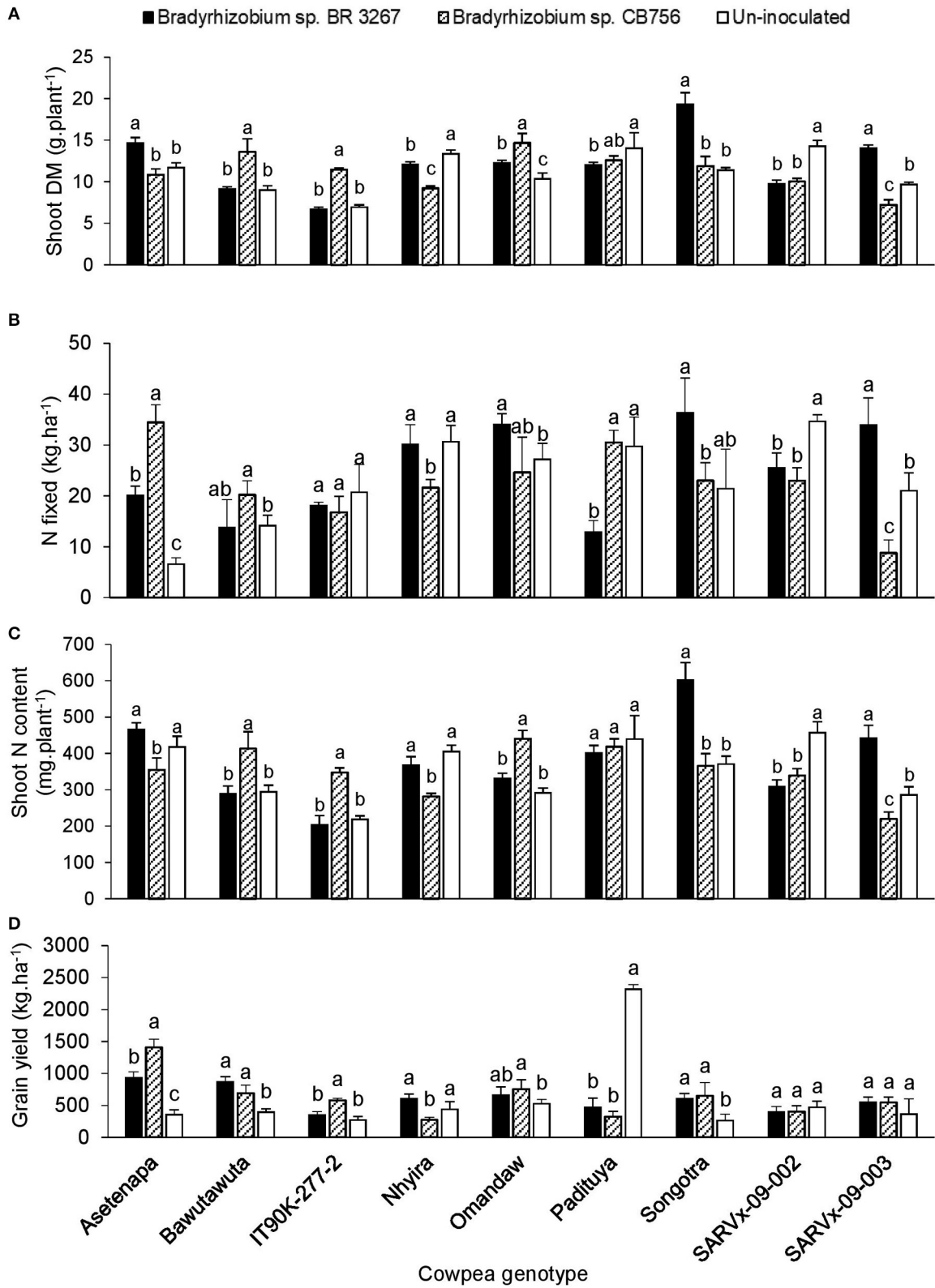
FIGURE 2 | The interactive effect of genotype x inoculation on **(A)** shoot dry matter, **(B)** N-fixed, **(C)** N content, and **(D)** grain yield of 9 cowpea genotypes planted in Savelugu in 2013. For each genotype, bars with dissimilar letters are significantly different at $p \leq 0.05$. Error bars represent standard errors.
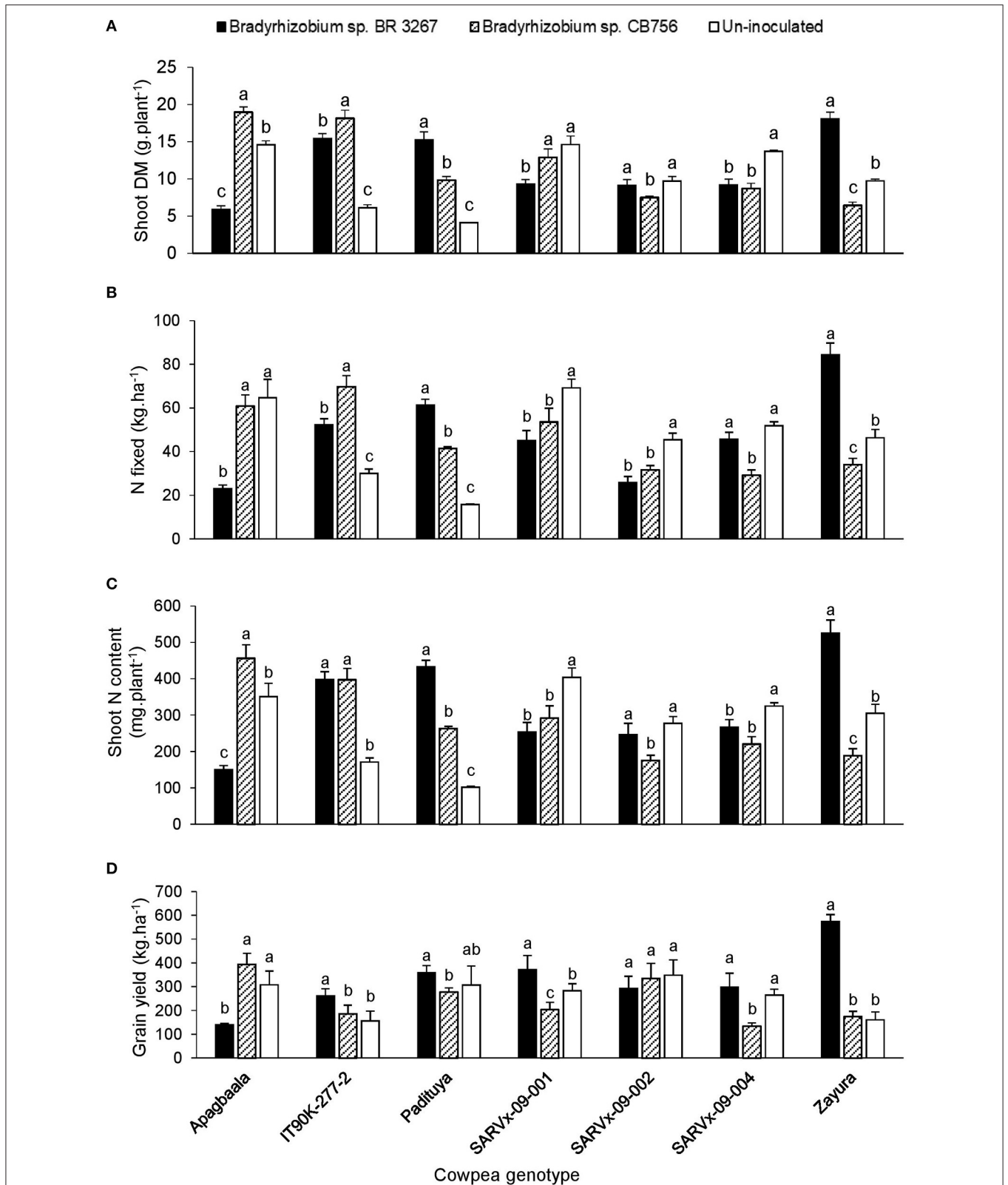
FIGURE 3 | The interactive effect of genotype x inoculation on **(A)** shoot dry matter, **(B)** N-fixed, **(C)** N content, and **(D)** grain yield of 7 cowpea genotypes planted in Gbalahi in 2013. For each genotype, bars with dissimilar letters are significantly different at $p \leq 0.05$. Error bars represent standard errors.

TABLE 5 | The concentrations of ureides, nitrate, amino-N, and Relative ureide-N in xylem sap of cowpea genotypes inoculated with two *Bradyrhizobium* strains and planted in Nyankpala, Savelugu and Gbalahi in 2013.

