Review Paper

A meta-analysis of the effectiveness of diverse rhizobia inoculants on soybean traits under field conditions

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ABSTRACT

Soybean is the most widely grown legume in the world. A beneficial feature of soybean is its ability to associate with rhizobia bacteria in its root nodules to fix atmospheric nitrogen through symbiotic nitrogen (N) fixation. Here, we provide a meta-analysis of 28 peer-reviewed studies examining the effectiveness of diverse rhizobia inoculants under field conditions, with the goal of understanding the underlying factors that affect inoculant success or failure. The data demonstrate that a diversity of *Bradyrhizobium* and *Sinorhizobium* species/isolates can be effective inoculants, including some indigenous strains. These inoculants varied in their efficacy for nodule number (-28 to $+178$ nodules), grain yield (-34% to $+109\%$), and grain-N yield (-6% to $+176\%$) compared to uninoculated controls. The greatest increase in nodule numbers occurred when background nodulation by indigenous soil rhizobia was absent or extremely low. Some studies demonstrated that indigenous rhizobia strains may be better adapted to local environmental stress conditions compared to introduced rhizobia, suggesting native rhizobia may have potential for local commercialization. There was a positive but moderate correlation between inoculant-mediated increases in nodulation and grain yield/grain-N. Moderate pH conditions were critical for inoculants to improve nodulation. Inoculant success was affected by the soybean genotype and soybean x rhizobia strain interactions, inoculant titre, formulation, and application method. Potential additional explanations from the literature for the failure/success of inoculants pertain to the persistence of rhizobia (inoculants) in soil, since seed-coated rhizobia colonize roots through the rhizosphere, and furthermore stress may favor indigenous, locally-adapted competitors. Rhizobia survival in soil is strain dependent and affected by soil organic matter, nutrients, pH, salinity, agricultural practices (e.g. organic, no till, rotations, application of pesticides) as well as temperature and drought. We conclude by proposing new studies to fill current research gaps.

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1. Introduction

Soybean (*Glycine max* (L.) Merrill) originated in China and has been cultivated for more than 5000 years (Li et al., 2008). It is the top legume crop globally, representing 50% of the world's legume crop growing area (Herridge et al., 2008). Furthermore soybean is the top source of plant based protein (Nishinari et al., 2014). Major soybean producing countries in the world are the United States, Brazil, Argentina, China, and India (Biate et al., 2014; Chang et al., 2015). Soybean fixes atmospheric nitrogen through symbiotic nitrogen fixation (SNF), resulting in 16.4 Tg of nitrogen (N) fixed

annually, accounting for 77% of the total N fixed by legume crops (Herridge et al., 2008). On average, SNF can meet 50—60% of the N demand of soybeans (Salvagiotti et al., 2008).

The process of SNF of soybean is complex, mediated by chemical communication between rhizobia and soybean to facilitate nodule formation and nitrogen fixation. Soybean plants produce isoflavones (genistein, daidzein) through the phenylpropanoid pathway which are released to the rhizosphere, where they trigger the nodulation (*nod*) genes in rhizobia (Subramanian et al., 2006). Once the *nod* genes are activated, rhizobia-to-plant signaling is mediated by the release of nodulation (*nod*) factors (D'Haeze and Holsters, 2002), causing root hair deformation wherein root hairs trap the rhizobia between the cell walls (Gage, 2004). Rhizobia enter into the plant roots with the initiation of an infection thread, which grows and directs bacteria towards the inner cortex of the roots (Jones et al., 2007). The infection thread releases bacteria into

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the cortical cells of the nodule primordium enveloped by a plant-derived membrane called the peribacteroid membrane (Mylona et al., 1995). Soybeans produce determinate type nodules, defined as nodules that lose meristematic activity after they initiate (Subramanian et al., 2007). Within each nodule, the released bacteria differentiate into bacteroids, which are the basic N fixing units enclosed by the peri-bacteriod membrane (PBM) and termed the symbiosome. Rhizobia multiply inside soybean root nodules, and some of the rhizobia can enter back into the soil once nodules senesce (Denison and Kiers, 2011).

For decades, it was thought that soybean could form nodules only in association with *Bradyrhizobium japonicum* (Rodríguez-Navarro et al., 2010). However, in time it was reported that soybean can also be nodulated by different species of *Bradyrhizobium*, as well as *Rhizobium*, *Mesorhizobium*, and *Sinorhizobium fredii* (Biate et al., 2014). Soybean nodulators include both slow growing rhizobia (*Bradyrhizobium* spp.) and fast growing rhizobia (e.g. *R. tropici*, *R. oryzae* and *M. tianshanense*) (Neves and Rumjanek, 1997; Hungria et al., 2001; Biate et al., 2014). According to de Almeida Ribeiro et al. (2015), currently there are 29 *Bradyrhizobium* species described in the literature: *B. japonicum*, *B. elkanii*, *B. liaoningense*, *B. yuanmingense*, *B. betae*, *B. canariense*, *B. denitrificans*, *B. pachyrhizi*, *B. jicamae*, *B. iriomotense*, *B. cytisi*, *B. lablabi*, *B. daqingense*, *B. huanghuaihaiense*, *B. oligotrophicum*, *B. rifense*, *B. arachidis*, *B. retamae*, *B. diazoefficiens*, *B. ganzhouense*, *B. paxllaeri*, *B. icense*, *B. manausense*, *B. ingae*, *B. valentinum*, *B. neotropicale*, *B. ottawaense*, *B. erythrophlei*, and *B. ferriligni*. The efficiency of SNF in soybean depends on the type of rhizobia strain that occupies the root nodules. Rhizobial strains vary in their nodulation ability, N fixation efficiency, compatibility with different crop cultivars, and adaptations to local environmental conditions (Biate et al., 2014).

Inoculation of soybean with rhizobia inoculants helps to improve soybean yield with low financial risk, as inoculants are cheaper than inorganic N fertilizers (Ronner et al., 2016). Soybean is increasing in importance in many developing countries, where poverty limits fertilizer use (Giller et al., 2011; Thilakarathna and Raizada, 2015). According to Giller (2001), there are three situations where introduction of rhizobia are necessary to ensure effective nodulation and SNF: (1) in the absence of compatible rhizobia; (2) when there is a low population of compatible rhizobia resulting in slow nodulation; and/or (3) ineffective or less effective indigenous rhizobia than the selected inoculants for a particular legume host variety. Inoculants may be especially required when soybean is introduced into a new geographic area, as compatible rhizobia may not be available in the soil. For example, when soybean was first introduced to North America (1765) and South America (1880–1882) (Chang et al., 2015), SNF was very low due to the lack of compatible and efficient rhizobia in local soils. However, with the introduction of promiscuous soybean varieties (those that nodulate freely with indigenous soil rhizobia), soybean may now be introduced into different environments even in the absence of suitable inoculants (Mpepereki et al., 2000).

Inoculant response is directly proportional to the available mineral N in the absence of local soil rhizobia (Thies et al., 1991a). However, inoculants also vary in their ability to compete against local rhizobia (Alves et al., 2003), as nodule occupancy varies between different rhizobia strains (Thies et al., 1992). Ineffective local rhizobia can compete with introduced rhizobia, leading to lower inoculant nodule occupancy (Sanz-Sáez et al., 2015). It was found that at least 66% of nodules must be occupied by a rhizobia inoculant in order to show a yield response in the presence of ineffective local rhizobia (Thies et al., 1991b). Furthermore, introduced rhizobia may not survive in the soil system due to competition from local, free living rhizobia, thus frequent inoculation may be required. However, the need for re-inoculation depends on the environment and particular rhizobia strain (Hungria and Vargas, 2000; Giller, 2001; Albareda et al., 2009b).

The native soil rhizobia population and its effectiveness for SNF can be highly variable between different soils (Singleton and Tavares, 1986; Thies et al., 1992, 1991b). Models based on analyses of field trial data suggest that the yield response is inversely related to the titre of the indigenous, compatible soil rhizobia population (Thies et al., 1991a). Therefore inoculation with effective rhizobia may not always improve SNF in some soil conditions, especially when the soil rhizobia population is above 20 cells per gram of soil and the population contains some effective strains (Singleton and Tavares, 1986). Thies et al. (1991b) have shown that rhizobia inoculants only show SNF or a positive yield response when the native rhizobia population is below 50 cells per gram of soil. The authors observed an 85% yield increase when the soil rhizobia population was below 10 cells per gram of soil.

The success of an introduced inoculant also depends on the quality of the inoculant (Rodríguez-Navarro et al., 2010; Ronner et al., 2016), wherein critical for successful nodulation are the number of viable rhizobia per unit of inoculant and the number of introduced rhizobia that result in root infection. Since the mobility of rhizobia in soil is limited under real field conditions, inoculation methods must ensure that sufficient rhizobia are present around the seeds for successful nodulation (Giller, 2001). However, the plant demand for N is determined by the yield potential of a crop in a given environment. If the N demand of soybean can be matched by the indigenous rhizobia population, inoculation with even efficient rhizobia strains may not show any improvement in yield or SNF (Thies et al., 1991a, 1991b). We have summarized the situations where inoculants are necessary and how legumes respond to inoculants under different soil conditions (Fig. 1) based on several key observations from the literature.

The survival and persistence of rhizobia are affected by soil and environmental factors (Hungria and Vargas, 2000). The survival of rhizobia in soil has been shown to be affected by extreme soil pH, desiccation, nutrient deficiencies, salinity/alkalinity, extreme temperatures, toxicities (Zahran, 1999; Hungria and Vargas, 2000; Giller, 2001), and predation by protozoa (Danso et al., 1975). Interestingly rhizobia can survive in the soil through formation of biofilms on biotic or abiotic surfaces (Hirsch, 2010; Denison and Kiers, 2011). The survival and titre of rhizobia in soil are positively correlated with soil clay content, percentage carbon, and moisture availability (Zengeni et al., 2006). Application of organic manure improves the survival of rhizobia in soil by improving the soil carbon and moisture content, thus minimizing the need for repeated inoculation (Zengeni et al., 2006). Screening and selection of rhizobia strains for different abiotic stresses under laboratory conditions may not work under field conditions, rather the laboratory findings provide possible explanations for the different SNF responses by rhizobia strains under abiotic stresses (Giller, 2001).

