Olivia Laggner, B.Sc.

Exploring the microbiome of novel leafy greens in Eastern Africa

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Zusammenfassung

Die Blattgemüsesorten Okra (*Abelmoschus esculentus*), Nightshade (*Solanum scabrum*), Spiderplant (*Cleome gynandra*) und Black Jack (*Bidens pilosa*) haben aufgrund ihrer besonderen medizinischen Eigenschaften und ihres hohen Nährstoffgehalts eine lange Tradition in der ländlichen Medizin und Ernährung in Ostafrika. Verglichen mit kultivierten Nutzpflanzen sind diese Pflanzen weniger anfällig für Schädlinge und in der Regel resilienter gegenüber abiotischen Belastungen. Das pflanzenassoziierte Mikrobiom leistet einen großen Beitrag für diese Resilienz. Eine Übertragung dieser Eigenschaften auf andere Nutzpflanzen zur Steigerung der Produktivität, ist vor allem in Ländern wie Uganda, in dem ein hoher Anteil der Bevölkerung direkt von ihrer Ernte abhängig ist, von existenzieller Bedeutung.

Um die Rolle des Mikrobiomes zu verstehen und bakterielle Schlüssel-Spezies zu identifizieren, wurde das Mikrobiom von Okra, Nightshade, Spiderplant und Black Jack basierend auf Amplicon Sequenzierung des 16S rRNA Gens und anschließenden bioinformatischen Analysen charakterisiert. Zusätzlich wurden Bakterien aus den Pflanzen isoliert und auf antagonistische Aktivitäten gegen die wichtigsten phytopathogenen Pilze (*Botrytis cinerea, Fusarium oxysporum, Fusarium verticillioides, Sclerotium rolfsii* und *Verticillium dahliae*) untersucht. Die identifizierten Antagonisten wurden weiter auf ihre Fähigkeit getestet, abiotischem Stress wie hohem Salzgehalt, Dürre und reaktivem Sauerstoff zu widerstehen.

Dabei konnte festgestellt werden, dass die Vielfalt des Mikrobioms von einheimischem Blattgemüse deutlich höher ist, als von kultiviertem Gemüse. Diese mikrobielle Vielfalt steht im direkten Zusammenhang mit Pflanzengesundheit. Sechs Isolate, die *Sphingomonas* sp. und *Bacillus* sp. zugeordnet werden konnten, erwiesen sich als vielversprechende Kandidaten für die weitere Entwicklung zu einem Biokontrollmittel. *In vitro* zeigten diese Isolate hochantagonistische Effekte gegen alle fünf pathogenen Pilze. Diese Antagonisten waren außerdem sehr resistent gegen abiotischen Stress. Die Ergebnisse dieser Studie dienen als Grundlage zur Entwicklung eines Biokontrollmittels und somit zur biologischen Optimierung der Gemüseproduktion, als Beitrag zur Ernährungssicherheit für Kleinbauern in Ostafrika.

Abstract

Indigenous leafy greens, such as Okra (*Abelmoschus esculentus*), Nightshade (*Solanum scabrum*), Spiderplant (*Cleome gynandra*), and Black Jack (*Bidens pilosa*) were recently rediscovered for rural medicine and cuisine in East Africa, due to their special medicinal properties and high level of nutrients. Compared to cultivated crops, those plants struggle less from pests and are generally more robust to abiotic stresses. Robustness of crops is very important to ensure food security, especially in countries like Uganda, where a high proportion of people is depending on subsidiary agriculture. The robustness of the host plant is strongly depending on its microbiome.

In order to better understand the robustness of these plants and the role of beneficial bacteria, the microbiome of the four leafy greens Okra, Nightshade, Spiderplant and Black Jack was characterized based on 16S rRNA gene fragment amplicon sequencing and bioinformatics analysis. Additionally, bacteria from each plant were isolated and screened for antagonistic activity against main phytopathogenic fungi (*Botrytis cinerea*, *Fusarium oxysporum*, *Fusarium verticillioides*, *Sclerotium rolfsii* and *Verticillium dahliae*). The identified antagonists were further tested for their ability to resist abiotic stresses, such as salinity, drought, and reactive oxygen.

The microbiome of indigenous leafy greens from Uganda was revealed to be significantly more diverse than of cultivated crops, which is reportedly in direct correlation with plant health. The microhabitat of the plants was found to be rather the driving force of microbial diversity than the plant species. Isolated *Sphingomonas* sp. and *Bacillus* sp. were selected as promising candidates for application as biocontrol agents as they showed highly antagonistic effects on main pathogenic fungi *in vitro*. Additionally, those isolates have strategies to stand abiotic stresses such as salinity and drought. This study shall further be used to develop a biocontrol agent, to optimize vegetable production and yield in order to ensure food security especially for smallholders in East Africa.

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Introduction

Situated in Eastern Africa, Uganda is rich in natural resources like fertile soils and regular rainfalls. The climate is relatively humid with moderate temperatures throughout the year. Total annual rainfall varies from 500 mm to 2800 mm and is compressed within rainy seasons (CIAT 2017).

Agricultural situation and challenges in Uganda

More than 80% of Ugandan population work in the agricultural sector, with a large share of smallholders, producing for their livelihoods. Almost 40% of Uganda's land area is used as agricultural land (Mungyereza 2016). Nevertheless, Uganda is not able to produce food self-sufficiently. A high portion of the population suffers from food insecurity and malnutrition (CIAT 2017). The most grown crops in Uganda are maize, bean and cassava, followed by sweet potato, coffee, rice, soy bean and banana (CIAT 2017). Harvest is endangered by biotic and abiotic environmental factors.

Main abiotic factors that cause crop stresses are drought, salinity, temperature and heavy metal accumulation (Venkarteswarlu et al. 2011). Crops in Uganda are mainly fed by rain and therefore vulnerable to climate hazards like droughts and weather variabilities threatening food security of farmers. Only about 0.1% of total cultivated land is irrigated (CIAT 2017). Climate change challenges farmers as weather effects come more abrupt and are more extreme like hail storms and floods. As long-term changes droughts are prolonged and soils degraded (Venkarteswarlu et al. 2011).

Biotic factors comprise animals, pathogens and weeds. Plant pathogenic fungi harm yield, quality and potentially reduce shelf-life through post-harvest diseases (Venkarteswarlu et al. 2011). In the presented experiments the focus was on *Botrytis cinerea* (Persoon 1801), *Fusarium oxysporum* (Schlechtendal 1824), *Fusarium verticillioides* (Nirenberg and O'Donnell 1998), *Sclerotium rolfsii* (Tu and Kimbrough 1978) and *Verticillium dahliae* (Klebahn 1913) as phytopathogenic fungi, causing biotic stress to plants. The choice of fungal plant pathogens was due to their high impact on harvest and post-harvest losses in Eastern Africa. *B. cinerea* and *Fusarium* sp. are listed among the top 10 fungal pathogens with scientific and economic importance (Dean et al. 2012).

Botrytis cinerea is a plant pathogen that invades plants by their wounded tissue but can also grow and reproduce on plant remains (Venkarteswarlu et al. 2011). *Fusarium oxysporum* causes vascular wilt or crown and root rot, mostly on tomatoes but also on variety of other crops (Larkin and Fravel 1998). The fungi favour warm temperatures (Mui-Yun 2003). *Fusarium verticillioides* can live as symptomless endophyte within the plant but a change of biotic and abiotic factors can provoke the fungi to become pathogenic, and thereby producing the harmful mycotoxin fumonisin (Bacon et al. 2008). *Sclerotium rolfsii* is a soil borne plant pathogen that mainly appears in tropics and subtropics and has a broad host range. It forms sclerotia that remain infective many years in the soil (Punja 1985). *Verticillium dahliae* is a plant pathogen, causing verticillium wilt diseases in crops (Inderbitzin et al. 2011).

To counteract food insecurity, pests need to be controlled. Chemical pesticides are used predominantly for treating pests. Pesticides are meant to kill pests like insects, weeds, rodents and fungi. Besides their effects on pests, pesticides can also have poisonous effects on other organisms – also on humans ('WHO | Pesticides' n.d.). The World Health Organisation (WHO) reported 272,000 pesticide poisonings per year in Uganda during the 1980s (Jeyaratnam 1990), with tendencies to higher numbers nowadays as worldwide sales of pesticides – and especially those in developing countries - are rising each year (Northoff 1999).
A survey carried out in Uganda in 2011 evaluated the knowledge and practices of pesticide use at homes. The authors concluded that less then every second respondent reads the manufacturer's instructions and relies on friends and sales points for information about which pesticide to use and how to apply them (Nalwanga and Ssempebwa 2011). Although this survey focussed on application of pesticides in households, the assumption that the inappropriate pesticide application on the fields is comparable, was suggested. This is also manifested by the white pesticide-staining residues our group found during the sampling on vegetables on markets in Uganda. Inappropriate pesticide use includes the usage of inappropriate products, wrong dosage, timing or targeting and poor application equipment. Using unauthorised or banned products, mixes of products, splashing pesticides onto crops and application without wearing protective clothes is considered extremely harmful (Williamson et al. 2008). Acute pesticide poisoning mostly concerns farmers that use pesticides uncontrolled and unregulated and causes substantial morbidity and mortality. In dependence of the pesticide type used, chronic effects can range from cancer over adverse reproductive outcome to immunological effects and neuropsychological dysfunctions (al-Saleh 1994; Rosenstock et al. 1991). The US National Research Council reports oncogenic and other chronical risks of pesticides too, but highlights that the loss of pesticides comes with an adverse economic impact due to lack of alternatives to pest management (National Research Council 1987). Besides the threat for humans, the environment is also challenged by inappropriate pesticide use and leaking pesticide stocks. Deteriorated stocks are a result of excessive donations, inadequate storage, wrong pesticide formulations or aggressive sales practices (Northoff 1999). The leaking chemicals contaminate soil, irrigation systems, ground water and drinking water. Various accidents with those residing stocks are reported as they are stored next to food and markets and have no barrier for children (Northoff 1999).

Due to the problems arising with the use of chemical pesticides for environment and human health, it is important to find alternatives, especially in countries where a big share of people is farming for their livelihood, like in Uganda.

Robust indigenous leafy greens with beneficial traits in nutrition and medicine

One alternative could be to switch to more robust plants for agriculture. Commercial crops were bred for an increase in productivity and sometimes in quality. Incidentally, those plants lost resistance to stress factors (Venkarteswarlu et al. 2011). On the other hand, some indigenous plants are found to struggle much less with pests and are generally more robust.

Due to their beneficial traits, popularity of indigenous leafy greens grew within the last years in Eastern Africa. Production area of such leafy greens raised by 25% between 2011 and 2013 in Kenya (Cernansky 2015). Especially the leafy greens Okra (*Abelmoschus esculentus,* (Kumar et al. 2010)), Nightshade (*Solanum scabrum,* (Ronoh et al. 2019)), Spiderplant (*Cleome gynandra,* (Onyango et al. 2013)) and Black Jack (*Bidens pilosa,* (Bartolome et al. 2013)) show many beneficial traits such as a higher incidence of valuable nutrients and the ability to stand biotic and abiotic stresses.

Leaves and shoots of Black Jack are used in rural cuisine for sauces and teas. In rural medicine, the plant as a whole or different parts of it are used to treat more than 40 disorders, like tumours, inflammations, diabetes, immune disorders and malaria to name some of them. Compounds are shown to have antibacterial and antifungal activities. The fresh plant is also thought to cure snake bites (Bartolome et al. 2013).

Nightshade is a valuable resource of proteins, vitamin A, iron and calcium. Traditional medicinal applications use the plant to treat stomach upsets, duodenal ulcers, swollen glands and teething problems (Mwai et al. 2007). Besides the use in kitchen and medicine, Nightshade is also used as ink dye (Lehmann et al. 2007).

Besides provitamin A, vitamin C and some minerals as magnesium, calcium and potassium Okra is also rich in dietary fiber (Kumar et al. 2010). In the kitchen, leaves and fruits show a high versatility. For medicinal applications, poultices made from leaves and immature fruits of Okra were used to relieve pain (Council 2006).

Whereas tender leaves of Spiderplant are consumed, whole leaves, roots and stem find application in medicine with anti-malarial, antioxidant and anti-microbial traits. Nutritional benefits are due to the plant's high micronutrient and phytochemical content (Onyango et al. 2013).

Out of those four, Okra is the only traditionally cultivated plant. Okra is easy to grow and withstands many pests and diseases. It still shows quite a robust nature as until now, no effort has been put, neither into genetically improving the traits of plants, nor resistance breeding (Kumar et al. 2010). The other three plants are usually collected from the wild and considered as weeds, due to their invasiveness (Bartolome et al. 2013; Onyango et al. 2013).

Interplay of plants and their microbiome

Plants host distinct microbial communities. Every plant consists of different microhabitats like phyllosphere, rhizosphere and endosphere. Each of these has particular characteristics and therefore harbours specific microbes (Bulgarelli et al. 2013). The microbiome is stable and host specific. Plants and microbiota are highly connected to each other through metabolism and morphology. This ensemble of host and microbiome is described as holobiont and is of special importance for plant health. Whereas the host-genome is static, the genome of the microbiota is also influenced by the host and supports adaption and survival under varying environmental conditions (Singh et al. 2013).

Microbiota vary among microhabitats but to some extend bacterial communities in and on roots, which appear also in and on leaves, are influenced by inoculation from soil and

migration of microbes through the plant (Wagner et al. 2016). The rhizosphere builds the interface between soil and roots and is in exchange with the surrounding soil. The same is valid for the phyllosphere, which is in direct contact with the surrounding air (Berg et al. 2016). Plant colonization is species specific but also influenced by multiple factors like plant age, developmental stage and health status (Berg and Smalla 2009).
Some plant-associated bacteria antagonize pathogens, support stress resistance in plants, provide nutrients and thus increases growth, yield and quality of crops. The microbiome of both, phyllosphere and rhizosphere contain microbes protecting the plant from antagonists. Their mechanisms are either direct like niche colonization or production of substrates antagonizing pathogens (like volatile organic compounds). Roots also harbour species supporting plant growth by providing metabolites or facilitate nutrient uptake (Bulgarelli et al. 2013). An indirect mechanism of antagonism is the stimulation of the plant's immune system (Lugtenberg and Kamilova 2009). Selection of microorganisms in rhizosphere and phyllosphere by the plant is substrate-driven and can thus be shaped through trans-kingdom interactions by the plant (Hartmann and Schikora 2012). Phyllospheric microbiota is of special interest, as phyllosphere of leafy greens is the eaten part.
Our hypothesis is that the robustness of those selected leafy greens is not only due to their species but also based on the plant's microbiome.

