Promoting effect of *Fusarium oxysporum* [f.sp. *strigae*] on abundance of nitrifying prokaryotes in a maize rhizosphere across soil types

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Abstract

The integrated application of resistant crop varieties with biological control agents (BCAs) such as the *Fusarium oxysporum* [f.sp. *strigae*] strain “Foxy-2” has shown to be effective in fighting off the weed *Striga hermonthica* which is parasitic to several cereals cultivated in Sub-Saharan Africa (Schaub et al., 2006; Venne et al., 2009). “Foxy-2” proliferates in the rhizosphere and has been mainly studied for its virulence and mode of action. Contrary, no understanding is available regarding its interactions with key rhizosphere microorganisms steering relevant nutrient cycles in soils including nitrogen (N). In this study, we tested the hypothesis that “Foxy-2” displaces indigenous prokaryotic, N cycling communities in the maize rhizosphere due to competition for organic resources. Consequently, we evaluated if the application of an N-rich organic residue (i.e., *Tithonia diversifolia* with C/N ratio = 13, lignin content = 8.9%, polyphenol content = 1.7%) compensates these presumed competition effects. In a rhizobox experiment, quantitative polymerase chain reaction was used to follow the response of rhizosphere ammonia-oxidizing archaea (AOA) and bacteria (AOB) as well as total bacteria and archaea following “Foxy-2” inoculation in two physico-chemically contrasting soils (sandy Ferric Alisol versus clayey Humic Nitisol). Soils were treated with or without “Foxy-2”, *S. hermonthica* seeds, and *T. diversifolia* residues. Contrary to our expectations, we observed a distinct soil texture dependent, promoting effect of “Foxy-2” on rhizosphere prokaryotes. Abundance of AOA and total prokaryotic communities increased in response to “Foxy-2” in the sandy soil, while AOB remained unaffected. This effect on AOA was accelerated when *T. diversifolia* residues were incorporated. Further, in the clayey soil, AOA abundance was promoted when exposed to *S. hermonthica* infestation of maize. This suggested their capability to adapt to this biotic stress situation. It was concluded that “Foxy-2” did not pose a negative effect on targeted indigenous microorganisms, but the underlying mechanisms for the observed promoting effect of AOA abundance by “Foxy-2” inoculation are yet to be understood.

Keywords: *Fusarium oxysporum* [f.sp. *strigae*]; *Striga hermonthica*; Organic resource competition; Nitrifying prokaryotes; Rhizosphere; Quantitative PCR.

1. Introduction

The weed *Striga hermonthica* represents a major constraint to food security in Sub-Saharan Africa (Midega et al., 2013; Packer, 2014). It inflicts yield losses (ranging from 30 to 90%) on staple crops such as maize, pearl millet, sorghum, and rice (Ejeta, 2007; Watson et al., 2007; Rebeka, 2013). In order to reduce the economic impacts caused by *S. hermonthica*, it has been widely accepted that a single control option is not effective, and, hence, a combination of sustainable multi-pronged technologies is recommended (Berner et al., 1996; Hearne et al., 2008; Atera et al., 2012).

Integration of biological control agents (BCAs) and tolerant crop varieties provide effective and environmentally sound benefits for *S. hermonthica* control (Beed et al., 2007; Venne et al., 2009; Rebeka 2013). *Fusarium oxysporum* [f.sp. *strigae*] “Fos” strain “Foxy-2” was approved for biological control of *S. hermonthica* on maize and sorghum and it is one of the most extensively studied model strains of “Fos” (Elzein and Kroschel, 2004; Ndambi et al., 2011; Zarafi et al., 2014). A seed treatment technology for field application of “Foxy 2” has been developed by the University of Hohenheim (Stuttgart, Germany) in collaboration with the seed company SUET Saat-und Erntetechnik GmbH (Eschwege, Germany) (Kroschel et al., 2010; Elzein et al., 2012). “Foxy-2” was reported to be effective in reducing *S. hermonthica* germination by up to 90% under field (e.g., Benin, Burkina Faso, Nigeria) and greenhouse conditions (Ciotola et al., 2000; Elzein et al., 2006; Venne et al., 2009). On the other hand, recent findings have indicated that “Foxy 2” did not achieve biological control of *S. hermonthica* parasitizing maize in Western Kenya further raising questions on the effectiveness and adaptation mechanisms of “Foxy-2” as a BCA (Avedi et al., 2014). Previous studies also reported that “Foxy-2” was specific to its host *S. hermonthica* (Elzein and Kroschel, 2006; Ndambi et al., 2011). Conversely, recent host range studies by Zarafi et al. (2014) found solanaceous crops (e.g., Irish potato, tomato, eggplant) to be susceptible to “Foxy-2”, while biological control of *S. hermonthica* was only realized in cereal crops (e.g.,

sorghum, millet, maize). These contradictory findings disclose that advanced in depth understanding on rhizosphere processes are essential to understand the obvious, but underestimated interactions between “Foxy 2”, crops and root associated soil microorganisms taking also the local environmental conditions such as varying soil types and climate into account (Avedi et al., 2014; Zarafi et al., 2014).