The genotypic variability among different rhizobia strains isolated from soybean growing soils and root nodules has been characterized primarily using molecular taxonomic analysis: Rep-PCR (Loureiro et al., 2007), RAPD characterization (Hungria et al., 2006), DNA microarrays (Itakura et al., 2009), amplified rDNA restriction analysis (ARDRA), multilocus sequence analysis (MLSA) (Li et al., 2011), BOX-PCR, Enterobacter repetitive intergenic consensus (ERIC) assays (Saldaña et al., 2003), 16S rDNA sequencing (Li et al., 2008), horizontal, fluorophore-enhanced, repetitive extragenic palindromic-PCR (HFERP) DNA fingerprinting (Wongphatcharachai et al., 2015), taxonomic analysis of various housekeeping genes (de Almeida Ribeiro et al., 2015), and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (Noel and Brill, 1980). Further sequencing of different symbiosis-requiring genes, including *nodA*,
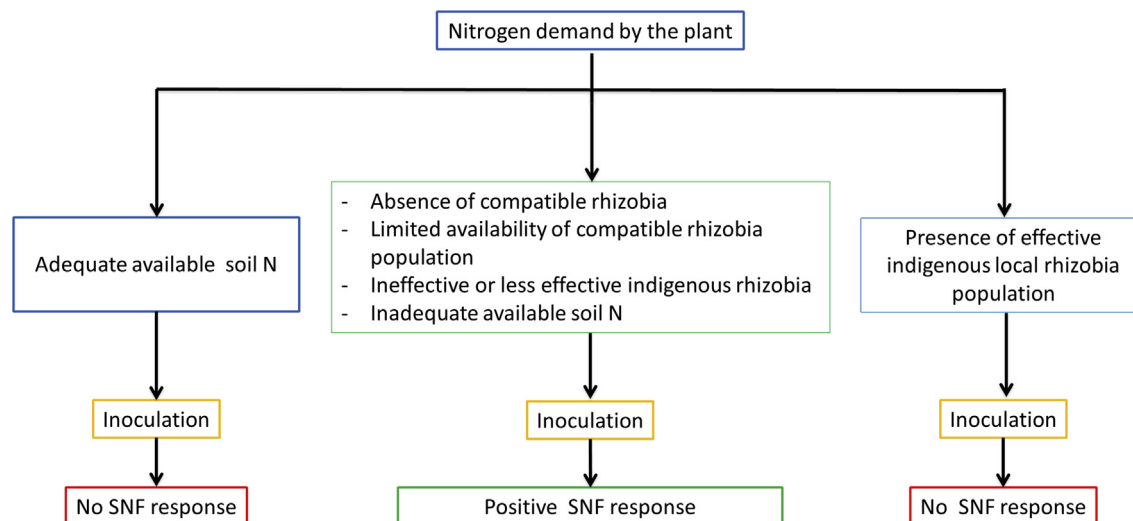
Fig. 1. Conceptual model for predicting the symbiotic nitrogen fixation (SNF) response of legumes to inoculation with rhizobia.

nodD1, *nifH* and *nifD* (Risal et al., 2010; Li et al., 2011; Youseif et al., 2014), and antibiotic resistance testing (Dowdle and Bohlool, 1985) have also been used to characterize soybean nodulating rhizobia.

There are a limited number of field trials in the literature that have tested the large diversity of soybean nodulating rhizobia now available. The objective of this paper is to provide a meta-analysis of peer-reviewed studies (since 1980) that have examined the effectiveness of different rhizobial strains on soybean traits under field conditions, with the goal of understanding the underlying factors that affect inoculant success or failure. Robust studies were selected that directly compared multiple rhizobia strains to background native soil rhizobia under field conditions across a variety of climatic zones. The reader is also encouraged to look at previous, excellent reviews related to this topic (Hungria and Vargas, 2000; Mpepereki et al., 2000; Van Kessel and Hartley, 2000; Giller, 2001; Alves et al., 2003; Salvagiotti et al., 2008; Miransari et al., 2013).

2. Effectiveness of different rhizobia strains on nodulation, yield and grain nitrogen content in soybean under field conditions

We have summarized the major findings of 28 field studies in Table 1, which compares the effect of different rhizobia strains on nodulation and yield traits in soybean under field conditions organized by the climatic zone. Most of these studies note that soybean had not been grown at the test sites in previous years. The nodule and yield values reported here were from treatments in which N fertilizer was not added. From these findings, we have outlined the effect of different rhizobia strains on soybean nodulation (Supplementary Fig. 1A) (14 studies, representing 45 rhizobia strains), grain yield (Fig. 2) (26 studies, representing 97 rhizobia strains), and grain N content (Supplementary Fig. 1B) (12 studies, representing 34 rhizobia strains). For normalization, the effects of different rhizobia strains on yield and grain N content were calculated as the percentage increase or decrease over the corresponding uninoculated control.

With respect to nodule number, the effects of the inoculants ranged from the average nodule number per plant decreasing by 28 to the nodule number increasing by 178, in comparison to uninoculated control plants (Supplementary Fig. 1A). There was high variability for nodulation between locations even for a particular

rhizobia strain. As an example, plants inoculated with standard *B. japonicum* strain USDA 110 showed increases in the number of nodules per plant that ranged from +7 to +106 relative to the uninoculated control. The impact on soybean grain yield ranged from −34% to +109% compared to uninoculated controls (Fig. 2). The impact of inoculants on soybean grain N yield was observed to be highly variable based on the rhizobia strain introduced and the particular study, ranging from −6% to +176% compared to the uninoculated controls (Supplementary Fig. 1B). These results show that although many reported rhizobia inoculants have the potential to show dramatic impacts on soybean traits in the field, they may fail or have negative impacts in other cases (McLoughlin et al., 1991; Wiersma and Orf, 1992; Bai et al., 2003), and it is possible that many other negative field trial results are simply unreported in the literature.

2.1. Factors affecting the efficacy of rhizobia inoculants in field soybean

To elucidate the underlying causes of the variability in soybean response observed following inoculation, a meta-analysis was performed in which the data was sorted based on potential causes (presence of compatible indigenous rhizobia in soil, soil pH, soil-N, available phosphorus). Nodulation numbers were used to measure the impact of rhizobia objectively and in part because this trait is typically reported in the literature in contrast to direct measures of N fixation. However it is important to note that nodule number is only one factor that contributes to final N fixation (e.g. along with nodule biomass, rhizobia activity) and hence has limited value.

2.1.1. Rhizobia taxonomy

The largest fraction of reported field studies employed *B. japonicum*, along with other *Bradyrizobium* species. A few studies employed *Sinorhizobium* (*Ensifer*) or *Rhizobium*, and a number of studies used local isolates, which were not taxonomically described (Fig. 2). The data suggests that a diversity, albeit limited, of rhizobia species can be effective inoculants. Sinorhizobia inoculants in the field increased crop response traits to a similar extent (yield) or possibly greater extent (nodule number, seed N) than *Bradyrhizobium* inoculants (Fig. 2, Supplementary Fig. 1). When *B. japonicum* inoculants were used, compared to uninoculated controls, the change in nodule numbers per plant ranged from −28 to +106

Table 1
Effect of rhizobia strains on soybean growth, nodulation, yield, and symbiotic nitrogen fixation under field conditions.

Area/Country	Soil characters	Rhizobia strains	Inoculation method	Major findings	References
Temperate region					

Madison, USA	Silty clay loam	- *B. japonicum* strains
		  - I17
		  - 110
		  - 61A76
		- Indigenous strain 0336
			Seeds coated with finely ground peat inoculants.
			5.9 g (low rate) − 31.4 g (high rate) of inoculant per 800 g seeds.
				- Generally strain I17 was more competitive than other strains, and strain 61A76 was better than strain 110 in terms of nodule occupancy.
				- Indigenous strain 0336 was very competitive and formed 80% of the nodules occupied by the indigenous strains.
					Kamicker and Brill (1987)

Maryland, USA	Adelphia fine sandy loam classified as Aquic Hapludult
	Total soil N (TSN) = 0.25 g N kg^{-1} soil
		- *B. japonicum* strains
		  - I-110ARS
		  - TA-11NOD$^+$
			5 m row received one liter of a 5×10^{-2} dilution of rhizobia culture containing 6.0 or 5.5×10^7 cells per ml^{-1}	- In comparison to strain I-110ARS, plants inoculated with TA-11NOD$^+$ produced a higher nodule number (36 *vs.* 25 plant^{-1}), nodule mass (65 *vs.* 44 mg plant^{-1}), nodule occupancy (33 *vs.* 20%), nitrogenase activity (1.6 *vs.* 1.0 μmol h^{-1} plant^{-1}), shoot dry weight (4.8 *vs.* 3.5 g plant^{-1}), and seed yield (3007 *vs.* 2827 kg ha^{-1}). Altered indole biosynthesis in TA-11NOD$^+$ was possibly associated with the higher observed nodulation and SNF.	Kuykendall et al. (1996)

QC, Canada	Chicot fine sandy loam	- *B. japonicum* 532C - *B. japonicum* USDA 30 - *B. japonicum* USDA 31	20 ml bacteria solution (YEM) per 1 m row was applied on seeds along the furrow. (10^8 cells ml^{-1})	- Soybean plants inoculated with USDA 30 and 31 strains increased the nodule number (91−94 *vs.* 81−83 nodules plant^{-1}), nodule dry weight (450−467 *vs.* 402−416 mg plant^{-1}), shoot N yield (319−336 *vs.* 286−291 kg ha^{-1}), and SNF (152−161 *vs.* 134−146 kg N ha^{-1}) compared to strain 532C at the R4 growth stage of soybean.	Zhang et al., 2003)
QC, Canada	Chateauguay clay loam and Chicot fine sandy loam	- *B. japonicum* 532C - *B. japonicum* USDA110	Inoculants sprayed into open furrows (10^8 cells ml^{-1}, 1 ml per seed)	- Although both the rhizobia strains improved the total weight, seed weight, total plant N, and seed N compared to uninoculated control, no significant difference found between the two *Bradyrhizobium* strains.	Bai et al. (2003)

Ontario, Canada	London loam
	Available soil N (ASN) = 11−13 mg NO$_3^-$ kg^{-1}soil
		- *B. japonicum* strains
		  - 532C
		  - USDA 110
		  - CB 1809
		- *S. fredii*- HH303
			Granular inoculants (1.3×10^7 - 9.4×10^7 g^{-1} inoculant)	- Significant strain-by-genotype interactions were found for seed yield and grain protein content.
				- 11−17% higher seed yield was recorded with strain 532C compared to the USDA 110 and CB 1809.
				- Lower yield was associated with HH303 compared to USDA 110 and 532C.
					Ravuri and Hume (1992)

