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Department of Soil and Environment

Need for seed re-inoculation in Swedish soybean cropping sequences

Emelie Andersson

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Faculty of Natural Resources and Agricultural Sciences
Department of Soil and Environment

Emelie Andersson

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Återkommande behov av ympning inom svensk sojabönsodling

Supervisor: Anna Mårtensson, Department of Soil and Environment, SLU
Assistant supervisor: Fredrik Fogelberg, JTI
Examiner: Sebastian Håkansson, Department of Microbiology, SLU

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Abstract

Soybean is a tropical legume that is widely used world-wide as a food product. Its advantage is its ability to fix nitrogen from the atmosphere to produce a high amount of protein, making soybean suitable as food and animal feed. Sweden imports large quantities of soybean, since domestic production is far below the demand. However, imports have negative impacts on the global environment, owing *e.g.* to deforestation in countries where soybeans are produced and long transportation routes. By increasing the production of soybean in Sweden, these negative effects could be avoided.

In order to carry out nitrogen fixation, soybeans need to co-exist with the nitrogen-fixing bacterial species *Bradyrhizobium japonicum*. This requires inoculation of soybean seed with different cultures of the bacterium before sowing, a practice which is currently performed every time soybean is grown in Sweden.

This study examined if annual re-inoculation is necessary, or if *B. japonicum* can survive in the soil after a soybean crop grown in field which is inoculated with this bacterium. Other questions examined were how long *B. japonicum* can survive in the soil, how different soil factors and cultivation measures affect *B. japonicum*, if there is a difference in survival rate of *B. japonicum* in different soil types and if the inoculation cultures Hi-Stick and E11 are suitable as inoculation material and result in similar nitrogen fixation.

These issues were studied in a greenhouse experiment with soybeans cultivated in different soils collected from fields in which soybeans had/had not been grown previously. It was found that *B. japonicum* was able to survive in soils in which soybean had been grown previously and that there was no need for re-inoculation of soybean seeds during a period of at least two years. The soil factors pH, nitrogen content and carbon content had varying and inconsistent effects on the survival rate of *B. japonicum*, making it difficult to estimate their overall influence. However, application of nitrogen (as manure) and high amounts of phosphorus in soil appeared to promote *B. japonicum* survival. The amount of potassium in soil had varying effects, but high amounts appeared to impair *B. japonicum* survival. As regards to soil type, *B. japonicum* survived better in light soils than in clayey soils. Both the inoculant cultures tested Hi-Stick and E11, proved to be suitable as inoculants of soybeans in this greenhouse study.

Sammanfattning

Sojabönan är en tropisk baljväxt som används runt om i världen som livsmedel och foder åt djur. Fördelen med denna gröda är att den har förmågan att fixera kväve från atmosfären och av detta producera höga mängder protein. Sverige importerar stora mängder sojabönor eftersom den odling som sker i landet inte räcker till det som efterfrågas. Importen har i sin tur negativa effekter på miljön på grund av skogsskövling i de länder som odlar soja och de långa transportsträckorna. Genom att öka den inhemska produktionen av soja, kan dessa negativa effekter undvikas.

För att sojabönor ska kunna fixera kväve, måste denna leva tillsammans (symbios) med en kvävefixerande bakterie vid namn *Bradyrhizobium japonicum*. Denna symbios blir möjlig då sojabönor ympas med denna bakterie innan sådd. Det anses vara nödvändigt att ympa fröna varje gång sojabönor ska odlas för att garantera en kvävefixering.

Denna studie undersökte om årlig ympning är nödvändig och om *B. japonicum* kan överleva i jorden efter en odling av sojabönor som tidigare blivit inokulerad med denna bakterie. Andra frågor som undersöktes var hur länge *B. japonicum* kan överleva i jorden, hur olika jordfaktorer och odlingsåtgärder påverkar *B. japonicum*, om det finns någon skillnad i överlevnadsgrad hos *B. japonicum* i olika jordtyper och om ympningskulturerna Hi-Stick och E11 är lämpliga som ympningsmaterial till sojabönor.

Dessa frågor undersöktes i ett växthusförsök med sojabönor som odlades i olika jordar som samlats från fält där man tidigare har haft/inte har haft någon odling av sojabönor. Resultaten visade att *B. japonicum* kan överleva i jorden i två år efter en sojabönsodling, vilket betyder att en återympning inte är nödvändig under denna tidsperiod. pH, kväve och kol halten i jorden hade olika påverkan på överlevnadsgraden hos *B. japonicum*, vilket gjorde det svårt att komma fram till hur de påverkar bakterien. Däremot visade det sig att en kväve-giva och höga mängder av fosfor i marken gynnar överlevnaden hos *B. japonicum*. Mängden kalium i jorden gav olika påverkan, men det verkade som att höga mängder kalium missgynnar överlevnaden hos *B. japonicum*. Dessutom visade det sig att *B. japonicum* överlevde bättre i de lättare jordarna än i de jordar som innehöll lera. Angående ympningskulturerna Hi-Stick och E11, så är båda dessa lämpliga som ympningsmaterial till sojabönor.