Treatment	Nyankpala				Savelugu				Gbalahi			
	Ureides	Nitrate	Amino	RU-N	Ureides	Nitrate	Amino	RU-N	Ureides	Nitrate	Amino	RU-N
	mg.ml^{-1}			%	mg.ml^{-1}			%	mg.ml^{-1}			%
GENOTYPE												
Apagbaala	156.7 ± 12.60cde	50.3 ± 10.28a	277.8 ± 10.76a	44.7 ± 1.55g	56.4 ± 11.35de	25.1 ± 2.07ab	81.2 ± 14.92c	43.8 ± 5.57d	65.1 ± 4.84b	11.7 ± 3.01b	37.7 ± 7.04bcd	71.9 ± 3.32b
Asetenapa	188.7 ± 9.56b	28.7 ± 4.80cd	327.1 ± 20.14a	50.0 ± 1.86d–g	87.0 ± 7.12b	16.5 ± 2.69cde	115.7 ± 22.38ab	55.9 ± 5.13bc	72.4 ± 7.94a	6.6 ± 0.44de	58.7 ± 4.64a	65.2 ± 3.14de
Bawutawuta	181.6 ± 6.01bc	35.1 ± 12.07bc	161.1 ± 13.36fg	63.0 ± 3.15a	38.1 ± 4.80fgh	20.4 ± 1.60bcd	87.8 ± 17.83bc	39.5 ± 4.68d	47.0 ± 11.19cd	ND	37.1 ± 5.74bcd	ND
IT90K-277-2	173.4 ± 16.10bc	25.1 ± 3.07de	171.3 ± 19.93efg	60.5 ± 1.57ab	101.0 ± 10.08a	21.0 ± 3.20bc	100.1 ± 9.88bc	58.1 ± 3.65abc	36.8 ± 3.42efg	7.4 ± 1.54cd	50.7 ± 7.57ab	52.6 ± 4.15h
Marfotuya	118.2 ± 12.64fgh	8.2 ± 1.11f	159.9 ± 14.16fg	56.3 ± 4.08bc	45.5 ± 3.85ef	7.6 ± 1.39gh	38.5 ± 6.16d	63.5 ± 4.57ab	37.8 ± 5.88ef	5.8 ± 0.77ef	23.9 ± 4.03f	64.2 ± 2.85e
Nhyira	101.3 ± 28.75h	28.4 ± 10.57cd	153.4 ± 14.22g	44.2 ± 7.80g	92.4 ± 8.73ab	3.8 ± 1.07h	94.9 ± 11.94bc	64.4 ± 3.46a	39.2 ± 6.50ef	3.0 ± 0.61i	42.7 ± 6.14b	58.0 ± 3.00fg
Omandaw	281.1 ± 24.68a	42.1 ± 4.87b	361.1 ± 43.70a	56.0 ± 2.87bcd	44.3 ± 6.58efg	11.2 ± 0.92efg	134.8 ± 16.84a	36.4 ± 5.17de	30.0 ± 3.78g	4.6 ± 1.10gh	40.2 ± 5.15bc	53.0 ± 3.28h
Padituya	162.9 ± 19.64bcd	19.8 ± 4.18e	172.8 ± 23.61efg	58.5 ± 5.59abc	72.0 ± 3.86c	15.3 ± 2.36c–f	94.7 ± 15.28bc	55.5 ± 3.68bc	66.8 ± 7.10ab	5.4 ± 0.45fg	33.1 ± 2.30de	73.0 ± 3.15b
Songotra	136.4 ± 9.38def	41.3 ± 6.11b	258.2 ± 21.86bc	45.3 ± 3.00g	93.4 ± 13.16ab	10.0 ± 2.70bc	88.4 ± 24.34bc	63.7 ± 7.97ab	48.6 ± 11.98cd	ND	58.5 ± 7.79a	ND
Soronko	105.9 ± 3.92gh	10.5 ± 2.02f	206.3 ± 11.99de	48.6 ± 1.46fg	58.5 ± 6.75d	14.7 ± 2.30a	129.3 ± 15.31a	42.2 ± 3.02d	32.0 ± 3.70fg	4.0 ± 0.77h	11.0 ± 1.47g	77.9 ± 1.88a
SARVx-09-001	181.3 ± 13.73bc	27.2 ± 6.06d	233.9 ± 15.32cd	55.2 ± 2.29b–e	94.3 ± 6.35ab	13.1 ± 1.81efg	107.3 ± 18.49abc	59.7 ± 2.70abc	36.0 ± 4.92efg	ND	26.1 ± 1.01f	ND
SARVx-09-002	130.7 ± 19.29efg	11.9 ± 2.57f	196.4 ± 22.65def	52.3 ± 3.91c–f	39.4 ± 4.13fgh	8.7 ± 1.29gh	96.7 ± 10.92bc	41.0 ± 3.64d	42.7 ± 7.97de	7.7 ± 1.25c	38.7 ± 3.36bcd	56.3 ± 5.89g
SARVx-09-003	152.9 ± 14.49cde	40.8 ± 5.90b	217.8 ± 15.91d	49.4 ± 2.45efg	30.8 ± 4.14h	29.4 ± 3.94a	94.5 ± 18.70bc	29.6 ± 2.87e	50.2 ± 5.23c	4.6 ± 0.30gh	62.3 ± 10.62a	59.8 ± 5.23f
SARVx-09-004	159.2 ± 12.93b–e	35.4 ± 7.09bc	272.2 ± 10.45b	47.4 ± 1.40fg	32.1 ± 5.44gh	16.9 ± 3.82cde	76.2 ± 15.97bc	38.1 ± 5.58d	65.6 ± 7.56ab	13.4 ± 3.28a	29.5 ± 3.08ef	68.4 ± 4.98c
Zayura	98.2 ± 12.58h	49.9 ± 9.29a	209.1 ± 26.15de	37.7 ± 2.53h	62.0 ± 4.78cd	19.8 ± 3.55bcd	81.4 ± 14.89bc	51.8 ± 4.56c	64.4 ± 6.61b	4.2 ± 0.90h	34.4 ± 2.28cde	73.5 ± 2.22b
INOCULATION												
Bradyrhizobium sp. BR 3267	143.8 ± 10.36b	40.4 ± 4.26a	197.7 ± 10.80b	49.3 ± 1.94b	54.9 ± 4.21c	16.4 ± 1.36a	124.5 ± 7.86a	41.1 ± 2.60c	39.7 ± 2.62c	7.3 ± 1.05a	39.6 ± 2.36a	58.6 ± 1.98c
Bradyrhizobium sp. CB756	159.3 ± 8.27a	24.6 ± 2.88b	237.8 ± 9.38a	51.6 ± 1.42ab	63.4 ± 3.98b	14.8 ± 1.40a	57.0 ± 4.77c	57.6 ± 2.19a	48.1 ± 4.03b	7.5 ± 0.85a	42.0 ± 3.43a	63.5 ± 2.32b
Un-inoculated	162.6 ± 7.77a	25.9 ± 2.37b	240.2 ± 14.24a	52.9 ± 1.79a	71.1 ± 4.87a	15.5 ± 1.39a	102.8 ± 6.87b	49.9 ± 2.06b	59.2 ± 3.30a	4.8 ± 0.53b	35.3 ± 2.91b	71.4 ± 1.80a
F STATISTICS												
Genotype (G) **[df = 14]**	17.94***	32.58**	20.02***	11.29***	35.57***	12.82***	5.93***	18.43***	38.84**	109.68**	42.94***	53.64**
Inoculation (I) **[df = 2]**	4.27*	68.44**	14.32***	3.72*	18.50***	0.95ns	66.58***	47.72***	91.44**	96.73**	12.44***	120.95**
G x I **[df = 28]**	7.26***	38.56**	6.77***	11.37***	11.72***	5.09***	7.81***	9.38***	43.49**	139.63**	32.25***	46.38**

Xylem sap was collected at early podding stage of the genotypes. Values (mean ± standard error) with dissimilar letters in a column are significantly different at ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$. ns, not significant. ND, not determined.

natural abundance, the cowpea genotypes Bawutawuta, IT90K-277-2, and Padituya also recorded higher RU-N in xylem sap at Nyankpala. In contrast, genotypes Apagbaala, Nhyira, Songotra, and Zayura recorded much lower RU-N at Nyankpala, although Nhyira and Zayura had high %Ndfa at the location based on ^{15}N natural abundance (**Tables 2**, **5**).

The main effect of inoculation was also significant for the concentrations of ureides, nitrate, amino acids, and RU-N in xylem sap of the test cowpeas at Nyankpala (**Table 5**). Here, as observed with the ^{15}N natural abundance, non-inoculation, together with bradyrhizobial inoculation with strain CB75, increased xylem ureides and amino acids, but decreased nitrate concentration, resulting in greater RU-N when compared to inoculation with strain BR 3267 (**Table 5**).

