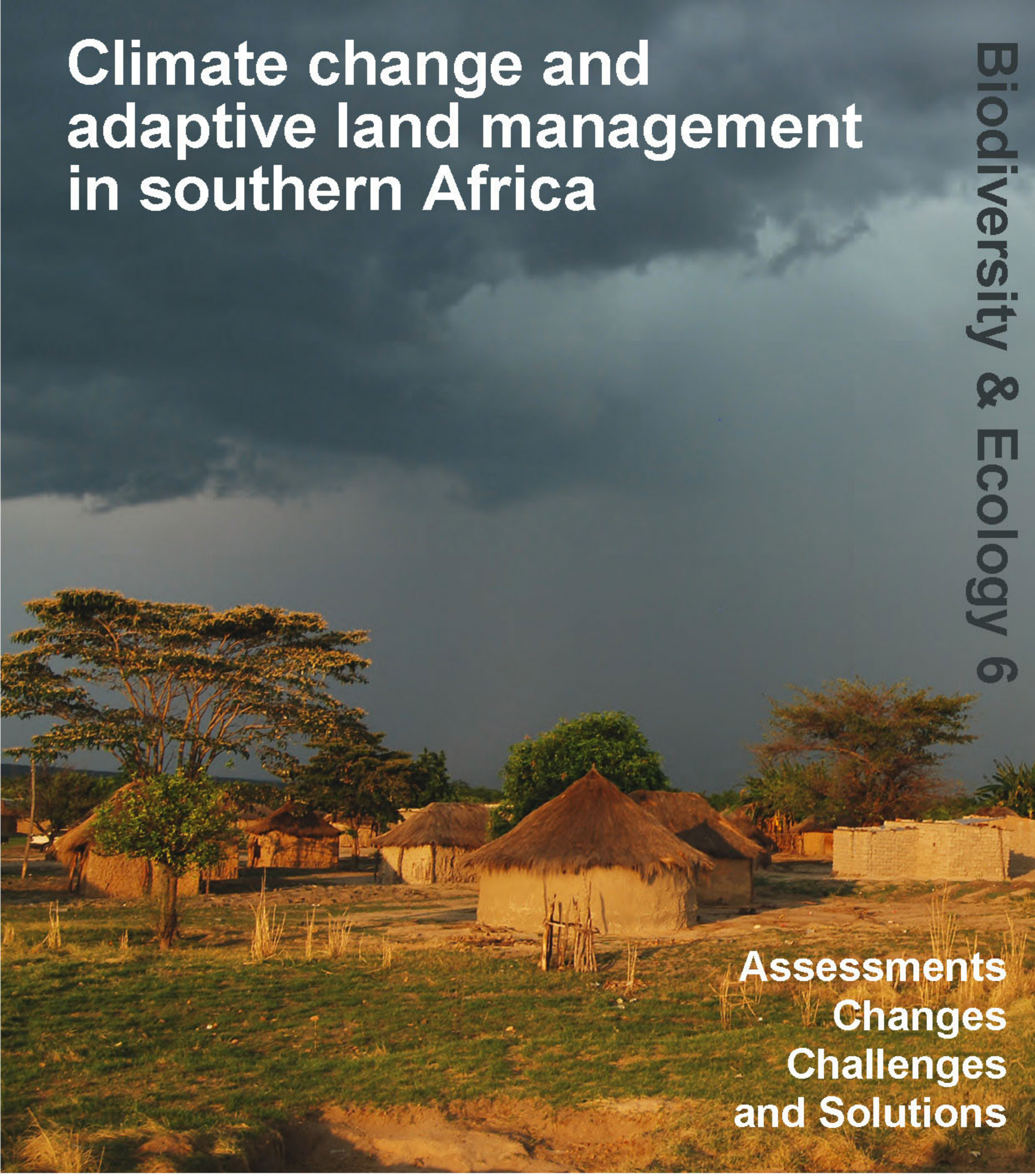
Climate change and adaptive land management in southern Africa

Biodiversity & Ecology 6



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Changes
Challenges
and Solutions

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Climate change and adaptive land management in southern Africa

Assessments, changes, challenges, and solutions

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Food security

Diversity of wild herbaceous legumes in Southern Africa, their associated root nodule bacteria, and insect pests

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Abstract: Climate change models predict that most parts of southern Africa including Botswana and Namibia will experience severe water stress and temperature increases as a result of climate change. Wild drought-tolerant nitrogen-fixing plants with heat-tolerant bacterial symbionts might be a source for mitigation, nutrient-rich grazing grounds, and soil fertility. Herbaceous legumes may be developed into forage plants that are resilient to climate change effects. Therefore, the purpose of the study was to assess the diversity of wild herbaceous legumes in the north-western and eastern parts of Botswana, northern parts of Namibia, and Northern Cape of South Africa. They were assessed for nodulation and insect damage, root nodule bacteria were isolated, and some were identified and authenticated on their homologous hosts. In Namibia, rhizosphere bacteria were isolated and characterised. For the first time, it was shown that a wide range of wild legumes in the study area were nodulated. Common plant species included, amongst others, *Chamaecrista bieinsis* (Stey.) Lock, *Chamaecrista absus* (L.) Irwin and Barneby, *Zornia glochidata* DC, and several *Crotalaria* and *Indigofera* species. The jewel beetle *Sphenoptera* sp. damaged over 90% of the *Indigofera* sp. in Lecheng. The bacteria isolated were typical plant growth–promoting bacteria mostly belonging to the *Bacillus* and *Brevibacillus* genera, with fewer rhizobial species. Such bacteria may be valuable inoculants for pulses and cereals, respectively. Taken together, the results of this study highlight the potential for herbaceous legumes in mitigating climate change effects through the use of inoculants as biofertiliser and through use in intercropping that modulates pest infestation, leading to low usage of chemical pesticides.

Resumo: Os modelos de alterações climáticas prevêem que a maior parte do Sul de África, incluindo o Botswana e a Namíbia, irá sofrer stress hídrico e um aumento da temperatura severos. Plantas silvestres fixadoras de azoto e tolerantes à seca, com bactérias simbiontes tolerantes ao calor, poderão ser uma fonte de mitigação para campos de pastagem ricos em nutrientes e fertilidade do solo. Leguminosas herbáceas poderão ser desenvolvidas em plantas forrageiras que são resilientes aos efeitos das alterações climáticas. Assim, o propósito do estudo foi avaliar a diversidade de leguminosas herbáceas silvestres no Noroeste e Nordeste do Botswana, Norte da Namíbia e Cabo Norte da África do Sul. Foram avaliadas quanto à nodulação, danos por insectos e bactérias dos nódulos radiculares foram isoladas, algumas identificadas e autenticadas nos seus hospedeiros homólogos. Na Namíbia, bactérias da rizosfera foram isoladas e caracterizadas. Pela primeira vez, foi demonstrado que uma grande variedade de legumes silvestres estava nodulada na área de estudo. As espécies de plantas comuns incluiram, entre outras, *Chamaecrista bieinsis* (Stey.) Lock, *Chamaecrista absus* (L.) Irwin e Barneby, *Zornia glochidata* DC., bem como diversas espécies de *Crotalaria e Indigofera*. O besouro *Sphenoptera* sp. danificou mais de 90% de *Indigofera* sp. em Lecheng. As bactérias isoladas eram bactérias típicas promotoras de crescimento vegetal, maioritariamente pertencentes aos géneros *Bacillus* e *Brevibacillus*, com menos espécies de rizóbios. Tais bactérias podem ser inoculantes valiosos para legumes e cereais, respectivamente. No geral, os resultados deste estudo destacam o potencial das leguminosas herbáceas na mitigação dos efeitos das alterações climáticas através do uso de inoculantes como biofertilizantes e de culturas alternadas, as quais modulam a infestação por pragas, levando a um menor uso de pesticidas químicos.