Northwest Minnesota, USA		- *B. japonicum* strains			Wiersma and Orf (1992)

Location	Soil	Strains	Inoculation method	Results	Reference
	Silt clay, silt loam, coarse loam, sandy loam, fine loam $ASN = 52-175$ kg NO_3^- ha^{-1}	- 61A118b - 61A152 - 61A212 - 61A148 - 61A196	Granular inoculant (5.5 g m^{-1} of row)	- *B. japonicum* strains 61A152 and 61A212 generally performed better compared to the other strains. - Significant strain-by-cultivar interactions were not detected for nodulation, yield, and SNF.	
Wisconsin, USA	Loamy sand $ASN = 31.6$ kg NO_3^- ha^{-1}	- *B. japonicum*- USDA 123spc - USDA 110 - USDA 110str - I-123spc - NJ2-lastr - USDA 138	Liquid inoculants (1×10^8 cells per 2.5-cm row)	No significant differences were observed for the seed yield by different rhizobia strains.	McLoughlin et al. (1991)
Oregon, USA	Silt loam soil $TSN = 0.06-0.10\%$	- *B. japonicum* Hup$^+$ strains - USDA 143 - USDA 6 - USDA 122 - USDA 110 - USDA 136 - SR - *B. japonicum* Hup$^-$ strains Wild-type strains: - USDA 117 - USDA 135 - USDA 120 - USDA 16 - Mutants (derived from Hup$^+$ SR): - SR2 - SR3	Seed treatment with peat based inoculants (30 g kg^{-1} seeds)	- Plants inoculated with wild-type Hup$^+$ strains (USDA 143, USDA 6, USDA 122) produced higher seed N% (6.02−7.04 *vs.* 5.69−6.65) than the wild-type Hup$^-$ strains.	Hanus et al. (1981)
Ontario, Canada	London loam and Chinguacousy clay soil (Adequate soil N for plant growth)	- *B. japonicum* Hup$^+$ strains - 61A89 - 61A133 - 61A148 - *B. japonicum* Hup intermediate strains - 61A149 - *B. japonicum* Hup$^-$ strains - 61A150 - 61A152	Granular inoculants (10 kg ha^{-1})	- Plants inoculated with Hup$^-$ 61A152 produced the highest yields numerically compared to other strains. However, no significant difference was found among the Hup$^+$ and Hup$^-$ strains for yield.	Hume and Shelp (1990)
Romania	Chernozem	Local isolates - *B. japonicum* SO30 - *B. japonicum* SO618	Seeds were inoculated with peat based water slurry just before sowing	- Plants inoculated with SO_{30} had greater nodule number (29−66 *vs.* 19−32 nodules plant^{-1}) compared to strain SO_{618} at R_2 growth stage. - SNF was slightly higher with SO_{30} (62−132 *vs.* 25−103 kg N ha^{-1}) compared to strain SO_{618}.	Danso (1987)
Tropics					
Pantnagar, India	Silty loam textured soil $TSN = 224-235$ kg N ha^{-1}	Rhizobia strain - SB-6 - SB-12 - SB-16 - SB-120 - SB-294 - SB-102 - SB-271 - SB-243 - SB-9	Seed treatment with carrier based inoculant (500 g inoculant per 75 kg seeds)	- In comparison to uninoculated controls, generally strains SB-12, SB-16 and SB-294 had higher shoot dry weight (20.3−22.2 *vs.* 14.8 g plant^{-1}) and grain yield (21.7−22.2 *vs.* 16.2 q ha^{-1}). - According to the efficiency ratio, SB-12, SB-16, SB-102, SB-9, SB-271 were efficient, SB-6, SB-120, SB-294 were moderately	Patra et al. (2012)

Table 1 (continued)

Area/Country	Soil characters	Rhizobia strains	Inoculation method	Major findings	References
Paraná, Brazil	TSN = 0.10–0.13 g dm^{-3}	- *B. japonicum* USDA 110 - *B. japonicum* USDA 122 - *B. japonicum* CB 1809 - *B. japonicum* CPAC 7 - *B. japonicum* S-370 - *B. japonicum* S-372 - *B. elkanii* SEMIA 587 - *B. elkanii* SEMIA 566 - *B. elkanii* USDA 31 - *B. elkanii* USDA 76 - *B. elkanii* SEMIA 566 - *B. elkanii* 29w - *B. elkanii* S-273 - *B. elkanii* S-381	100 ml of inoculant kg^{-1} of seeds at 10^9 cells ml^{-1}	efficient, and strain SB-243 was inefficient in SNF. - No difference was observed between *B. elkanii* and *B. japonicum* strains in for nodule number (38–69 *vs.* 44–65 nodules plant^{-1}), nodule dry weight (115–168 *vs.* 140–162 mg plant^{-1}), or nodule occupancy (18–63 *vs.* 41–81%) with soybean BR-37 cultivar. Same trend was observed with cultivar BR-16 for nodule number (25–53 *vs.* 38–54 nodules plant^{-1}), nodule dry weight (95–155 *vs.* 118–144 mg plant^{-1}), and nodule occupancy (15–65 *vs.* 32–78%). - Cultivar BR-16 inoculated with *B. japonicum* strains had higher yield (3248–3789 *vs.* 2880–3186 kg ha^{-1}) and total grain N (211–248 *vs.* 170–197 kg N ha^{-1}) compared to *B. elkanii* strains. - Same trend was observed with cultivar BR-37 for yield (2880–3215 *vs.* 2010–2850 kg ha^{-1}) and total grain N (190–209 *vs.* 119–176 kg N ha^{-1}).	Hungria et al. (1998)

Paraná, Brazil	N/A	- *Rhizobium* spp.
		  - Strain 5
		  - Strain 22
		- *Bradyrhizobium* spp.
		  - Strain 8
		  - Strain 16
		- *S. fredii*- USDA 205
		  - HH103-2
		  - SMH12
		- Brazilian *B. japonicum/B. elkanii* commercial strains
		  - SEMIA 587
		  - SEMIA 5019
		  - SEMIA 5079
		  - SEMIA 5080
			100 ml of inoculant kg^{-1} of seeds, at 10^9 cells ml^{-1}	- Highest grain yield and N content were achieved with plants inoculated with commercial strains SEMIA 5080 (3.53 t ha^{-1} and 193 kg ha^{-1}) and SEMIA 5079 (3.50 t ha^{-1} and 195 kg ha^{-1}).
				- Nodule occupancy was higher with Brazilian commercial inoculants (35–56%) compared to the other rhizobia strains used (5–11%).
					Hungria et al. (2001)

Awka, Nigeria	Sandy loam soil
	TSN = 0.08–0.1%
		- *B. japonicum* strains
		  - USDA 136
		  - USDA 138
		  - USDA 110
		  - USDA 122
			Seeds were inoculated with decomposed rice husk based media containing *Bradyrhizobia* before sowing. (10^9 cells g^{-1})	- Nodulation parameters (number and dry weight) and nodule occupancy increased with strains 110, 122, and 138. Strains 122 and 110 resulted in higher shoot dry matter (2900–3100 *vs.* 2700 kg ha^{-1}), total N (84–92 *vs.* 61–80 kg ha^{-1}), and seed yield (1.5 *vs.* 1.1–1.3 tons ha^{-1}) compared to strains 138 and 136.	Okereke et al. (2000)

Igbariam and Awka, Nigeria	Loamy sand (Igbariam) and sandy loam (Awka)
	TSN = 0.14–0.18%
		- *B. japonicum* strains
		  - USDA136
		  - TAL 122
			Seed were inoculated with decomposed rice husk based media	- Nodule number and nodule dry weight increased with introduced foreign rhizobia	Okereke et al. (2001)

		- USDA 6 - TAL 377 - TAL 102	containing *Bradyrhizobia* before sowing. (10^9 cells g^{-1})	strains compared to the local soil rhizobia, but was dependent on the soybean cultivar as well. Increase in shoot dry matter, N%, and total N was highly influenced by the interaction effect of rhizobia strain, soybean cultivar, and location.	
Zaria, Mokwa, Fashola, Nigeria	TSN = 0.06%	- *B. japonicum* IRj 2180A - *Bradyrhizobium* isolate R25B - *Bradyrhizobium* isolate IRc 461	Seed treatment at 1×10^7 cells seed^{-1}	- Plants inoculated with R25B produced highest nodule number (52 *vs.* 18 nodules plant^{-1}), nodule fresh weight (2.88 *vs.* 0.82 g plant^{-1}), and shoot dry weight (36 *vs.* 24 g plant^{-1}) compared to IRj 2180A. However, rhizobial strains had no significant effect on grain weight.	Sanginga et al. (2000)
Zaria, Mokwa, Fashola, Nigeria	TSN = 0.06%	- Control (local rhizobia) - *Bradyrhizobium* isolate R25B - *B. japonicum* IRj 2180A + R25B	Seed treatment at 1×10^7 cells seed^{-1}	- Plants inoculated with R25B + IRj2180A produced highest nodule occupancy (30 −36 *vs.* 17−29%) and shoot N (115 *vs.* 100 kg ha^{-1}) compared to strain R25B alone.	Okogun and Sanginga (2003)
Mokwa and Yandev, Nigeria	Coarse-textured Paleustaff soils (low N soil)	- *B. japonicum* strains - 110 - 110-M - 61A76 - SM-31 - SM-35	Seeds were imbibed for 30 min in rhizobia containing YEM broth (10^7 cells ml^{-1}) immediately before planting. Excess media was applied into the furrow.	- In Mokwa, soybean cultivar TGm 294 inoculated with strains 61A76, SM-31, and SM-35 increased nodule mass (790 −1545 *vs.* 292−570 mg plant^{-1}), shoot growth (13.3−16.6 *vs.*12.9 −13.3 g plant^{-1}), N accumulation (7.6−13.3 *vs.* 2.3−6.5 mg plant^{-1} per day), and seed yield (1695 −2530 *vs.* 381−1415 kg ha^{-1}). - The same trend was found in Yandev, with significant interaction between rhizobia strains x soybean cultivars.	Pulver et al. (1982)
Sub Tropics					
Varanasi, India (subtropics, mild)	N/A	- *B. japonicum* strains - CB 1809 (Australia) - USDA123 (USA) - ASR011 - ASR031 - ISR076 (India)	Seed treatment with peat based inoculants	- Generally plants inoculated with strain ASR011 produced higher plant biomass and grain yield compared to other strains. - Nodule number and their nitrogenase activities were affected by rhizobia strains and soybean variety.	Appunu et al. (2008)
Nyabeda, West Kenya	Red clay loam TSN = 0.21%	- *B. japonicum* USDA 110 - *Rhizobia* isolates - NAK 84 - NAK 89 - NAK 115 - NAK 117 - NAK 128 - NAK 135	Seeds treatment with sterilized sugar mill filter mud based media. 10 g kg^{-1} seed	- In comparison to USDA 110, some local rhizobia isolates (NAK 117, 115, 128, 84, 89) resulted in higher nodulation (370-600 $\times 10^2$ *vs.* 790-6910 $\times 10^2$ nodules ha^{-1}) and nodule dry weight (2.9−6.7 *vs.* 7.8 −53.8 kg ha^{-1}).	Waswa et al. (2014)
Panzhihua and Suining, China	Savanna red soil and Purpli-Udic Cambosols. TSN = 0.7−1.0 (g N kg^{-1})	- *B. japonicum* SCAUs36 - *B. diazoefficiens* SCAUs46 - *Ensifer fredii* SCAUs65	Seed treatment with peat based inoculants (5×10^8 cells g^{-1})	- In comparison to the uninoculated control, SCAUs65 and SCAUs46 increased nodule number (55, 62 *vs.* 35 nodules plant^{-1}), shoot dry weight (613,	Chen et al. (2015)