In Savelugu, there was a significant effect ($p \leq 0.05$) of genotype on the concentrations of ureides, nitrate, and amino acids and RU-N in the xylem sap of cowpeas planted at Savelugu (**Table 5**). Here, genotype IT90K-277-2 recorded the highest ureide concentration in xylem sap despite having lower N-fixed measured from ^{15}N natural abundance (**Tables 3**, **5**). However, the genotypes Nhyira, Songotra, and SARVx-09-001 exhibited high ureide levels and relatively lower nitrate concentrations, which resulted in greater RU-N and %Ndfa measured from ^{15}N natural abundance (**Tables 3**, **5**). The least concentration of ureides was recorded in the xylem sap of SARVx-09-003 followed by SARVx-09-002, SARVx-09-004, Marfotuya, Bawutawuta, and Omandaw which also had low ureides in xylem sap (**Table 3**). With low ureide concentration, the genotypes Soronko, SARVx-09-003, and Apagbaala recorded the highest level of nitrate in xylem sap (**Table 3**). Moreover, the genotypes Omandaw and Soronko recorded the highest concentration of amino acids in xylem sap at Savelugu, followed by Asetenapa and SARVx-09-001 also with high concentrations of amino acids (**Table 3**). The least RU-N was recorded in genotype SARVx-09-003 followed by Omandaw, SARVx-09-004, Bawutawuta, SARVx-09-002, and Apagbaala in that order (**Table 3**). Other genotypes which coupled high RU-N with relatively high %Ndfa from ^{15}N natural abundance include Marfotuya and IT90K-277-2 (**Tables 3**, **5**).

The main effect of inoculation at Savelugu was also significant for xylem ureides, amino acid concentrations, and RU-N but not for nitrates (**Table 3**). Overall, non-inoculation or bacterial inoculation with *Bradyrhizobium* sp. strain CB756 each increased xylem ureide concentration when compared to inoculation with strain BR 3267 (**Table 3**). The concentration of nitrate in the xylem sap was however unaltered by bradyrhizobial inoculation at Savelugu. However, the concentration of amino acids was greater in plots inoculated with *Bradyrhizobium* sp. BR 3267 followed by the uninoculated plots, and least in plots inoculated with strain CB756. Non-inoculation and bradyrhizobial inoculation with strain CB756 each increased RU-N when compared to inoculation with strain BR 3267 at Savelugu although %Ndfa from ^{15}N natural abundance was unaltered by the inoculation treatment (**Table 3**).

In Gbalahi, there was a marked effect of genotype on the concentrations of ureides, nitrate, amino acids, and RU-N in xylem sap of cowpea (**Table 5**). Here, genotype Asetenapa recorded the highest concentration of ureides in xylem sap followed by Padituya and SARVx-09-004. Other genotypes with high ureide levels in the location include Apagbaala and Zayura. The least concentration of ureide was recorded in genotype Omandaw followed by Soronko, IT90K-277-2, Marfotuya, and Nhyira also with low xylem ureide concentration (**Table 5**). With high ureides however, genotypes Apagbaala and SARVx-09-004 also recorded higher concentration of nitrate in xylem sap. Together with genotypes Apagbaala, Soronko, and Zayura which recorded high levels of ureides, Padituya also recorded greater RU-N despite having reduced concentration of ureides in xylem sap (**Table 5**). Moreover, genotypes Soronko and Zayura recorded high RU-N as well as greater %Ndfa measured using ^{15}N natural abundance (**Table 5**).

In Gbalahi, there was a marked effect of inoculation on the concentration of ureides, nitrate, amino acids, and RU-N in xylem sap (**Table 5**). Overall, the highest concentration of ureides in xylem sap in Gbalahi was obtained by non-inoculation followed by bacterial inoculation with strain CB756. Non-inoculation also reduced overall nitrate and amino acids in xylem sap when compared to inoculation with either *Bradyrhizobium* strains in Gbalahi. Here, together with non-inoculation, bacterial inoculation with strain CB756 each increased RU-N and %Ndfa measured from ^{15}N natural abundance when compared to inoculation with strain BR 3267 (**Tables 4**, **5**).

N and C Assimilation in Cowpea Genotypes

The 15 cowpea genotypes markedly varied in their shoot N and C concentrations (%) and N and C contents (mg.plant^{-1}) in Nyankpala, Savelugu, and Gbalahi (**Table 6**).

In Nyankpala, the genotype Asetenapa recorded the highest %N followed by Marfotuya and SARVx-09-004 also with high %N. On the other hand, genotypes IT90K-277-2 and Omandaw recorded the least %N at the location (**Table 6**). Further, genotype Apagbaala recorded the least %C (36.5%) in Nyankpala, while the remaining genotypes exhibited markedly greater but similar %C values (38.8–40.6%) in that location (**Table 6**). With high %N and %C, the genotype Asetenapa coupled higher shoot N accumulation with greater C content in Nyankpala. Other genotypes with high N and C contents in Nyankpala include Omandaw and SARVx-09-001 (**Table 6**).

The main effect of inoculation was significant for N and C contents in Nyankpala, with non-inoculation resulting in greater shoot N and C accumulation when compared to inoculation with either *Bradyrhizobium* strains at the location. However, the overall %N and %C were unaltered by inoculation (**Table 6**).

In Savelugu, there was a significant effect of genotype on %N, %C, and N and C contents (mg.plant^{-1}) (**Table 6**). Here, %N was highest in genotype Zayura followed by Asetenapa, SARVx-09-004, and then Marfotuya and Padituya, but least in Omandaw and Apagbaala (**Table 6**). Genotype Marfotuya recorded the highest %C in Savelugu, followed by IT90K-277-2, Omandaw, Soronko, SARVx-09-001, SARVx-09-002, and Zayura also with high %C. The genotypes SARVx-09-004, Songotra, Padituya, and Asetenapa exhibited high N and C contents in Savelugu while Omandaw recorded higher C content but lower N content (**Table 6**). On the other hand, genotypes such

TABLE 6 | Shoot N and C assimilation in 15 cowpea genotypes inoculated with two *Bradyrhizobium* strains and planted in Nyankpala, Savelugu, and Gbalahi in 2013.