The goal of this study is to characterize the microbiome of indigenous leafy greens, with focus on the specificity of the core microbiome for the plants and their microhabitats. By using diversity indices, differences to cultivated crops shall be revealed. The knowledge about the role of microorganisms within the plant's microbiome opens possibilities to specifically induce such key species to crops, making them as robust as indigenous leafy greens. After characterizing species with antagonistic traits against biotic stresses as well as robustness towards challenging abiotic factors, the aim is to develop a biocontrol agent, supporting smallholders in rural areas of Uganda. Therefore, the plant-associated microbiome was studied to reveal the host-microbiome interplay and the core microbiome characterized. Additionally, bacteria from the leafy greens were isolated and tested for their antagonistic potential against main pathogenic fungi (*Botrytis cinerea*, *Fusarium oxysporum*, *Fusarium verticillioides*, *Sclerotium rolfsii* and *Verticillium dahliae*) and for their ability to resist abiotic stress, such as salinity, drought, and reactive oxygen.

Materials and Methods

Experimental design and sampling procedure

The leafy greens Black Jack (*Bidens pilosa*), Okra (*Abelmoschus caillei*), Nightshade (*Solanum scabrum*) and Spiderplant (*Cleome gynandra*) were sampled in Kasangati, Uganda (0° 26' 33''N, 32° 36' 19''E) in April 2017. Four samples of each leafy green consisting of one individual specimen were harvested. Except for Nightshade, where 3 specimens per sample were harvested. Throughout the study plant leaves and stalks were termed as phyllosphere regarding the habitat description. Additionally, 4 bulk soil samples were collected as reference. Plant and soil samples were placed in air-tight plastic bags, kept cool and transferred to the laboratory. 300 g of soil were assessed for pH, nutrient content (K, P, Mg, and organic matter) and soil type by "AGROLAB Agrar und Umwelt GmbH" (Sarstedt, Nümbrecht, Germany). All further experiments described were performed at the Institute of Environmental Biotechnology, University of Technology, Graz. In order to homogenize the samples, 3 g of the phyllosphere and 5-10 g of the rhizosphere and the soil per replicate were physically disrupted in a Bag Mixer with 15 ml of 0.85% NaCl. 2 x 2 ml of the suspension were transferred to a 2 ml Eppendorf tube, and subsequently centrifuged (16500 g, 20 min, 4°C). Rhizosphere samples were further surface sterilised with 4% NaCl for 3 min, washed 4 times with 0.85 % NaCl pestled with 15 ml NaCl, and finally 3 x 2 ml of the suspension were transferred to a 2 ml Eppendorf tube and centrifuged. All DNA extracts were stored at -70°C for further processing.

Isolation of total community DNA and bacterial cultures

During the sampling procedure 100 µl of the 15 ml 0.85% NaCl suspensions of each habitat-sample of the four leafy green was plated onto NBII agar plates in dilutions from 10^0 to 10^{-5}. In total 512 strains were isolated for further screening. Community DNA pellets from the habitats soil, rhizosphere, rhizo-endosphere and phyllosphere of the four leafy greens were subjected to PCR-based barcoding. First, extraction of the DNA pellets was conducted using "FastDNA Spin Kit for soil" (MP Biomedical, Eschwege, Germany). PCR-products were cleaned with GENECLEAN TurboTM Kit (MP Biomedicals, Eschwege, Germany) following the manufacturer's instructions for genomic DNA. The PCR approach was carried out in 3 x 30 µl reactions with the Illumina barcode universal bacterial primer set 515f-806r (Caporaso et al. 2011) and PNA Mix (Lundberg et al. 2013) to remove plastid DNA. PCR products of barcoded samples were pooled to equimolarity. Sequencing was carried out by Eurofins MWG Operon (Eurofins, Ebersberg, Germany) with an Illumina HiSeq 2500 system [for details, see Additional file 11 – Doc S2].

Illumina sequencing and bioinformatics processing of 16S rRNA gene amplicons

The generated 16S rRNA gene libraries were pre-processed using the bioinformatics tool Quantitative Insights Into Microbial Ecology (QIIME) release 1.9.1 and analysed with QIIME 2 core (2017.12). First the read quality was checked with fastqc and barcodes were extracted. The length- and quality-filtered sequences were assembled into operational taxonomic units

(OTUs) with a 97% similarity cut-off. As a classifier, SILVA reference data base version 128 was used. OTUs containing mitochondria or chloroplasts were removed. Data analysis was performed according to the "moving pictures" tutorial from QIIME 2, provided at the QIIME2 homepage (https://docs.qiime2.org/2018.2/). For evaluating alpha diversity, Kruskal-Wallis all groups and pairwise with boxplots, alpha rarefaction and Shannon diversity index were calculated. Beta diversity was analysed by Principal coordinate analysis (PCoA) plots and ANOSIM test. The PCoA plot was based on phylogenetic distance metrics of weighted UniFrac and visualised with EMPEROR. The non-parametric ANOSIM test was evaluated on the basis of 999 permutations.

Growth and culture media

The culture media used for cultivation of bacterial isolates and fungal strains as well as for the antagonistic tests were Potato Dextrose Agar (PDA), Waksman Agar (WA) from Carl Roth, and Nutrient Agar (NA) from sifin Diognostics. For reproduction of *V. dahliae*, Czapek Dox from Duchefa Biochem, was used.

Waksman Agar (WA)

Trypton	5 g/l	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Glucose	10 g/l	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Yeast extract	3 g/l	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
NaCl	5 g/l	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Agar-Agar	20 g/l	Carl Roth GmbH + Co. KG, Karlsruhe, Germany

Botanical Research Institute's phosphate growth media (NBRIP)

Glucose	10 g/l	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
$Ca_3(Po_4)_2$	5 g/l	Scharlau Chemie, Barcelona, Spain
$MgCl_2x6H_2O$	5 g/l	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
$MgSO_4x7H_2O$	0.25 g/l	Merck KGaA, Darmstadt, Germany
KCl	0.2 g/l	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
$(NH_4)_2SO_4$	0.1 g/l	neoLab Migge GmbH, Heidelberg, Germany
Agar	15 g/l	Carl Roth GmbH + Co. KG, Karlsruhe, Germany

Screening for and identification of antagonists

For antagonistic screening four bacterial isolates were streaked onto a WA-plate and exposed to a fungal pathogen in the middle. Bacterial isolates originate from four different plants that are Okra, Nightshade, Spiderplant and Black Jack, thereof three different parts comprising rhizosphere, rhizo-endosphere and phyllosphere as well as soil samples from two different sites. The fungal strains utilized were *Botrytis cinerea*, *Fusarium oxysporum*, *Fusarium verticillioides*, *Sclerotium rolfsii* and *Verticillium dahliae*. The fungi were cultivated on PDA plates at room temperature and kept in the dark.

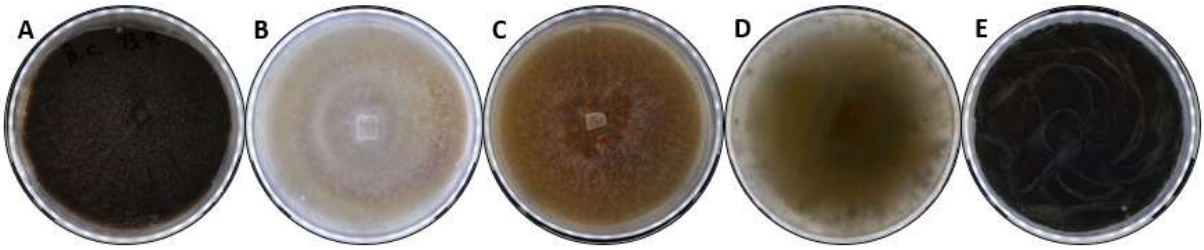

Figure 1: Plant pathogenic fungi on PDA. *Botrytis cinerea* (A), *Fusarium oxysporum* (B), *Fusarium verticillioides* (C), *Sclerotium rolfsii* (D) and *Verticillium dahliae* (E). Picture credits Lea Gibitz-Lambert.

The respective fungi were grown on separate PDA plates, which were cut with a razor blade to quadrats of a side length of around 5 mm. Those were further placed upside down in the centre of the WA-plates pre-colonized by bacteria. For *Verticillium dahliae* the procedure was slightly different: Using 3 ml of Czapek Dox per PDA plate containing *V. dahliae*, the spores were collected in the media by scraping with a trigalski spatula. 100 µl were plated on each WA-plate. After the solution was dried completely, bacterial isolates were streaked onto the plate with sterilized tooth picks. Those tests were performed in triplicates.
After incubation at room temperature in the dark for 3 to 7 days (thereof two days in the fridge), depending on the growth of the fungi, the plates were evaluated using the categories listed in table 1:

Table 1: Description of categories to which bacterial isolates were assigned depending on their interaction with fungi.

Category	Description
0	No antagonistic effect; fungi overgrows bacteria
1	Bacteria is not overgrown but in touch/surrounded by the fungi
2	Fungi and bacteria do not touch; halo is visible but small (< 5 mm)
3	Clear halo between fungi and bacteria of at least 5 mm

DNA of 20 identified bacterial isolates with strong antagonistic traits against all tested fungi was extracted by ribolysing. Therefore, colonies were solubilized, transferred into glass-beads filled tubes, ribolysed and centrifuged. The supernatant was used as template for BOX-PCR. BOX-PCR was performed according to the protocol of Rademaker & De Bruijn (1997), using the BOXA1R primer 5'-CTA CGG CAA GGC GAC GCT GAC G-3'. 12 µl aliquots of amplified PCR products were separated by gel electrophoresis and the resulting band pattern were compared with "Gel Compar II" V.5.1 (Applied Maths, Kortrijk, Belgium). Based on similar BOX pattern, bacterial isolates were grouped into 5 similarity categories. One isolate out of each BOX similarity group was further sequenced based on the whole genome by Evogene (Evogene Ltd. Rehovot, Israel).

Abiotic stress assays and phosphate-solubilization test

Potential antagonistic isolates were screened for resistance to abiotic stress such as drought, salinity and reactive oxygen. The antagonist's ability to solubilize phosphate was also

evaluated. As inoculum for all assays, bacterial isolates were cultivated as overnight culture (ONC) in Luria broth (LB) media. Bacterial isolates, capable of solubilizing phosphate were screened in a plate assay with National Botanical Research Institute's phosphate growth media (NBRIP: Glucose 10 g/l, $Ca_3(Po_4)_2$ 5 g/l, $MgCl_2x6H_2O$ 5 g/l, $MgSO_4x7H_2O$ 0.25 g/l, KCl 0.2 g/l, $(NH_4)_2SO_4$ 0.1 g/l and Agar 15 g/l). After incubation of 10 and 14 days at room temperature bacteria were screened for the formation of a halo. Isolates with a halo > 0.5 cm are categorized as phosphate solubilizing positive. For reactive oxygen species tests, bacterial isolates were cultivated in LB media with different tellurite concentrations (1, 3, 5, 7, 9, 10, 13, 15, 18 and 20 µg/ml). In an additional test, bacterial isolates were cultivated in LB media with different hydrogen peroxide concentrations (100, 300, 500, 700, 900, 1000, 1300, 1500, 1750, 2000, 2250, 2500, 2750, 3000, 3500, 3800 and 4000 µmol). For both tests 5 µl overnight culture were mixed with 195 µl LB media containing the respective tellurite concentration. Growth has been measured after 24 h at 30°C under agitation in four replicates using the plate reader (Infinite 200, Tecan Trading AG, Switzerland) at a wavelength of 600 nm. For evaluation of the tolerated osmolarity level, bacterial isolates were cultivated in LB media with various sodium chloride concentrations (steps of 1% from 0%-15%). 5 µl overnight culture were mixed with 195 µl LB media containing the respective sodium chloride concentration. Growth has been measured in four replicates after 24 h, 48 h, 72 h and 144 h using the plate reader (Infinite 200, Tecan Trading AG, Switzerland) at a wavelength of 600 nm. For the desiccation assay 20 µl of bacterial overnight culture were dried under sterile conditions in a 96-well plate and resuspended after 24 h, 48 h, 5d (120 h), 7d (168 h), 14 d (336 h), 30 d (720 h), 60 d (1440 h) and 88 d (2112 h) in 20 µl 0.9% NaCl. 10 µl of the resuspended cells were dropped onto LB-agar plates in a dilution series. Growth was evaluated by counting colonies and calculating the Colony Forming Units (CFU).

Screening for antifungal VOCs production

The screening for antifungal VOCs-producing strains was carried out using a two-clamp VOC assay (Cernava et al. 2015): Bacterial isolates and pathogenic fungi were streaked onto 6-well plates containing NA (for bacteria) or WA (for fungi). Bacteria were put up side down onto fungal growth plates, separated by a sterile, perforated silicone foil. The arrangement was fixed with clamps. After 7 d of incubation the diameter of fungal hyphae was measured and compared to a reference.

Screening for plant growth-promoting activities

Growth-promoting activities of bacterial isolates were tested on tomato (*Lycopersicon esculentum* cv. moneymaker) plants. To prime the tomato seeds, bacterial cultures from three agar plates were suspended in 20 ml sterile water and incubated with the seeds for 4 h under agitation. CFU and OD_{600} were determined. Two pouches were prepared per strain with 8-9 seeds each. After 15 d the plants were harvested, leaves and roots weighted, and roots mortared for CFU determination.

Results

Microbial diversity associated to leafy greens

Using the information of 16S rRNA amplicon sequencing, the microbiome of the four leafy greens was analysed. With these dataset diversity metrics based on phylogeny were calculated with QIIME 2. Figure 1 shows the visualization by principal coordinate analysis (weighted UniFrac).

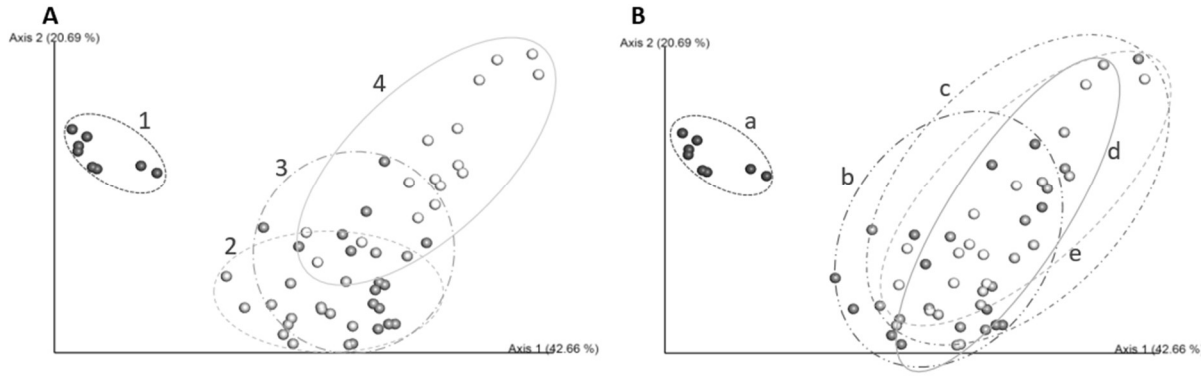

Figure 2: Microbial diversity of samples visualized by principal coordinate analysis (PCoA) plots. PCoA plots of the 16S rRNA amplicon datasets of the four leafy greens (Okra, Nightshade, Spiderplant and Black Jack) were constructed based on phylogenetic distance metrics (weighted UniFrac). The distance between the data points negatively correlates with the similarity of the communities. **A** clusters the communities based on habitat (1=soil, 2=rhizosphere, 3=root-endosphere and 4=phyllosphere) and **B** based on organisms (a=nan/soil, b=Nightshade, c=Spiderplant, d=Okra and e=Black Jack).