Introduction

Nitrogen is unique among the other essential plant nutritional elements because N_2 from the atmosphere can be fixed via biological nitrogen fixation (BNF), carried out exclusively by prokaryotes that possess the enzyme nitrogenase. Nitrogen input by symbiotic plant-microbe interactions not only improves soil fertility (Grönemeyer et al., 2014) but also enhances the productivity of the ecosystem and provides protein-rich plant biomass for grazing. However, there is very little published knowledge on rhizobial symbionts of crops from Namibia, Angola, and Botswana (Pule-Meulenberg & Dakora, 2007). In addition to nodulated legumes and N_2-fixing legumes, in this study it is appreciated that there are many microorganisms that reside in the root nodules of legumes and generally in the rhizosphere of all plants. The role of such organisms is that of plant growth promotion through various mechanisms.

There are many wild legume species that are important to local communities. Examples include use as grazing resources for livestock (Nezomba et al., 2008; Madibela et al., 2018), building material, food, medicinal resources (Pule-Meulenberg & Dakora, 2007), and green manure (Nezomba et al., 2008) for rural and urban communities. Nevertheless, the economic importance of many plant species is still largely unknown. In natural ecosystems where wild legumes grow, in addition to cycling N, they provide food to insect herbivores. Naturally, plants interact with an array of arthropod species, microbes, and other plants both below and above ground. Although some of these interactions may be detrimental, such as instances where insects feed on translocated nutrients from the plant, there are cases where mutualism occurs. Detrimental effects of plant-insect interaction may be major constraints if wild legumes are to be utilized as sources of nutrients and livestock feed in future.

These wild legumes are well adapted to the harsh environmental conditions such as high temperature, low soil moisture, winter drought, high incidence of pests, soil acidity, and soil salinity that are prevalent in Botswana, Namibia, and other countries in southern Africa. This may also extend to their symbionts, as they have to survive in the soil under these conditions. For example, in Namibia, where high soil temperatures prevail, novel *Bradyrhizobium* isolates were found to be also well adapted, showing extraordinarily high temperature tolerance (Grönemeyer et al., 2014). Projections of consequences of climate change at the local scale indicated that the Kavango basin will become warmer (1.5–2.5°C) and receive less mean annual precipitation (50–100 mm) until 2045 (Pröpper et al., 2015). Hotter and drier climates, loss and degradation of soil, crop failure, and increase in population are the main issues that have to be dealt with.

In addition to harbouring N_2-fixing bacteria, both wild and domesticated legume species are known to harbour bacterial strains with plant growth–promoting traits, phosphate solubilisation, siderophore production, phytohormone production, or triggering of plant defence responses by various mechanisms (Grönemeyer et al., 2012; Burbano et al., 2015; Chimwamurombe et al., 2016).

Generally in Africa, farmers continue to obtain low yields because of the non-replacement of nutrients year after year, leading to declining soil fertility in smallholder farms under dryland conditions. Unfortunately, the majority of African small farmers are not able to afford the high mineral fertilizer prices (Yanggen et al., 1998). Low-cost and sustainable technical solutions compatible with the socioeconomic conditions of small farmers are needed to solve soil fertility and yield problems (Chianu et al., 2011). Also in the northern regions of Namibia and in the North-West District of Botswana, rainfed agriculture is practised by smallholder farms with very low crop yields and also poorly developed value chains and food processing. The resource-poor farmers do not use chemical fertilizers, manure, or other inputs but are prone to slash-and-burn agriculture. Previous soil analyses have shown that the key mineral nutrients N and P are very low in these sandy soils (Gröngröft et al., 2013), and dryland farming lowers carbon pools and nutrient levels (Wisch et al. 2008; Luther-Mosebach et al., 2015). Arbuscular mycorrhiza might be useful for P-acquisition, but inoculant production from the nonculturable fungi is challenging. The use of herbaceous legumes as green manure and as a source of elite biofertiliser strains for plant growth promotion can be regarded as sustainable agriculture. The use of inorganic fertilisers has been associated with greenhouse gas emission (Linquist et al., 2012) and pollution of underground water resources, particularly nitrate pollution (Gao et al., 2012). Polycultures using legumes are known to reduce pest population pressure on crops, thereby reducing the use of chemical pesticides (Amoako-Atta et al., 1983). Furthermore, over-reliance on pesticides increases environmental pollution, which contributes to environmental change. Hence, the use of herbaceous legumes and their associated microorganisms can be used as a strategy to improve soil fertility and reduce pests sustainably with minimal environmental pollution. Therefore, the purpose of the study was to assess the diversity of wild herbaceous legumes in some regions of Botswana, South Africa, and Namibia and their associated root bacteria and insect pests. The specific objectives were to (1) identify wild legume species in the target region, (2) assess whether they formed effective N_2 fixation symbioses in nature and isolate and characterise their bacteria, (3) identify pests of legumes and assess the damage they caused, and (4) assess rhizosphere microbiomes along the Kavango River in Namibia.

Methods

Description of study sites

Field surveys were conducted around the Okavango Delta in Botswana, in several regions of Nambia, and in the Northern Cape region of South Africa. Sampling in farmers' fields and in nature was carried out when both herbaceous wild and cultivated legumes were at the peak of flowering. In the Okavango panhandle, the survey was conducted at Seronga (18°50′11″S; 22°18′06″ E), Ngarange

(18°24′31″ S; 22°01′25″ E), and Xakao (18°18′17″ S; 21°53′16″ E). In Namibia, plant surveys were carried out in Kavango Province near Rundu and Mashare, in the Kunene region near the Grootberg Pass, and in the Omaheke region near Gobabis. All of the sites in the three countries where legume surveys were undertaken are covered by Kalahari aeolian sand deposits, and hence the soils are very sandy with very low fertility, low soil moisture, and high soil temperatures (Schulze & Kruger, 2007; Grönemeyer et al., 2014; Bernard et al., 2017).

Plant sampling

At each of the sites in Botswana, grain legumes were sampled from farmers' fields by digging them up with intact roots and nodules. In the wild, herbaceous legumes were sampled from within a radius of 50 m at each of the sites (Botswana), at smallholders' fields near Mashare (Namibia), or at natural sites free of cropping (Namibia, South Africa). Plants from Botswana were identified by the BUAN Herbarium, from Namibia by NBRI (Leevon Nanyeni) and from South Africa by Ute Schmiedel.