(continued on next page)

Table 1 (*continued*)

Area/Country	Soil characters	Rhizobia strains	Inoculation method	Major findings	References
				556 *vs.* 403 kg ha^{-1}) and seed yield (1810, 1773 *vs.* 1428 kg ha^{-1}) respectively with local variety. -Similarly with Yushi No.8 variety, nodule number (40, 22 *vs.* 4 nodules plant^{-1}), shoot dry weight (3338, 3380 *vs.* 2714 kg ha^{-1}), and seed yield (4000, 3579 *vs.* 2567 kg ha^{-1}) increased with SCAUs46 and SCAUs65 isolates compared to the uninoculated control, respectively. - Strains SCAUs36 did not perform well compared to the above two strains.	
Bako, Western Ethiopia (cool subtropics, mid-altitude)	Nitisols TSN = 0.14%	- *B. japonicum* TAL 378 - *B. japonicum* TAL 379	Thick slurry of inoculant mixed with seeds 10 g kg^{-1} seeds	- In comparison to TAL 378, plants inoculated with TAL 379 produced higher number of nodules (12.0 *vs.* 0.1 nodules plant^{-1}), nodule dry weight (180 *vs.* 4 mg plant^{-1}), plant dry matter (15.5 *vs.* 10.8 t ha^{-1}), N uptake (2700 *vs.* 1665 mg plant^{-1}), and yield parameters.	Solomon et al. (2012)
Chihwai and Masenyama, Zimbabwe (subtropics, mid altitude)	Sandy soil TSN = 0.05%	- Soybean isolates from nodules of the Magoye variety (M1-M5) - Soybean isolates from nodules of the Hernon variety (H1-H5) - Commercial inoculants (MAR 1491 and 1495)	Seed treatment with liquid inoculants at 1×10^7 cells ml^{-1}	- Plants inoculated with isolate M3 had higher plant N content (74–93 *vs.* 57–75 kg N ha^{-1}) in the Magoy variety compared to the reference strains. Strains H1, H3 and H5 gave better plant N content with crop variety Solitaire (60–72 *vs.* 50–60 kg N ha^{-1}).	Zengeni and Giller (2007)
Mediterranean zone					
Greece	Clay loam	Commercial strain - *B. japonicum* Nitragin Local isolate - *B. japonicum* strain D	Seeds were inoculated with peat based water slurry just before sowing	- Plants inoculated with Nitragin had greater nodule number (57–110 *vs.* 8–37 nodules plant^{-1}), nodule dry weight (0.45–0.65 *vs.* 0.13–0.24 g plant^{-1}), and SNF (65–236 *vs.* 22–197 kg N ha^{-1}) compared to local strain D under low N supply.	Danso (1987)
Las Torres-Tomejil and La Orden, southern Spain	Silty loam and sandy loam soil Kjeldahl N = 0.7–1.0 g kg^{-1}	*B. japonicum* USDA110 *Sinorhizobium (Ensifer) fredii* - HH29 - S5 - S6 - S17 - S18 - S30 - S31 - S51 - SMH12	Seed treatment with peat based inoculants (1×10^6 cells seed^{-1})	- In Las Torres-Tomejil, *S. fredii* produced higher number of nodules (105–171 nodules plant^{-1}) compared to USDA 110 (37–46 nodules plant^{-1}). However, nodule mass was not significantly different between the plants inoculated with the two groups of rhizobia. Strain SMH 12 produced higher seed N content (314–347 kg ha^{-1}) similar to USDA 110 strain (296–371 kg ha^{-1}).	Albareda et al. (2009a)
Las Torres-Tomejil, southern Spain		- *B. japonicum* USDA110			Albareda et al. (2009b)

	Silty loam and sandy loam soil Kjeldahl N = 0.7–1.0 g kg^{-1}	- *Sinorhizobium (Ensifer) fredii* SMH12	Seed treatment with peat based inoculants (1×10^6 cells seed^{-1})	- Inoculation with SMH 12 had higher number of nodules (131 *vs.* 46 nodules plant^{-1}) and nodule dry weight (492 *vs.* 310 mg plant^{-1}) compared to USDA 110 under no N fertilizer addition. However, inoculations with USDA 110 produced slightly higher grain yield (6318 *vs.* 6071 kg ha^{-1}) and seed-N (376 *vs.* 347 kg N ha^{-1}) compared to SMH 12.	

Semi-arid zone

Somali region of Ethiopia	Sandy clay soil TSN = 0.25%	- *B. japonicum* (TAL-379 isolate) - *Bradyrhizobium* sp. (UK-Isolate) - *Bradyrhizobium* sp. (local-isolate)	20 g inoculant per 200 g seeds	- Generally, nodule number (53 *vs.* 32–44 plant^{-1}) and nodule dry weight (0.44 *vs.* 0.33–0.41 g plant^{-1}) were higher with plants inoculated with the local *Bradyrhizobium* sp. compared to the other two strains. However, grain yield was higher with the UK isolate (2766 kg ha^{-1}) compared to either the local isolate (2398 kg ha^{-1}) or TAL-379 (1882 kg ha^{-1}). - The rhizobia strain type did not affect plant tissue N%.	Argaw (2014)
Khouzestan province, Iran	TSN = 0.07%	*Bradyrhizobium* isolates - BR-9 - BR-12 - BR-14 - BR-15 - BR-35 - S-10 - Soya-1 - Rhizoking	Seed treatment with perlite based inoculants (150 ml per 50 g perlite)	In comparison to uninoculated control, thermotolerant isolate BR-12 significantly increased shoot dry weight (75%), N content (145%), and grain yield (70%).	Rahmani et al. (2009)