Regarding the differences between microhabitats (Fig. 1, A), a trend from rhizosphere to phyllosphere was seen, whereas the community of rhizosphere (Fig. 1, A2) was overlapping to some extend with root-endosphere (Fig. 1, A3); differences to phyllosphere (Fig. 1, A4) were much higher. Root-endospheric communities were situated within both clusters, rhizosphere and phyllosphere, but shared more similarity with rhizosphere. The reference soil (Fig. 1, A1) showed a high distance to the other habitats with quantitative measures. ANOSIM test over all habitats confirmed significant differences in diversity with R=0.504 and p-value=0.001 (all values are provided in supplementary). The R-value is a ratio of dissimilarities between communities within a group and dissimilarities between communities of different groups. The higher the R-value, the more similar are communities within a group and dissimilar to communities of other groups. When assigning the same communities to their respective plants (Fig. 1, B), the clusters were more overlapping. Communities of Spiderplant, Okra and Black Jack (Fig. 1, B3, 4 and 5) were located closely and overlapped to a high extend. Only Nightshade (Fig. 1, B2) showed in some parts a more different clustering pattern. The ANOSIM test validated that there is no significant difference between diversity of plants, with R=0.048 and p-value=0.064. When taking a closer look by pairwise comparison, only Nightshade and Okra showed significant differences in diversity (R=0.111, p-value=0.049) (all values are provided in supplementary). Further, habitats were analysed for specificity of plants. Distinct distribution patterns of diversity of plants within habitats could only be seen within

rhizosphere. Nightshade formed a separate cluster, whereas the other clusters of plants were overlapping. Communities within root-endosphere and phyllosphere formed overlapping clusters. The clusters of phyllosphere of Black Jack and Nightshade were wider spread (should have more samples for evidence) but in parts overlapping with the other clusters. Thus, the only specificity that could be seen within habitats was the cluster of Nightshade in the rhizosphere (Fig. 3, PCoA-plots for all habitats are provided in supplementary).

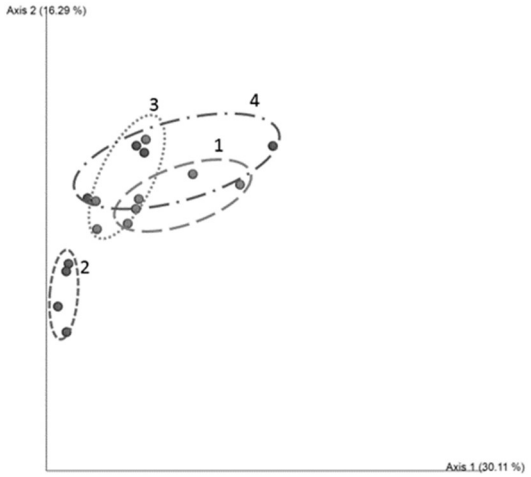

Figure 3: Microbial diversity of plants within rhizosphere visualized by principal coordinate analysis (PCoA) plots. PCoA plots were constructed based on phylogenetic distance metrics (weighted UniFrac). The closer the points, the more similar the communities. 1=Black Jack (long interrupted lines), 2=Nightshade (short interrupted lines), 3=Spiderplant (dots) and 4=Okra (line-dot-line).

Community structures were more different between habitats in quantitative measures, whereas community structures of different plants formed overlapping patterns and were more similar. Therefore, bacterial communities were specific for habitats but not plant type-specific for leafy greens. For statistical investigation of relationships between plants (within-sample), the following alpha-diversity indices were calculated: Kruskal-Wallis all groups and Kruskal-Wallis pairwise as well as Shannon index. Species diversity within a sample was evaluated using the non-parametric Kruskal-Wallis test. For comparison of the groups, all values were ranked without reference to the group they belong to. The H-value in Kruskal-Wallis statistics describes the discrepancy among the rank sums. The higher the H-value, the higher the discrepancy. P-values > 0.05 mean that no dominance between samples can be recognized (not significant). Lower p-values reveal that at least one sample stochastically dominates another sample (significant): populations have different distributions and differences due to random sampling can be excluded. The following table (Tab. 2) evaluates specificity of microbial diversity for habitat and plants.

Table 2: Microbial diversity investigated with Kruskal-Wallis all groups. Significant differences in diversity are highlighted in grey.

Category	Groups	H-value	p-value
Habitat	Rhizosphere, root-endosphere, phyllosphere	13.095	0.001
Organism	Black Jack, Nightshade, Okra, Spiderplant	6.758	0.080

Diversity was significantly different between the habitats (p-value=0.001), whereas the differences in diversity were not significant between the four plants (p-value=0.080). This was also reflected by the higher discrepancy (H-value=13.095) of habitat samples among rank sums. The discrepancy of organism samples was only about half as high (H-value=6.758). Thus, microbial diversity was specific for each habitat but not specific for the either one of the plants Black Jack, Nightshade, Okra and Spiderplant.
Table 2 gives a closer look on specificity of diversity by comparing the samples of habitats as well as samples of plants pairwise.

Table 3: Microbial diversity investigated with Kruskal-Wallis pairwise. Significant differences in diversity are highlighted in grey.

Group 1	Group 2	H-value	p-value
Rhizosphere	Root-endosphere	0.115	0.734
Root-endosphere	Phyllosphere	7.363	0.007
Phyllosphere	Rhizosphere	12.023	0.001
Black Jack	Nightshade	1.470	0.225
Black Jack	Okra	2.803	0.094
Black Jack	Spiderplant	0.003	0.954
Nighshade	Okra	6.453	0.011
Nightshade	Spiderplant	0.853	0.356
Okra	Spiderplant	1.763	0.184

The overall group of habitats differed in diversity, but with respect to pairwise investigations, this was due to differences of rhizosphere as well as root-endosphere to phyllosphere. Microbial diversity in the rhizosphere and root-endosphere did not differ significantly from each other (p-value=0.734). All group statistics showed that diversity of these leafy greens was not plant type-specific. Although, pairwise comparison showed that there were significant differences in microbial diversity of Nightshade compared to Okra. However, principal coordinate analysis and Kruskal-Wallis test revealed that microbial diversity was habitat-specific albeit rhizosphere and root-endosphere shared common elements. But microbial diversity was not specific for each of the four plants. Only the plants Nightshade and Okra differed significantly.

Shannon diversity indices

A mathematical measure for species diversity in a community was provided by Shannon's diversity index. The Shannon diversity index H is based on the number of OTUs and takes also the number of clones within those OTUs into account. The higher the value, the higher the species diversity. Species richness is also a function of sequencing depth. The number of species rises with every step of sequencing depth until a certain plateau is reached. To compare samples of different reads with another, rarefaction was performed. Within this experiment a random collection of samples with a specified depth was taken and analysed at ten different depths between 0 and 6000 with ten repeats each. Figure 3 shows two

rarefaction-curves: OTU numbers per sequencing depth (Fig. 4A) and Shannon diversity index H per sequencing depth (Fig. 4B).

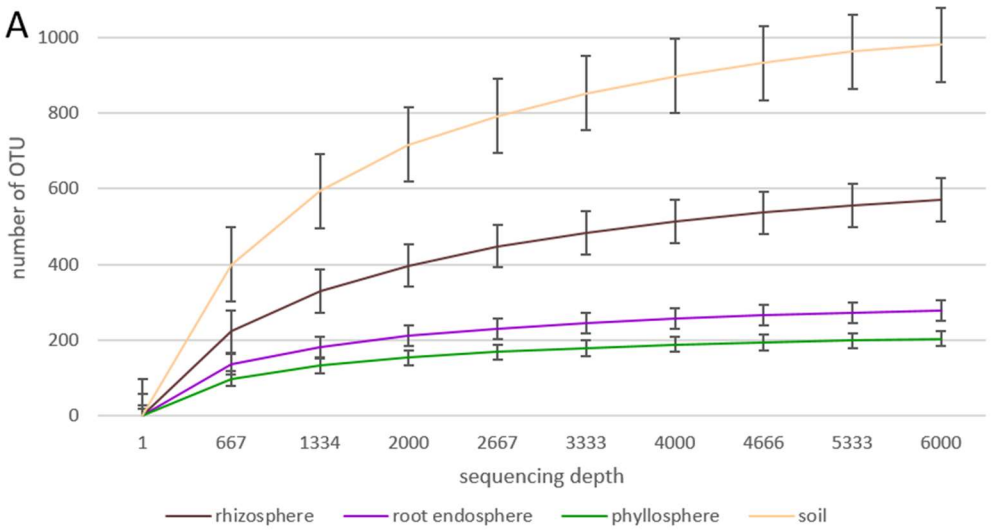

Figure 4A: Visualization of OTU numbers of habitats as function of sequencing depth. The method applied is alpha rarefaction with 10 repeats at 10 different points of sequencing depths. Legend: ochre=soil, brown=rhizosphere, purple=root-endosphere, green=phyllosphere.

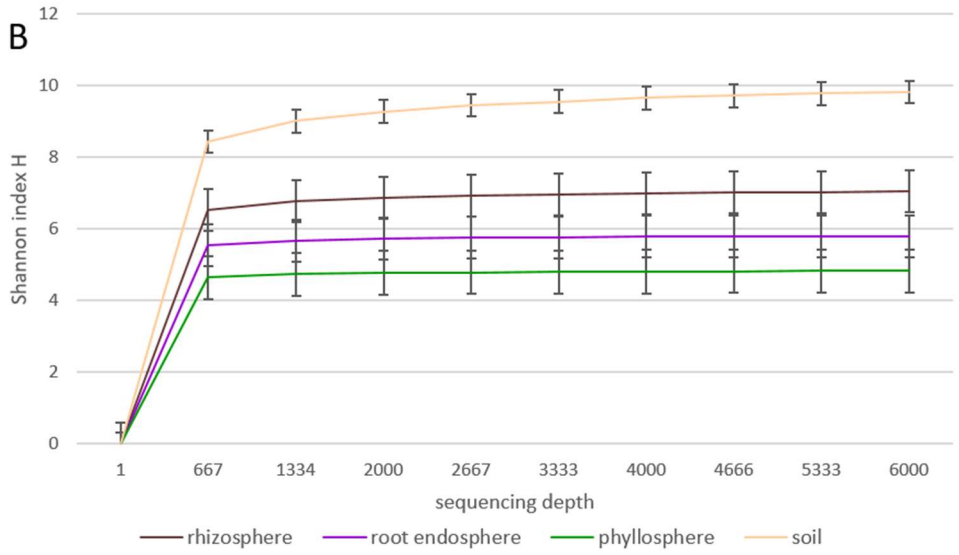

Figure 4B: Visualization of Shannon diversity index H of habitats as function of sequencing depth. The method applied is alpha rarefaction with 10 repeats at 10 different points of sequencing depths. Legend: ochre=soil, brown=rhizosphere, purple=root-endosphere, green=phyllosphere.

The curves of OTU numbers and Shannon diversity followed a comparable shape: soil had the highest values for OTU numbers and Shannon diversity index, followed by rhizosphere, root-endosphere and phyllosphere. Standard deviations of diversity (Fig. 4B) of rhizosphere and root-endosphere were close with a few overlaps, whereas root-endosphere and phyllosphere were overlapping.

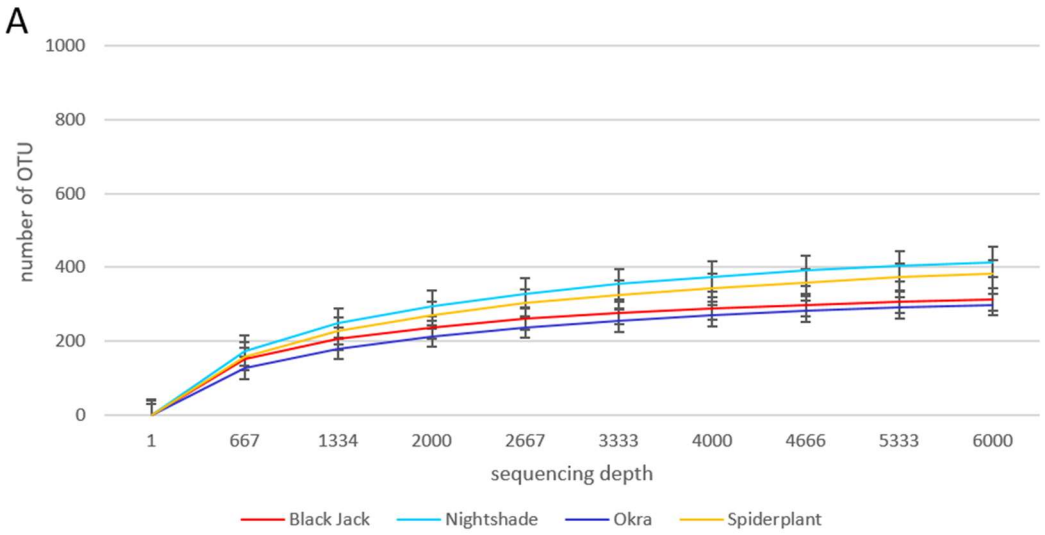

Figure 5A: Visualization of OTU numbers of plants as function of sequencing depth. The method applied is alpha rarefaction with 10 repeats at 10 different points of sequencing depths. Legend: yellow=Spiderplant, turquois=Nightshade, red=Black Jack and dark blue=Okra.