Insect sampling and damage assessment

Insects associated with herbaceous legumes were sampled and the incidence of damage on the plants assessed in Botswana. At each location, 20 herbaceous leguminous plants per species were randomly sampled in an area covering 40 m^2 at each of the above-mentioned villages. The insect infestation was assessed above and below the ground. The assessment of insect damage on foliage was done using in situ counts. To assess the infestation by soil-dwelling insects, plants were dug up with intact roots and inspected for the presence of insects and damage. Tiny insects were observed under the microscope and larger ones with the naked eye. The assessment was based on the number of insects per plant, proportion of plants damaged, and severity of damage based on symptoms. The insects collected were identified to taxonomic class, order, family, and species where possible. In this paper, we report only on the jewel beetle (*Sphenoptera* sp.) because it was the most widely distributed.

Table 1: Nodulated herbaceous legumes detected in Botswana

Okavango	**Tswapong**	**Kgalagadi**
Indigofera flavicans Baker	*Zornia glochidiata* DC.	*Indigofera* spp.
Indigofera tinctoria L.	*Chamaecrista bieinsis* (Stey.) Lock	*Cyamopsis dantata* (N.E. Br.) Torre
Chamaecrista biensis (Stey.) Lock		*Cullen tometosum* (Thunb.) J.W. Grimes
Indigofera astragalina DC.	*Vigna unguiculata* subsp. *dekindtiana* (Harms) Verdc.	*Arachis hypogea* L.
Indigofera daeloides Harv.	*Chamaecrista absus* (L.) Irwin and Barneby	*Vigna unguiculata* (L.) Walp
Tephrosia purpurea Pers.	*Tephrosia purpurea* Pers.	*Vigna subterranean* (L.) Verdc.
Tephrosia lupinifolia DC.	*Rhyncosia totta* DC.	
Chamaecrista absus (L.) Irwin and Barneby	*Arachis hypogea*	
Crotalaria astragalina Hochst.	*Vigna unguiculata* (L.) Walp	
Crotalaria sphaerocarpa DC.	*Vigna subterranean* (L.) Verdc.	
Crotalaria pisicarpa Baker		
Vigna unguiculata subsp. *dekindtiana* (Harms) Verdc.		
Rhyncosia totta DC.		
Zornia glochidiata DC.		
Arachis hypogea L.		
Vigna unguiculata (L.) Walp		
Vigna subterranean (L.) Verdc.		

Table 2: Nodulated wild legumes detected in South Africa and Namibia

South Africa, Northern Cape	**Namibia, Kavango**	**Namibia, Omaheke**
Leobordea digitata (Harv.) B.-E. van Wyk & Boatwr.	*Chamaecrista absus* (L.) Irwin and Barneby	*Indigofera alternans* DC.
Leobordea polycephala (E. Mey.) B.-E. van Wyk & Boatwr.	*Chamaecrista biensis* (Stey.) Lock	*Indigofera flavicans* Baker
	Chamaecrista sp.	
	Crotolaria flavicarinata Baker f.	
Lotononis falcata (E. Mey.) Benth.	*Crotolaria heidmanii* Schinz	*Tephrosia burchellii* Burtt Davy
Lotononis leptoloba Bolus	*Crotalaria podocarpa* DC.	*Tephrosia dregeana* E. Mey.
Medicago laciniata (L.) Mill.	*Crotalaria platysepala* Harv.	
Medicago polymorpha L.	*Crotalaria sphaerocarpa* DC.	
Wiborgia monoptera E. Mey.	*Indigastrum parviflorum* Jaub. & Spach	**Namibia, Kunene**
Lessertia diffusa R. Br.	*Indigofera astragalina* DC.	*Crotalaria podocarpa* DC.
	Indigofera charlieriana Schinz	*Indigofera auricoma* E. Mey.
	Indigofera rautanenii Baker f.	
Lessertia capitata E. Mey.	*Rhynchosia venulosa* (Hiern) K. Schum.	
	Tephrosia burchelii Burtt Davy	
	Tephrosia dregeana E. Mey.	
	Zornia glochidata DC.	

Isolation and characterisation of root nodule and rhizosphere bacteria

Root nodules were transported to the laboratory under dried conditions with silica gel dessicant (Grönemeyer et al., 2013), and bacteria were isolated and characterised from root nodules using standard procedures (Vincent, 1970;Grönemeyer et al., 2013). Briefly, the bacterial isolation procedure was as follows: After the soil had been removed from the nodules, they were immersed for 10 s in 70% ethanol, followed by immersion in 3% sodium hypochlorite for 2–3 min depending on the size of the nodule, and then rinsed 10 times in sterile distilled water. Each nodule was dissected with a blade and squashed with forceps. The milky liquid from the nodules was plated on yeast mannitol agar and incubated at 28°C for about 15 days. Plates were checked daily and new growth reported. After pure colonies had been isolated, bacteria were plated on different media to assess their phosphate solubilisation, siderophore production, and cellulase activity. Bacteria were also isolated from the rhizosphere of indigenous legumes using standard procedures in Namibia (Kandjimi et al., 2015).

Authentication of bacterial isolates

To satisfy Koch's postulates, bacteria were authenticated on their homologous hosts under sterile conditions in the greenhouse. Eleven isolates were tested for their ability to form root nodules on *Crotalaria sphaerocarpa*. Seeds were sterilised by immersion for 3 min in household bleach solution (3% NaOCl), followed by 12 rinses in sterile distilled water and soaking in the last rinse for 2 h. The sterilised seeds were then placed on 1% water agar and incubated at 28ºC for 2 d to produce seedlings. The seedlings of *C. sphaerocarpa* were raised in sterile autoclaved sand pots under greenhouse conditions. Two seedlings were planted in the pot and inoculated with 1 mL of bacterial cells suspended in sterile distilled water. Three pots per strain were inoculated. Plants were fed with N-free Hoagland solution twice a week and were grown for up to 5 weeks. For each strain, a negative control without fertiliser and inoculation and a positive control with 5 mM of potassium nitrate were included. At harvest, each plant was separated into shoots, roots, and nodules. The shoots and roots were dried at 60ºC for 48 h and weighed on a balance to determine their dry matter.

Table 3: Bacteria species isolated from grain and indigenous legume species from Okavango Delta and Tswapong region in Botswana

Bacteria with Highest Similarity from NCBI BLAST	Host Legume Plant	Place of Origin
Bacillus acidiceler	*Vigna unguiculata*	Mmoo Kokonye
Rhizobium tropici	*Vigna unguiculata* subsp. *dekindtiana*	Xakao
Brevibacilllus brevis	*Crotalaria sphaerocarpa*	Ngarange
Bacillus acidiceler	*Vigna unguiculata* subsp. *dekindtiana*	Qabo
Rhizobium sp.	*Arachis hypogaea*	Lekobeng
Pseudomonas aeruginosa	*Arachis hypogaea*	Mmoo Kokonye)
Brevibacillus agri strain	*Vigna unguiculata*	Mmoo Kokonye
Brevibacillus brevis	*Vigna subterranea*	Lekadiba

DNA sequencing of root nodule bacteria

The 16S rDNA gene fragment was amplified using universal bacterial 16S primers 27f (5'-AGAGTTGATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTTACGACTT-3') (Lane, 1991). The names of the primers denote the positions of their recognition sites on the 16S rDNA gene. The 16S rDNA amplicons were sent to Inqaba Laboratories (Pretoria, South Africa) for sequencing.