For large scale use of microbial BCAs in agricultural soils, their role and potential impact on the soil ecosystem (e.g., rhizosphere) needs to be thoroughly studied as part of ecological risk assessment (Mezzalama et al., 1997). There has been certain concern regarding the potential of BCAs to displace or suppress resident soil microorganisms with key functions in element cycles (e.g., nitrogen (N)) due to competition for resources in terrestrial ecosystems (Mezzalama et al., 1997; Edel-Hermann et al., 2009; Laurent et al., 2013). Hence, it is essential to assess if microbial BCAs such as “Foxy-2”, which primarily performs in the rhizosphere, results in undesired side-effects on crop root-associated microbial communities. This includes those involved in nitrification (i.e., ammonia oxidation), a central component of soil N cycling (Hallin et al., 2009; Jia and Conrad, 2009; Wessén et al., 2010).

Previous studies on “Foxy-2” have mainly focused on virulence, efficacy, specificity and its mode of action (Schaub et al., 2006; Elzein et al., 2004; Ndambi et al., 2011; Avedi et al., 2014; Zarafi et al., 2014). But there is little information available regarding its potential ecological impacts on key rhizosphere microorganisms steering the N cycle. It has been reported that fungi draw nutrients from their host through the production of growth regulators (Strange, 2005). Accordingly, such altered nutrient availability in a crop rhizosphere inoculated with a fungal BCA such as “Foxy-2” may therefore affect the indigenous soil microbial population (Gullino et al., 1995; Mezzalama et al., 1997; Martinuz et al., 2012a). However, there remains a considerable knowledge gap on the actual influence of “Foxy-2” on ammonia-oxidizing bacteria (AOB) and archaea (AOA) which have been shown to be sensitive to soil disturbance including the exposure to fungi (Pereira e Silva et al., 2013). In

addition, Wessén and Hallin (2011) showed that abundance and community structure of ammonia-oxidizing soil microorganisms were suitable bio-indicators for measuring the effect of soil perturbations and soil health.

A few years ago, the *amoA* gene, encoding the α -subunit of the enzyme ammonia monooxygenase which catalyzes ammonia oxidation, was thought to be unique to AOB (Treusch et al., 2005). Recent findings, however, indicated that AOA also play a major role in ammonia oxidation in agricultural soils (Leininger et al., 2006). Several reports suggested a terrestrial niche differentiation of both AOA and AOB (Jia and Conrad, 2009; Höfferle et al., 2010; Wessén et al., 2010). In this respect, potential niches for AOA were found in low nutrient (e.g., low ammonia substrate conditions) and low pH soils. Contrastingly, AOB responded sensitively to high ammonia concentrations (Valentine, 2007; Erguder et al., 2009; Höfferle et al., 2010).

Accordingly, it can be hypothesized that the saprophytic fitness of “Foxy-2” enabling its survival under low resource conditions may render it aggressively competitive over AOA in low nutrient soils (Elzein et al., 2010). On the other hand, AOB may be competitive against “Foxy-2” in nutrient rich soil environments fertilized with e.g., N-rich organic residues. Hence, there is a critical need to study the potential side-effects of “Foxy-2” on both AOB and AOA to underline that no negative consequences on indigenous microorganisms occur in the soil ecosystem after “Foxy-2” inoculation. The primary objective of this study was thus to assess potential impacts of “Foxy-2” on the abundance of total bacteria and total archaea as well as their ammonia oxidizing counterparts (i.e., AOB, AOA) in *S. hermonthica* affected maize rhizospheres. We compared the effects of “Foxy-2” inoculation alone and its combination with an N-rich organic resource (i.e., *Tithonia diversifolia*) on abundance of respective genes in a low (sandy Ferric Alisol) and high (clayey Humic Nitisol) nutrient resource soil taken from the central highlands of Kenya. We postulated that application of *T.*

diversifolia residues redeems the presumed competition effects of “Foxy-2” via additional N resource availability in soils sustaining the abundance of ammonia oxidizing prokaryotes.

2. Material and methods

2.1 Fungal biocontrol agent

The fungal strain *Fusarium oxysporum* [f.sp. *strigae*] (“Foxy-2”) used in this study was isolated from diseased *S. hermonthica* collected in North Ghana (Abbasher et al., 1995). Identification of the isolate was done by the Julius-Kühn-Institute, Berlin, Germany (accession number: BBA-67547-Ghana). However, the true identity of “Foxy-2” remains debatable as it did not cause disease on *S. hermonthica* (Avedi et al., 2014) but was pathogenic to solanaceous crops (Zarafi et al., 2014). The isolate was preserved at -80°C at the Institute of Plant Production and Agroecology in the Tropics and Subtropics, University of Hohenheim, Stuttgart, Germany.