Treatments	Nyankpala				Savelugu				Gbalahi			
	N conc'n %	C conc'n %	N content mg.plant^{-1}	C content g.plant^{-1}	N conc'n %	C conc'n %	N content mg.plant^{-1}	C content g.plant^{-1}	N conc'n %	C conc'n %	N content mg.plant^{-1}	C content g.plant^{-1}
GENOTYPE												
Apagbaala	2.8 ± 0.09bcd	36.5 ± 1.29b	240 ± 28.7bcd	3.03 ± 0.32c–e	3.0 ± 0.07de	35.4 ± 0.68d	331 ± 22.17de	3.90 ± 0.25cd	2.45 ± 0.08d	37.2 ± 0.88b	319 ± 41.5a	4.95 ± 0.67ab
Asetenapa	3.7 ± 0.08a	40.6 ± 0.18a	532 ± 103.3a	5.84 ± 1.10a	3.3 ± 0.10ab	36.8 ± 1.03cd	413 ± 20.03bc	4.58 ± 0.26ab	2.84 ± 0.10ab	39.5 ± 0.29a	242 ± 19.9de	3.36 ± 0.24g
Bawutawuta	2.8 ± 0.09bcd	39.4 ± 0.46a	230 ± 14.4bcd	3.22 ± 0.22b–e	3.2 ± 0.09bcd	37.6 ± 0.42bc	332 ± 23.81de	3.98 ± 0.33cd	2.85 ± 0.08ab	38.7 ± 0.45a	324 ± 39.3a	4.33 ± 0.42cde
IT90K-277-2	2.5 ± 0.07e	40.3 ± 0.22a	187 ± 14.7d	3.04 ± 0.20c–f	3.1 ± 0.10cde	38.8 ± 0.27ab	257 ± 21.46f	3.25 ± 0.26e	2.52 ± 0.09cd	39.4 ± 0.30a	322 ± 34.3a	5.18 ± 0.62a
Marfotuya	3.1 ± 0.08b	40.1 ± 0.38a	233 ± 14.85bcd	3.03 ± 0.22c–f	3.3 ± 0.05abc	39.5 ± 0.30a	325 ± 27.90de	3.91 ± 0.33cd	2.72 ± 0.07bcd	38.7 ± 0.77a	271 ± 27.3cd	3.82 ± 0.35efg
Nhyira	3.0 ± 0.10bcd	39.2 ± 0.50a	186 ± 11.9d	2.45 ± 0.08f	3.0 ± 0.06cde	37.8 ± 0.39abc	351 ± 18.19de	4.36 ± 0.22abc	2.45 ± 0.13d	40.1 ± 0.23a	205 ± 25.9e	3.42 ± 0.44g
Omandaw	2.7 ± 0.11de	38.8 ± 0.88a	271 ± 23.89b	3.85 ± 0.34b	2.9 ± 0.08e	38.4 ± 0.60abc	354 ± 21.08de	4.78 ± 0.30a	2.51 ± 0.05cd	38.7 ± 0.49a	245 ± 25.0de	3.75 ± 0.38efg
Padituya	3.0 ± 0.09bcd	39.8 ± 0.26a	259 ± 20.4bc	3.48 ± 0.24bcd	3.3 ± 0.09abc	37.3 ± 0.44bc	420 ± 21.96b	4.79 ± 0.22a	2.68 ± 0.08bcd	39.3 ± 0.22a	266 ± 41.2cd	3.81 ± 0.55efg
Songotra	3.0 ± 0.13bcd	40.0 ± 0.35a	258 ± 23.0bc	3.41 ± 0.30b–e	3.2 ± 0.06bcd	37.7 ± 0.30bc	446 ± 38.35b	5.35 ± 0.46a	2.65 ± 0.08bcd	39.1 ± 0.33a	301 ± 15.06abc	4.44 ± 0.18bcd
Soronko	3.0 ± 0.18bcd	39.4 ± 0.97a	227 ± 24.3bcd	2.96 ± 0.26def	3.2 ± 0.06a–d	38.5 ± 0.34abc	300 ± 18.31ef	3.55 ± 0.18de	2.75 ± 0.13abc	39.8 ± 0.38a	258 ± 13.5cd	3.75 ± 0.13efg
SARVx-09-001	2.7 ± 0.11cde	38.9 ± 0.52a	258 ± 12.1bc	3.66 ± 0.15bc	3.2 ± 0.08a–d	38.2 ± 0.60abc	345 ± 17.39de	4.08 ± 0.21bcd	2.58 ± 0.09bcd	39.2 ± 0.46a	317 ± 24.4ab	4.80 ± 0.33abc
SARVx-09-002	2.8 ± 0.10bcd	39.0 ± 0.44a	235 ± 20.4bcd	3.18 ± 0.20cde	3.2 ± 0.05a–d	38.2 ± 0.34abc	368 ± 23.00cd	4.34 ± 0.29abc	2.62 ± 0.10bcd	37.3 ± 1.02b	233 ± 17.4de	3.28 ± 0.18g
SARVx-09-003	3.0 ± 0.07bcd	39.1 ± 0.30a	246 ± 24.2bc	3.21 ± 0.31b–e	3.0 ± 0.09cde	37.4 ± 0.54bc	315 ± 30.96de	3.84 ± 0.31cd	2.61 ± 0.09bcd	39.3 ± 0.36a	236 ± 16.9de	3.56 ± 0.25fg
SARVx-09-004	3.1 ± 0.05bc	39.1 ± 0.38a	222 ± 25.5bcd	2.84 ± 0.32def	3.3 ± 0.10ab	37.7 ± 0.74bc	524 ± 43.34a	5.94 ± 0.47a	2.61 ± 0.09bcd	38.7 ± 0.49a	271 ± 15.7bcd	4.09 ± 0.30def
Zayura	3.0 ± 0.09bcd	40.6 ± 0.34a	204 ± 10.2cd	2.75 ± 0.09ef	3.5 ± 0.07a	38.6 ± 0.44abc	323 ± 11.72de	3.59 ± 0.10de	3.00 ± 0.11a	40.0 ± 0.23a	340 ± 44.5a	4.54 ± 0.59bcd
INOCULATION												
Bradyrhizobium sp. BR 3267	2.9 ± 0.05a	39.1 ± 0.32a	222 ± 8.2b	2.96 ± 0.10b	3.2 ± 0.04a	37.4 ± 0.26a	383 ± 16.38a	4.50 ± 0.18a	2.75 ± 0.04a	38.3 ± 0.30b	312 ± 14.8a	4.37 ± 0.20a
Bradyrhizobium sp. CB756	2.9 ± 0.05a	39.8 ± 0.16a	233 ± 8.4b	3.17 ± 0.10b	3.2 ± 0.04a	38.0 ± 0.28a	349 ± 9.69b	4.18 ± 0.13b	2.54 ± 0.04b	39.4 ± 0.18a	253 ± 12.6b	3.97 ± 0.21b
Un-inoculated	3.0 ± 0.06a	39.2 ± 0.32a	302 ± 26.8a	3.87 ± 0.28a	3.2 ± 0.04a	38.1 ± 0.22a	349 ± 13.76b	4.17 ± 0.15b	2.68 ± 0.05a	39.4 ± 0.21a	264 ± 11.5b	3.88 ± 0.17b
F STATISTICS												
Genotype (G) [df = 14]	6.61***	2.71**	19.81***	14.42***	3.89***	3.29***	15.92***	17.46***	3.41***	3.50***	7.24***	10.63***
Inoculation (I) [df = 2]	0.49ns	1.60ns	28.47***	26.71***	0.11ns	2.93ns	6.92**	6.08**	8.08***	10.5***	21.05***	9.74***
G x I [df = 28]	0.91ns	0.43ns	12.35***	12.49***	0.94ns	1.16ns	8.34***	12.09***	1.85*	2.20***	14.44***	23.31***

Plants were sampled at early podding stage of genotypes. Values (mean ± standard error) with dissimilar letters in a column are significantly different at ***p < 0.001, **p < 0.01, and *p < 0.05. ns, not significant.

as Apagbaala and Soronko had lower N and C contents in Savelugu (**Table 6**).

The main effect of inoculation was also significant for N and C contents in Savelugu but not %N and %C. Here, bacterial inoculation with strain BR 3267 increased overall N and C contents when compared to non-inoculation or inoculation with strain CB756 in Savelugu (**Table 6**).

At Gbalahi, the genotypes showed marked variations in %N, %C, N, and C contents (**Table 6**). The genotype Zayura had the highest %N in shoot followed by Asetenapa and Bawutawuta also with high %N. In contrast, Apagbaala and Nhyira recorded the least %N at Gbalahi (**Table 6**). The least %C was recorded in genotypes Apagbaala (37.2%) and Nhyira (37.3%) while the remaining genotypes recorded higher but similar %C ranging between 38.7 and 40.1%. In Gbalahi, genotypes Apagbaala, IT90K-277-2, and SARVx-09-001 coupled high N contents with greater C content. On the other hand, genotypes Bawutawuta and Zayura recorded relatively lower C contents despite greater N content in Gbalahi. The least N and C contents at Gbalahi were recorded in genotypes SARVx-9-002, SARVx-09-003, Nhyira, Omandaw, and Asetenapa (**Table 6**).

The main effect of *Bradyrhizobium* inoculation markedly influenced %N, %C, as well as N and C contents in Gbalahi (**Table 6**). Here, together with non-inoculation, bacterial inoculation with strain BR 3267 increased overall %N compared to inoculation with strain CB756. On the other hand, non-inoculation or inoculation with strain CB756 each increased %C when compared to inoculation with strain BR 3267. However, bacterial inoculation with strain BR 3267 increased shoot N and C contents at Gbalahi when compared to non-inoculation or inoculation with strain CB756 at the location (**Table 6**).

THREE-WAY ANOVA COMPARISON OF PARAMETERS AMONG LOCATIONS

Plant Growth, Symbiotic Parameters, δ^{13}C, and Grain Yield

A three-way ANOVA revealed marked effect of planting location on nodule mass, shoot biomass, symbiotic parameters, soil N uptake, δ^{13}C, and grain yield of the cowpea genotypes (**Supplementary Table 2**). Overall, nodule mass of cowpea was highest in Gbalahi, followed by Nyankpala, and least in Savelugu. However, overall shoot biomass was highest in Savelugu despite reduced nodulation by plants at the location followed by plants in Gbalahi (**Supplementary Table 2**).

The least δ^{15}N was recorded by plants in Nyankpala followed by those at Gbalahi while plants in Savelugu recorded the highest overall δ^{15}N (**Supplementary Table 2**). As a result, average %Ndfa and N-fixed were both higher in plants in Nyankpala and Gbalahi when compared to the plants in Savelugu (**Supplementary Table 2**). With higher δ^{15}N and lower %Ndfa, plants in Savelugu recorded the highest soil N uptake while those in Nyankpala recorded the least. The overall C/N ratio was highest in plants in Gbalahi followed by those in Nyankpala, and least in plants in Savelugu (**Supplementary Table 2**). On the other hand, %N was highest in plants in Savelugu, whilst %C

was least in the plants at the same location. The shoot N and C contents of cowpea closely mirrored patterns in their δ^{13}C values across locations, with the highest values being recorded in Savelugu (**Supplementary Table 2**). Overall, average δ^{13}C values of cowpea was highest in Savelugu followed by Gbalahi, and least in the plants grown in Nyankpala. With greater δ^{13}C, plants in Savelugu also recorded the highest grain yield while those in Gbalahi recorded the least (**Supplementary Table 2**).