Results

Diversity of herbaceous legumes

Table 1 presents the results of the legume survey that was undertaken in the Okavango Delta and the Tswapong region. In both cases the survey was conducted at four locations. Table 1 shows that more herbaceous legume plant species were found in the higher-rainfall Okavango Delta compared to Tswapong area. In Namibia and South Africa, a broad range of wild legumes were found, 33 plant types were nodulated at their natural sites, and most of them could be taxonomically identified (Tab. 2). This is the first report of nodulation of these wild legumes in these areas of Botswana, Namibia, and South Africa. Several species were commonly found in the different surveys (Tab. 1 and 2) in the Kavango region of Namibia and Botswana, such as *Chamaecrista bieinsis* (Stey.) Lock, *Chamaecrista absus* (L.) Irwin and Barneby, *Zornia glochidata* DC., and several *Crotalaria* and *Indigofera* species. Root nodule bacteria were isolated and characterized for some plants (Tab. 3).

Authentication of root nodule bacteria

Table 3 shows the authentication of root nodule bacterial isolates on *Crotalaria sphaerocarpa* (DC.) Benth. under sterile greenhouse conditions. The various isolates come from root nodules of *C. sphaerocarpa* from Lekadiba (LKD) in Tswapong, Ngarange (NGA), Qabo (QAB), Grootlagte (GRT), and Xauga in the Okavango Delta. Eight out of eleven isolates were authenticated as rhizobia since they formed N_2-fixing root nodules on their homologous host. Some rhizobia such as strain BUAN316/XAU-Sc70B induced biomass production similar to 5 mM of potassium nitrate. Interestingly, some non-N_2-fixing strains — for example, BUAN316/NGA-Cs7C — were able to induce plant growth better than some N_2-fixing strains such as BUAN316/LKD-Cs4.

Diversity of root nodule bacteria isolated from herbaceous legumes

The majority of the bacteria isolated from the root nodules of herbaceous legumes sampled from various regions in Botswana showed that few were rhizobia (Tab. 4). Their 16S rDNA identified them as belonging to the genera *Bacillus* and *Brevibacillus*. *Rhizobium tropici* was isolated from *Vigna unguiculata* subsp. *dekindtiana*.

Table 4: Bacteria species isolated from identified indigenous legume species along Kavango River, Namibia

Isolate number	Bacteria with Highest Similarity from NCBI BLAST	Host Legume Plant	Place of Origin
3	*Shigella flexneri*	*B. petersiana*	Mabushe
4	*Acinetobacter pitti*	*Acacia hebeclada*	Nkurenkuru
5	*Bacillus aryabhattai*	*B. petersiana*	Nkurenkuru
8	*Ochromobacterium intermedium*	*B. petersiana*	Mabushe
9	*Azospirillum oryzae*	*B. petersiana*	Mabushe
11	*Klebsiella oxytoca*	*A. hebeclada*	Nkurenkuru
12	*Ochromobacterium intermedium*	*A. hebeclada*	Mashare
13	*Acinetobacter pitti*	*A. hebeclada*	Mashare
14	*Lysinibacillus pakistanensis*	*A. erioloba*	Katwitwi
15	*Trabulsiella guamensis*	*A. erioloba*	Katwitwi
21	*Brevibacillus reuszeri*	*A. hebeclada*	Mashare
25	*Pseudomonas xanthomarina*	*A. erioloba*	Katwitwi
30	*Enterobacter cloacae* subsp. *dissolvens*	*Brachystegia boehmii*	Katwitwi
40	*Arthrobacter oxydans*	*B. petersiana*	Mabushe
52	*Bacillus vallismortis*	*B. petersiana*	Mabushe
55	*Streptomyces* sp.	*B. petersiana*	Mashare
64	*Brevibacillus formosus*	*A. erioloba*	Rundu
66	*Bacillus aryabhattai*	*A. erioloba*	Mabushe

Similarly, various bacteria from different genera such as *Bacillus*, *Ochromobacterium*, *Pseudomonas*, and *Brevibacillus* were isolated from the rhizosphere of leguminous shrubs and trees in Namibia (Tab. 4).

Insect incidence and damage

The flat-headed borer, *Sphenoptera* sp. [Buprestidae: Chrysochroinae] was widely distributed across the study areas, having been collected from the Tswapong, Kgalagadi South, Okavango, and Gantsi regions of Botswana. *Sphenoptera* sp. beetles were collected from the roots of *Crotalaria sphaerocarpa, C. diateri, T. pumila, T. dregeana, T. lupinifolia*, and *Indigofera* sp. It was recorded on *C. absus* and *Senna obtusifolia* during this study. The highest percentage of plants damaged was recorded at Lecheng on *Indigofera* sp. The infestation also differed between plant species. The highest infestation was on *C. sphaerocarpa,* followed by *Tephrosia* sp. and *Indigofera* spp. The larval stage of the *Sphenoptera* jewel beetle bored into stems and tunneled into root systems (Fig. 1).

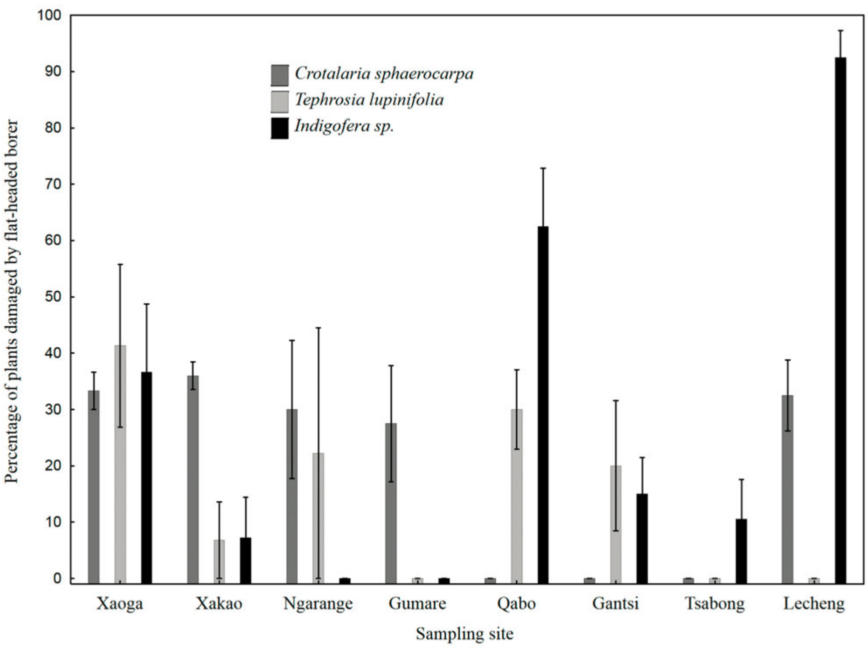

Figure 1: Percentage of plants damaged by flat-headed borers at the different study sites.

Discussion

The predominant agriculture paradigm based on improved varieties of common staple crops in high-input systems has not succeeded in addressing food insecurity and malnutrition in places such as sub-Saharan Africa (Rudebjer et al., 2013), where over 200 million people (28% of the population) were undernourished in 2005–2007 (FAO, 2010). Yield variability and risk for crop failure in Africa's rainfed agriculture systems contribute to factors that explain difficulties in adopting new technologies (Ogada et al., 2010). This will even become more problematic as climate change leads to a decline in land area suitable for the cultivation of crops under dryland conditions in Sub-Saharan Africa (Lane & Jarvis, 2007). Population pressure and traditional food production through dryland agriculture without nutrient inputs are leading to a reduction of soil carbon pools and available nutrients (Luther-Mosebach et al., 2015) as well as causing soil degradation and increased fire frequency (Carreira et al., 1996).