2.2 Rhizobox experiment

Maize (*Zea mays* L., variety ‘WH507’, provided by Western Seed Company Ltd., Kitale, Kenya) was selected as the test crop as it is preferred by smallholder farmers in Western Kenya due to its tolerance to *S. hermonthica*. Maize seeds were coated with “Foxy-2” (1.15×10^5 colony forming units per seed) using gum Arabic (40%) as an adhesive providing a uniform inoculum coverage (Elzein et al., 2006; SUET GmbH). After coating, coated and uncoated (controls) maize seeds were allowed to germinate on wet filter paper to ensure that only germinated seeds were used for the rhizobox experiment. Meanwhile, *S. hermonthica* seeds were surface sterilized according to Elzein et al. (2010) and germination ability of seeds (75%) was checked as described by Kroschel (2002).

Rhizoboxes (3 x 7 x 20 cm) were filled with dry soils (165 g) derived from two contrasting non *S. hermonthica* infested field sites in the central highlands of Kenya: Embu (0° 30' S, 37°

30' E; 1380 m above sea level (a.s.l.)) and Machanga (0° 47' S, 37° 40' E; 1022 m a.s.l.). Soils differed in physical properties: the Embu soil was a clayey Humic Nitisol (17% sand, 18% silt, 65% clay) derived from basic volcanic rocks, while the Machanga soil was a sandy Ferric Alisol (67% sand, 11% silt, 22% clay) derived from granitic gneisses (FAO, 1998). Each rhizobox was filled with 1 cm ground layer of vermiculite (grain size 3-8 mm) for drainage improvement. On top of this layer, the soil was added and adjusted to 50% water holding capacity.

Both soils were artificially infested with the sterilized *S. hermonthica* seeds (20 mg seeds 165 g dry soil⁻¹). *S. hermonthica* seeds were thoroughly mixed with the moist soils and pre-conditioned at 28°C in the dark for 7 days (Kroschel, 2002). After this step, germinated maize seedlings were introduced into the rhizoboxes. A 1 cm layer of vermiculite was placed as a top layer after planting the maize seedling to reduce evaporation and facilitate water drainage. Boxes were placed in an incubation chamber (12 hours artificial light (1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and darkness at 28/21°C (day/night) for 6 weeks. Two and 4 weeks after incubation start, fertilization with inorganic liquid fertilizer (0.2% Wuxal N-P-K (8-8-6), Aglukon GmbH, Düsseldorf, Germany) was applied to avoid nutrient deficiency. In addition, a treatment with organic residues was included by incorporating air-dried and ground (particle size 1 to 3 mm) leaf and stem material of *T. diversifolia* (1 g dry matter 100 g dry soil⁻¹; 3.2% N) into soils. Non-fertilized treatments were included as controls.

The rhizobox experiment was arranged as a completely randomized design with 6 treatments and 3 replicates each: i) uncoated maize with no *S. hermonthica* (C), ii) uncoated maize and *S. hermonthica* (C+S), iii) coated maize with “Foxy-2” (F), iv) coated maize with “Foxy-2” and *S. hermonthica* (F+S), v) coated maize with “Foxy-2” and *T. diversifolia* (F+T), and vi) coated “Foxy-2”, *S. hermonthica* and *T. diversifolia* (F+S+T).

Rhizosphere samples were taken 14, 28 and 42 days after planting (DAP). For this step, the rhizobox was opened carefully and approximately 2 g of root adhered soil was taken with care

not to destruct the rooting system. Rhizosphere soil was gently scraped off with sterile forceps and transferred into sterile sampling bags. Chemical analyses were done using bulk soil samples at 42 DAP. Rhizosphere soil samples were freeze dried and stored at -20°C until molecular analysis, while bulk soils for chemical analyses were directly maintained at -20°C.

2.3 Quantification of prokaryotic abundance

Four hundred mg of freeze dried rhizosphere soil was used for DNA extraction from each of the 3 replicates per treatment. Soil DNA was extracted using the FastDNA® Spin for Soil Kit (MP Biomedicals, Solon, Ohio, USA) following the manufacturer's instructions. Quality of extracted DNA was checked on 1.5 % (w/v) agarose gels. DNA extracts were quantified (Nanodrop ND-1000, NanoDrop Technologies, Wilmington, USA) and stored at -20°C.