Ureides, Nitrate, Amino-N, and Relative Ureide-N in Xylem Sap

The concentration of xylem N solutes (ureides, nitrate and amino-N) and RU-N of the cowpea genotypes markedly varied between the test locations (**Supplementary Table 3**). The concentrations of ureides, nitrate, and amino-N in xylem sap were highest in plants grown in Nyankpala followed by those in Savelugu, and least in Gbalahi. However, RU-N was highest in plants in Gbalahi followed by those in Nyankpala due to relatively lower concentrations of nitrate and amino-N in xylem sap of cowpea in the former location (**Supplementary Table 3**). The plants grown in Savelugu coupled low RU-N with the least overall %Ndfa measured from ^{15}N natural abundance (**Supplementary Table 3**).
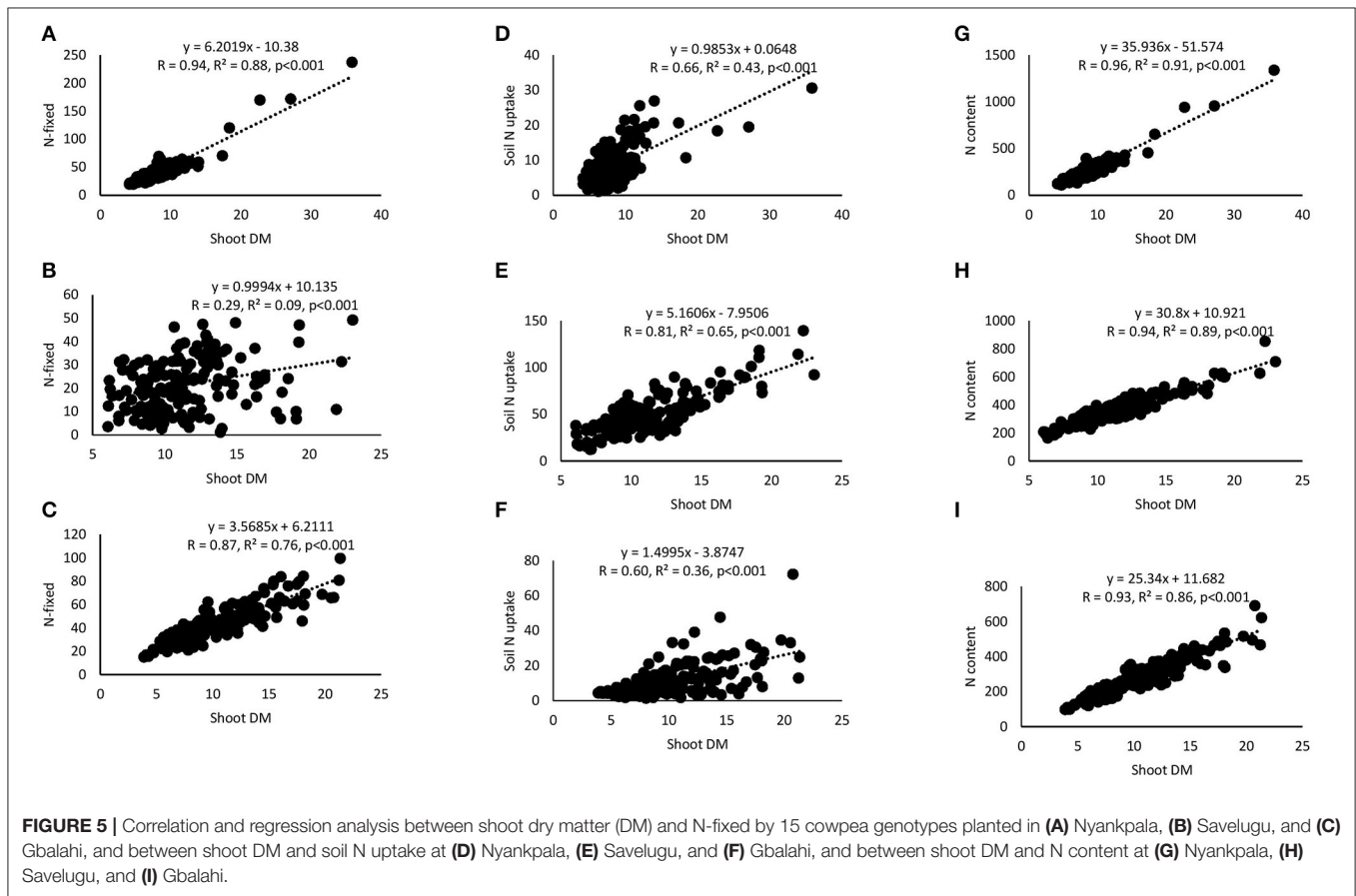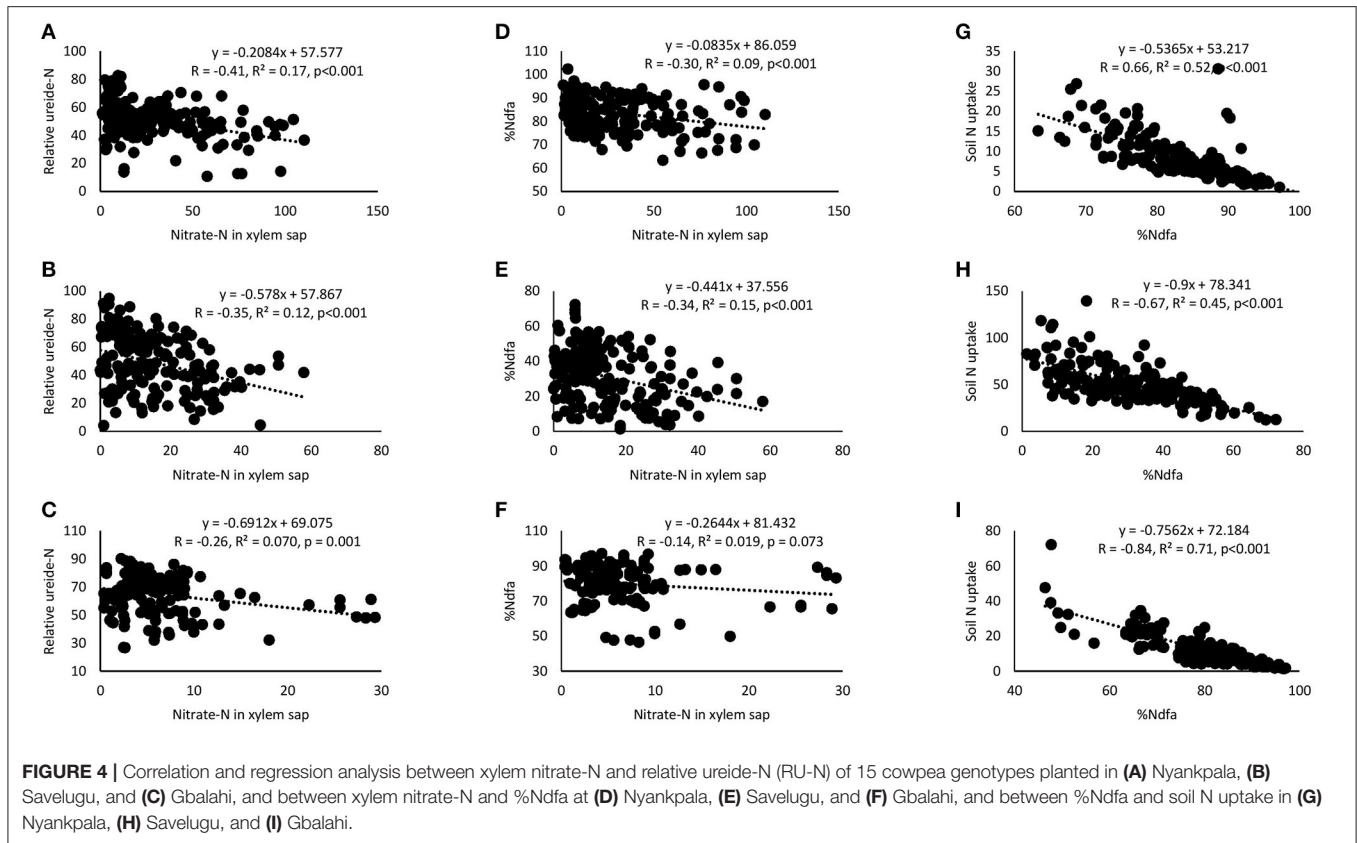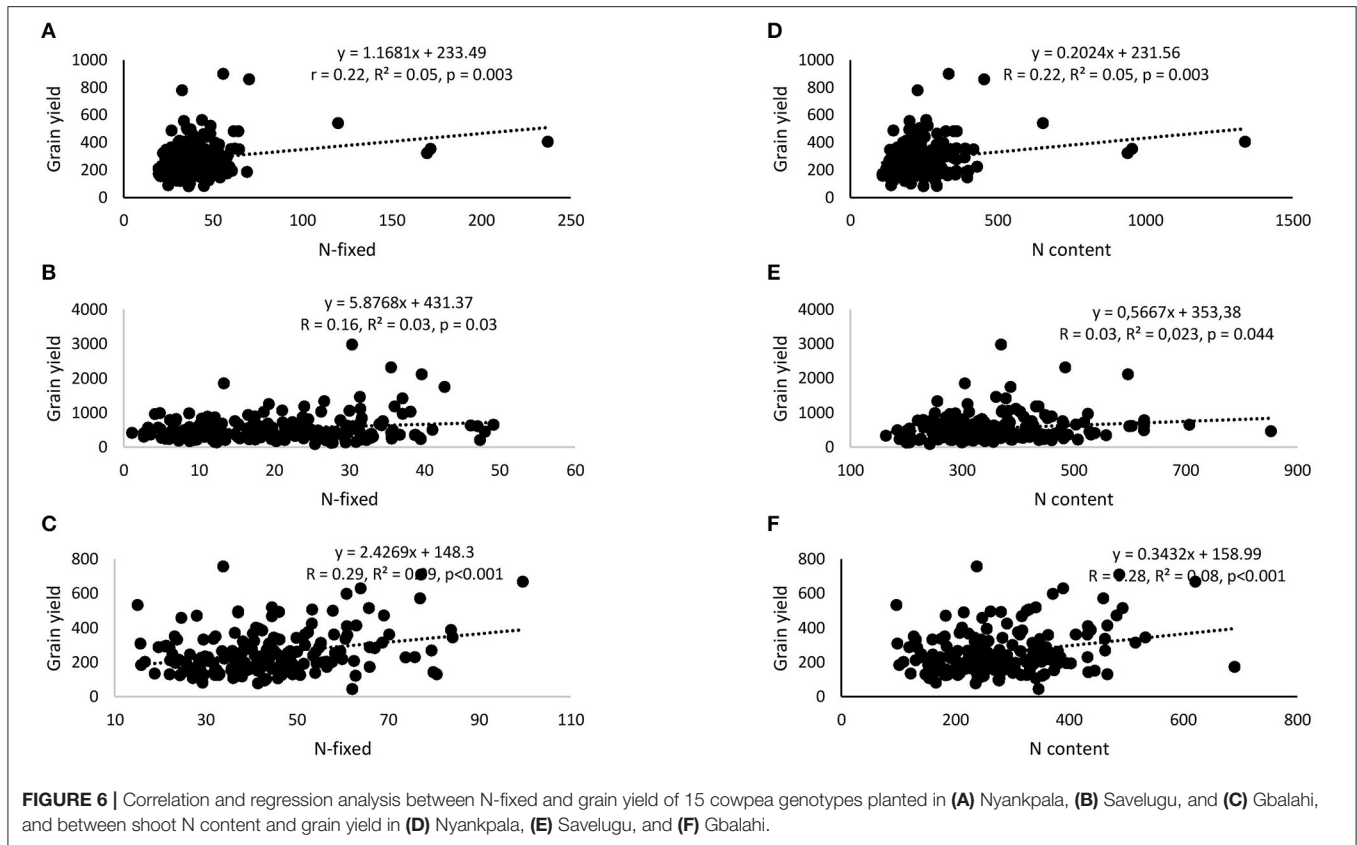
CORRELATION AND REGRESSION ANALYSIS