N/A, not available; TSN, total soil nitrogen; ASN, available soil nitrogen.
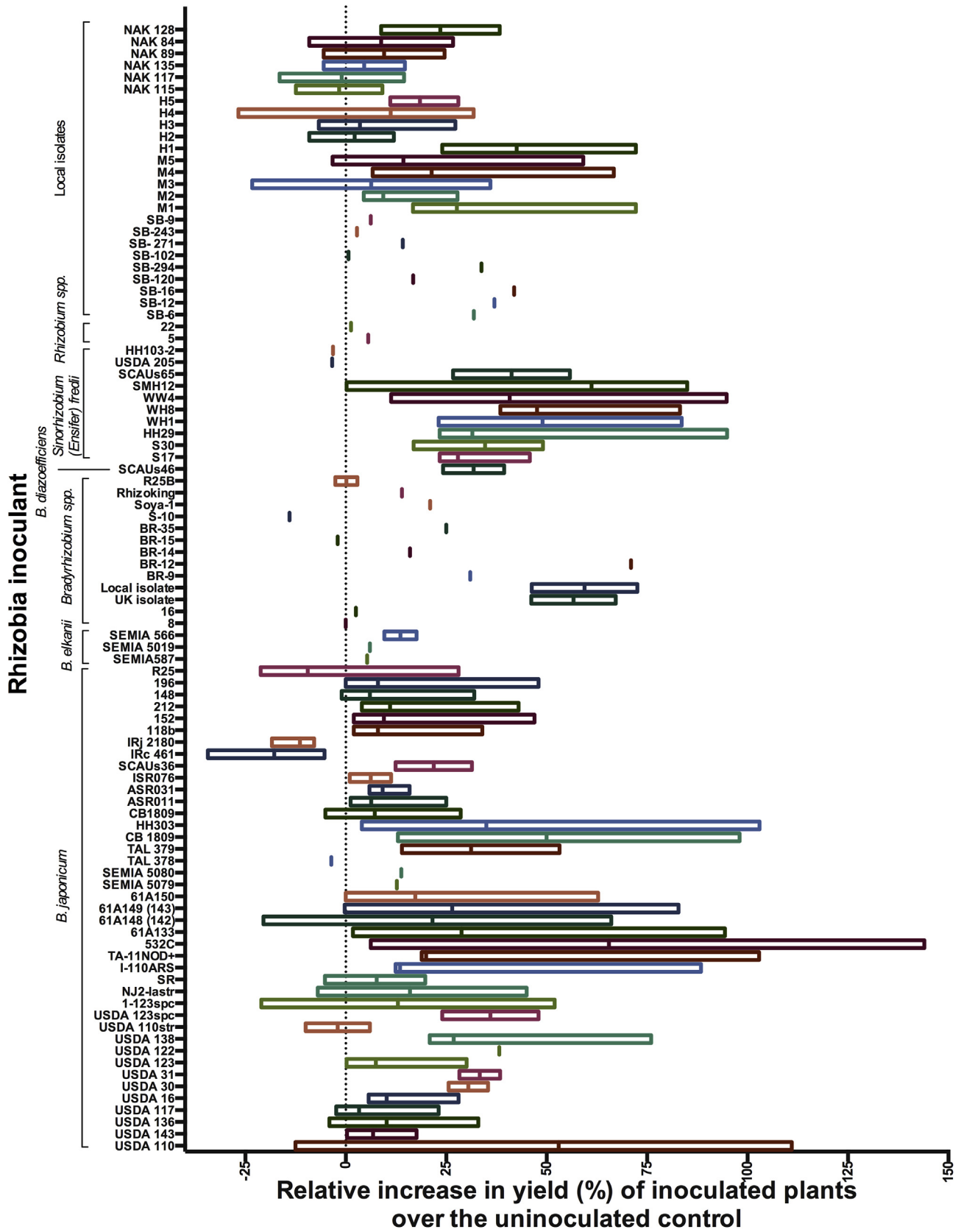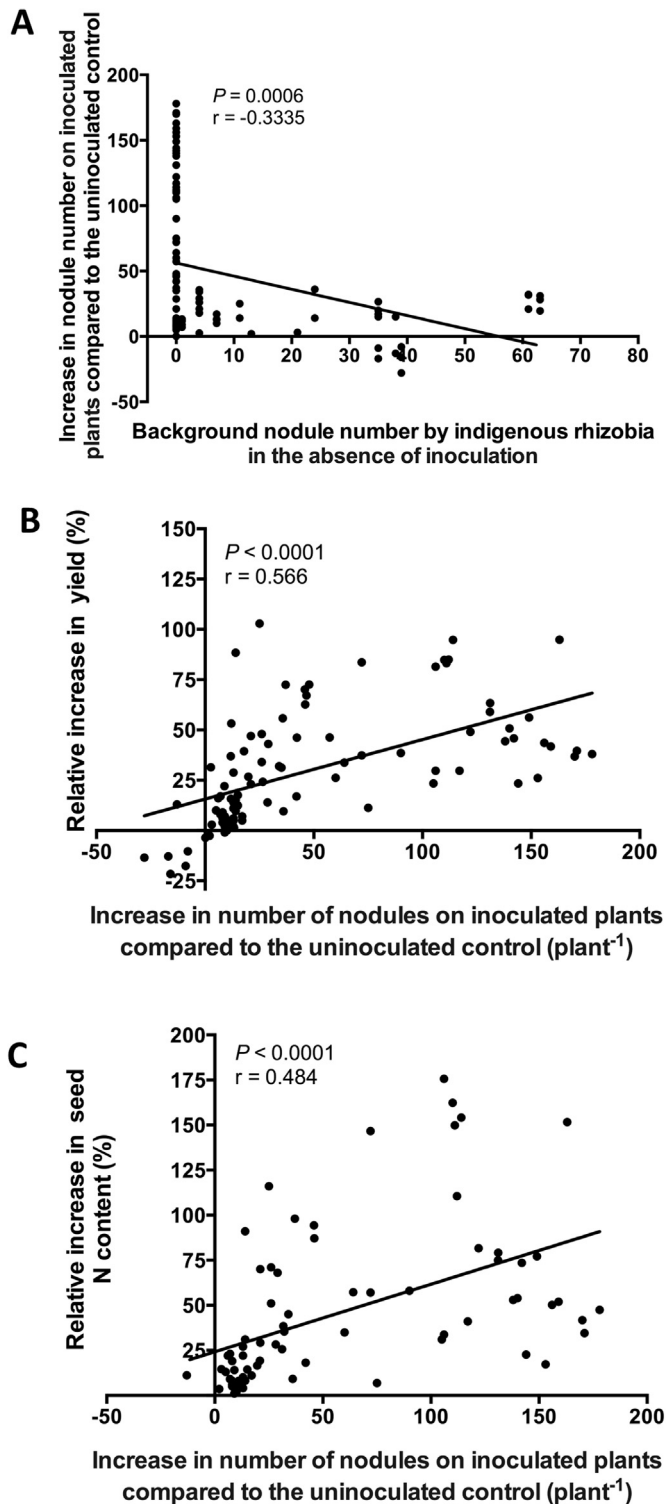
Fig. 2. Relative increases in grain yield [(inoculated value − uninoculated value)/uninoculated value x 100] of soybean under field conditions following inoculation with different rhizobia strains, based on an analysis of results from the published literature.

Fig. 3. Rhizobia inoculant responses to nodulation, grain yield, and grain nitrogen as an indicator of the level of background indigenous, compatible rhizobia in the soil. The data is based on an analysis of results from the published literature. **A**, correlation between the increase in nodule number by the inoculants *vs.* the corresponding background nodule number by indigenous rhizobia. **B**, correlations between the relative increase in grain yield or **C**, grain nitrogen content by the inoculants *vs.* the increase in nodule number by the inoculants, compared to the uninoculated controls. r, Pearson correlation coefficient.

(Supplementary Fig. 1A), the yield response ranged from −34% to +144% (Fig. 2), and the seed N response ranged from −5% to +176% (Supplementary Fig. 1B). Inoculation with *Sinorhizobium*

(*Ensifer*) *fredii* showed changes in nodule number ranging from +20 to +178 (Supplementary Fig. 1A), yield from −3% to +95% (Fig. 2), and seed N from −21% to +162% (Supplementary Fig. 1B). Substantial changes in yield (from −27% to +72%) were also reported when indigenous rhizobial strains were used as inoculants (Fig. 2).

2.1.2. Effect of indigenous soil rhizobia

To measure whether compatible indigenous rhizobia were present in the soil, the nodule number of uninoculated plants was used as a proxy. The greatest increases in nodule numbers per plant occurred when the background nodulation by local soil rhizobia was absent or extremely low (i.e. 0–1 nodules) (Supplementary Fig. 2A). There was a weak but significant inverse correlation between the nodule number of uninoculated plants compared to inoculated plants (Fig. 3A). Relative increases in yield and grain N of inoculated plants were moderately correlated to absolute increases in nodule number compared to uninoculated controls (Fig. 3B and C). However, both yield and grain N could increase substantially without substantial increases in nodule number (e.g. M1-M6 and H1-H5 inoculants, Supplementary Figs. 2B and 2C) (Zengeni and Giller, 2007). High variability for these traits, at the field level, between treatments of a particular rhizobia strain (Supplementary Figs. 2A–2C) might be due to the variable field conditions, treatments and phenotyping methods used. These results suggest that a soybean inoculant has the greatest chance of having a positive impact when the soil does not possess compatible indigenous rhizobia or contains a very low population of compatible rhizobia, which support the earlier reports by Singleton and Tavares (1986), Thies et al. (1991a) and Giller (2001).

2.1.3. Effect of soil characteristics

In the various field experiments reported in the literature, the soil traits varied considerably with respect to texture, soil organic matter, pH and nutrients. Given this trial-to-trial variability, it was difficult to interpret the effect of specific soil characteristics. There were additional technical challenges. For example, though starter N is required to develop good N fixation capacity in soybean (Van Kessel and Hartley, 2000; Osborne and Riedell, 2006), initial mineral N concentrations were not measured in many published studies. Furthermore, whereas the concentration of available N is known to be critical for successful SNF, most studies reported total soil N, which includes the (less available) organic N pool in soil. Phosphorus (P) is also known to limit legume productivity (Zahran, 1999). Nevertheless, for the interested reader, the impacts of inoculants were arranged by total soil N concentration (Supplementary Fig. 3A, y-axis, in brackets, prior to the onset of the experiment or addition of fertilizers) as well as increasing P availability (Supplementary Fig. 3B, y-axis, in brackets, prior to the onset of the experiment or addition of fertilizers).

Despite the challenge of comparing results from different trials, we felt more confident to interpret the impacts of soil pH as it is known to be a major driver of inoculant success across agroecological environments (Zahran, 1999; Hungria and Vargas, 2000). When the impacts of rhizobia inoculants were arranged by increasing soil pH (Fig. 4, y-axis, in brackets), the published studies demonstrate that inoculants resulted in the greatest increases in nodule number when the soil pH was moderate (pH 6.6–7.8), declining substantially when the soil was more acidic or basic (Fig. 4 and inset). This result represents one of the most striking observations of this meta-analysis.

2.2. Additional lessons from inoculant studies using field soybean

2.2.1. Indigenous versus commercial inoculants

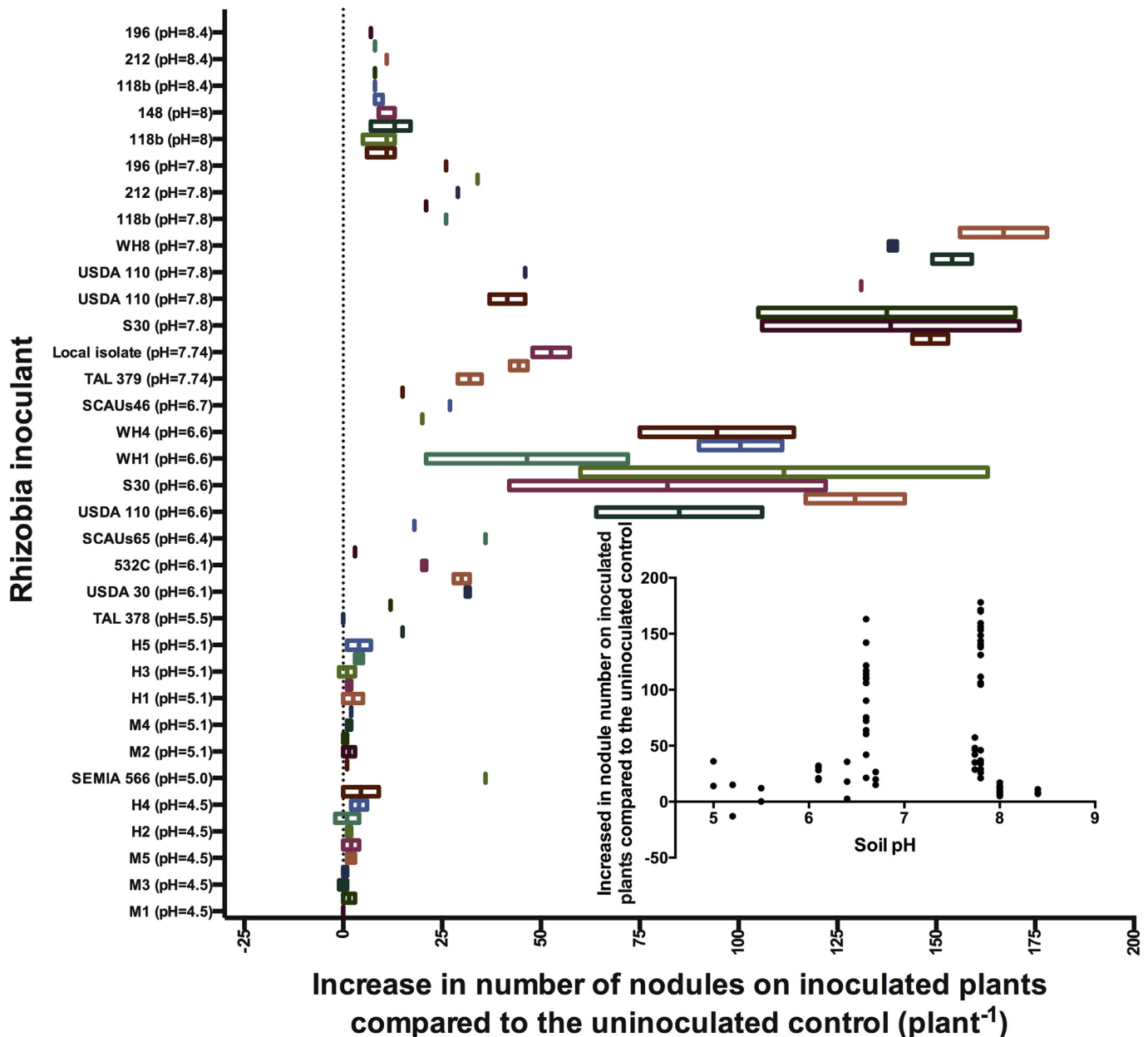Various studies have demonstrated that sometimes inoculants