Abundance of both total and ammonia oxidizing prokaryotic communities was determined by DNA-based quantitative PCR (qPCR) using bacterial and archaeal 16S rRNA genes (total community) as well as bacterial (AOB) and archaeal (AOA) *amoA* genes as molecular markers (Table 1) (Rasche et al., 2011). For standard preparation, amplicons from each target gene were purified (Invisorb Fragment CleanUp kit, Stratec Molecular GmbH, Berlin, Germany), ligated into the StrataClone™ PCR cloning vector pSC-A (Stratagene, La Jolla, CA, USA) and ligation products were transformed with StrataClone Solopack competent cells (Stratagene).

The qPCR assays were carried out in a 25 µl reaction containing 12.5 µl of Power SYBR® green master mix (Applied Biosystems, Foster City, CA, USA), 1 µl primer (each 0.4 µM), 0.25 µl T4 gene 32 protein (500 ng µl⁻¹, MP Biomedicals) and 10 ng template DNA. For Machanga soils, 20 ng were used as template for quantification of AOB and AOA abundance. The qPCRs were performed on a StepOnePlus™ Real-Time PCR System (Applied Biosystems) and were started with 10 min at 95°C, followed by amplification cycles specific for each target gene (Table 1). Melting curve analysis of amplicons was conducted to confirm

that fluorescence signals originated from specific amplicons and not from primer-dimers or other artifacts. Each DNA sample was processed in triplicate reactions, whereas the standard curves were generated using duplicate serial dilutions of isolated plasmid DNA containing the genes studied (Rasche et al., 2011).

2.4 Measurement of soil chemical parameters

Total carbon (TC), total nitrogen (N_t), extractable organic C (EOC), extractable N (EON) and pH of soils were recorded using bulk soils from each rhizobox shortly after the last rhizosphere soil sampling (DAP 42). The pH analyses were conducted in a soil water ratio of 1:2.5 using a pH meter (inoLab® Labor-pH-Meter, WTW GmbH, Weilheim, Germany). TC and N_t were quantified by dry combustion (vario MAX CN analyzer, Elementar Analysensysteme GmbH, Hanau, Germany). For EOC measurement, 5 g of soil were extracted with 20 ml 0.5 M K_2SO_4 , shaken horizontally (250 rpm) for 30 min and filtered (Rotilabo-Rundfilter AP55.1 (retention of 2-3 μm), Carl Roth GmbH). EOC concentration in filtered extract was measured on an Analytik Jena Multi N/C 2100 analyzer (Analytik Jena AG, Jena, Germany). Ammonium (NH_4^+) and nitrate (NO_3^-) were extracted with 1 M KCl (soil to extractant ratio (w/v) of 1:4), shaken on a horizontal shaker for 30 min at 250 rpm and filtered (Rotilabo-Rundfilter AP55.1, Carl Roth GmbH). Concentrations of NH_4^+ and NO_3^- were measured on an auto-analyzer (Bran & Luebbe, Norderstedt, Germany) (Mulvaney, 1996), while EON was determined as the difference between N_t and mineral N (NH_4^+ and NO_3^-) according to Rousk and Jones (2010).

2.5 Statistical analysis

Statistical analyses were performed using R software (Software R 3.0.1, R foundation for Statistical Computing, Vienna, Austria, <http://www.R-project.org>). To assess effects of “Foxy-2”, organic N addition (*T. diversifolia*), *S. hermonthica*, sampling time and soil type on

abundance of assayed genes and soil chemical parameters, a multifactorial ANOVA was done in R. Homogeneity of variance and normality were tested on the residuals of each model. Logarithmic transformations were performed when required. Least squares means comparison between soil types, treatments and sampling time was done using the Tukey's range test ($P < 0.05$). For graphical visualization, gene abundance data were shown without standard errors due to back transformation of the least square means. In addition, Pearson's linear correlation coefficients were calculated for assessing relations between abundance of studied genes with soil chemical parameters.

3. Results

3.1 Microbial gene abundance

3.1.1 Total archaeal and bacterial communities

In the sandy soil (i.e., Machanga), archaeal 16S rRNA gene abundance in F and F+S treatments remained constant during the incubation period, while that in C and C+S decreased over time (Fig. 1A). Gene copies in soils treated with "Foxy-2" and *T. diversifolia* (F+S+T and F+T) were higher than treatments without *T. diversifolia* (C, C+S and F+S) in the sandy soils 28 days after planting (DAP) ($P < 0.05$). In the clayey soil (i.e., Embu), "Foxy-2" treatments (F, F+S) and the addition of the N-rich organic residue (i.e., F+S+T, F+T) did not influence the abundance of archaeal 16S rRNA genes ($P > 0.05$).