Correlation and regression analyses revealed significant functional relationships between several parameters (**Figures 4–7**). There were significant negative correlations between xylem nitrate-N and RU-N in all three locations, and xylem nitrate-N and %Ndfa measured from ^{15}N natural abundance at Nyankpala and Savelugu (**Figures 4A–F**). %Ndfa and soil N uptake also showed negative correlation. Further, there were significant positive correlations between shoot biomass and N-fixed, soil N uptake, and shoot N content in all test locations (**Figures 5A–I**). Grain yield also showed significant positive correlations with N-fixed and shoot N content in all three locations (**Figures 6A–F**) while %N and %C were also positively correlated in Nyankpala and Savelugu. As to be expected, shoot biomass and C content correlated positively in all three locations (**Figures 7A–E**).

DISCUSSION

Cowpea Genotypic Differences in Growth, Symbiotic N$_2$ Fixation and Grain Yield

Although several interaction effects were significant within test locations, analysis of the main effects also revealed marked variations in nodulation, plant growth, and symbiotic performance of the test cowpeas, a finding similar to previous reports among cowpea germplasm grown in contrasting environments in Africa (Belane and Dakora, 2010; Belane et al., 2014). Although greater nodulation was expected to promote symbiosis and plant growth, instances of higher nodulation were not always accompanied by increases in plant growth and symbiotic performance by the test cowpeas. For example, even
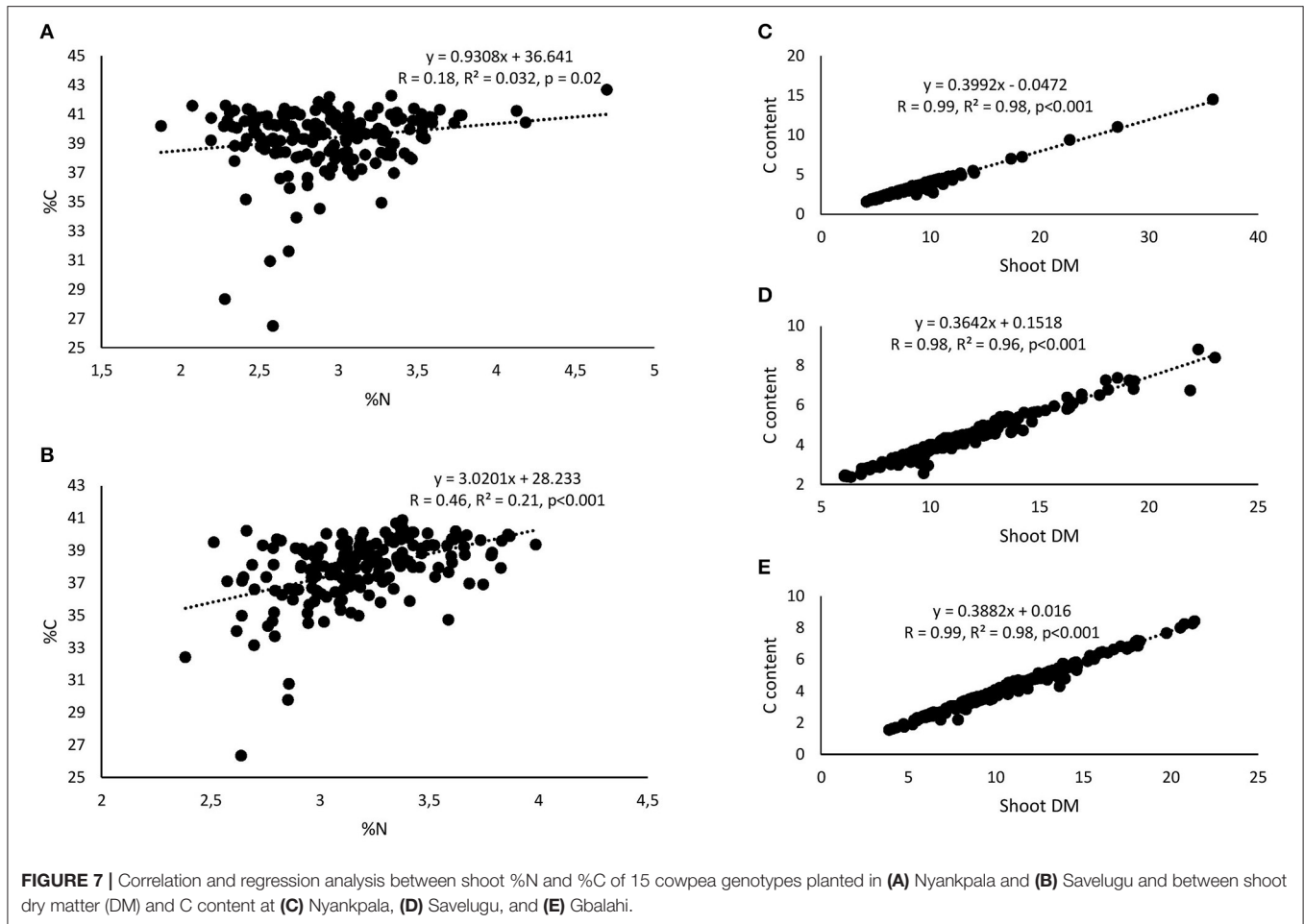
FIGURE 4 | Correlation and regression analysis between xylem nitrate-N and relative ureide-N (RU-N) of 15 cowpea genotypes planted in **(A)** Nyankpala, **(B)** Savelugu, and **(C)** Gbalahi, and between xylem nitrate-N and %Ndfa at **(D)** Nyankpala, **(E)** Savelugu, and **(F)** Gbalahi, and between %Ndfa and soil N uptake in **(G)** Nyankpala, **(H)** Savelugu, and **(I)** Gbalahi.