Fig. 4. Rhizobia inoculant responses to nodulation in field-grown soybean under different levels of soil pH. The data is based on an analysis of results from the published literature. Increases in nodule number by the inoculants compared to the uninoculated controls (inoculated value − uninoculated value) organized by the initial soil pH. The corresponding inset represents the correlation between the increase in nodule number by the inoculants relative to the corresponding uninoculated control *vs*. the initial soil pH values.

consisting of indigenous rhizobia result in greater soybean yields than non-local, improved rhizobia under field conditions (Pulver et al., 1982; Zengeni and Giller, 2007; Waswa et al., 2014). Compared to improved strains, local rhizobia could sometimes produce greater nodule numbers and nodule biomass as in a study from Kenya (Waswa et al., 2014), or greater plant N as in a study from Zimbabwe (Zengeni and Giller, 2007) (Table 1). It is interesting to note that sometimes indigenous rhizobia were more competitive than the improved rhizobia (USDA 110) in terms of nodule occupancy (Kamicker and Brill, 1987) (Table 1). These studies suggest that native rhizobia may have potential for commercial inoculant production at a local level. Selection for inoculants that are more competitive than indigenous strains in the soil in terms of nodule occupancy may be a key factor for improving SNF in soybean (Okereke et al., 2000, 2001).

2.2.2. Inoculants adapted to the local environment

Some of the studies suggested that locally adapted rhizobia strains are capable of performing better under environmental stress conditions (low/high temperature) compared to introduced rhizobia (Zhang et al., 2003; Rahmani et al., 2009). For example, Zhang et al. (2003) showed that strains selected from a northern adapted climate were more effective under cool conditions, a finding that shows the benefits of testing strains under similar environmental conditions as their origin. Similarly in Iran, local rhizobia originally isolated from a high temperature environment resulted in greater soybean yield traits under hot field conditions compared to inoculants isolated from a moderate temperature environment (Table 1) (Rahmani et al., 2009). Identification of thermotolerant soybean rhizobia strains are especially important in semi arid regions, which have high soil and air temperatures,

stresses that lead to poor nodulation and SNF (Zahran, 1999; Hungria and Vargas, 2000). Selection of adapted rhizobia strains for various environmental stress factors will enable higher SNF and grain yield in soybean compared to the currently available inoculants (Hungria and Vargas, 2000).

2.2.3. Effect of soybean genotype

Several studies from around the world demonstrate that subsequent to inoculation, traits such as nodulation, nodule occupancy, SNF and soybean yield can be affected by the soybean genotype (Pulver et al., 1982; Danso, 1987; Okereke and Unaegbu, 1992; Albareda et al., 2009a; Mapope and Dakora, 2016) and strain-by-genotype interactions (Ravuri and Hume, 1992; Argaw, 2014; Zimmer et al., 2016). In one noteworthy field study conducted in Nigeria, local soybean cultivars did not respond significantly to inoculants from the United States compared to U.S. bred cultivars (Pulver et al., 1982) (Table 1). As locally bred cultivars were more promiscuous than the U.S. bred cultivars in this study, plants were nodulated with local soil rhizobia strains rather than the foreign inoculants. In a study conducted in southern Spain, the nodule occupancy of several introduced strains was dependent on the soybean genotype, again highlighting the importance of considering the host genotype for efficient SNF (Albareda et al. (2009a) (Table 1). A recent study conducted by Zimmer et al. (2016) found that protein content and protein yield of soybean were significantly affected by inoculant-by-host genotype interactions.

2.2.4. Lack of correlation between nodulation and nitrogen fixation

Our meta-analysis demonstrated significant but moderate correlations between nodulation and yield/grain N. Despite this general finding, it is important to recognize that some studies have reported poor correlation between nodulation parameters compared to grain yield/grain-N content (Hungria et al., 1998; Sanginga et al., 2000; Albareda et al., 2009a, 2009b). For example, in Nigeria, some inoculants significantly affected nodulation and nodule occupancy in soybean, but did not affect grain yield (Sanginga et al., 2000). Conversely, in Brazil, Hungria et al. (1998) demonstrated that rhizobia inoculants affected grain yield, but did not influence nodulation parameters (nodule number, nodule dry weight and nodule occupancy). In a study conducted in southern Spain, several introduced strains resulted in very low nodule occupancy (1—18%) under field conditions compared to indigenous *Bradyrhizobium*, yet surprisingly nodulation and N yield traits showed significant improvements (Albareda et al., 2009a). In contrast to these findings, other studies have suggested a strong correlation between nodulation and yield traits. For example, in a field study conducted in Ethiopia, Argaw (2014) found that nodule number and nodule dry weight of soybean were highly correlated with grain yield, especially with late maturing genotypes (Table 1).

2.2.5. Inoculant formulation, titre and application method

The soybean field trial literature shows different methods of inoculant application including seed coating with a solid/liquid carrier, imbibing seeds in liquid solution containing rhizobia, and application of rhizobia to open furrows (Table 1). Peat was the most common inoculant carrier material used, however different local materials were also used including sterilized sugar mill filter mud and decomposed rice husk based media (Table 1). It was found that a higher percentage of nodules were occupied with inoculant when applied as high titres on seed and soil compared to seed-only treatments or low titres on seed and soil, showing the importance of optimizing the inoculant formation and application methodology (Kamicker and Brill, 1987) (Table 1). Combined application of different rhizobia strains into a single inoculant has also been shown to improve nodule occupancy compared to

individual rhizobia (Okogun and Sanginga, 2003). It is difficult to generalize as to what might constitute the best formulation as it depends on many factors. In general, in order to achieve maximum efficacy under field conditions, an inoculant must be simple to apply, contain the highest possible number of viable rhizobia (at least 10^9 rhizobia g^{-1}), survive after introduction to soil, nodulate plants under various soil conditions, and be compatible with farmer practices (Howieson and Dilworth, 2016). The N2Africa project which works across a diversity of field sites has observed that survival of rhizobia in inoculants depends on the carrier material as well the type of rhizobia strain.

2.2.6. Usefulness of hydrogenase activity for strain selection

During the N fixation reaction, hydrogen gas is produced as a by-product, consuming 5% of net photoassimilates (Dong and Layzell, 2001). Some rhizobia are capable of recycling released hydrogen due to the presence of an active hydrogenase enzyme; these strains are termed Hup$^+$. Rhizobia that lack the hydrogenase uptake activity are called Hup$^-$. Based on field experiments conducted with Hup$^+$ and Hup$^-$ strains of *B. japonicum* at four locations in Oregon, USA, Hanus et al. (1981) observed that Hup$^+$ strains were efficient in SNF, resulting in an 8.9% increase in seed crude protein content in soybean compared to the Hup$^-$ strains (Table 1). Based on these results, the authors further highlighted the importance of using Hup$^+$ strains for inoculum preparation. However, contradictory results were reported by Hume and Shelp (1990) (Table 1), wherein the authors suggested that Hup$^+$ activity is not a sufficient trait for screening rhizobia strains with high N fixation activity. Therefore, hydrogenase activity may not be a reliable tool for strain selection for SNF.

3. Factors that affect persistence of soybean-compatible rhizobia in soil

Some of the results from our meta-analysis of field studies may have been influenced by the impacts of environmental stress on the survival of rhizobia inoculants once in soil, since seed-coated rhizobia must colonize roots through the rhizosphere. Alternatively, environmental stress may have favored indigenous competitors adapted to the local soils, thus indirectly reducing the efficacy of the inoculants, as already alluded to above. Rhizobia strains vary in their environmental resiliency which affects their survival in soil (Giller, 2001). While some studies have tracked the survival of individual strains, others have used soil rhizobial diversity to measure the impacts of stress and management practices. Rhizobia diversity in soil is affected by many factors such as soil pH (Hungria et al., 2001; Yang et al., 2001; Giongo et al., 2008; Li et al., 2011; Wongphatcharachai et al., 2015), clay content (Loureiro et al., 2007), organic matter content (Hungria et al., 2006), available soil nitrate content (Wongphatcharachai et al., 2015), available phosphorus (Li et al., 2011), potassium (Li et al., 2011), salinity/alkalinity (Zhang et al., 2011; Youseif et al., 2014), and different agricultural practices (tillage, fertilization, crop rotations, pesticides) (Ferreira et al., 2000; Hungria et al., 2006; Yan et al., 2014). These factors are further described below:

3.1. Soil fertility (N, P, K)

Soil N concentrations were shown to be significant factors affecting the survival, abundance, and diversity of soybean rhizobia in soil. Available soil N is shown to have either positive/negative or neutral effects on survival, abundance, and diversity of soybean nodulating rhizobia in soil (Zhang et al., 2011; Yan et al., 2014; Wongphatcharachai et al., 2015) (Supplementary Table 1). Nitrogen fertilization may not have significant effects on soybean

rhizobia diversity (based on the Shannon diversity index) when a soil contains a limited rhizobia population and diversity (Herrmann et al., 2014). However, our meta-analysis of the soybean literature clearly showed that inoculants only succeeded when at least minimal soil N was present (Supplementary Fig. 3A).