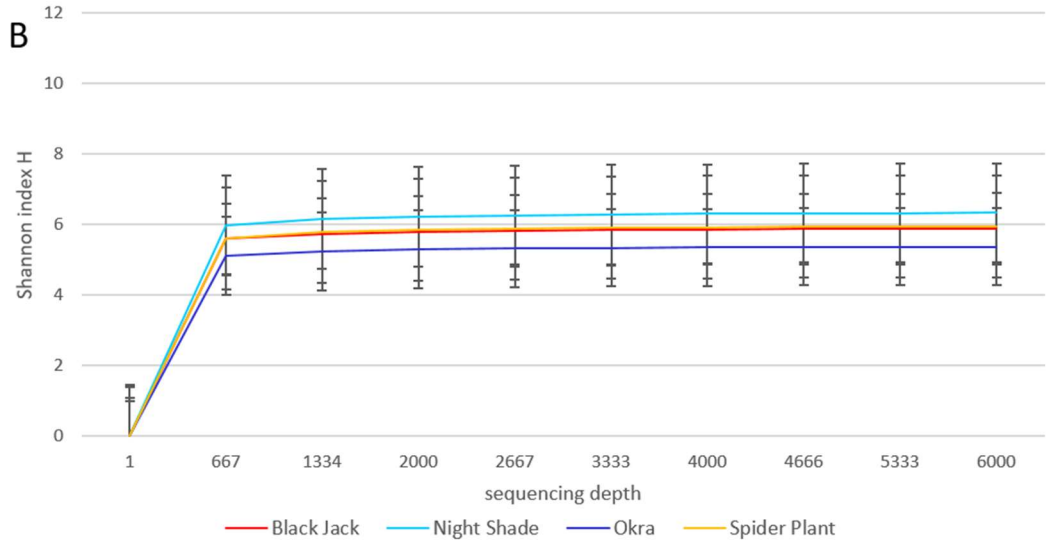

Figure 5B: Visualization of Shannon diversity index of plants as function of sequencing depth. The method applied is alpha rarefaction with 10 repeats at 10 different points of sequencing depths. Legend: yellow=Spiderplant, turquois=Nightshade, red=Black Jack and dark blue=Okra.

Comparing plants, the OTU numbers and Shannon diversity indexes were more compact. Nightshade had the highest values, followed by Spiderplant and Black Jack and Okra, the lowest line. Whereas Spiderplant samples were tended to have higher OTU numbers (Fig. 5A) than Black Jack, both shared similar values for diversity (Fig 5B). Standard deviations were all overlapping.

After a certain sequencing depth (667 reads per sample), the Shannon diversity index H remained constant for both, habitats and plants. Thus, the whole diversity of the amplicon sequencing could be captured. Only the reference sample soil still increased slightly with sequencing depth. Only values from the plateau were used for determining diversity indices. Table 3 lists up Shannon diversity index H of organisms and habitats.

Table 4: Shannon diversity index H of plants and habitats. The higher the value, the higher the species diversity within-samples. Shannon diversity index H of **soil** is **9.41 ± 0.42**.

	Okra	**Black Jack**	**Spiderplant**	**Nightshade**	average
Phyllosphere	4.40 ± 0.06	4.83 ± 0.07	4.15 ± 0.07	5.74 ± 0.11	4.78 ± 0.08
Root-endosphere	5.13 ± 0.10	6.21 ± 0.11	6.43 ± 0.12	5.18 ± 0.05	5.74 ± 0.09
Rhizosphere	6.39 ± 0.14	6.39 ± 0.13	7.04 ± 0.15	5.81 ± 0.21	6.91 ± 0.16
average	5.31 ± 0.10	5.81 ± 0.10	5.87 ± 0.12	6.24 ± 0.12	

A clear trend of diversity increase could be followed from phyllosphere over root-endosphere to rhizosphere. The only plant that did not follow this trend was Nightshade with the highest overall diversity. Nightshade showed the least diversity within its root-endosphere (table 3, highlighted in grey), surpassed by phyllosphere. Anyways diversity within Nightshade's rhizosphere was the highest comparing all plants. Diversity within soil samples that were not associated with plants was far higher (9.41 ± 0.42) compared to rhizosphere of plants (average=6.91 ± 0.16, highest Nightshade=7.81 ± 0.21). Whereas the plants Black Jack and Spiderplant were quite similar in their overall Shannon diversity index H (Black Jack=5.81 ± 0.10, Spiderplant=5.87 ± 0.12), Okra (lowest diversity, 5.31 ± 0.10) and Nightshade (highest diversity 6.24 ± 0.12) were further spread.

Composition of the microbiome associated to leafy greens in Uganda

The core microbiome (Fig. 6) was defined on family level with a threshold of 1% of total prokaryotic abundance captured within the 16S amplicon data set. Families with lower abundance were summarized within "others" (all values are provided in supplementary).

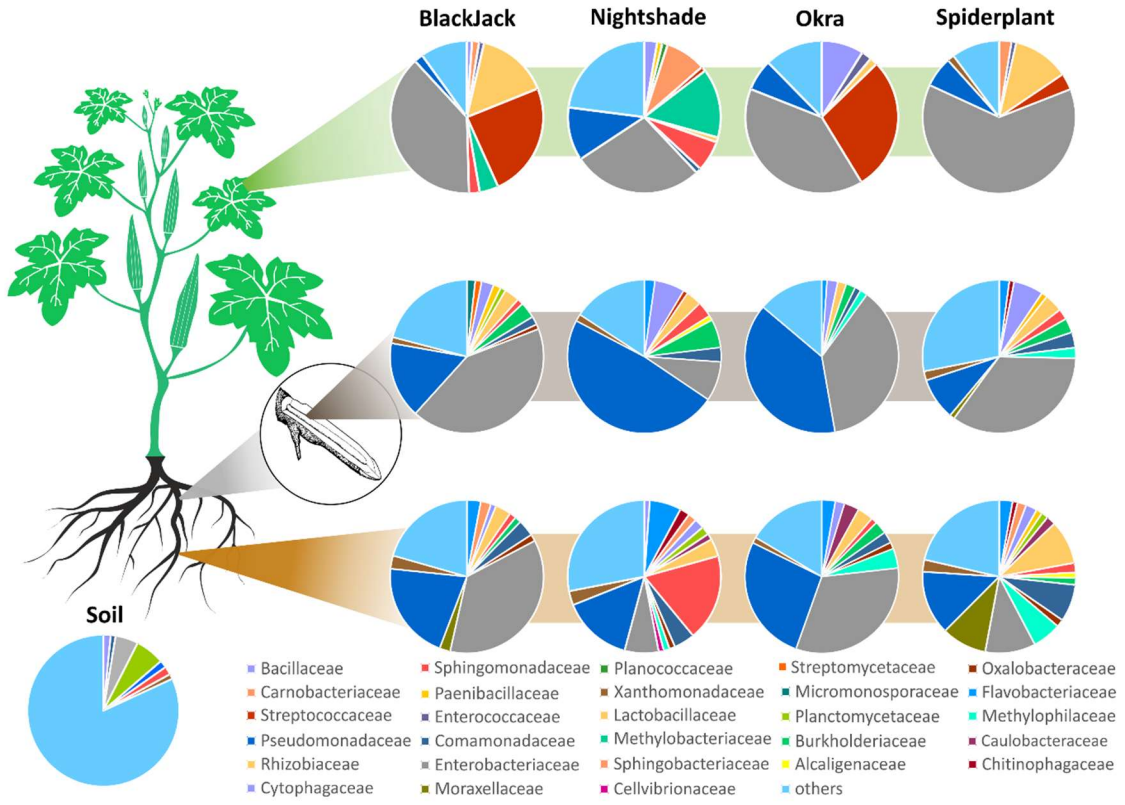

Figure 6: Core microbiome of leafy greens. The composition of the microbiome of Okra, Black Jack, Nightshade and Spiderplant of their following microhabitats are displayed at family level: phyllosphere (green stripe), root-

endosphere (gray stripe) and rhizosphere (brown stripe). Families with abundances below 1% of total microbiome are captured within “others”.

In the phyllosphere of Okra and Black Jack *Streptococcaceae* are dominant with around one fourth of the core microbiome. Black Jack and Spiderplant harboured both *Lactobacillaceae* with over 10% in the phyllosphere. *Enterococcaceae* was broadly spread throughout all habitats. *Bacillaceae* and *Pseudomonadaceae* were part of the core microbiome of each plant in each habitat. Families harbouring plant growth-promoting bacteria, such as *Serratia plymuthica, S. proteamaculans, Pantoea ananatis* belonging to the family *Enterobacteriaceae*, *Pseudomonas trivialis, P. florescens, P. aurantiaca, P. putida, P. tauricus, P. filiscendens* part of *Pseudomonadaceae* and *Bacillus subtilis, B. cereus* from Bacillaceae, were ubiquitous throughout all plants and all habitats. The first two mentioned families, *Enterobacteriaceae* and *Pseudomonadaceae* comprised a larger portion within the core microbiome. *Xanthomonadaceae*, another family, comprising plant growth-promoting bacteria such as *Stenotrophomonas rhizophila* and *S. maltophilia*, is distributed throughout all plants but only found in rhizosphere and root-endosphere. Additionally, *Xanthomonadaceae* were found within the phyllosphere of Spiderplant. *Paenibacillaceae*, the family to which *Paenibacillus amylolyticus* belongs to, was part of the core microbiome of the phyllosphere of Nightshade and of the root-endosphere as well as the rhizosphere of Spiderplant.

Feature-network analysis of core microbiome

Microbial core communities across Nightshade, Okra, Spiderplant and Black Jack were cross-linked based on taxonomic analysis at family level and visualized as a network using Cytoscape. Space was used to depict similarities or dissimilarities of the four plants, based on their core microbiome, where each node represented a taxonomic family. The feature-network is depicted in figure 7.

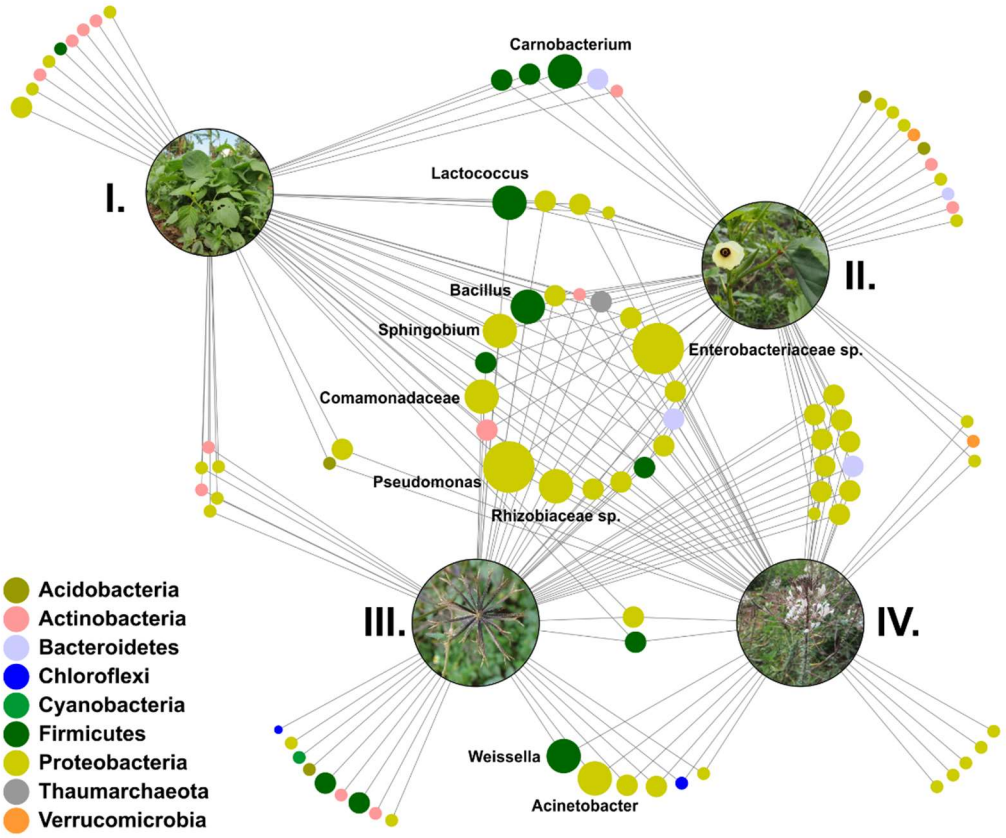

Figure 7: Feature-network based on feature-table and taxonomic analysis at family level. Each node stands for a family of the core microbiome. Cross-linked nodes express families shared between the plants Nightshade, Okra, Black Jack and Spiderplant. Legend: I: Nightshade, II: Okra, III: Black Jack and IV. Spiderplant.

In total 91 features were identified, whereas only one out of them belonged to Archaea. A big core microbiome of 18 families (including the archaeal soil *Crenarchaeotic* group), most assigned to Proteobacteria, were shared between all four plants and additional 11 families, also dominated by Proteobacteria, were common between Okra, Spiderplant and Black Jack, thus communities of Nightshade were further apart. Each plant showed some specific bacterial families that were unique in the core microbiome of the respective plant. The number of such distinctive communities ranged from 5 (Spiderplant; IV) over 9 (Black Jack and Nightshade; III and I) to 11 (Okra; II).

Screening and characterisation of bacterial antagonists against main pathogenic fungi

Bacteria were isolated from four leafy greens thereof three different parts: rhizosphere, root-endosphere and phyllosphere, as well as soil samples from two different sites. These 512 bacterial isolates were then tested regarding their antagonistic activity against plant pathogenic fungi *Botrytis cinerea*, *Fusarium oxysporum*, *Fusarium verticillioides*, *Sclerotium rolfsii* and *Verticillium dahliae*. Bacteria-fungi-interactions under laboratory conditions were quite divers, ranging from bacterial isolates completely overgrown by the fungi over joint growth of bacteria and fungal hyphae to a clear boundary between both, sometimes even accompanied by a shield of metabolites (see supplementary). Out of 512 bacterial isolates, only 108 showed antagonistic activity of category 3 (clear halo between fungi and bacteria of

at least 5 mm) against at least one pathogenic fungi. Out of them only 23 showed strong antagonistic activity against all four fungi. Screening tests results against *V. dahliae* needed different categories as cultivation of the fungi demanded a different procedure and was therefore not included into Venn diagram (Fig. 8; excluding *V. dahliae*).