Diversity of wild herbaceous legumes and N_2 fixation

In this study, we have shown that herbaceous legumes were nodulated (Tab. 1 and 2). Bernard et al. (2017) have shown that in Botswana, the legumes in Table 1 fixed N_2 in their locales. Furthermore, results from the current study indicated that the diversity of legumes decreased with increasing aridity in Botswana. For example, whereas 15 herbaceous species were recorded in the wetter Okavango Delta (rainfall average of over 500 mm), only six were found in the drier Kgalagadi region around Tsabong (rainfall average of about 300 mm). With the increasing effects of climate change, under which southern Africa is predicted to become drier (Serdeczny et al., 2017), the diminishing number of nodulated legumes with aridity has a bearing on nutrient cycling in ecosystems. For example, *Chamaecrista rotundifolia* fixed 144 kg N/ha in Nigeria (Sanginga et al., 2003), *Crotalaria pallida* fixed 173 kg N/ha in Domboshawa in Zimbabwe, both seeded at 120 seeds/m^2 (Nezomba et al., 2008) and fixed 140 kg N/ha in Chinyika in Zimbabwe

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(Tauro et al., 2009), and *Sesbania sesban* could fix between 43 and 102 kg N/ha in Senegal (Ndoye & Dreyfus, 1988). In Botswana, studies on the amount of N_2 fixed per hectare of herbaceous legumes are scarce. In the study by Bernard et al. (2017), an estimation of N-fixed per plant was made using the natural abundance technique. For example, *Chamaecrista absus* fixed 134.8 mg N/plant while *Tephrosia* sp could fix 143.4 mg N/plant. N_2 fixation is an important process for the sustainable maintenance of soil fertility. The legumes in this study have the potential to be green manures and fodder crop species (see Madibela et al., 2018) because of their N_2 fixing ability. With less rainfall and higher temperatures as predicted by climate change models (Pröpper et al., 2015), it is important to select such crops and plants that can withstand the harsh environmental conditions.

Characteristics and diversity of bacteria isolated from root nodules and the rhizosphere of legumes

During the plant surveys, root nodules were collected and bacteria were isolated from them. In Botswana, the reason for isolating bacteria was to prospect for elite rhizobial strains with the potential for development into inoculants. It is noteworthy that most of the bacteria isolated from the root nodules of herbaceous legumes harvested from the wild were not rhizobia, but instead mostly species of *Bacillus* and *Brevibacillus*, known plant growth–promoting bacteria (Tab. 3). Interestingly, when examined, the nodules were pinkish red in their interior, showing the presence of leghaemoglobin. It is possible that the rhizobia enter a viable but not culturable (VBNC) state. Bacterial cells enter the VBNC state as a response to some form of natural stress, such as starvation, incubation outside the temperature range of growth, elevated osmotic concentrations (e.g., seawater), oxygen concentration, or exposure to white light (Oliver, 2000). It is not clear whether most rhizobia enter into VBNC during stress periods since Oliver (2005) listed only *Rhizobium leguminosarum* and *Sinorhizobium meliloti* as being capable of entering the VBNC state. What is clear is that in addition to rhizobia, root nodules harbour other bacteria that promote plant growth in different ways. For example, *Brevibacillus brevis* has been shown to promote growth in cotton through positive traits such as indole-3-acetic acid (IAA), acetylene reduction assay (ARA), antifungal activity, and ammonia production (Nehra et al., 2016). In Table 5, some non-rhizobial bacterial strains have been shown to promote plant growth. For example, BUAN316/LKD-Cs4, an N_2-fixing strain, induced significantly less biomass compared to BUAN316/NGA-Cs7C, a non-rhizobial strain. What needs to be ascertained is the mechanism through which plant growth is promoted. In Namibia, a number of bacteria were isolated from the roots of some nodulated and non-nodulated leguminous plants (Tab. 4). Some of the listed bacteria such as species of *Brevibacillus, Bacillus*, and *Arthrobacter* have been shown to have various traits of plant growth promotion (de Souza et al., 2015).

The potential of biological nitrogen fixation (BNF) that can be realized in nitrogen-fixing symbioses between legumes and rhizobia can be applied for a more sustainable agricultural practice. Current research and extension efforts need to be directed towards an integrated nutrient management approach in which legumes play a crucial role. Inoculation with compatible rhizobia resistant to harsh environmental conditions can make BNF a key resource for farmers with little income (Smaling et al., 2008). Legumes or pulses can provide protein- and nutrient-rich food and feed, providing marketable products and a long-lasting value chain. Furthermore, plant residues of nitrogen-fixing legumes, if added to soil, enhance nitrogen status and organic matter content in intercropping and crop rotation schemes.

Insect incidence and damage

In addition to the soil fertility issue, pests such as stemborers are a real challenge. The prevalence of and damage by the widely distributed flat-headed borer (*Sphenoptera* spp.) on the roots of wild herbaceous legumes negatively affect these plants' biological nitrogen-fixing efficiency and reduce their biomass

Table 5: Nodulation and dry matter of *Crotalaria sphaerocarpa* during authentication of bacterial strains

Strain	Nod	N_2 Fix	Shoot DM ($g.plant^{-1}$)	Root DM ($g.plant^{-1}$)	Nodules $plant^{-1}$	Nodule weight ($mg.plant^{-1}$)
BUAN316/LKD-Cs4	+	+	0.10 ± 0.02bc	0.01 ± 0.00cd	4.00 ± 0.88cd	13.30 ± 3.34b
BUAN316/LKD-Cs6	+	+	0.33 ± 0.08abcd	0.01 ± 0.00cd	5.00 ± 0.67cd	16.70 ± 5.78b
BUAN316/NGA-Cs7C	-	+	0.18 ± 0.05bdc	0.01 ± 0.00cd	0.00 ± 0.00e	0.00 ± 0.00c
BUAN316/QAB-Cs36	+	-	0.02 ± 0.01d	0.01 ± 0.00cd	2.00 ± 0.33cd	5.00 ± 0.00b
BUAN316/QAB-Cs36*	-	-	0.19 ± 0.04bcd	0.04 ± 0.00ab	0.00 ± 0.00e	0.00 ± 0.00c
BUAN316/NGA-Cs38	+	+	0.37 ± 0.11abc	0.03 ± 0.01abcd	13.00 ± 3.67b	46.70 ± 12.03b
BUAN316/GRT-Cs52	+	+	0.36 ± 0.07abc	0.03 ± 0.01abcd	5.00 ± 0.33cd	30.00 ± 5.78b
BUAN316/GRT-Cs52*	-	-	0.15 ± 0.02bcd	0.01 ± 0.00cd	0.00 ± 0.00e	0.00 ± 0.00c
BUAN316/XAU-Cs68B	+	+	0.30 ± 0.03abcd	0.05 ± 0.01a	26.00 ± 2.91a	650.00 ± 276.08a
BUAN316/XAU-Cs70A	+	+	0.26 ± 0.07bcd	0.02 ± 0.00bcd	6.00 ± 1.15c	43.30 ± 8.83b
BUAN316/XAU-Cs70B	+	+	0.46 ± 0.09ab	0.04 ± 0.01ab	8.00 ± 1.73c	50.00 ± 5.78b
BUAN316-CsC	+	-	0.63 ± 0.12a	0.03 ± 0.00abc	0.00 ± 0.00e	0.00 ± 0.00c
BUAN316-CsC	-	-	0.11 ± 0.02cd	0.02 ± 00bcd	0.00 ± 0.00e	0.00 ± 0.00c
F statistics			**6.44*** **	**2.22***	**24.05*** **	**4.72** **

production. Direct feeding on root nodules by *Sphenoptera* sp. was not established, and currently no literature attests to that. However, feeding on root systems may affect the physiology of the plants and consequently interfere with a legume's ability to fix nitrogen, as in case of nodule-feeding weevils, *Stona* spp. (Quinn & Hower, 1986). Feeding on the roots may reduce translocation of water and nutrients up the plants, consequently reducing the biomass needed for livestock feed.