Available soil P has been reported to be one of the possible determinants of the geographic distribution of soybean rhizobia (Han et al., 2009). Based on canonical correspondence analysis (CCA) of 12 soybean nodulating rhizobia groups in China, it was observed that available P in soil influenced soybean rhizobia diversity compared to the available N, potassium, and organic matter (Li et al., 2011) (Supplementary Table 1). In other studies, however, available soil P was shown to have a slight influence on the distribution of soil rhizobia (Zhang et al., 2011; Yan et al., 2014) (Supplementary Table 1). Our meta-analysis of the soybean literature demonstrated that the success of inoculants was not predictably correlated to starting soil P concentrations (Supplementary Fig. 3B), perhaps due to other confounding variables.

Available soil K is shown to have a weak impact on soybean rhizobia diversity and abundance in soil (Li et al., 2011; Zhang et al., 2011; Yan et al., 2014) (Supplementary Table 1).

3.2. Soil pH

It has been reported that at least some soybean rhizobia can tolerate a wide range of soil pH conditions ranging from pH 5—11 (Youseif et al., 2014). Generally soil pH is a major factor that drives *Bradyrhizobium* survival (Giongo et al., 2008). In general, nodule occupancy by fast growing soybean rhizobia was greater under moderate pH conditions (6.8—7.9) compared to acidic pH (5.1—5.4) (Hungria et al., 2001; Yang et al., 2001; Albareda et al., 2009a). However, based on rhizobia collected from nodules in Egypt, it was found that even some fast growing rhizobia can tolerate low soil pH (pH 4) (Youseif et al., 2014). Soil pH favors or inhibits the distribution or population of soil rhizobia, which is highly dependent on the species (Li et al., 2011; Zhang et al., 2011; Adhikari et al., 2012; Wongphatcharachai et al., 2015) (Supplementary Table 1). These results demonstrate that compatible soybean strains can vary significantly in their optimal pH, which would be consistent with our meta-analysis of field results (Fig. 4).

3.3. Clay and organic matter content

High clay content in soil has been shown to reduce rhizobia diversity (based on the abundance-based coverage estimator, and traditional and modified Shannon indices) due to soil compaction and lack of aeration compared to sandy soil (Loureiro et al., 2007). However, based on *Bradyrhizobium* strains isolated from soil in southern Brazil, it was shown that both clay and organic matter content in soil have low influence on rhizobia diversity (based on the Shannon diversity index) (Giongo et al., 2008). Soil organic matter content was shown to have both positive and negative effects on the distribution of soil rhizobia, which varies by rhizobia species (Li et al., 2011; Yan et al., 2014) (Table 1). However, the effect of organic matter on soybean rhizobia was shown to be minimal when the soil contained a limited number of rhizobia (Herrmann et al., 2014). One interpretation of these contradictory results is that clay and organic matter may not play significant roles in soybean rhizobia survival compared to other factors such as pH and temperature.

3.4. Soil salinity/alkalinity

Salinity can have significant negative effects on rhizobia survival, their diversity and distribution (Elsheikh, 1998; Han et al.,

2009), as salt stress is more chronic than drought stress. The negative impacts of salt on rhizobia have been more attributed to direct toxicity by ions, but salt also causes associated declines in soil osmotic potential and causes the soil to be more alkaline (Elsheikh, 1998). The effect of salinity/alkalinity on the distribution or population of soil rhizobia was highly variable and dependent on the rhizobia species (Chen et al., 2002; Zhang et al., 2011; Youseif et al., 2014). Indigenous rhizobia strains with resistance to salinity have also been isolated and characterized from Indian soil (Singh et al., 2013). Since salinity/alkalinity is becoming aggravated as a result of climate change, identification of resistant rhizobia strains is a timely need.

3.5. Temperature

Temperature is one of the main factors controlling growth, survival, nodulation and efficiency of SNF in soybean. High soil temperatures can negatively affect the growth and survival of both local and inoculated rhizobia populations (Hungria and Vargas, 2000), but genotypic variability also exists among different rhizobia strains for tolerance to high temperatures (Hungria et al., 2006; Rahmani et al., 2009; Youseif et al., 2014). Temperature dependent genotypic variability also exists among different rhizobia strains for nodulation and SNF (Zhang et al., 2003) (Table 1), nodule occupancy (Shiro et al., 2013; Suzuki et al., 2014), rhizobia abundance and occupancy in soil (Saeki et al., 2010; Adhikari et al., 2012; Shiro et al., 2012) (Supplementary Table 1). It was found that the yield performance of soybean under high temperature conditions was greater when the plants were inoculated with rhizobia that had been pre-screened for higher heat tolerance and SNF (Rahmani et al., 2009). The minimum temperature of the soil was also shown to have a direct influence on soybean nodule occupancy by introduced rhizobia, as some rhizobia strains (USDA 110) competed better under warm temperatures and others competed well under cooler temperatures (USDA 138, 136b) (Thies et al., 1992). When multiple strains were allowed to compete with one another, temperature was shown to affect which rhizobia dominate soybean nodules (Suzuki et al., 2014). Mechanistically, for at least some rhizobia, low temperatures negatively affect nod factor formation, which is important for root hair curling, resulting in delayed and reduced nodule formation in soybean (Zhang et al., 2003). These results show that soybean-compatible rhizobia strains show significant variation with respect to their temperature optimal. Therefore, temperature may have affected the success of soybean inoculants when applied in different climatic zones (Table 1).

3.6. Drought

Drought is one of the major environmental constraints affecting the survival of rhizobia in soil (Lowendorf, 1980; Hungria and Vargas, 2000). However some rhizobia can survive in soil even under low moisture conditions (Zahran, 1999). Although many studies have been conducted to evaluate the effect of drought stress on SNF, limited research has been conducted to evaluate the effect of drought on soybean rhizobia diversity, abundance and survival in soil. Based on a greenhouse study, it was observed that the population of *B. japonicum* was not affected by exposure to 4—8 weeks of drought (percent soil water content <1%) (Barthelemy-Delaux et al., 2014). Contrary to these results, in a field study conducted in central Kenya, it was shown that some *Bradyrhizobium* strains are sensitive to drought conditions, where the population size decreased following exposure to prolonged drought (>2 months of drought) (Herrmann et al., 2014). However, the authors found genotypic differences between rhizobia for

drought stress, wherein some strains were resistant to drought stress and persisted from one season to another. In a study conducted in Zimbabwe, reductions in the rhizobia population (10 cells per gram of soil) in soybean-grown soil were reported under low soil moisture conditions (<2% soil moisture) (Zengeni et al., 2006). These results suggest that genetic variability may exist among different soybean rhizobia strains for drought resistance, and hence seasonal precipitation may have affected the success of soybean inoculants in different climatic zones in our meta-analyiss (Table 1).

3.7. Agricultural practices

3.7.1. Organic, no-till, and crop rotation practices

Rhizobia diversity in soybean-grown soil can change significantly following the introduction of certain agricultural practices. Interestingly higher soybean rhizobia diversity was observed in organically managed fields (Grossman et al., 2011), undisturbed soil (Hungria et al., 2006), and no-till conditions (Ferreira et al., 2000; Hungria and Vargas, 2000; Loureiro et al., 2007; Bizarro et al., 2011; Grossman et al., 2011) compared to tilled fields. High soil moisture content, low soil temperature, high carbon content, slightly acidic soil pH (pH 6.4) and preservation of soil aggregates under no-till conditions and organically managed fields may favor soil rhizobia diversity (Hungria and Vargas, 2000; Grossman et al., 2011). Furthermore, higher nodule occupancy was observed with rhizobia inoculants compared to local rhizobia strains in conventionally managed fields (Grossman et al., 2011).

With respect to the cropping system, it was found that soil persistence of rhizobia including *Bradyrhizobium* spp. increased when soybean was included in the crop rotation compared to monocropping with non-legumes (Ferreira et al., 2000; Hungria and Vargas, 2000; Grossman et al., 2011). Further, soybean rhizobia diversity was shown to increase under crop rotations compared to monocropped soybean (Bizarro et al., 2011; Yan et al., 2014; Tiemann et al., 2015) (Supplementary Table 1), perhaps by enriching the soil with crop residues (quantity and quality), which are also more chemically diverse (Tiemann et al., 2015).

These results have practical implications for inoculant technologies: since indigenous rhizobia can survive in soil for long periods of time even in the absence of the host legume (Kamicker and Brill, 1987), beneficial practices that favor soil rhizobia (e.g. organic management) may cause local rhizobia to outcompete introduced rhizobia inoculants (Osunde et al., 2003; Melchiorre et al., 2011; Sanz-Sáez et al., 2015). In our meta-analysis of field soybean (Table 1), the various studies differed in the crop history of the fields, which may have added to the variability of the results.

3.7.2. Application of fungicides, herbicides and insecticides

Insecticides, herbicides, and fungicides can have negative effects on soybean rhizobia in soil. Based on 122 rhizobia strains tested for tolerance against different agrochemicals, it was observed that rhizobia were least tolerant to fungicides, followed by herbicides and then insecticides (Drouin et al., 2010). Generally, pesticides can have negative effects on soil rhizobia diversity, their activity, and plant-microbial interactions (Fox et al., 2007; Hussain et al., 2009; Ahemad and Khan, 2013).