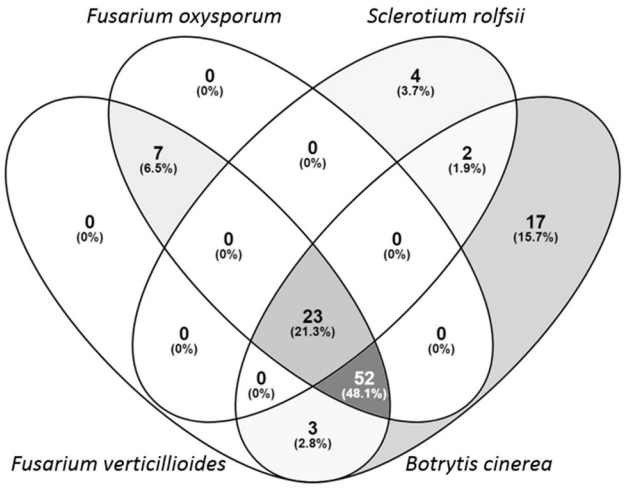

Figure 8: Bacterial isolates with antagonistic effects against fungal pathogens. Fungal pathogens considered are *F. oxysporum*, *S. rolfsii*, *F. verticillioides* and *Botrytis cinerea*. Only bacterial antagonists of category 3 were assigned to their respective fungi.

Additionally, to those 23 isolates, another 52 isolates showed antagonistic effects on *F. oxysporum*, *F. verticillioides* and *B. cinerea* but not on *S. rolfsii.* Overall there was a broad range of the number of active antagonistic isolates regarding their hostplant, ranging from 29 antagonists against *S. rolfsii* to 97 against *B. cinerea.* The two *Fusarium* sp. were antagonized by 82 (*F. oxysporum*) and 85 (*F. verticillioides*). Just 44 highly active antagonists (category 3) against *V. dahliae* could be found. Based on the results of the biotic screening of bacterial antagonists against plant pathogenic fungi, a selection of 24 antagonists, mostly active against all pathogens, was chosen for further characterization. Out of those 24 selected bacterial antagonists, 12 isolates originated from soil samples. Okra harboured 7 antagonistic bacteria, 3 were isolated from Nightshade and 2 from Spiderplant. These plant-associated antagonists were mainly isolated from root-endosphere (9 isolates) and rhizosphere (3 isolates).

Antagonistic isolates were further characterized using BOX-PCR and 16S sequencing, to identify bacterial species based on their specific pattern. After excluding similar replicates, samples were sequenced. 16 isolates were identified as *Bacillus* sp. with suggested species *B. siamensis, B. velenzensis, B. amyloliquefaciens, B. methylotrophicus, B. vallismortis* and *B. subtilis.* The other 8 isolates were assigned to *Sphingomonas* sp. with hits for *S. echinoides, S. glacialis, Shphingomonas* uncultured and uncultured marine bacterium. Combining the alignment results with similarity pattern of BOX PCR bands, isolates were clustered into 5 similarity groups. 5 isolates, one from each similarity group, were selected for whole genome sequencing. An additional 6th strain (originating from Okra's root-endosphere, assigned to *Sphingomonas* sp.) was included for further abiotic stress tests, as a BOX PCR and sequencing did not result in a clear classification.

Abiotic stress assays and phosphate-solubilisation test

In order to characterize the antagonistic bacterial strains to evaluate their potential for application as future biocontrol agent (BCA), abiotic stress tests were conducted. The abiotic stress confrontation assays comprised reactive oxygen species stress tests with tellurite (TeO_2) and hydrogen peroxide (H_2O_2), osmolarity stress with sodium chloride (NaCl), a desiccation assay and a test for the ability to solubilize phosphate. None of the listed isolates showed ability to solubilize phosphate, neither could any of the isolates grow in presence of tellurite (TeO_2) concentrations between 1 and 20 µg/ml. Therefore, those tests were not included into the summarized results of table 5.

Table 5: Abiotic stress confrontation assays. Growth after desiccation was measured by CFU/ml: 0=CFU below 10^5 after drought for 2112h, 1= CFU above 10^5 after drought for 2112h. Reactive oxygen species test performed with hydrogen peroxide (H_2O_2): 0=growth lies below the threshold OD of 0.3. Other values show the highest concentration of H_2O_2, the culture could still tolerate. Osmolarity stress was tested with sodium chloride for various incubation times and concentrations: 0=growth lies below the threshold OD of 0.4. Other values show the highest concentration of NaCl, the culture could still tolerate. Outstanding results are highlighted in grey.

Origin	Microhabitat	Species	Drought	H_2O_2	NaCl 24 h	NaCl 48 h	NaCl 72h	NaCl 6d
Soil	Soil	*Bacillus sp.*	1	0	0	0	8%	6%
Soil	Soil	*Bacillus sp.*	1	2000 µmol	5%	7%	7%	7%
Soil	Soil	*Sphingomonas sp.*	1	0	0	0	8%	11%
Okra	Root-endosphere	*Sphingomonas sp.*	1	100 µmol	0	0	8%	10%
Okra	Root-endosphere	*Sphingomonas sp.*	1	0	0	0	8%	11%
Nightshade	Root-endosphere	*Bacillus sp.*	1	900 µmol	0	0	0%	0

The desiccation assay showed that all tested isolates were highly resistant to drought with CFU/ml of above 10^5 after almost 3 months (2112 h) (Supplementary). The ability to resists reactive oxygen could not be approved for any isolate with a tellurite test. Using hydrogen peroxide for the same purpose, three isolates were able to grow on media with H_2O_2. Whereas the *Sphingomonas* sp. only tolerated 100 µmol one *Bacillus* sp. grew still at a concentration of 2000 µmol H_2O_2. This species, isolated from soil, showed additionally high toleration of sodium chloride, already after 24 h. Other species needed longer to adapt to higher NaCl concentrations and showed tolerance only after an adaption phase of 72 h. To further characterize and investigate the mechanism of antagonism of the isolates, two clamp VOC assays (TCVA) were performed. With this assay, antagonistic effects of bacteria against fungi, based on volatile organic compounds, could not be reproduced. Fungal growth was not restricted significantly (Supplementary). Isolated bacteria identified to antagonize fungal pathogens were additionally tested for their ability to promote plant growth. Therefore, tomato seeds (*Solanum lycopersicum*) were primed with those isolates. The growth performance of seeds primed with bacterial isolates versus sterile plants showed promising

results for some samples (Supplementary). Further tests of bacterial consortia are required for detailed information about plant growth promotion activities.

Abiotic stress tests were additionally performed with antagonistic isolates originating from a previous study on root-knot-nematode infected tomatoes from Uganda, in order to develop a highly effective BCA. Those isolates were effectively tested against the same phytopathogenic fungi (same procedure) and additionally against nematodes. Out of 11 antagonists, 7 were isolated of infected galls, one additional strain from infected rhizosphere, whereas the remaining 3 antagonists were isolated from the rhizosphere of healthy galls. The identified antagonists comprised the families *Pseudomonadaceae* (*P. soli*, *P. koreensis* and *P. monteilli*) *Comamonadaceae* (*Comamonas sediminis*, *Variovorax paradoxus*) and *Bacillaceae* (*Bacillus* sp.). Whereas *Bacillus* sp. was found to have antagonistic effects only against fungi, the other isolates, belonging to *Pseudomonadaceae* and *Comamonadaceae*, showed antagonistic traits against nematodes too. Pseudomonads were all able to solubilize phosphate and showed also resistance to tellurite. Only *Bacillus* sp. resisted drought, and none of the isolates produced volatile-organic compounds, which inhibited fungal growth (detailed results are provided in supplementary).

Antagonistic families within the microbiome

Identified antagonistic families were *Bacillaceae*, *Comamonadaceae*, *Pseudomonadaceae* and *Sphingomonadaceae*. Identified species of those antagonistic families were *Bacillus* sp. (*Bacillaceae*), *Comamonas sediminis and Variovorax paradoxus* (*Comamonadaceae*)*, Pseudomonas koreensis, P. soli and P. monteilli* (*Pseudomonadaceae*) *and Sphingomonas* sp. (*Sphingomonadaceae*). Whereas *Sphingomonas* sp. and *Bacillus* sp. were tested effective against phytopathogenic fungi, the other antagonistic families showed additionally antagonistic traits against nematodes and were isolated from tomatoes. Fungi-antagonizing *Bacillus* sp. are isolated from both, leafy greens and tomatoes. The abundance of all four mentioned families within the microbiome of leafy greens is depicted in figure 9. As a reference, abundance within soil is also shown.

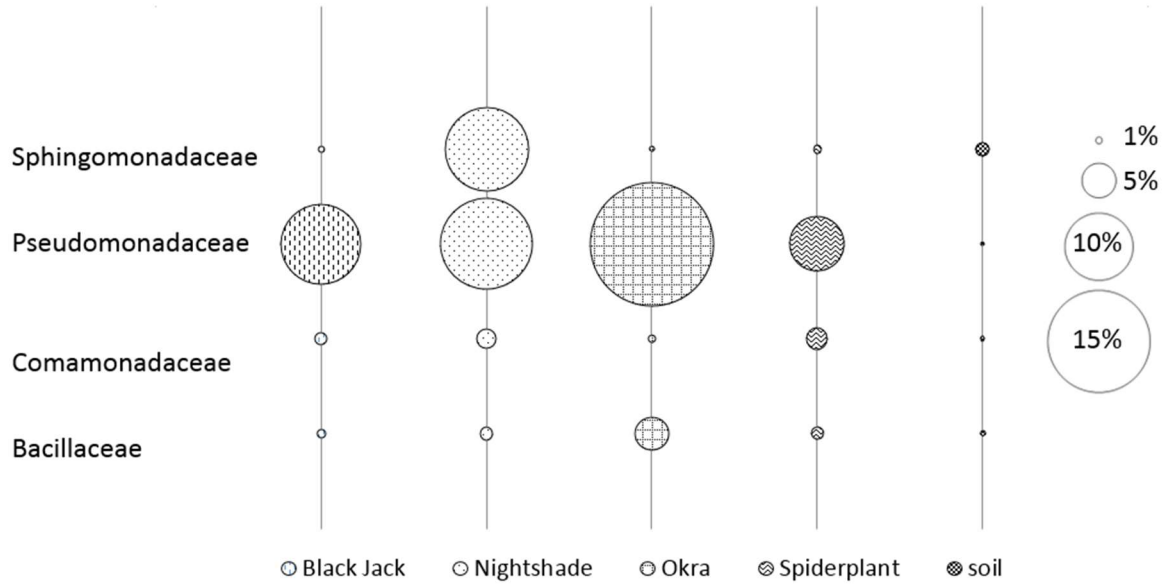

Figure 9: Abundance of antagonistic families. The diameter of the bubbles represents the abundance of each family within the microbiome of leafy greens and soil.

Pseudomonadaceae was the most abundant antagonistic family within the microbiome of each plant, with the highest relative portion in Okra. All antagonistic families were found in the soil but at lower levels, only *Sphingomonadaceae* was relatively more abundant in the soil than in the microbiome of all plants except Nightshade which was showing the highest relative abundance of *Sphingomonadaceae*. Most antagonistic families were relatively enriched within the plant's microbiomes compared to their relative abundance within soil. The microbiome of Nightshade consisted of the highest share of antagonistic families (31.2%), followed by Okra (25.5%), Black Jack (16.3%) and Spiderplant (14.8%). Within soil, antagonistic families comprised only 4.5% of all occurring microorganisms.

Discussion

Diversity indices of leafy greens are higher compared to cultivated crops

The plant associated microbiome is crucial for the fitness and robustness of plants. Plants and their respective microbiome are highly connected through metabolism, hence plants are also able to influence the composition of their microbiome (Garbeva et al. 2004). Even though certain species fulfil certain functions, the microbiome with its interactions between the members is important for plant health. Thus, a higher species diversity is linked to robustness as more metabolic pathways can be performed (Latz et al. 2012). Approving its pivotal role, the microbiome is often referred to as the "second genome" of the plant (Berendsen et al. 2012). To gain insight into the microbial diversity associated with Okra, Nightshade, Spiderplant and Black Jack, the Shannon diversity index H, as universally used term for diversity indices was calculated (Spellerberg and Fedor 2003). In the presented study, neither analysis of microbial diversity nor OTU numbers revealed significant differences between the four leafy greens on the taxonomic level of family. In further studies, species-specificity might be detected within other phylogenetic groups or to be restricted only to endophytes. Black Jack, Nightshade, Okra and Spiderplant grew under the same conditions on soil with the same consistency. All four showed similarities in their microbiome, with respect on diversity, dominant bacterial families and bacterial abundance. Meanwhile the surrounding soil formed distinct clusters and showed a higher diversity by a lower number of bacteria. The composition of microbiomes varied in some parts but had a strong core with irreplaceable key-species. Thus, plant species could not be identified as significant driving force of microbial diversity, but the microbiome was found to be habitat-specific. Besides soil, the highest diversity was found in the rhizosphere followed by root-endosphere and phyllosphere. With respect on bacterial abundance, the highest OTU numbers were found in rhizosphere, followed by root-endosphere and phyllosphere. This indicates that microbes were distinctively enriched by the plant around its roots and microbes further migrated from rhizosphere to endosphere. This specific selection of microbes by the plant is also referred to as "rhizosphere effect". A selective enhancement of certain microbes is conjoined with a loss of overall diversity. Thus the microbiome of rhizosphere is generally less divers than bulk soil (Berendsen et al. 2012), which was also substantiated with the presented study. Additionally to the migration through the plant, microbes from the phyllosphere could be introduced from the surrounding air or through contact with animals (Berg et al. 2016).

Comparing the diversity of phyllosphere from the four leafy greens with cultivated vegetables, like spinach (*Spinacia oleraceae*), the diversity of the cultivated leafy green was lower. The spinach' phyllosphere had a Shannon diversity index H of 3.15 ± 0.51 (Lopez-Velasco et al. 2013), compared to diversity indices of indigenous leafy greens, ranging from 4.40 ± 0.06 (Okra) to 5.74 ± 0.11 (Nightshade) with an average of 4.78 ± 0.08. One of the main cultivated crops worldwide is maize (Development 2013). Its Shannon diversity index for the rhizosphere was found to be 3.42, which was far lower than the diversity of leafy green's rhizosphere (García-Salamanca et al. 2013). Indigenous leafy greens were not overbred as they used to be collected in the wild and just recently found their way into agriculture. The assumption that

intensive agriculture is a main factor for the loss of diversity in the microbiome of crops is suggested. Further our hypothesis was substantiated by studies investigating the influence of alternative agricultural practices. Although a conventional tillage and crop rotation from wheat to field peas resulted into an increased diversity within the rhizosphere (H= 4.43), diversity of rhizosphere of uncultivated leafy greens was still far higher (H= 6.39 ± 0.14 and 7.81 ± 0.21) (Lupwayi et al. 1998). Further organic farming led to increased diversity and abundance of the microbiome compared to conventional farming practices with the employment of pesticides. Comparing organic farming with conventional farming significant differences in the microbiome of corn, melon, pepper and tomato (p-value=0.049) were found (Xia et al. 2015). Comparing the relative species abundance of endophytes, it was consistently higher for organically grown plants than for conventionally grown ones. Those effects of increased richness and decreased evenness within low-input agricultural systems were also seen in soil microbial diversity (Hartmann et al. 2015).