Total bacterial 16S rRNA gene copy numbers were promoted by F+S in comparison to C in the sandy soil ($P < 0.05$; Fig. 1C). On the contrary, gene copies in treatment F under the same soil were similar to C and C+S. Treatments with *T. diversifolia* addition (F+S+T, F+T) strongly increased gene copy numbers in comparison to treatments that did not receive *T. diversifolia* (i.e., C, C+S, F, F+S). Similarly, in the clayey soil, F+S+T and F+T increased total bacterial abundance across all sampling time points although to a lesser extent ($P < 0.05$;

Fig. 1D). On the other hand, total bacterial abundance in C+S and C did not show any difference ($P>0.05$).

3.1.2 Archaeal *amoA* gene abundance

Archaeal *amoA* gene copies showed higher abundance in F compared to C and C+S in the sandy soil (Fig. 2A). Similarly, F+S+T and F+T revealed increases of archaeal *amoA* gene abundance in comparison to C, C+S and F+S ($P<0.05$). However, when combined with *S. hermonthica* (F+S), there were no significant differences between F+S, C and C+S ($P>0.05$). There were also no differences in archaeal *amoA* gene copy numbers between C and C+S ($P>0.05$).

In the clayey soil, F had higher archaeal *amoA* gene copy numbers compared to C, F+S and F+S+T ($P<0.05$; Fig. 2B). An exception was at 14 DAP, where a transient decline was observed. F+T had higher *amoA* gene abundance in comparison to C, F+S and F+S+T at the last two sampling dates ($P<0.05$). Treatment F+S+T was not different from C and F+S at 28 and 42 DAP ($P>0.05$). Archaeal *amoA* gene abundance remained stable in *S. hermonthica* infested soils (C+S) and was higher than C, F, F+S and F+S+T ($P<0.05$).

3.1.3 Bacterial *amoA* gene abundance

In the sandy soil, bacterial *amoA* gene abundance was not influenced by F or F+S compared to C and C+S ($P>0.05$; Fig. 2C). Overall, bacterial *amoA* gene abundance was by tendency higher in F+S+T than in C, C+S and F+S. Treatment F+T only showed higher abundance at 14 DAP in comparison to C ($P<0.05$). Only at 14 DAP, there was an abundance difference between C and C+S in bacterial *amoA* gene abundance ($P<0.05$).

In the clayey soil, bacterial *amoA* gene abundance in F and F+S treatments was not different from C and C+S ($P>0.05$; Fig. 2D). However, a different pattern was observed at incubation

end with a lower abundance in the F treatment than in C, C+S, F+S and F+S+T ($P<0.05$). No difference was determined between C+S and C ($P>0.05$).

3.2 Treatment effects on soil chemical parameters

Addition of *T. diversifolia* residues (F+S+T and F+T) increased soil pH in both sandy and clayey soils ($P<0.001$; Table 2). In comparison to the initial soil pH (4.70 and 4.74 for the clayey and the sandy soil, respectively), soil pH remained unchanged under C, C+S, F, F+S treatments ($P>0.05$). A similar pattern was observed for TC and N_t (Table 2). With respect to EON and EOC, F+S+T and F+T showed in both soils higher values compared to C, C+S, F and F+S (Table 2). EOC in the clayey soil was lower under F+S than F, F+T and F+S+T ($P<0.001$), but not different from both controls (C, C+S) ($P>0.05$; Table 2). NH_4^+ contents were higher in treatments F+S+T and F+T than in C, C+S, F, and F+S in the sandy soil ($P<0.001$), while in the clayey soil, F+S+T and F+T revealed higher NH_4^+ in comparison to C+S and F+S ($P<0.01$; Table 2). In the clayey soil, NO_3^- contents were higher in C and F than C+S and F+T ($P<0.01$), while no treatment effects were determined for NO_3^- in the sandy soil ($P>0.05$).

3.3 Linear correlations between gene abundance and soil chemical parameters

Linear correlations between gene abundance and soil chemical parameters were calculated at 42 DAP (Table 3). TC, N_t , EOC and EON were positively correlated with bacterial 16S rRNA gene copy numbers in both soils. In addition, archaeal 16S rRNA gene copies revealed a positive correlation with N_t and EON in the sandy soil. NH_4^+ correlated positively with bacterial and archaeal 16S rRNA gene abundance in the sandy soils. On the contrary, negative correlations were determined in the clayey soil between NH_4^+ and NO_3^- with bacterial *amoA* gene copy numbers, while in the sandy soil, NO_3^- was positively correlated with archaeal *amoA* gene abundance. Soil pH revealed positive correlations with bacterial 16S rRNA gene

copies in both soils, while bacterial *amoA* genes only showed positive correlation with soil pH in the sandy soil.