Seed treatment with fungicides is a common practice, but it can negatively affect soil rhizobia populations in soybean fields, resulting in low SNF and yield reduction (Revellin et al., 1993; Campo et al., 2009). The negative effects of fungicide-treated seeds on rhizobia were even greater in fields where rhizobia were newly introduced. The type of fungicide used has also been shown to be important. For example, it was found that the fungicide Captan had less influence on native soybean rhizobia compared to

Carbendazim, when applied as seed treatments (Kaur et al., 2007). Carbendazim is a biochemically specific inhibitor and persists in soil compared to Captan. In another study, it was shown that seeds treated with Mancozeb reduced the survival of *B. japonicum* on seeds compared to a mixture of Carbendazim and Thiram fungicides, resulting in poor nodulation (Martyniuk et al., 2002). Quinolate Pro (carbendazim and oxine copper), Vitavax 200FF (carboxin and thiram), and Monceren (pencycuron) were also shown to be compatible with soybean seed inoculation, whereas Germipro UFB (carbendazim and iprodione), Apron 35J (metalaxyl), and Tachigaren (hymexazol) negatively affected soybean rhizobia survival and nodulation (Revellin et al., 1993), and thus were not compatible with soybean seed inoculation. Similarly, nodulation and nodule activity were also influenced by the type of pesticide used (Mallik and Tesfai, 1985; Yueh and Hensley, 1993). The concentration of fungicides is a critical factor as seeds treated with fungicides at high concentrations negatively affect rhizobia and SNF (Mallik and Tesfai, 1985). For example, reduced nodulation and nitrogenase activity was reported above 100 μg ml^{-1} of Thiram as a seed treatment (Bikrol et al., 2005). Therefore special attention has to be paid when soybean seeds are treated with different fungicides in order to minimize negative effects on nodulation, SNF and the soil rhizobia population. Interestingly it was found that some fungicides, which were applied as either a seed treatment (thiophanate-methyl) (Lakshmi and Gupta, 1997) or foliar application (pyraclostrobin) (Joshi et al., 2014) increased the number and activity of soybean nodules.

With respect to herbicides, application of sulfentrazone herbicide was shown to have negative effects on rhizobia performance in soybean, resulting in reductions in growth, yield and SNF (Vieira et al., 2007). However, it was shown that application of chlorimuron-ethyl herbicide [an acetolactate synthase (ALS) inhibitor] at a standard rate had a low impact on the survival of *B. japonicum* (Zawoznik and Tomaro, 2005). Although transgenic soybean varieties have been widely commercialized with resistance to the well known herbicide Glyphosate, exposure to Glyphosate has been shown to inhibit *B. japonicum* growth and even causes rhizobia death and reduction in nitrogenase activity at high concentrations, as *B. japonicum* possesses a glyphosate-sensitive enzyme (Zablotowicz and Reddy, 2004, 2007). Being a strong metal chelator, Glyphosate has a negative effect on SNF by reducing the availability of nickel, which is very important for rhizobia bacteria (Zobiole et al., 2010). The effect of Glyphosate depends on the commercial formulation used: one commercial form of Glyphosate (Cerdeira et al., 2007), Roundup Transorb®, was shown to have more negative effects on *Bradyrhizobium* survival compared to pure Glyphosate, primarily due to the presence of ethylamine as a surfactant in the formulation. Selection and application of less harmful pesticides at recommended concentrations will minimize the negative effects on rhizobia as well as the soybean-rhizobia symbiosis.

The effect of insecticides on the legume-soybean symbiosis has been given less attention than that of fungicides and herbicides. By contrast to the above agrochemicals, application of certain insecticides at recommended rates was shown to have no influence on rhizosphere rhizobia populations in fields cultivated with soybean; these insecticides included phorate, carbofuran, carbosulfan, thiomethoxam, imidacloprid, chlorpyriphos, monocrotophos (Sarnaik et al., 2006), a mixture of chlorpyrifos and lindane (Revellin et al., 1992) and disulfoton and carbaryl (Kapusta and Rouwenhorst, 1973). Soil acts as a buffer for many chemical compounds; agrochemicals can be hydrolyzed, absorbed by clay/ organic matter or degraded by the soil microbial population (Kapusta and Rouwenhorst, 1973).

3.8. Climate change and elevated CO_2

Climate change, which is associated with elevated CO_2, temperature, and drought, is predicted to have a significant influence on soil rhizobia and SNF in legumes (Compant et al., 2010; Classen et al., 2015). Since nodule rhizobia are fueled by carbon from the host plant, changes in carbon fixation due to climate change may enhance rhizobia metabolism and/or populations. However, based on recent findings from a field study conducted under elevated CO_2 concentrations, the authors found that even soybeans inoculated with an enhanced nitrogen fixing *B. japonicum* strain (USDA 110) failed to enhance plant growth and SNF (Sanz-Sáez et al., 2015). Furthermore, as elevated CO_2 may lead to elevated plant carbon fixation, there may be higher carbon inputs into the soil via decomposition of plant matter, which may in turn affect soil rhizobia populations and diversity. If climate change alters root exudates (Morgan et al., 2005), there may be an impact on rhizobia-soybean symbiotic associations, as initiation of plant-microbial symbiotic interactions is governed by compounds found in root exudates (Subramanian et al., 2006). In future years, it will be useful to measure the impact of climate change on different rhizobia strains and soybean varieties.

4. Summary of factors that affect inoculant success in soybean fields

In conclusion, our meta-analysis demonstrates that a number of factors affect the success of rhizobia inoculants on soybean under field conditions. Soybeans can be nodulated under field conditions by an apparently limited taxonomic range of rhizobia isolated from around the world, with both *Bradyrhizobium* and *Sinorhizobium* species being successful. Inoculants caused the largest absolute increases in nodule number and grain N when local compatible rhizobia were apparently absent or extremely low in soil. Some studies suggested that indigenous rhizobia strains may be better adapted to local environmental stress conditions (low/high temperature) compared to introduced rhizobia. There was a positive but moderate correlation between inoculant-mediated increases in grain yield/grain-N and the number of nodules. Available soil P caused unpredictable impacts when considered in isolation. Soil pH was one of the strongest drivers of inoculant success, with inoculant-mediated increases in nodulation favored by moderate pH conditions. Finally, inoculant success depended on the soybean genotype and soybean x rhizobia strain interactions, inoculant formulation, titre and application method. Additional explanations for the success or failure of inoculants could be found in literature that describes the survival of rhizobia (inoculants) in soil, as seed-coated rhizobia must colonize roots through the rhizosphere. The soil literature showed that diverse factors (environmental, soil composition, agricultural practices) can all impact rhizobia survival in soil.

5. Research gaps and future studies

Based on our review of the existing literature, it is clear that there is a need to target future studies to overcome current research gaps or limitations:

5.1. Need for more effective indoor and field studies

Some rhizobia strains that performed better under laboratory or greenhouse conditions were shown not to perform similarly under field conditions (McLoughlin et al., 1991; Sanginga et al., 2000; Hungria et al., 2001; Zengeni and Giller, 2007; Argaw, 2014; Chen et al., 2015). Different environmental constraints under real field

conditions (low/high pH, inadequate moisture, salinity, competition by local rhizobia) may have masked SNF performance. Indoor studies can be improved by testing rhizobia in undisturbed soil cores, for example to evaluate whether an inoculant will survive in the local soil and compete successfully with indigenous rhizobia (Giller, 2001). More critically, however, evaluation of soybean rhizobia strains for nodulation and SNF under field conditions is always needed in order to select optimal strains for commercial inoculant production (Rodríguez-Navarro et al., 2010). It is also important to include both negative controls (no inoculation, no N fertilizer) and positive controls (no inoculation and with N fertilizer) in field trials. For example, if soybean growth is limited when N is applied, it demonstrates that other factors are limiting plant growth and hence rhizobia inoculants may not be beneficial.

5.2. Need for functional studies using indigenous rhizobia

Based on the literature, it is clear that limited research has been conducted to evaluate indigenous soybean rhizobia strains for SNF especially under field conditions (Fig. 2). The literature demonstrates that different factors, such as soil pH, soil fertility, temperature, clay and organic matter content influence rhizobia survival. Unlike local strains, foreign strains may not be adapted to local soil, climatic conditions, and cropping systems, and thus may not persist in the soil. Identification of local rhizobia strains with high SNF may therefore be a promising path forward. However, it is important to note that some indigenous soybean rhizobia have failed to nodulate even promiscuous soybean varieties especially when the soil and environmental factors are not favorable (Herrmann et al., 2014).

5.3. Need for research on soybean variety x rhizobia strain interactions

Based on the above literature, it is clear that soybean SNF depends on the soybean variety x rhizobia strain interaction (Pulver et al., 1982; Danso, 1987; Ravuri and Hume, 1992; Sanginga et al., 2000; Appunu et al., 2008; Argaw, 2014). Therefore, inoculant strain testing must be considered in the context of the soybean variety in order to improve SNF. It is especially critical to select appropriate rhizobia with optimal SNF in late maturing soybean genotypes which have high biomass and hence high N demand (Argaw, 2014).

5.4. Need for research on micronutrient x soybean-rhizobia interactions

Soil micronutrients such as molybdenum, boron, zinc, nickel, cobalt, selenium, manganese, and copper are important for rhizobia symbiotic interactions with legumes (Giller, 2001; O'Hara, 2001). However, based on our meta-analysis, it is clear that the effect of micronutrients on soybean-rhizobia interactions under field conditions has been poorly studied. More studies in this area are needed in order to optimize SNF in soybean.

5.5. Need to optimize inoculation methodologies

Successful nodulation of soybean depends not only on the type of rhizobia strain introduced, but also on the inoculation methodology used. When rhizobia are introduced as a seed coating, the inoculant is concentrated mostly around the seeds even after sowing in the field (López-García et al., 2002). Interestingly it was found that rhizobia cell position in the soil profile is more important for successful nodule occupancy with introduced rhizobia compared to the inherent competitiveness of local rhizobia present in the soil (López-García et al., 2002). Repeated inoculation and a

higher rate of inoculation may be required especially in areas subject to environmental stress (Hungria and Vargas, 2000). Therefore it may also be important to optimize the method of rhizobia strain inoculation in the field in order to maximize nodulation and SNF.

5.6. Need to measure SNF under field conditions

Finally, the literature demonstrates that various parameters have been used to evaluate the symbiotic performance of different rhizobia strains under field conditions, such as nodule number, nodule biomass, growth traits, yield and tissue/grain N content. However, most of these studies have not measured the N fixation capacity (percentage nitrogen derived through SNF) or the amount of N fixed through SNF. It will be beneficial to evaluate more rhizobia inoculants at the field level using standard SNF techniques such as ^{15}N isotope analysis, ureide quantification, the acetylene reduction assay, and/or the nitrogen difference method (Peoples et al., 1989). However, there is a need for the research community to develop simple and low cost methods for measuring SNF under field conditions in order to fulfill this requirement, especially in developing countries.

6. Conclusions

Improving the reliability and responses of rhizobia inoculants on soybean is a timely need due to expected increases in the price of synthetic fertilizers, and environmental concerns related to their application. Improved SNF in soybean will help to meet the increased demand for high quality protein in developing nations as a result of population increases at a time of climate change. It is hoped that this meta-analysis will provide important lessons to assist in these efforts.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.11.022.

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