By comparing diversity indices of uncultivated leafy greens from Uganda with crops grown in intensive agriculture, the conclusion that diversity in plant-associated microbiomes gets lost due to intensive agriculture, breeding and cultivation was made. Even efforts in agricultural practices like tillage, crop rotation or organic farming do not achieve comparable diversity indices. The less input is given in agricultural systems, the higher is the diversity of its microbiome. Collected, wildly grown vegetables, like leafy greens, have the highest microbial diversity. Microbial diversity is directly correlated to healthy plants, less vulnerable to pathogenic outbreaks (Berg et al. 2015). The assumption that the high diversity of the leafy greens is mainly contributing to their increased robustness compared to other crops is suggested.

The core microbiome and its colonization pattern

Enterobacteriaceae and *Pseudomonadaceae* were the most dominant taxonomic families within the core microbiome of leafy greens. The dominance of those two taxa was also found in the microbiome of other crops, such as sugarcane (de Souza et al. 2016) or *Zea* (Johnston-Monje and Raizada 2011). The microbiome of most plants is dominated by the four phyla *Firmicutes*, *Bacteriodetes*, *Proteobacteria* and *Actinobacteria*, of which *Proteobacteria* and *Actinobacteria* are important for plant protection against fungal infections (Mendes et al. 2011). *Streptomycetaceae*, belonging to the phyla *Actinobacteria* as well as *Sphingomonadaceae*, *Pseudomonadaceae* and *Enterobacteriaceae*, belonging to *Proteobacteria* were broadly distributed throughout the core microbiome of leafy greens which highlighted the healthy microbiome, capable of protecting the leafy greens from fungal pathogens.

Enterobacteriaceae play an important role within the microbiome of their host plant. Many members of *Enterobacteriaceae* are reported to have plant-growth promoting activities and a potential as biocontrol agent, as they compete pathogens such as *Rhizoctonia solani* (Shoebitz et al. 2009). The dominant distribution of *Enterobacteriaceae* throughout all habitats of Ugandan leafy greens was striking. Plants are able to shape their microbiome by the selective enrichment of microbes from the surrounding soil (Berendsen et al. 2012). Some microbes

could further migrate from the roots to the phyllosphere, or even in the opposite direction top-down. A species of *Enterobacteriaceae*, *Enterobacter asburiae,* was revealed to move from stem to the roots (Johnston-Monje and Raizada 2011). The family *Enterobacteriaceae* also includes human enteric pathogens (Brandl 2006), which is of interest, as leafy greens are also eaten raw and thus humans are in direct contact with the microbes. A small fraction of human enteric pathogens within human diet was recently considered to have health promoting effects through stimulation of the immune system. In previous studies it was emphasized that *Enterobacteriaceae* are ubiquitous in plants and therefore have always been part of human diet. Traditionally, food was neither processed nor sterilized and therefore humans were always in direct contact with the plant's microbes. This everyday contact underlines that the pathogenic role of *Enterobacteriaceae* is a secondary one, contrary to its function of immune stimulant or "natural vaccination" (Berg et al. 2015).

Besides *Enterobacteriaceae*, *Pseudomonadaceae* was a substantial fraction of the microbiome of all four leafy greens, whereas they were relatively more abundant in root-endosphere and rhizosphere compared to phyllosphere. Pseudomonads are well-adapted to colonizing the habitats of soil and rhizosphere. Some selected strains are even endophytic, colonizing the intercellular spaces (Couillerot et al. 2009). Pseudomonads, such as *P. fluorescens*, *P. aeroginosa P. denitrificans*, *P. rathonis*, *P. putida* and *P. tolaasii* besides others, were reported to have plant growth promoting activities, as they positively influence growth, physiology and health of the plant (Hayat et al. 2010). Additionally, *Pseudomonadacea* were broadly researched antagonists of phytopathogenic fungi but to a lesser extend of bacteria or nematodes (Couillerot et al. 2009). Their potential as biocontrol agent is based on their aggressive colonisation of the rhizosphere, where they compete with root pathogens for nutrients and root surface colonisation and produce antimicrobial secondary metabolites (Haas and Défago 2005).

Besides the dominant families mentioned, *Xanthomonadaceae* (*Proteobacteria*), *Bacillaceae* and *Paenibacillaceae* (both *Firmicutes*) were also ubiquitous in the leafy green's microbiomes. *Bacillaceae* were mainly present in phyllosphere and root-endosphere and comprise growth-promoting bacteria such as *Bacillus subtilis*, *B. amyloliquefaciens* and *B. cereus* (Hayat et al. 2010). Furthermore, the families *Xanthomonadaceae* and *Paenibacillaceae* were reported to comprise plant growth-promoting species such as *Stenotrophomonas rhizophila*, *S. maltophilia* and *Paenibacillus amylolyticus* besides others (Hariprasad et al. 2014).

The microbiome is essential for the robustness of plants. In this study it could be proved that the core microbiome of the four leafy greens was not plant specific but matched with healthy microbiomes found in other plants. The core microbiome harboured *Proteobacteria* and *Actinobacteria*, that are reportedly important for plant protection against fungi. Many dominant families, such as *Enterobacteriaceae* and *Pseudomonadaceae*, comprise several species, which are plant-growth promoting and some are even successfully tested as biocontrol agents.

Sphingomonas sp. and *Bacillus* sp. are promising key-species for biocontrol agents

For the robustness of plants, it is important that the microbiome is divers. To unravel key-species, able to antagonize main pathogenic fungi, bacterial isolates from the four leafy greens were tested *in vitro*. Thereby *Bacillus* sp. and *Sphingomonas* sp. could be identified for playing a pivotal role in suppressing *Botrytis cinerea*, *Fusarium oxysporum*, *Fusarium verticillioides*, *Sclerotium rolfsii* and *Verticillium dahlia,* main phytopathogenic fungi causing food insecurity in Uganda.

Bacilli are aerobic, gram-positive bacteria that form highly resistant endospores. They were present throughout all habitats of Black Jack, Nightshade, Okra and Spiderplant. *Bacillus sp.* are reported to have plant growth-promoting properties and to produce antimicrobial substances. *B. subtilis* e.g. produces mycosubtilin, which antagonizes *B. cinerea* and *Fusarium oxysporum* (Leclère et al. 2005), and lipopeptides that harm a variety of pathogenic fungi and bacteria. Another *Bacillus* species, *B. amyloliquefaciens,* has also been shown to have antagonistic effects towards *S. sclerotiorum* and *Fusarium oxysporum* through bacillomycin D, a variant of iturin group of molecules (Koumoutsi et al. 2004; Kumar and Johri 2012).

Sphingomonads are aerobic, gram-negative bacteria, mainly known for their ability to degrade refractory contaminants (White et al. 1996). Therefore, *Sphingomonas* sp. are interesting candidates for bioremediation but may also support plant health by eliminating contaminants from the plant's environment. *Sphingomonas* sp. have also been reported to antagonize *Verticillium dahliae*, causing verticillium wilt, and *Pseudomonas syringae* (White et al. 1996; Innerebner et al. 2011). Further Sphingomonads are effective against several Fusarium species (*F. avenaceum, F. culmorum, F. tricinctum and F. graminearum*) (Wachowska et al. 2013). Endophytic *Sphingomonas* sp. additionally promote plant growth in tomatoes by producing gibberellins (GA) and indole acetic acid. Indole 3-acetic acid (IAA) is a plant hormone, regulating plant growth by stimulating rapid responses of cell division and elongation as well as differentiation of cells and tissue. Different *Bacillus* sp. have also been found to produce GA and IAA (Khan et al. 2014; Chowdappa et al. 2013). Gibberellins are important phytohormones improving agricultural and horticultural productivity with effects on earlier flowering, higher crop yield and bigger fruit size (Khan et al. 2014).

Even though microbiomes are quite complex, *Bacillus* sp. and *Sphingomonas* sp. were identified for their ability to antagonize all five tested fungi. Additionally, many related species are reported for further antagonism and plant growth-promoting activity. This antagonistic activity makes them promising candidates for application against fungal infections in Ugandan agriculture and may be the missing link to robustness of crops towards pests.

Performance of antagonistic isolates under abiotic stresses and development of a biocontrol agent

For better characterizing and evaluating future biocontrol agents, abiotic stress tests were conducted. Microbes, able to tolerate abiotic stresses may also be able to support the robustness of crops. The abiotic stress tests for antagonistic isolates comprised reactive oxygen species stress tests, osmolarity stress, a desiccation assay and a test for the ability to solubilize phosphate. Although strains belonging to the genera *Bacillus* were previously

reported as solubilizers of inorganic phosphate (Hayat et al. 2010), phosphate solubilizers could not be identified among the reported isolates.
During aerobic respiration reactive by-products of O_2 are generated and when exceeding the cell's defence capacity, they cause oxidative stress (Cabiscol et al. 2000). Tellurite and hydrogen peroxide were chosen as trigger for oxidative stress to test for bacterial resistance. Only one *Bacillus* sp. candidate was able to tolerate a higher load of hydrogen peroxide.
In dry and warm regions, the natural soil formation process comes along with salinity. This causes problems for agriculture as salt reduces plant's efficiency to use water and suppresses photosynthesis. Microorganisms capable of dealing with osmolarity stress may also confer resistance in plants to salt stress (Mayak et al. 2004). All candidates were able to grow under saline conditions. Meanwhile an adaption time was needed to tolerate up to 10% salinity.
Episodic drying and re-wetting of soil cause fluctuations in the soil's water potential and challenges microbes. Microorganisms with adaption strategies to low water potentials are favourable for application as biocontrol agents. All the candidates were highly resistant to desiccation.
The described candidates for biocontrol agents were chosen due to their strong antagonistic activities against main pathogenic fungi. Additionally, abiotic stresses such as salinity and desiccation do not cause problems for them *in vitro*. There is still a need to test if those abiotic resistances can enhance the robustness of the host plants *in vivo*.

Conclusion

In the presented study the microbiome of indigenous leafy greens from Uganda was found in general to be significantly more diverse compared to literature values found from cultivated crops. Microbial diversity is directly correlated with plant health. The habitat could be identified to be rather the driving force of microbial diversity than plant species. The core microbiome of robust Okra, Nightshade, Spiderplant and Black Jack harboured microbes with strong antagonistic activities against main pathogenic fungi and mechanisms to stand abiotic stresses. Especially 6 isolates assigned to the families *Sphingomonadaceae* and *Bacillaceae* showed to be promising key-candidates for future biocontrol agents supporting smallholders in rural areas of Uganda. The biocontrol approach is a possibility to reduce or even replace excessive pesticide use for crops in Eastern Africa and support smallholders by ensuring harvest and reducing risks associated with pesticides, for human and environmental health.

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Figure 1: Plant pathogenic fungi on PDA. Botrytis cinerea (A), Fusarium oxysporum (B), Fusarium verticillioides (C), Sclerotium rolfsii (D) and Verticillium dahliae (E). Picture credits Lea Gibitz-Lambert.

Figure 2: Microbial diversity of samples visualized by principal coordinate analysis (PCoA) plots. PCoA plots of the 16S rRNA amplicon datasets of the four leafy greens (Okra, Nightshade, Spiderplant and Black Jack) were constructed based on phylogenetic distance metrics (weighted UniFrac). The distance between the data points negatively correlates with the similarity of the communities. **A** clusters the communities based on habitat (1=soil, 2=rhizosphere, 3=root-endosphere and 4=phyllosphere) and **B** based on organisms (a=nan/soil, b=Nightshade, c=Spiderplant, d=Okra and e=Black Jack).

Figure 4: Microbial diversity of plants within rhizosphere visualized by principal coordinate analysis (PCoA) plots. PCoA plots were constructed based on phylogenetic distance metrics (weighted UniFrac). The closer the points, the more similar the communities. 1=Black Jack (long interrupted lines), 2=Nightshade (short interrupted lines), 3=Spiderplant (dots) and 4=Okra (line-dot-line).

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Figure 6: Core microbiome of leafy greens. The composition of the microbiome of Okra, Black Jack, Nightshade and Spiderplant of their following microhabitats are displayed at family level: phyllosphere (green stripe), root-endosphere (gray stripe) and rhizosphere (brown stripe). Families with abundances below 1% of total microbiome are captured within “others”.

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Figure 10: Microbial diversity of plants within habitats visualized by principal coordinate analysis (PCoA) plots. PCoA plots were constructed based on phylogenetic distance metrics (weighted UniFrac). The closer the points, the more similar the communities. **A** clusters the communities of plants within rhizosphere, **B** within root-endosphere and **C** within phyllosphere. 1=Black Jack (long interrupted lines), 2=Nightshade (short interrupted lines), 3=Spiderplant (dots) and 4=Okra (line-dot-line).

Figure 11: Selection of antagonistic tests. Four different bacterial isolates are streaked onto WA plates with space for fungi in the middle. Antagonistic traits are evaluated due to growth performance of fungi. A = bacterial isolates from phyllosphere of Nightshade and Okra against Fusarium verticillioides, B = bacterial isolates from rhizosphere of Okra against Fusarium oxysporum, C = bacterial isolates from rhizosphere of Okra against Fusarium verticillioides, D = bacterial isolates from soil against Sclerotium rolfsii and E = bacterial isolates from phyllosphere of Black Jack against Fusarium oxysporum. Picture credits: Lea Gibitz-Lambert.

Figure 52: Bacterial growth after desiccation. Growth after 0 h, 24 h, 48 h, 120 h, 168 h, 336 h, 720 h and 1440 h of drought is given in CFU/ml.

Figure 13: Fungal growth under exposure of bacterial VOCs. With the method of two clamps VOCs assay (TCV), fungi are exposed to VOCs produced by bacteria. The growth is depicted in %; 100% growth is the reference value of a control strain growing without exposure to fungal VOCs.

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Figure 15: Growth of tomatoes primed with bacterial isolates. Tomato seeds (*Solanum lycopersicum*) were primed with bacterial isolates originating from Ugandan soil samples (SI-11, SI-14, SI 45), root endosphere from Okra (ORE-30, ORE-44) and root endosphere from Nightshade (NSRE-2). The growth performance of primed plants and sterile plants (K1 and K2) is depicted as harvested g of roots and leaves.

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Table 17: Bacterial growth after desiccation. Growth after 0 h, 24 h, 48 h, 120 h, 168 h, 336 h, 720 h and 1440 h of drought is given in CFU/ml.

Table 18: Abundance of antagonistic families in % of the microbiome.

Table 6: Diversity of habitat samples based on ANOSIM. Distance matrix used is weighted UniFrac.