FIGURE 5 | Correlation and regression analysis between shoot dry matter (DM) and N-fixed by 15 cowpea genotypes planted in **(A)** Nyankpala, **(B)** Savelugu, and **(C)** Gbalahi, and between shoot DM and soil N uptake at **(D)** Nyankpala, **(E)** Savelugu, and **(F)** Gbalahi, and between shoot DM and N content at **(G)** Nyankpala, **(H)** Savelugu, and **(I)** Gbalahi.

FIGURE 6 | Correlation and regression analysis between N-fixed and grain yield of 15 cowpea genotypes planted in **(A)** Nyankpala, **(B)** Savelugu, and **(C)** Gbalahi, and between shoot N content and grain yield in **(D)** Nyankpala, **(E)** Savelugu, and **(F)** Gbalahi.

though increased nodulation in genotype Asetenapa promoted greater biomass accumulation at Nyankpala, other genotypes, including Nhyira, Songotra, and SARVx-09-001, elicited reduced plant growth and N-fixed despite higher nodulation at the location. Similar trends between cowpea nodulation, plant growth, and N_2-fixing parameters were also observed in Savelugu and Gbalahi, clearly suggesting that the extent of nodulation alone did not always explain plant growth and legume N_2 fixation in this study. These results agree with previous reports that rather than the number of nodules, it is the N_2-fixing efficiency of the rhizobial microsymbionts which is a key determinant of legume symbiotic performance (Westhoek et al., 2017; Mohammed et al., 2018).

As expected, the cowpea genotypes in this study varied in their symbiotic dependence, with %Ndfa ranging between 74.4 and 89.8% in Nyankpala, 11.7 and 50.5% in Savelugu and 71.9 and 90.3% in Gbalahi. Similarly, previous reports have shown that some genotypes can derive up to 96% of their N requirements from the atmosphere (Belane and Dakora, 2010). The observed high symbiotic dependence by cowpeas in this study partly accounts for the wider adaptation of the crop to nutrient depleted soils in which other crops may fail to survive (Belane and Dakora, 2010; Hall, 2012). The low symbiotic dependence by most cowpeas in Savelugu resulting from relatively high soil N (0.16% N) in the location was augmented by greater soil N uptake leading to grain yield increases, a finding supported by significant positive correlations when N-fixed and N content were each

plotted against shoot biomass and grain yield within the test locations. The observed soil N inhibition of symbiosis (Mbah and Dakora, 2017) was also evidenced by significant negative correlations between xylem nitrate-N and RU-N, xylem nitrate-N and %Ndfa measured from ^{15}N natural abundance, and between %Ndfa and soil N uptake in all test locations (**Figures 4A–I**).

With only few exceptions however, the xylem N solute measure of symbiosis in individual cowpea genotypes did not always follow the same pattern as that measured using the ^{15}N natural abundance method, an observation attributed to the instantaneous nature of the ureides technique in estimating legume symbiotic dependence (Unkovich et al., 2008). Nevertheless, the overall symbiotic dependence measured from the ureides (RU-N) and ^{15}N natural abundance (%Ndfa) were both least in plants grown in Savelugu compared to those in Nyankpala and Gbalahi, indicating the usefulness of both techniques in estimating legume N_2 fixation.

Grain yield of cowpea in this study ranged between 212.4 and 419.9 kg.ha^{-1} in Nyankpala, 338.2 and 1036.1 kg.ha^{-1} in Savelugu, and 139.2 and 440.3 kg.ha^{-1} in Gbalahi. Despite the much lower %Ndfa and N-fixed by the test cowpeas grown at Savelugu, those genotypes augmented their low symbiotic N with increased soil N uptake, leading to higher grain yields. The complementary roles of symbiosis and soil N uptake in promoting the growth and yield of the test cowpeas was evidenced by significant positive correlations when N-fixed and N content were each plotted against shoot biomass and grain

FIGURE 7 | Correlation and regression analysis between shoot %N and %C of 15 cowpea genotypes planted in **(A)** Nyankpala and **(B)** Savelugu and between shoot dry matter (DM) and C content at **(C)** Nyankpala, **(D)** Savelugu, and **(E)** Gbalahi.

yield (**Figures 6A–F**). The genotypes Asetenapa and Omandaw (local landrace) respectively recorded higher grain yields in Nyankpala (419.9 and 411.4 kg.ha^{-1}) and Savelugu (896.7 and 646.5 kg.ha^{-1}), suggesting possible wider adaptation of those genotypes to the different test environments. The highest grain yield in Savelugu (1036.1 kg.ha^{-1}) was however recorded in genotype Padituya which also recorded relatively high grain yield (314.2 kg.ha^{-1}) in Gbalahi when compared to other genotypes in the location. The fact that most cowpeas in Gbalahi had relatively lower grain yields despite deriving up to 90% of N from symbiosis was unexpected. However, other location specific factors including lower rainfall/soil moisture or its distribution (**Supplementary Figure 1**) might have contributed to the observed lower grain yield at the location.

Genotypic Variations in N and C Assimilation, and Water Use Efficiency (δ^{13}C) in Field-Grown Cowpea

Despite the widely reported drought tolerance of cowpea (Hall, 2012), most cultivated varieties are yet to be assessed for their water relations in the field. This study revealed marked differences in the N and C concentrations of the test cowpea genotypes. For example, except for Apagbaala in Nyankpala, and

together with SARVx-09-002 in Gbalahi which recorded much lower %C in shoots (36.5–37.6%), the remaining genotypes had higher but similar shoot %C (38.7–40.6%) (**Table 3**). Moreover, the fact that genotype Asetenapa in Nyankpala, and Marfotuya, Padituya, SARVx-09-004, and Zayura in Savelugu, each coupled greater %N with relatively higher %C in those locations suggest that increased C gain *via* photosynthesis stimulated N nutrition (Rogers et al., 2006). The functional link between N and C nutrition *via* photosynthesis was previously reported in field grown cowpeas (Belane and Dakora, 2011), and was further supported by positive correlations between %N and %C in this study (**Figures 7A,B**). Given the rising atmospheric CO_2 levels with climate change, incorporating legume genotypes eliciting greater N and C accumulation in cropping systems could help in C sequestration, and therefore reduce greenhouse gas emissions. Moreover, the typically low C/N ratio (11.1–16.9 g.g^{-1}) recorded for cowpeas across the three locations is characteristic of N_2-fixing legumes, and partly accounts for their ease of decomposition which makes them valuable in conservation agriculture where they improve C sequestration and soil microbial activities (Jarecki and Lal, 2003).

Aside their potential N contribution to cropping systems, the test cowpeas also showed marked variation in plant water

use efficiency ($\delta^{13}C$) within the test locations. Interestingly, the cowpea genotypes Soronko, Asetenapa, Nhyira, Omandaw, and IT90K-277-2 elicited greater water use efficiency in both Nyankpala (−28.67 to −28.27‰) and Savelugu (−28.12 to −28.12‰). Further, the fact that genotypes Asetenapa and Omandaw in Nyankpala together with Padituya and Soronko in Savelugu coupled greater water use efficiency ($\delta^{13}C$) with relatively higher grain yields in the respective locations are traits that could be exploited for crop improvement programs aimed at screening for enhanced plant-water relations and grain yield. The robustness of carbon isotope discrimination in estimating plant water relations as observed in this study has been reported in other field-grown legumes (Mohale et al., 2014; Mapope and Dakora, 2016).