4. Discussion

Fusarium oxysporum [f.sp. *strigae*] strain “Foxy-2” has been undergoing an extended evaluation for possible commercialization as a mycoherbicide in Sub-Saharan Africa. The four main components of this evaluation include (1) its ability to suppress *S. hermonthica* in varying agro-ecological conditions that was only achieved in Western Kenya (Avedi et al., 2014), (2) its non-pathogenicity to maize and other cereal crops (Avedi et al., 2014; Zarafi et al., 2014), (3) its non-pathogenicity to other food crops which is not the case for solanaceous crops that succumb to its infection (Zarafi et al., 2014), and (4) lack of negative impacts on indigenous soil microorganisms with beneficial functions colonizing the rhizosphere of the host crop, which was the subject addressed in the presented study.

4.1 Effect of “Foxy-2” on abundance of indigenous rhizosphere prokaryotes

Contrary to our expectations, abundance of nitrifying prokaryotes and particularly that of archaea increased in “Foxy-2” treatments in the assayed maize rhizosphere treatments. However, the observed effects differed greatly depending on soil type and organic N fertilization (i.e., *Tithonia diversifolia* residues). Further, we did not observe the hypothesized resource competition effect between the abundance of indigenous rhizosphere prokaryotes and “Foxy-2”.

AOA were apparently better adapted to coexist with “Foxy-2” in the less fertile sandy soils as noted by a consequent abundance boost. This finding was supported by Valentine (2007) who showed that archaea and their nitrifying counterparts (i.e., AOA) possess a higher competitive advantage under reduced organic resource availabilities. Accordingly, we found positive

correlations between AOA abundance and “Foxy-2” propagation (J. Zimmermann, personal communication).

In contrast to the sandy soil, we determined only minor differences of archaeal abundance in the clayey soil (i.e., Embu) providing a greater resource pool of soil organic matter. This was corroborated by higher TC and EOC contents in the clayey soils offering an important resource back-up sufficiently fueling both, indigenous rhizosphere archaea and also “Foxy-2” (Chivenge et al., 2011; Rasche et al., 2014). Bacterial abundance increased when organic N was added. This corresponded to other studies observing similar responses of bacterial abundance to easily available organic resources (Wessén et al., 2010).

Overall, we did not observe the hypothesized reduced abundance of indigenous rhizosphere prokaryotes by “Foxy-2” which suggested a likely niche differentiation, but not a direct resource competition (Valentine, 2007; Nicol et al., 2008, Wessén et al., 2010). On the other hand, we point out that the mechanisms driving the differences in prokaryotic gene abundance may include other, indirect drivers such as induced plant systemic resistance (i.e. production of secondary metabolites) and altered root exudation patterns of the maize crop after “Foxy-2” inoculation (Martinuz et al., 2012b; Wang et al., 2013). However, these speculative interpretations require further research to clarify the mode of actions of AOA responses to “Foxy-2” inoculation in crop rhizospheres.

4.2 Imperative considerations when implementing “Foxy-2” as BCA

There has been increasing concern that research on *F. oxysporum* including “Foxy-2” and other potential BCAs provided inconsistent information on their performance and safety (Ochieno, 2010; Avedi et al., 2014). Recent findings from an efficacy study on “Foxy-2” by Avedi et al. (2014) did not achieve biological control of *S. hermonthica* parasitizing maize in Western Kenya. Their report raised questions on effectiveness, mechanisms and conditions under which “Foxy-2” performs as a BCA. One of the major limitations of seed treatment

with microorganisms is their narrow range of climatic or agronomic conditions as indicated in a recent review by Parker (2014). Given that the strain was originally isolated from Ghana; its inefficiency in Western Kenya may be explained by such limitations. Further adaptation studies are hence required which will contribute to advanced understanding on the behavior of “Foxy-2” in foreign environments.

There exist also certain concerns that some misidentified strains of *F. oxysporum* could be intentionally used as “agro-terrorist weapons” against crop production around the world (Suffert et al., 1999; Avedi et al., 2014). Most recent host range studies on “Foxy-2” (Zarafi et al., 2014) reported that this strain is also pathogenic to solanaceous crops implying that it is not highly specific to *S. hermonthica* as was earlier suggested (Ciotola et al., 1995; Elzein and Kroschel, 2006; Ndambi et al., 2012). These contradicting results have led to further questioning of the true identity of the strain (Avedi et al., 2014). Accordingly, it was recommended to avoid intercropping of “Foxy-2” treated maize with selected solanaceous crops (Zarafi et al., 2014).

Moreover, it was suggested that “Foxy-2” undergoes further re-identification as it may not be a true *forma specialis* attacking *S. hermonthica* (Avedi et al., 2014). Re-identification of “Foxy-2” as well as identification of other *Fusarium* sp. strains re-isolated from diseased solanaceous crops will contribute to a better understanding on the inconsistency surrounding the specificity and identity of “Foxy-2”. This will be a critical prerequisite to use this strain as BCA for *S. hermonthica*. Moreover, additional identification studies will determine if “Foxy-2” is actually suitable for release to farmers by addressing the raised concerns (Ajanga and Avedi, 2013; Avedi et al., 2014).