Habitat	
method name	ANOSIM
sample size	50
number of groups	4
R	0.504
p-value	0.001
number of permutations	999

Table 7: Diversity of samples based on ANOSIM. Habitat samples are compared pairwise. Distance matrix used is weighted UniFrac.

Habitat pairwise						
Group 1	Group 2	Sample size	Permutations	R	p-value	q-value
phyllosphere	rhizosphere	32	999	0.626	0.001	0.002
phyllosphere	root-endosphere	32	999	0.401	0.001	0.002
phyllosphere	soil	18	999	0.763	0.012	0.012
rhizosphere	root-endosphere	32	999	0.333	0.001	0.002
rhizosphere	soil	18	999	0.994	0.006	0.009
root-endosphere	soil	18	999	0.849	0.008	0.010

Table 8: Diversity of plant samples based on ANOSIM. Distance matrix used is weighted UniFrac.

Plants	
method name	ANOSIM
sample size	48
number of groups	4
R	0.048
p-value	0.064
number of permutations	999

Table 9: Diversity of samples based on ANOSIM. Plant samples are compared pairwise. Distance matrix used is weighted UniFrac.

Plants pairwise						
Group 1	Group 2	Sample size	Permutations	R	p-value	q-value
Black Jack	Nightshade	24	999	0.014	0.332	0.398
Black Jack	Okra	24	999	0.041	0.161	0.315
Black Jack	Spiderplant	24	999	-0.014	0.525	0.525
Nightshade	Okra	24	999	0.111	0.049	0.294
Nightshade	Spiderplant	24	999	0.056	0.119	0.315
Okra	Spiderplant	24	999	0.035	0.21	0.310

Microbial diversity within habitats rhizosphere (A), root-endosphere (B) and phyllosphere (C), visualized by principal coordinate analysis.

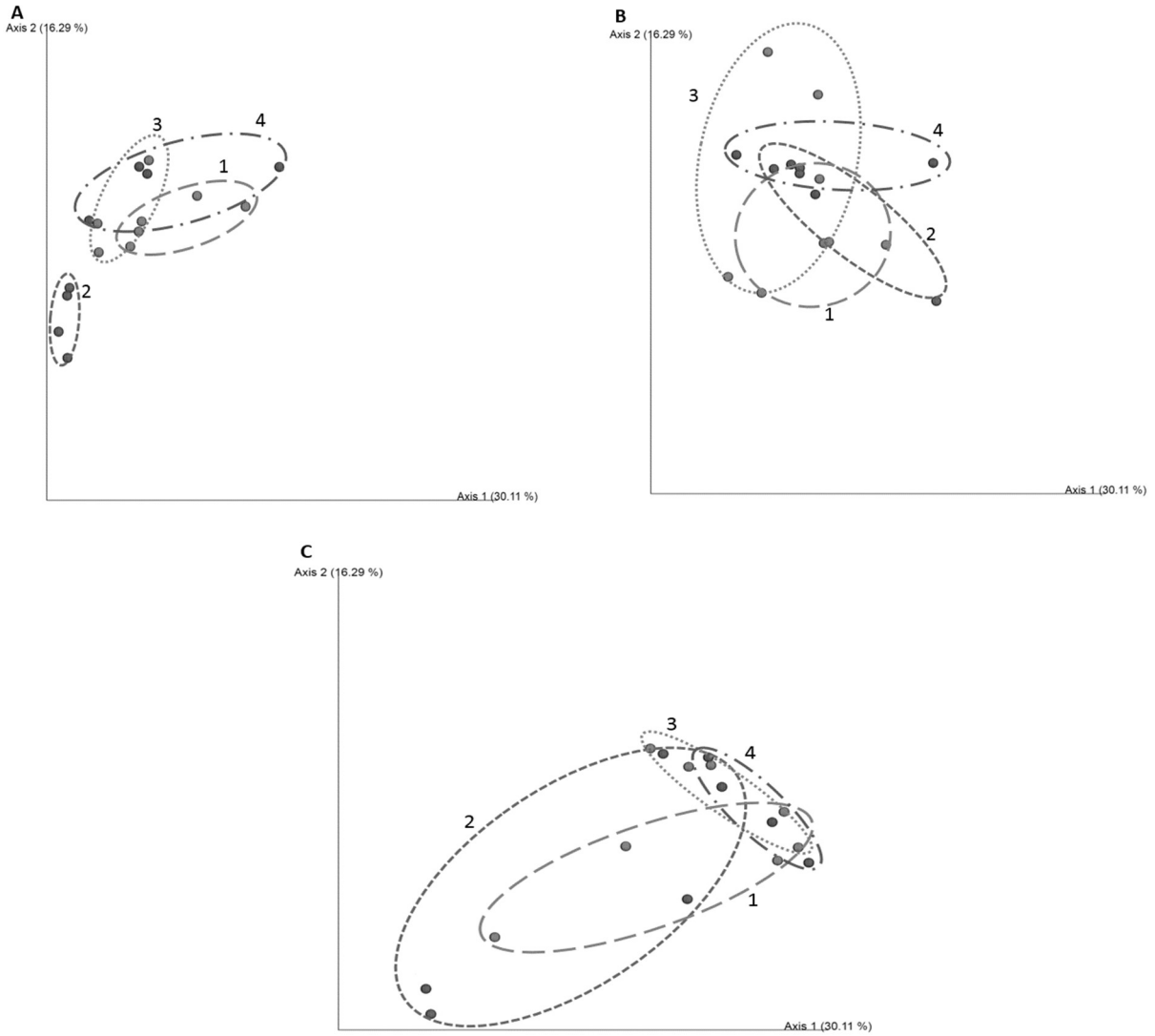


Figure 10: Microbial diversity of plants within habitats visualized by principal coordinate analysis (PCoA) plots. PCoA plots were constructed based on phylogenetic distance metrics (weighted UniFrac). The closer the points, the more similar the communities. **A** clusters the communities of plants within rhizosphere, **B** within root-endosphere and **C** within phyllosphere. 1=Black Jack (long interrupted lines), 2=Nightshade (short interrupted lines), 3=Spiderplant (dots) and 4=Okra (line-dot-line).

ad Composition of the microbiome associated to leafy greens in Uganda

Data behind figure 3 Core microbiome of four leafy greens. Only families exceeding 1% in abundance are listed separately. The rest is summarized under “others”.

Table 10: Microbial abundance within the microbiome of Black Jack. The habitats phyllosphere, rhizosphere and root-endosphere are listed. Values are given in % of the whole community captured.

Black Jack [%]	phyllosphere	rhizosphere	root-endosphere
Alcaligenaceae			
Bacillaceae	1.1	1.1	2.6
Burkholderiaceae		1.3	3.4
Carnobacteriaceae	1.5		

Caulobacteracea			
Cellvibrionaceae			
Chitinophagaceae			
Comamonadaceae		3.5	1.8
Cytophagaceae			
Enterobacteriaceae	38.6	36.2	42.5
Enterococcaceae	1.1		
Flavobacteriaceae		2.9	
Lactobacillaceae	15.3		
Methylobacteriaceae	4.0		
Methylophilaceae			
Micromonosporaceae			1.8
Moraxellaceae		2.3	
Oxalobacteraceae		1.4	1.0
Paenibacillaceae			1.6
Planctomycetaceae			1.1
Planococcaceae			
Pseudomonadaceae	1.9	20.9	16.1
Rhizobiaceae		3.3	3.2
Sphingobacteriaceae		2.2	
Sphingomonadaceae	2.3	1.4	1.3
Streptococcaceae	24.4		
Streptomycetaceae			1.3
Xanthomonadaceae		2.8	1.3
others	10.0	20.6	20.9

Table 11: Microbial abundance within the microbiome of Okra. The habitats phyllosphere, rhizosphere and root-endosphere are listed. Values are given in % of the whole community captured.

Okra [%]	phyllosphere	rhizosphere	root-endosphere
Alcaligenaceae			
Bacillaceae	8.9	2.0	2.3
Burkholderiaceae		2.7	2.0
Carnobacteriaceae			
Caulobacteracea		3.2	
Cellvibrionaceae			
Chitinophagaceae			
Comamonadaceae		2.4	1.3
Cytophagaceae			
Enterobacteriaceae	39.7	32.2	37.2
Enterococcaceae	2.2		
Flavobacteriaceae		2.9	1.1
Lactobacillaceae	1.6		
Methylobacteriaceae			

Methylophilaceae		4.2	1.6
Micromonosporaceae			
Moraxellaceae			
Oxalobacteraceae		1.4	
Paenibacillaceae			
Planctomycetaceae			
Planococcaceae			
Pseudomonadaceae	6.6	26.8	38.9
Rhizobiaceae		3.4	1.8
Sphingobacteriaceae			
Sphingomonadaceae		1.2	
Streptococcaceae	28.5		
Streptomycetaceae			
Xanthomonadaceae		1.2	
others	12.4	16.5	13.8

Table 12: Microbial abundance within the microbiome of Nightshade. The habitats phyllosphere, rhizosphere and root-endosphere are listed. Values are given in % of the whole community captured.

Nightshade [%]	phyllosphere	rhizosphere	root-endosphere
Alcaligenaceae			1.0
Bacillaceae	2.7	2.1	6.3
Burkholderiaceae			6.0
Carnobacteriaceae	8.6		
Caulobacteracea		1.3	
Cellvibrionaceae		1.1	
Chitinophagaceae		2.1	
Comamonadaceae	1.1	4.4	3.0
Cytophagaceae		1.2	
Enterobacteriaceae	27.7	7.2	8.3
Enterococcaceae			
Flavobacteriaceae		6.7	2.2
Lactobacillaceae			
Methylobacteriaceae	14.7		
Methylophilaceae		1.2	
Micromonosporaceae			
Moraxellaceae			
Oxalobacteraceae		1.2	
Paenibacillaceae	1.1		
Planctomycetaceae		1.8	
Planococcaceae	1.1		
Pseudomonadaceae	11.3	14.8	48.3
Rhizobiaceae	1.0	3.7	3.3
Sphingobacteriaceae		1.8	3.3
Sphingomonadaceae	6.5	18.3	

Streptococcaceae	1.0		1.0
Streptomycetaceae			
Xanthomonadaceae		3.0	1.5
others	23.0	28.0	15.8

Table 13: Microbial abundance within the microbiome of Spiderplant. The habitats phyllosphere, rhizosphere and root-endosphere are listed. Values are given in % of the whole community captured.

Spiderplant [%]	phyllosphere	rhizosphere	root-endosphere
Alcaligenaceae		1.1	
Bacillaceae		2.4	6.3
Burkholderiaceae		1.5	3.0
Carnobacteriaceae	2.6		
Caulobacteracea		1.9	
Cellvibrionaceae			
Chitinophagaceae		1.1	1.0
Comamonadaceae		7.9	3.3
Cytophagaceae			
Enterobacteriaceae	62.8	10.6	34.7
Enterococcaceae	1.0		
Flavobacteriaceae		2.8	2.1
Lactobacillaceae	12.0		
Methylobacteriaceae			
Methylophilaceae		6.1	2.3
Micromonosporaceae			
Moraxellaceae		9.5	1.0
Oxalobacteraceae		1.6	
Paenibacillaceae		1.3	1.2
Planctomycetaceae		1.5	
Planococcaceae			
Pseudomonadaceae	6.5	13.5	8.9
Rhizobiaceae		9.3	3.8
Sphingobacteriaceae		1.9	
Sphingomonadaceae		2.0	2.3
Streptococcaceae	1.5		
Streptomycetaceae			
Xanthomonadaceae	1.5	2.5	1.9
others	10.2	21.4	28.1

Table 14: Microbial abundance in soil. Values are given in % of the whole community captured.

soil	**[%]**
Alcaligenaceae	
Bacillaceae	1.6
Burkholderiaceae	
Carnobacteriaceae	
Caulobacteracea	

Cellvibrionaceae	
Chitinophagaceae	
Comamonadaceae	1.0
Cytophagaceae	
Enterobacteriaceae	4.9
Enterococcaceae	
Flavobacteriaceae	
Lactobacillaceae	
Methylobacteriaceae	
Methylophilaceae	
Micromonosporaceae	
Moraxellaceae	
Oxalobacteraceae	
Paenibacillaceae	
Planctomycetaceae	6.2
Planococcaceae	
Pseudomonadaceae	1.6
Rhizobiaceae	
Sphingobacteriaceae	
Sphingomonadaceae	1.8
Streptococcaceae	
Streptomycetaceae	
Xanthomonadaceae	1.1
others	81.9

ad Screening and characterisation of bacterial antagonists against main pathogenic fungi

Figure 11 shows some selected petri dishes, showing the divers interactions of fungi and bacterial isolates.