Here we identified a broad range of wild legumes which were reported for the first time to be nodulated in Botswana, Namibia, and South Africa. In Botswana, data on the diversity of wild herbaceous legumes suggested that diversity decreased with increasing aridity. Our data have also shown that the majority of the inhabitants of the root nodules were not rhizobia, leading to the suggestion that they were in a state of VBNC. Furthermore, known plant growth–promoting bacteria such as species of *Bacillus* and *Brevibacillus* were common in the root nodules of herbaceous legumes. Finally, the flat-headed jewel beetle (*Sphenoptera* sp.) caused a lot of damage on herbaceous legumes, suggesting that if some of the plants were to be developed into fodder crops in the future, pest control would be needed.

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References

Amoako-Atta, B., Emolo, E.O. & Kidega, E.K. (1983) Influence of maize, cowpea and sorghum intercropping systems on stem-/pod-borer infestations. *International Journal of Tropical Insect Science*, **4**, 39–46.

Bernard, N., Losologolo, M., Batlang, U., Ngwako, S., Mashungwa, G.N., Tselaesele, N.M. & Pule-Meulenberg, F. (2017) Symbiotic performance of grain and wild herbaceous legumes in the Okavango Delta and Tswapong region of Botswana. *Symbiosis*, 1–10.

Burbano, C.S., Grönemeyer, J.L., Hurek T. & Reinhold-Hurek, B. (2015) Microbial community structure and functional diversity of nitrogen-fixing bacteria associated with *Colophospermum mopane*. *FEMS Microbiology Ecology*, **91**, fiv030. doi:10.1093/femsec/fiv1030

Carreira, J.A., Arevalo, J.R. & Niell, F.X. (1996) Soil degradation and nutrient availability in fire-prone Mediterranean shrublands of southeastern Spain. *Arid Land Research and Management*, **10**, 53–64.

Chianu, J.N., Nkonya, E.M., Mairura, F.S., Chianu, J.N. & Akinnifesi, F.K. (2011) Biological nitrogen fixation and socioeconomic factors for legume production in Sub-Saharan Africa. *Sustainable agriculture 2* (ed. by E. Lichtfouse, M. Hamelin, M. Navarrete and P. Debaeke), pp. 309–329. Springer, Dordrecht.

Chimwamurombe, P.M., Grönemeyer, J.L. & Reinhold-Hurek, B. (2016) Isolation and characterization of culturable seed-associated bacterial endophytes from gnotobiotically grown Marama bean seedlings. *FEMS Microbiology Ecology*, **92(6)**, fiw083. http://dx.doi.org/10.1093/femsec/fiw1083

FAO (2010) *The state of food insecurity in the world. Addressing food insecurity in protracted crises.* Food and Agriculture Organization of the United Nations, Rome, Italy.

Gao, Y., Yu, G., Luo, C. & Zhou, P. (2012) Groundwater nitrogen pollution and assessment of its health risks: a case study of a typical village in rural-urban continuum, China. *PLOS One*, **7(4)**, e33982. doi:10.1371/journal.pone.0033982

Grönemeyer, J., Berkelmann, D., Mubyana-John, T., Haiyambo, D., Chimwamurombe, P., Kasaona, B., Hurek, T. & Reinhold-Hurek, B. (2013) A survey for plant-growth-promoting rhizobacteria and symbionts associated with crop plants in the Okavango region or Southern Africa. *Biodiversity & Ecology*, 5, 287–294.

Grönemeyer, J.L., Burbano, C.S., Hurek, T. & Reinhold-Hurek, B. (2012) Isolation and characterization of root-associated bacteria from agricultural crops in the Kavango region of Namibia. *Plant Soil*, **356**, 67–82.

Grönemeyer, J.L., Kulkarni, A. Berkelmann, D., Hurek, T. & Reinhold-Hurek, B. (2014) Identification and characterization of rhizobia indigenous to the Okavango region in Sub-Saharan Africa. *Applied and Environmental Microbiology*, **80**, 7244–7257.

Gröngröft, A., Luther-Mosebach, J., Landschreiber, L. & Eschenbach, A. (2013) Cusseque Soils. *Biodiversity & Ecology*, **5**, 51–54.

Kandjimi, O.S., Uzabakiriho, J.D. & Chimwamurombe, P.M. (2015) Isolation and characterization of culturable bacteria from bulk soil samples and the rhizosphere of arid-adapted *Tylosema esculentum* (Burchell) A. Schreiber (Marama bean) in Namibia. *African Journal of Biotechnology*, **14**, 944–952.

Lane, A. & Jarvis, A. (2007) Changes in climate will modify the geography of crop suitability: agricultural biodiversity can help with adaptation. *SAT eJournal ICRISAT*, **4**, 1–12.

Lane, D. J., 1991. 16S/23S sequencing. In Stackebrandt, E., and Goodfellow, M. (Eds.). Nucleic acid techniques in bacterial systematics. John Wiley & Sons, Inc. New York, pp. 115–175.

Linquist, B.A., Adviento-Borbe, M.A., Pittelkow, M., van Kessel, C. & van Groenigen, K.J. (2012) Fertilizer management practices and greenhouse gas emissions from rice systems: a quantitative review and analysis. *Field Crops Research*, **135**, 10–21.

Luther-Mosebach, J., Gröngröft, A. & Eschenbach A**.** (2015) Influence of landscape and land-use on the soil sarbon stocks in the Okavango Catchment. *Böden im Wandel* (ed. by C. Knoblauch, C. Fiencke and K. Berger), pp. 155–166. Verein zur Förderung der Bodenkunde Hamburg 77. University of Hamburg, Hamburg, Germany.

Madibela, O.R., Kemiso, D. & Kwedibana, J. (2018) Quality of wild herbaceous legumes and its role in livestock nutrition. This volume.

Ndoye, I., Dreyfus, B., 1988. N_2 fixation by *Sesbania rostrata* and *Sesbania sesban* using ^{15}N and total N difference methods. Soil Biology & Biochemistry, **20**, 209-213.

Nehra, V., Saharan, B.S. & Choudhary, M. (2016) Evaluation of *Brevibacillus brevis*. *SpringerPlus*, **5**, 1–10.