Based on the results of the present study, “Foxy-2” is a suitable candidate for release to farmers in Africa as we did not observe the hypothesized resource competition effect between indigenous rhizosphere prokaryotes and “Foxy-2”. This implied that “Foxy-2” had no negative ecological impacts in relation to other microorganisms.

5. Conclusions

Based on our results, we concluded that “Foxy-2” is safe for selected members of the rhizosphere microbial community such as nitrifying prokaryotes emphasized in this study. Further, the capability of archaeal communities (total and AOA) to respond more sensitively to treatments than their bacterial counterparts supported their particular application as indicators for assessing perturbations in soils (Wessén and Hallin, 2011; Pereira e Silva et al., 2013).

Although negative effects of “Foxy-2” on non-target rhizosphere microorganisms were not determined in this study, its future application as BCA for *S. hermonthica* control has to undergo additional evaluation including a broader range of soils with contrasting physico-chemical characteristics to corroborate our assumptions. These future studies should also consider crop species other than maize (e.g., sorghum) and different cultivars of the same crop as these may pose distinct effects on microbial community dynamics in the rhizosphere (Grayston et al., 1998; Soderberg et al., 2002; Rasche et al., 2006).

The present study was based on a short-term, controlled rhizobox experiment. To fully understand the ecological effects of “Foxy-2” and its behaviour in cropping fields and its long-term persistence in soils, similar “Foxy-2” exposure experiments should be conducted under natural field conditions to account for relevant factors such as crop development, a broader range of (organic) fertilization regimes, as well as seasonal characteristics including rainfall and temperature patterns (Cavaglieri et al., 2009; Aira et al., 2010; Murphy and Foster, 2014).

Finally, the presented study was focused on the abundance of selected prokaryotic communities. It can be assumed that “Foxy-2” may, apart from abundance, also influence the composition of targeted prokaryotic populations. In this respect, we recommend advanced studies on potential effects of “Foxy-2” on the community composition of non-target

microbial populations in crop rhizospheres using molecular fingerprinting techniques (Rasche et al., 2006).

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Figure captions

Figure 1. Abundance of the total archaeal and bacterial community in the sandy (a, c) and clayey (b, d) soils as determined during the six weeks of incubation in the rhizobox experiment. Different letters within each graph at each sampling date indicate significant differences between treatments ($P < 0.05$). Treatments are: C, uncoated maize with no *S. hermonthica*, C+S, uncoated maize and *S. hermonthica*, F, coated maize with “Foxy-2”, F+S, coated maize with “Foxy-2” and *S. hermonthica*, F+T, coated maize with “Foxy-2” and *T. diversifolia*, F+S+T, coated “Foxy-2”, *S. hermonthica* and *T. diversifolia*.

Figure 2. Abundance of the archaeal and bacterial *amoA* genes in the sandy (a, c) and clayey (b, d) soils as determined during the six weeks of incubation in the rhizobox experiment. Different letters within each soil type at each sampling date indicate significant differences between treatments ($P < 0.05$). Treatments are: C, uncoated maize with no *S. hermonthica*, C+S, uncoated maize and *S. hermonthica*, F, coated maize with “Foxy-2”, F+S, coated maize with “Foxy-2” and *S. hermonthica*, F+T, coated maize with “Foxy-2” and *T. diversifolia*, F+S+T, coated “Foxy-2”, *S. hermonthica* and *T. diversifolia*.

Table captions

Table 1. Description of primer sets, PCR ingredients and amplification details used for qPCR analysis.

Table 2. Soil chemical parameters (mean $\pm$ standard deviation, n = 18) obtained from the rhizobox experiment. Different letters within columns indicate significant differences (P<0.05).

Table 3. Pearson's linear correlation coefficients between prokaryotic gene abundance (16S rRNA gene, *amoA* gene) and soil chemical data at 42 DAP.

Table 1.

Target group	Primer set	Thermal cycling profile	Reference
Bacterial 16 S rRNA gene	Eub338f	40 cycles	Lane (1991)
	Eub518	95°C 30s, 55°C 35s, 72°C 45s	Muyzer et al. (1993)
Archaeal 16S rRNA gene	Ar109f	40 cycles	Lueders and Friedrich (2000)
	Ar912	95°C 30s, 52°C 35s, 72°C 45s	Lueders and Friedrich (2000)
Bacterial <i>amoA</i> gene	amoA-1f	45 cycles	Rotthauwe et al. (1997)
	amoA-2r	95°C 45s, 57°C 60s, 72°C 60s	Rotthauwe et al. (1997)
Archaeal <i>amoA</i> gene	Arch-amoAF	45 cycles	Francis et al. (2005)
	Arch-amoAR	95°C 45s, 53°C 60s, 72°C 60s	Francis et al. (2005)

Table 2.