Effect of Planting Location (Environment) on Cowpea Symbiotic N Nutrition, N and C Assimilation and Water Use Efficiency ($\delta^{13}C$)

Although genotypic differences in plant growth and symbiotic relations exist among cowpea germplasm, these physiological traits are also influenced by environmental differences including soil and climate factors (Belane and Dakora, 2009; Nyemba and Dakora, 2010; Belane et al., 2011). Consequently, plant growth and symbiotic performance of the test cowpeas in this study was markedly influenced by planting location. For example, overall plant growth was greater in Savelugu despite reduced nodulation and symbiosis at the location due to high soil N, whereas cowpea plants in Gbalahi coupled greater nodulation with relatively higher plant growth when compared to those grown in Nyankpala. However, overall symbiotic dependence (%Ndfa) and N-fixed were much higher in Nyankpala and Gbalahi due to reduced $\delta^{15}N$ resulting from effective symbiosis in those locations when compared to Savelugu. Since cowpea exports fixed N as ureides (Baral et al., 2016), xylem N solutes (ureide, nitrate and amino-N) were also used as a measure of symbiotic dependence (Unkovich et al., 2008). The concentrations of all xylem N solutes were greater in Nyankpala followed by Savelugu, and least in plants grown in Gbalahi (**Supplementary Table 3**). As observed with %Ndfa from ^{15}N natural abundance, the relative ureide-N abundance was higher in Nyankpala and Gbalahi, and again, least in Savelugu, an indication that both techniques are robust in assessing legume N_2 fixation. The observed lower symbiotic dependence by cowpeas in Savelugu was probably due to its suppression by endogenous soil N in the location, which resulted in greater soil N uptake to augment the shortfall in N requirement, a finding supported by significant negative correlations between %Ndfa and soil N uptake, xylem nitrates and RU-N, and between xylem nitrates and %Ndfa measured from ^{15}N natural abundance (**Figure 4**). Despite the reduced symbiotic dependence (%Ndfa) by plants in Savelugu, C/N ratio was much lower in those plants due to greater %N and shoot N accumulation resulting increased soil N uptake (**Supplementary Table 2**). The lower grain yield recorded by plants in Gbalahi despite higher

symbiotic performance was probably due to reduced rainfall or soil moisture at the location during the pod filling stage (**Supplementary Figure 1**).

Variable Response of Cowpea to Inoculation With *Bradyrhizobium* Strains in the Field

To test for possible cowpea genotypic differences in response to inoculation with *Bradyrhizobium* sp. strains BR 3267 and CB756, 15 cowpea genotypes were assessed for N_2 fixation and grain yield at three locations in the Northern Region of Ghana. The test cowpeas were found to elicit contrasting responses to inoculation with either *Bradyrhizobium* strains within and between locations in relation to nodulation, shoot growth, symbiotic performance, and grain yield. For brevity, selected interaction effects within test locations are discussed for genotypes that showed consistent patterns in relation to most symbiotic parameters. Bacterial inoculation of cowpea respectively increased N-fixed and grain yield by up to 5- and 4-fold, depending on genotype-inoculant strain combinations and planting location. For example, inoculating genotype Apagbaala with *Bradyrhizobium* sp. BR 3267 in Nyankpala doubled N-fixed (47.7 kg.ha^{-1}), and slightly increased it in IT90K-277-2 when compared to inoculation with strain CB756. On the other hand, bacterial inoculation of Bawutawuta, Padituya, and SARVx-09-003 with strain CB756 significantly increased shoot biomass, resulting in 32 – 200% increases in the amounts of N-fixed and shoot N accumulation when compared to inoculation of the same genotype with strain BR 3267 (**Figure 2**). Consequently, grain yield was increased by 1.3- to 2-fold in those cowpea-inoculant strain combinations in Nyankpala (**Figure 1**). In contrast, non-inoculation of Apagbaala, Asetenapa, Padituya, and SARVx-09-003 in Nyankpala each increased N-fixed by 1.5- to 3.4-fold (36.5–55.0 kg.ha^{-1}), resulting in greater shoot N accumulation and 1.2- to 2-fold increase in grain yield in those cowpea-inoculant combinations (**Figure 1**), a finding that confirms the presence of effective cowpea-compatible rhizobia in the studied soils. Although this is the first report of inoculation response in genotypes Apagbaala and Asetenapa, the increased N_2 fixation and grain yield in uninoculated Padituya is in contrast with an earlier study that found up to 57% increase in grain yield of the genotype due to inoculation with *Bradyrhizobium* sp. strain BR 3267 (Boddey et al., 2017). The discrepancy in inoculation response could be attributed to differences in the study locations together with prevailing climatic conditions, a finding consistent with earlier reports which also found inoculation failures in cowpea in the same growing season due to differences in planting location (Ulzen et al., 2016). Although N_2 fixation was generally low in plants in Savelugu, bacterial inoculation of Asetenapa with either strain BR 3267 or CB756 resulted in 3 to 5-fold increase in N-fixed, and, respectively, increased grain yield by 2.6- (932 kg.ha^{-1}) and 4-fold (1403 kg.ha^{-1}). On the other hand, inoculating Bawutawuta with strain CB756 at Savelugu increased shoot biomass, N-fixed (by 143%), shoot N

accumulation, and, consequently, grain yield by 175% (**Figure 2**). However, non-inoculation increased N-fixed in genotype Nhyira ($30.6 \, kg.ha^{-1}$) and doubled it in Padituya when compared to inoculation with *Bradyrhizobium* sp. strain CB756 or BR 3267, respectively. As a result, grain yield was increased by 61% ($440.7 \, kg.ha^{-1}$) in uninoculated Nhyira relative to inoculation with *Bradyrhizobium* sp. CB756, and up to 5 to 7-fold in uninoculated Padituya ($2318.7 \, kg.ha^{-1}$) relative to inoculation with either *Bradyrhizobium* strains (**Figure 2**). At Gbalahi, up to a 2.5-fold increase in N-fixed ($84.5 \, kg.ha^{-1}$) and 3.6-fold increase in grain yield ($573.7 \, kg.ha^{-1}$) was obtained by inoculating IT90K-277-2, SARVx-09-004, and Zayura with *Bradyrhizobium* sp. strain BR 3267 and Apagbaala with strain CB756. Here, non-inoculation respectively increased N-fixed by 2.5-fold and 78% in genotypes Apagbaala ($60.8 \, kg.ha^{-1}$) and SARVx-09-004 ($51.8 \, kg.ha^{-1}$) in Gbalahi, and resulted in grain yield increases of 2-fold and 97% relative to inoculation with strain BR 3267 and CB756 at the location. These findings are consistent with an earlier study which reported up to 38% increase in grain yield of cowpea resulting from inoculation with *Bradyrhizobium* sp. BR 3267 relative to non-inoculation although the same inoculant failed to increase the parameter in another location (Ulzen et al., 2016).

Clearly, cowpea inoculation response in this study was markedly influenced by the cowpea-inoculant strain combinations used and the planting location, a finding consistent with previous reports that inoculation success is influenced by the compatibility between crop genotype and inoculant strain, location-dependent factors, such as the population and N_2-fixing efficiency of native rhizobia, and the adaptation and competitive ability of introduced strains against the prevailing soil microbiome (Catroux et al., 2001). The fact that some cowpea genotypes, including Apagbaala, Asetenapa, and Padituya in Nyankpala, Padituya in Savelugu, and Apagbaala and SARVx-09-004 in Gbalahi, formed effective symbiosis with native rhizobia in the respective locations leading to enhanced plant growth and grain yield indicates the presence of effective indigenous cowpea-compatible rhizobia in the studied soils (**Figures 2, 3**). These findings also agree with a recent report by Mohammed et al. (2018) who found the presence of highly effective rhizobial symbionts responsible for cowpea nodulation in the studied locations. Although his study is the first report of inoculation response in most of the test cowpea genotypes, Padituya was earlier found to respond to inoculation with *Bradyrhizobium* sp. BR 3267, leading to improved grain yield over non-inoculation (Ulzen et al., 2016). The observed interactions within the test locations in this study further reveal the complexity of the factors influencing legume inoculation response in the field (Catroux et al., 2001), which therefore suggests a need to study the functional diversity of cowpea nodule microsymbionts alongside their adaptive traits such as tolerance to biotic and abiotic stresses that often occur under field conditions.

CONCLUSION

The test cowpeas exhibited variable nodulation, plant growth, and symbiotic performance across the test environments. The fact that cowpeas in this study could obtain up to 90% of their N requirement from symbiosis stresses their survival advantage in the N limited soils in most parts of Africa. Although endogenous soil N suppressed cowpea symbiotic dependence in Savelugu, increased soil N uptake augmented the shortfall leading to grain yield increases. Grain yields of up to $1,036 \, kg.ha^{-1}$ was recorded without any inorganic inputs, suggesting that the cowpea-rhizobia symbiosis could be exploited for increased yield. The fact that genotypes such as Asetenapa, Omandaw, Soronko, and Padituya coupled greater $\delta^{13}C$ with higher grain yield is a desirable trait that could be harnessed for increased production of the crop under water limiting conditions, and for breeding programs to improve plant-water relations. The fact that inoculation response was markedly influenced by genotype-inoculant strain combination and location effects suggest that the introduction of inoculant strains into new areas require field testing with widely grown varieties to assess their feasibility.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

MM conducted field experiments, analyzed and interpreted data, and prepared the manuscript. GM took part in xylem N solute analyses. ES helped in field experiments. FD funded and coordinated the research and helped in manuscript preparation. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro. 2022.764070/full#supplementary-material

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