Nezomba, H., Tauro, T.P., Mtambanengwe, F. & Mapfumo, P. (2008) Nitrogen fixation and biomass productivity of indigenous legumes for fertility restoration of abandoned soils in smallholder farming systems. *South African Journal of Plant and Soil*, **25**, 161–171.

Ogada, M.J., Nyangena, W. & Yesuf, M. (2010) Production risk and farm technology adoption in the rainfed semi-arid lands of Kenya. *African Journal for Agricultural and Resource Economics*, **4**, 159–174.

Oliver, J. D. (2000). Problems in detecting dormant (VBNC) cells, and the role of DNA elements in this response. Tracking genetically-engineered microorganisms. Landes Biosciences, Georgetown, Tex, pp 1-15.

Oliver, J. D. (2005). The viable but nonculturable state in bacteria. The Journal of Microbiology, **43**, 93-100.

Pröpper, M., Gröngröft, A., Finckh, M., Stirn, S., De Cauwer, V., Lages, F., Masamba, W., Murray-Hudson, M., Schmidt, L., Strohbach, B. & Jürgens, N. (2015) *The future Okavango: findings, scenarios and recommendations for action.* University of Hamburg, Hamburg.

Pule-Meulenberg, F. & Dakora, F.D. (2007). Assessing the biological potential of N_2-fixing Leguminosae in Botswana for increased crop yields and commercial exploitation. *African Journal of Biotechnology*, **6**, 325–334.

Pule-Meulenberg, F. & Dakora, F.D. (2009) Assessing the symbiotic dependency of grain and tree legumes on N_2 fixation for their N nutrition in five agro-ecological zones of Botswana. *Symbiosis*, **48**, 68–77.

Quinn, M. A. and Hower , A. A. (1986). Effects of root nodules and taproots on survival and abundance of *Sitona hispidulus* (Coleoptera: Curculionidae) on *Medicago sativa. Ecological Entomology,* **11**, 391–400

Rudebjer, P., Chakeredza, S., Dans, A., Ekaya, W., Ghezae, N., Aboagye, L.N., Kwapata, M., Njoroge, M. & Padulosi, S. (2013) Beyond commodity crops: Strengthening young scientists' capacity for research on underutilized species in Sub-Saharan Africa. *Acta Horticulturae*, **979**, 577–589.

Sanginga, N., Dashiell, K.E., Diels, J., Vanlauwe, B., Lyasse, O., Carsky, R.J., Tarawali, S., Asafo-Adjei, B., Menkir, A., Schulz, S. & Singh, B.B., (2003). Sustainable resource management coupled to resilient germplasm to provide new intensive cereal–grain–legume–livestock systems in the dry savanna. Agriculture, Ecosystems & Environment, **100**, 305-314.

Food security

Serdeczny, O., Adams, S., Baarsch, F., Coumou, D., Robinson, A., Hare, W., Schaeffer, M., Perrette, M. & Reinhardt, J. (2017) Climate change impacts in Sub-Saharan Africa: from physical changes to their social repercussions. *Regional Environmental Change*, **17**, 1585–1600.

Schulze, R.E. & Kruger, G.P. (2007) Terrain methodology. *South African atlas of climatology and agrohydrology*, pp. 13–16.

Smaling, E.M.A., Roscoe, R., Lesschen, J.P., Bouwman, A.F. & Comunello, E. (2008) From forest to waste: assessment of the Brazilian soybean chain, using nitrogen as a marker. *Agriculture, Ecosystems & Environment*, **128**, 185–97.

de Souza, R., Meyer, J., Schoenfeld, R., da Costa, P. B., & Passaglia, L. M. (2015). Characterization of plant growth-promoting bacteria associated with rice cropped in iron-stressed soils. Annals of microbiology, **65**, 951-964.

Tauro, T.P., Nezomba, H., Mtambanengwe, F. & Mapfumo, P. (2009) Germination, field establishment patterns and nitrogen fixation of indigenous legumes on nutrient-depleted soils. *Symbiosis*, **48**, 92–101.

Wisch, U., Petersen, A., Gröngröft, A. & Eschenbach, A. (2009) Dryland farming in the Kavango region: effect of land use on soil properties. *Zentralblatt für Geologie und Paläontologie*, **1**, 189–211.

Yanggen, D., Kelly, V., Reardon, T. & Naseem, A. (1998) Incentives for fertilizer use in Sub-Saharan Africa: A review of empirical evidence on fertilizer response and profitability. International Development Working Paper 70. Department of Agricultural Economics, Michigan State University, East Lansing, MI, USA.

References [CrossRef]

Amoako-Atta, B., Emolo, E.O. & Kidega, E.K. (1983) Influence of maize, cowpea and sorghum intercropping systems on stem-/pod-borer infestations. *International Journal of Tropical Insect Science*, **4**, 39–46. CrossRef

Bernard, N., Losologolo, M., Batlang, U., Ngwako, S., Mashungwa, G.N., Tselaesele, N.M. & Pule-Meulenberg, F. (2017) Symbiotic performance of grain and wild herbaceous legumes in the Okavango Delta and Tswapong region of Botswana. *Symbiosis*, 1–10. CrossRef

Burbano, C.S., Grönemeyer, J.L., Hurek T. & Reinhold-Hurek, B. (2015) Microbial community structure and functional diversity of nitrogen-fixing bacteria associated with *Colophospermum mopane*. *FEMS Microbiology Ecology*, **91**, fiv030. CrossRef

Carreira, J.A., Arevalo, J.R. & Niell, F.X. (1996) Soil degradation and nutrient availability in fire-prone Mediterranean shrublands of southeastern Spain. *Arid Land Research and Management*, **10**, 53–64. CrossRef

Chianu, J.N., Nkonya, E.M., Mairura, F.S., Chianu, J.N. & Akinnifesi, F.K. (2011) Biological nitrogen fixation and socioeconomic factors for legume production in Sub-Saharan Africa. *Sustainable agriculture 2* (ed. by E. Lichtfouse, M. Hamelin, M. Navarrete and P. Debaeke), pp. 309–329. Springer, Dordrecht.

Chimwamurombe, P.M., Grönemeyer, J.L. & Reinhold-Hurek, B. (2016) Isolation and characterization of culturable seed-associated bacterial endophytes from gnotobiotically grown Marama bean seedlings. *FEMS Microbiology Ecology*, **92(6)**, fiw083. CrossRef

FAO (2010) *The state of food insecurity in the world. Addressing food insecurity in protracted crises.* Food and Agriculture Organization of the United Nations, Rome, Italy.