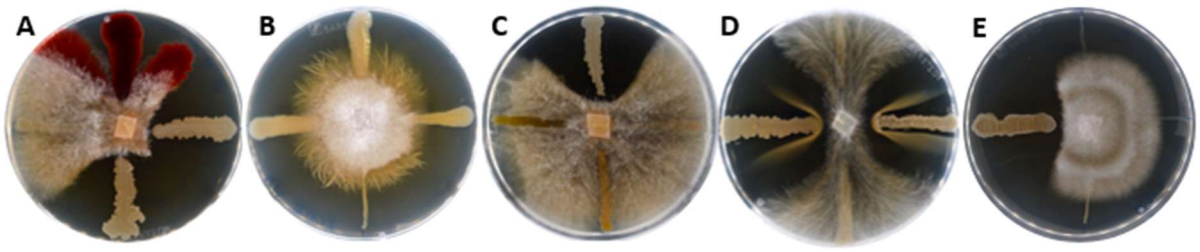
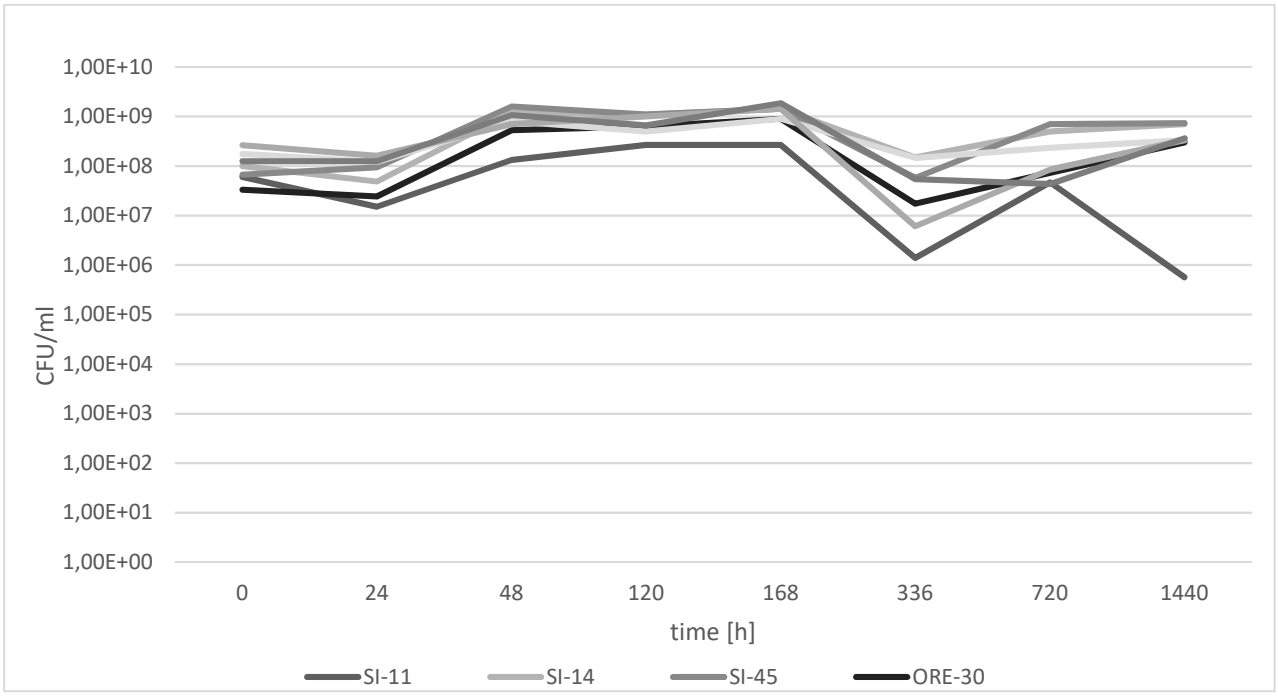


Figure 11: Selection of antagonistic tests. Four different bacterial isolates are streaked onto WA plates with space for fungi in the middle. Antagonistic traits are evaluated due to growth performance of fungi. A = bacterial isolates from phyllosphere of Nightshade and Okra against *Fusarium verticillioides*, B = bacterial isolates from rhizosphere of Okra against *Fusarium oxysporum*, C = bacterial isolates from rhizosphere of Okra against *Fusarium verticillioides*, D = bacterial isolates from soil against *Sclerotium rolfsii* and E = bacterial isolates from phyllosphere of Black Jack against *Fusarium oxysporum*. Picture credits: Lea Gibitz-Lambert

ad Abiotic stress assays and phosphate-solubilisation test

Table 15: Bacterial growth after desiccation. Growth after 0 h, 24 h, 48 h, 120 h, 168 h, 336 h, 720 h and 1440 h of drought is given in CFU/ml.

		0 h	24 h	48 h	120 h	168 h	336 h	720 h	1440 h
SI-11	*Bacillus* sp.	6,06E+07	1,52E+07	1,33E+08	2,67E+08	2,67E+08	1,39E+06	4,67E+07	5,67E+05
SI-14	*Bacillus* sp.	1,00E+08	4,85E+07	1,20E+09	1,10E+09	1,43E+09	1,48E+08	5,00E+08	7,00E+08
SI-45	*Sphingomonas* sp.	6,67E+07	9,39E+07	1,60E+09	1,10E+09	1,43E+09	5,76E+07	7,00E+08	7,33E+08
ORE-30	*Sphingomonas* sp.	3,33E+07	2,42E+07	5,33E+08	6,33E+08	9,00E+08	1,73E+07	7,33E+07	3,00E+08
ORE-44	*Sphingomonas* sp.	1,76E+08	1,21E+08	8,00E+08	5,00E+08	9,00E+08	1,45E+08	2,33E+08	3,33E+08
NSRE-2	*Sphingomonas* sp.	2,64E+08	1,61E+08	7,00E+08	1,00E+09	1,43E+09	6,06E+06	8,48E+07	3,33E+08
NSRE-27	*Bacillus* sp.	1,24E+08	1,24E+08	1,07E+09	6,67E+08	1,87E+09	5,45E+07	4,33E+07	3,67E+08



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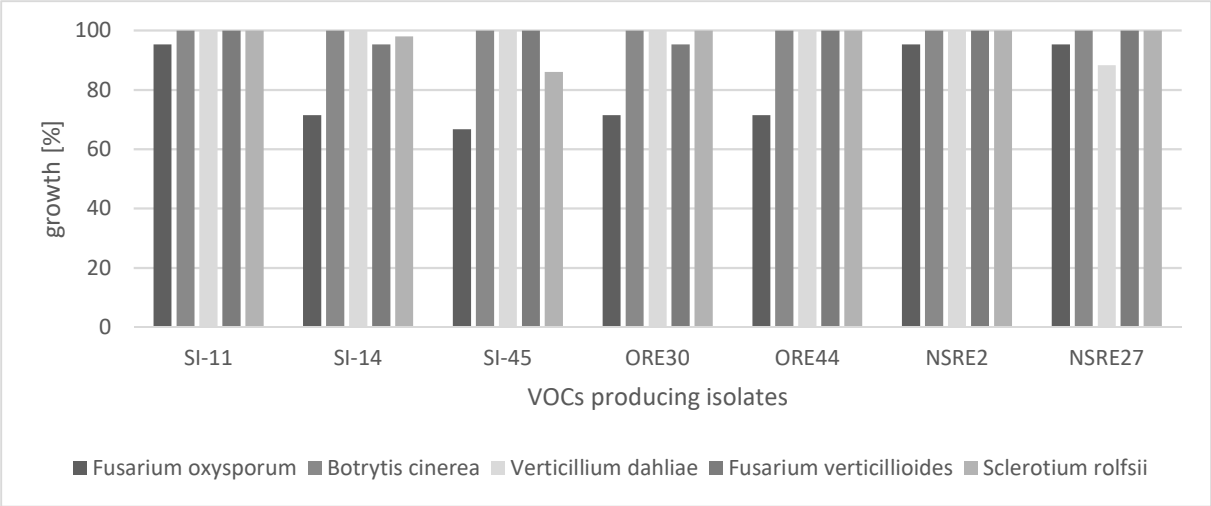

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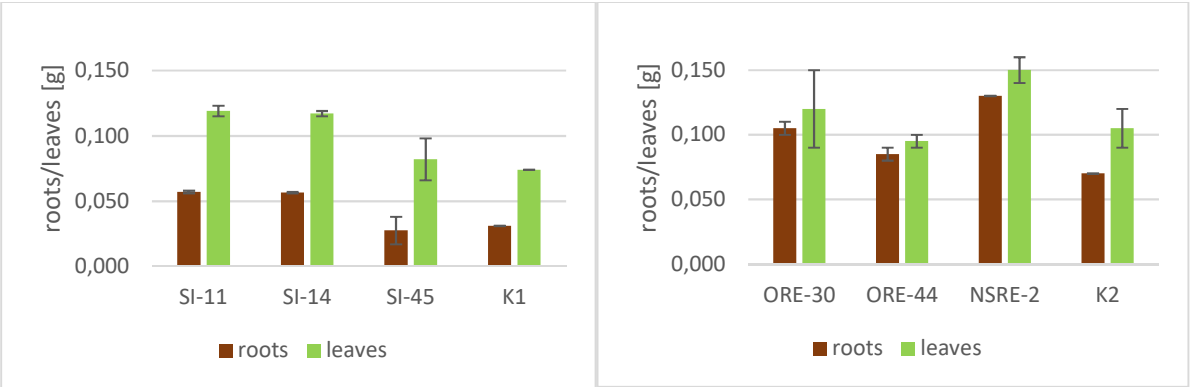

Figure 14: Growth of tomatoes primed with bacterial isolates. Tomato seeds (*Solanum lycopersicum*) were primed with bacterial isolates originating from Ugandan soil samples (SI-11, SI-14, SI 45), root endosphere from Okra (ORE-30, ORE-44) and root endosphere from Nightshade (NSRE-2). The growth performance of primed plants and sterile plants (K1 and K2) is depicted as harvested g of roots and leaves.

Bacterial isolates from tomato:

Table 16: Abiotic stress confrontation assay. The ability to solubilize phosphate was tested on NBRIP agar plates and evaluated after 7 d and 14 d, which resulted in the same: 0=no halo (phosphate solubilizing negative); 1=halo >0,5cm (phosphate solubilizing positive). Reactive oxygen species test performed with hydrogen peroxide (H_2O_2) and tellurite (TeO_2): 0=growth lies below the threshold OD of 0.3. Other values show the highest concentration of H_2O_2/TeO_2 the culture could still tolerate. Growth after desiccation was measured by CFU/ml: 0=CFU below 10^5 after drought for 2112h, 1= CFU above 10^5 after drought for 2112h. Osmolarity stress was tested with sodium chloride for various incubation times and concentrations: 0=growth lies below the threshold OD of 0.4. Other values show the highest concentration of NaCl, the culture can still tolerate. Outstanding results are highlighted in grey.

species	P solub.	TeO_2	H_2O_2	drought	NaCl 24 h	NaCl 48 h	NaCl 72h	NaCl 6 d
P. koreensis	1	9 µg/ml	3800 µmol	0	2%	3%	3%	4%

Comamonas sediminis	1	0	1500 µmol	0	3%	4%	3%	4%
Variovorax paradoxus	0	0	1300 µmol	0	3%	4%	3%	4%
P. soli	1	18 µg/ml	4000 µmol	0	4%	4%	5%	4%
P. monteilli	1	3 µg/ml	4000 µmol	0	4%	5%	3%	4%
P. monteilli	1	5 µg/ml	4000 µmol	0	5%	4%	4%	4%
Bacillus sp.	0	0	900 µmol	1	0	1%	7%	4%
Bacillus sp.	0	0	0	1	0	0	3%	4%
Bacillus sp.	0	0	0	1	0	0	8%	11%
Bacillus sp.	0	0	900 µmol	1	0	0	8%	10%

Table 17: Bacterial growth after desiccation. Growth after 0 h, 24 h, 48 h, 120 h, 168 h, 336 h, 720 h and 1440 h of drought is given in CFU/ml.

	0 h	24 h	48 h	120 h	168 h	336 h	720 h	1440 h
P. koreensis	7,00E+03	1,33E+03	2,00E+02	1,33E+02	1,33E+02	0,00E+00	0,00E+00	0,00E+00
Comamonas sediminis	1,60E+09	2,00E+05	0,00E+00	0,00E+00	1,00E+02	0,00E+00	0,00E+00	0,00E+00
Variovorax paradoxus	1,10E+06	2,33E+03	0,00E+00	0,00E+00	1,67E+02	0,00E+00	0,00E+00	0,00E+00
P. soli	8,33E+05	0,00E+00	2,00E+03	0,00E+00	1,33E+02	0,00E+00	0,00E+00	0,00E+00
P. monteilli	5,33E+05	1,33E+03	0,00E+00	0,00E+00	1,00E+02	0,00E+00	0,00E+00	0,00E+00
P. monteilli	2,00E+05	0,00E+00	0,00E+00	0,00E+00	1,00E+02	0,00E+00	0,00E+00	0,00E+00
Bacillus sp.	1,79E+08	2,00E+08	1,33E+08	1,23E+08	2,03E+09	1,30E+08	6,00E+08	4,00E+08
Bacillus sp.	1,24E+08	8,48E+07	8,00E+08	9,33E+07	2,10E+09	9,09E+07	9,33E+07	2,33E+08
Bacillus sp.	1,12E+08	4,55E+07	4,33E+08	8,33E+07	1,13E+09	1,15E+07	1,10E+08	5,00E+07
Bacillus sp.	2,55E+08	4,55E+07	1,03E+09	1,40E+08	2,00E+09	1,39E+08	7,33E+08	6,00E+08
Bacillus sp.	1,42E+08	1,33E+08	1,00E+08	4,33E+07	1,43E+09	8,48E+07	2,33E+07	2,33E+07

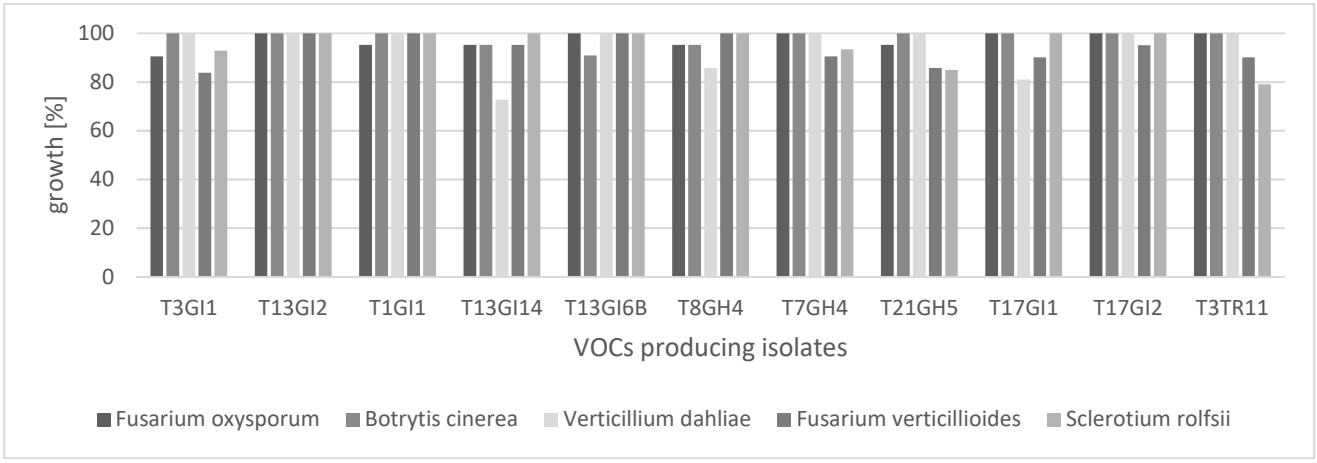

Figure 15: Fungal growth under exposure of bacterial VOCs. With the method of two clamps VOCs assay (TCV), fungi are exposed to VOCs produced by bacteria. The growth is depicted in %; 100% growth is the reference value of a control strain growing without exposure to fungal VOCs.

ad Antagonistic families within the microbiome

Data behind figure 8 abundance of antagonistic families.

Table 18: Abundance of antagonistic families in % of the microbiome.

	Black Jack	Nightshade	Okra	Spiderplant	soil
Bacillaceae	1.4%	2.0%	5.0%	1.9%	0.9%
Comamonadaceae	1.9%	3.1%	1.1%	3.2%	0.8%
Pseudomonadaceae	11.9%	13.6%	18.4%	8.3%	0.8%
Sphingomonadaceae	1.0%	12.5%	0.9%	1.3%	2.0%
total	16.3%	31.2%	25.5%	14.8%	4.5%