Soil	Treatment	TC [g kg ⁻¹]	N _t [g kg ⁻¹]	EOC [mg kg ⁻¹]	EON [mg kg ⁻¹]	NH ₄ ⁺ [mg kg ⁻¹]	NO ₃ ⁻ [mg kg ⁻¹]	pH
Clayey soil (Embu)	C	16.9 ±	1.51 ±	572 ±	1484 ±	3.9 ±	23.4 ±	4.5 ±
	C+S	17.2 ±	1.43 ±	583 ±	1426 ±	2.8 ±	3.9 ±	4.7 ±
	F	16.9 ±	1.47 ±	589 ±	1424 ±	6.8 ±	22.0 ±	4.6 ±
	F+S	17.4 ±	1.43 ±	551 ±	1419 ±	2.9 ±	10.1 ±	4.8 ±
	F+T	18.0 ±	1.57 ±	619 ±	1556 ±	6.4 ±	3.6 ±	4.9 ±
	F+S+T	18.6 ±	1.59 ±	619 ±	1581 ±	5.2 ±	4.3 ±	4.9 ±
Statistics		***	**	**	*	**	**	***
Sandy soil (Machanga)	C	3.70 ±	0.35 ±	103 ±	342 ±	3.6 ±	1.99 ±	4.7 ±
	C+S	3.88 ±	0.36 ±	109 ±	356 ±	3.7 ±	1.05 ±	4.7 ±
	F	3.67 ±	0.36 ±	100 ±	354 ±	3.5 ±	2.07 ±	4.7 ±
	F+S	4.06 ±	0.38 ±	110 ±	373 ±	5.1 ±	1.04 ±	4.8 ±
	F+T	5.64 ±	0.57 ±	207 ±	557 ±	15.0 ±	3.80 ±	5.6 ±
	F+S+T	5.46 ±	0.53 ±	194 ±	514 ±	8.7 ±	0.78 ±	5.7 ±
Statistics		***	**	***	**	***	n.s.	***

^aAbbreviations: TC, Total carbon, N_t; Total nitrogen, EOC; extractable organic carbon, EON; Extractable organic nitrogen, NH₄⁺; ammonia, NO₃⁻; nitrate, pH; soil pH.

^bSignificance levels: n.s.: P>0.05; *P<0.05; **P<0.01; ***P<0.001.

^cTreatment codes: uncoated maize with no *S. hermonthica* (C), uncoated maize and *S. hermonthica* (C+S), coated maize with “Foxy-2” (F) and coated maize with “Foxy-2” and *S. hermonthica* (F+S), as well as coated maize with “Foxy-2”, *S. hermonthica* and *T. diversifolia* (F+S+T) and without *S. hermonthica* (F+T).

Table 3.

Soil	Target gene	TC [g kg ⁻¹]	N _t [g kg ⁻¹]	EOC [mg kg ⁻¹]	EON [mg kg ⁻¹]	NH ₄ ⁺ [mg kg ⁻¹]	NO ₃ ⁻ [mg kg ⁻¹]	Soil pH
Clayey soil (Embu)	Total	0.84***	0.71**.	0.59*	0.73**	n.s.	n.s.	0.85***
	Total	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Bacterial	n.s.	n.s.	n.s.	n.s.	-	-	n.s.
	Archaeal	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Sandy soil (Machanga)	Total	0.90***	0.87***	0.91***	0.91***	0.83***	n.s.	0.99***
	Total	n.s.	0.52*	n.s.	0.51*	0.61*	n.s.	n.s.
	Bacterial	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.54*
	Archaeal	n.s.	n.s.	n.s.	n.s.	n.s.	0.53*	n.s.

^aAbbreviations: TC, Total carbon, N_t; Total nitrogen, EOC; extractable organic carbon, EON;

Extractable organic nitrogen, NH₄⁺; ammonium, NO₃⁻; nitrate.

^bSignificance levels: n.s.: P>0.05; *P<0.05; **P<0.01; ***P<0.001.

Rhizobox experiment	Control (C)	Foxy-2 (F)	<i>Striga hermonthica</i> (S)	F + S	<i>Tithonia diversifolia</i> (T) + F	F + S + T
Bacterial 16S rRNA gene	—	—	—	—	▲	▲
Archaeal 16S rRNA gene	—	—	—	—	—	—
Bacterial <i>amoA</i> gene (AOB)	—	—	—	—	—	—
Archaeal <i>amoA</i> gene (AOA)	—	▲	—	—	▲	—

▲ = Abundance stimulation; — = No treatment effect