Gao, Y., Yu, G., Luo, C. & Zhou, P. (2012) Groundwater nitrogen pollution and assessment of its health risks: a case study of a typical village in rural-urban continuum, China. *PLOS One*, **7(4)**, e33982. CrossRef

Grönemeyer, J., Berkelmann, D., Mubyana-John, T., Haiyambo, D., Chimwamurombe, P., Kasaona, B., Hurek, T. & Reinhold-Hurek, B. (2013) A survey for plant-growth-promoting rhizobacteria and symbionts associated with crop plants in the Okavango region or Southern Africa. *Biodiversity & Ecology*, 5, 287–294. CrossRef

Grönemeyer, J.L., Burbano, C.S., Hurek, T. & Reinhold-Hurek, B. (2012) Isolation and characterization of root-associated bacteria from agricultural crops in the Kavango region of Namibia. *Plant Soil*, **356**, 67–82. CrossRef

Grönemeyer, J.L., Kulkarni, A. Berkelmann, D., Hurek, T. & Reinhold-Hurek, B. (2014) Identification and characterization of rhizobia indigenous to the Okavango region in Sub-Saharan Africa. *Applied and Environmental Microbiology*, **80**, 7244–7257. CrossRef

Gröngröft, A., Luther-Mosebach, J., Landschreiber, L. & Eschenbach, A. (2013) Cusseque Soils. *Biodiversity & Ecology*, **5**, 51–54. CrossRef

Kandjimi, O.S., Uzabakiriho, J.D. & Chimwamurombe, P.M. (2015) Isolation and characterization of culturable bacteria from bulk soil samples and the rhizosphere of arid-adapted *Tylosema esculentum* (Burchell) A. Schreiber (Marama bean) in Namibia. *African Journal of Biotechnology*, **14**, 944–952.

Lane, A. & Jarvis, A. (2007) Changes in climate will modify the geography of crop suitability: agricultural biodiversity can help with adaptation. *SAT eJournal ICRISAT*, **4**, 1–12.

Lane, D. J., 1991. 16S/23S sequencing. In Stackebrandt, E., and Goodfellow, M. (Eds.). Nucleic acid techniques in bacterial systematics. John Wiley & Sons, Inc. New York, pp. 115–175.

Linquist, B.A., Adviento-Borbe, M.A., Pittelkow, M., van Kessel, C. & van Groenigen, K.J. (2012) Fertilizer management practices and greenhouse gas emissions from rice systems: a quantitative review and analysis. *Field Crops Research*, **135**, 10–21. CrossRef

Luther-Mosebach, J., Gröngröft, A. & Eschenbach A. (2015) Influence of landscape and land-use on the soil sarbon stocks in the Okavango Catchment. *Böden im Wandel* (ed. by C. Knoblauch, C. Fiencke and K. Berger), pp. 155–166. Verein zur Förderung der Bodenkunde Hamburg 77. University of Hamburg, Hamburg, Germany.

Madibela, O.R., Kemiso, D. & Kwedibana, J. (2018) Quality of wild herbaceous legumes and its role in livestock nutrition. This volume. CrossRef

Ndoye, I., Dreyfus, B., 1988. N_2 fixation by *Sesbania rostrata* and *Sesbania sesban* using ^{15}N and total N difference methods. Soil Biology & Biochemistry, **20**, 209-213. CrossRef

Nehra, V., Saharan, B.S. & Choudhary, M. (2016) Evaluation of *Brevibacillus brevis*. *SpringerPlus*, **5**, 1–10.

Nezomba, H., Tauro, T.P., Mtambanengwe, F. & Mapfumo, P. (2008) Nitrogen fixation and biomass productivity of indigenous legumes for fertility restoration of abandoned soils in smallholder farming systems. *South African Journal of Plant and Soil*, **25**, 161–171. CrossRef

Ogada, M.J., Nyangena, W. & Yesuf, M. (2010) Production risk and farm technology adoption in the rainfed semi-arid lands of Kenya. *African Journal for Agricultural and Resource Economics*, **4**, 159–174.

Oliver, J. D. (2000). Problems in detecting dormant (VBNC) cells, and the role of DNA elements in this response. Tracking genetically-engineered microorganisms. Landes Biosciences, Georgetown, Tex, pp 1-15.

Oliver, J. D. (2005). The viable but nonculturable state in bacteria. The Journal of Microbiology, **43**, 93-100.

Pröpper, M., Gröngröft, A., Finckh, M., Stirn, S., De Cauwer, V., Lages, F., Masamba, W., Murray-Hudson, M., Schmidt, L., Strohbach, B. & Jürgens, N. (2015) *The future Okavango: findings, scenarios and recommendations for action.* University of Hamburg, Hamburg.

Pule-Meulenberg, F. & Dakora, F.D. (2007). Assessing the biological potential of N_2-fixing Leguminosae in Botswana for increased crop yields and commercial exploitation. *African Journal of Biotechnology*, **6**, 325–334.

Pule-Meulenberg, F. & Dakora, F.D. (2009) Assessing the symbiotic dependency of grain and tree legumes on N_2 fixation for their N nutrition in five agro-ecological zones of Botswana. *Symbiosis*, **48**, 68–77. CrossRef

Quinn, M. A. and Hower , A. A. (1986). Effects of root nodules and taproots on survival and abundance of *Sitona hispidulus* (Coleoptera: Curculionidae) on *Medicago sativa*. *Ecological Entomology*, **11**, 391–400. CrossRef

Rudebjer, P., Chakeredza, S., Dans, A., Ekaya, W., Ghezae, N., Aboagye, L.N., Kwapata, M., Njoroge, M. & Padulosi, S. (2013) Beyond commodity crops: Strengthening young scientists' capacity for research on underutilized species in Sub-Saharan Africa. *Acta Horticulturae*, **979**, 577–589. CrossRef

Sanginga, N., Dashiell, K.E., Diels, J., Vanlauwe, B., Lyasse, O., Carsky, R.J., Tarawali, S., Asafo-Adjei, B., Menkir, A., Schulz, S. & Singh, B.B., (2003). Sustainable resource management coupled to resilient germplasm to provide new intensive cereal–grain–legume–livestock systems in the dry savanna. Agriculture, Ecosystems & Environment, **100**, 305-314. CrossRef

Serdeczny, O., Adams, S., Baarsch, F., Coumou, D., Robinson, A., Hare, W., Schaeffer, M., Perrette, M. & Reinhardt, J. (2017) Climate change impacts in Sub-Saharan Africa: from physical changes to their social repercussions. *Regional Environmental Change*, **17**, 1585–1600. CrossRef

Schulze, R.E. & Kruger, G.P. (2007) Terrain methodology. *South African atlas of climatology and agrohydrology*, pp. 13–16.

Smaling, E.M.A., Roscoe, R., Lesschen, J.P., Bouwman, A.F. & Comunello, E. (2008) From forest to waste: assessment of the Brazilian soybean chain, using nitrogen as a marker. *Agriculture, Ecosystems & Environment*, **128**, 185–97. CrossRef

de Souza, R., Meyer, J., Schoenfeld, R., da Costa, P. B., & Passaglia, L. M. (2015). Characterization of plant growth-promoting bacteria associated with rice cropped in iron-stressed soils. Annals of microbiology, **65**, 951-964. CrossRef

Tauro, T.P., Nezomba, H., Mtambanengwe, F. & Mapfumo, P. (2009) Germination, field establishment patterns and nitrogen fixation of indigenous legumes on nutrient-depleted soils. *Symbiosis*, **48**, 92–101. CrossRef

Wisch, U., Petersen, A., Gröngröft, A. & Eschenbach, A**.** (2009) Dryland farming in the Kavango region: effect of land use on soil properties. *Zentralblatt für Geologie und Paläontologie*, **1**, 189–211.

Yanggen, D., Kelly, V., Reardon, T. & Naseem, A. (1998) Incentives for fertilizer use in Sub-Saharan Africa: A review of empirical evidence on fertilizer response and profitability. International Development Working Paper 70. Department of Agricultural Economics, Michigan State University, East Lansing, MI, USA.