Finns det ett ympningsbehov hos sojabönor?

Sojaböna är en gröda som används runt om i världen till både livsmedel och foder. Denna gröda är känd för att innehålla mycket protein och anses därför vara en hälsosam produkt. I Sverige odlas sojabönor inte i någon större utsträckning och därför finns det ett behov av att importera soja, främst från Brasilien. Dock finns det negativa konsekvenser med att importera soja. Miljön påverkas negativt då sojan fraktas långa sträckor och skogar skövlas bort för att kunna odla mer soja.

Ett sätt att minska dessa negativa effekter är att minska importen av sojabönor till Sverige och istället öka den inhemska produktionen av soja. Försök har visat att sojabönor går att odla i Sverige, dock bör inte odlingen ligga längre norrut än till Mälardalen för att få bra resultat. Genom att odla inhemsk soja kan Sverige dessutom minska sina importkostnader. Det finns några få svenska lantbrukare som odlar soja, men det räcker inte till för att täcka efterfrågan på soja i Sverige. Om det i framtiden ska vara rimligt att Sverige ska ha en betydande inhemsk produktion av sojabönor, bör intresset för att odla soja öka bland lantbrukare.

För att sojabönan ska få sin höga proteinhalt, måste denna fixera kväve från luften. Detta kan inte grödan göra själv, utan måste ha hjälp av en kvävefixerande bakterie som hjälper grödan att fixera kväve. Denna bakterie heter *Bradyrhizobium japonicum*. Denna finns inte naturligt i Sveriges jordar och därför finns det ett behov av att behandla fröna med denna bakterie innan de ska sås. Denna metod kallas för ympning och har använts länge i jordbruket. Oftast säljer fröföretag sojabönor med ympkulturer som lantbrukaren själv ympar fröna med. Lantbrukaren ympar fröna varje gång då soja ska odlas för att garantera att det sker en kvävefixering hos sojabönan och därmed få en hög proteinhalt.

I ett växtförsök utfört 2013 på Sveriges Lantbruksuniversitet har det visat sig att *B. japonicum* kan överleva i jorden efter att en odling av sojabönor har skett. Försöket visade att bakterien kan finnas kvar i jorden i åtminstone två år. Den mängd bakterier som fanns kvar i marken var tillräcklig för att de nästkommande sojabönorna skulle få sin kvävefixering. Detta betyder att lantbrukare då inte behöver ympa sina frön med bakterier år efter år eftersom sojabönorna då kan använda de bakterier som finns kvar i marken efter föregående odling av soja. Detta i sin tur betyder att lantbrukarna inte behöver köpa nytt ympningsmaterial till sin nästkommande odling av soja, vilket skulle minska på lantbrukarens kostnad. Med denna fördel kan intresset för inhemsk produktion av soja öka i Sverige.

Frågan som kan ställas är om ympningsbakterien har förmågan att överleva i jorden under en längre period än två år. Då soja, precis som alla grödor, inte bör odlas alltför ofta på samma fält (detta för att minska risken för sjukdomsangrepp), vore det intressant att undersöka om bakterien kan överleva i kanske fem eller tio år efter en odling av sojabönor. Enligt en rapport från Spanien har *B. japonicum* hittats i ett fält där soja tidigare odlats för tio år sedan.

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Abbreviations
Värm = Värmland
V-man = Västmanland
Närk = Närke
Uppl. = Uppland
uninoc = non-inoculated
00 = soil with no cultivation of soybean
Harv. = harvesting
P-Al = plant-available phosphorus (mg P/100 g soil)
K-Al = plant-available potassium (mg K/100 g soil)
HCl-P = phosphorus bound in sediment (mg K/100 g soil)

HCl-K = potassium bound in sediment (mg K/100 g soil)
* = n = Only six replicates since some pots were deleted due to outlying values

Introduction

The soybean plant is a tropical legume that is widely used in the world as food and animal feed. The plant originates from China, where it has been used as a crop for more than 5000 years (Håstadius, 1949; Rankine, 1999; Heimer, 2010). The crop arrived in Europe during the 17^{th} century and about 200 years later people started to cultivate soybean to a larger extent in Europe (mainly Russia, Czechoslovakia and Romania) and the USA (Håstadius, 1949; Heimer, 2010). Soybean has the ability to fix nitrogen from the atmosphere and use it to produce high amounts of protein in the plant (~40% protein). This feature makes the crop suitable as a forage legume and food (Heimer, 2010). Soybean is considered to be healthy because of its high content of protein and its content of essential amino acids (Bengtsson & Larsson, 1979; Rankine, 1999; Altemose *et al.*, 2005; Jensen *et al.*, 2011).

In Sweden, soybean is not cultivated in any great extent (only 50-60 ha; Ljungdahl, 2013), and therefore Sweden relies heavily on imports of this crop. In 2011, soybean was the second largest food import to Sweden, comprising about 250 thousand tons (valued at approx. 125 million USD) (FAO, 2011b). Around 90% of the imported soybean is used as animal feed and the remaining 10% is used to produce food products. Soybean can also be used to produce material for technical purposes (ink, fibre, glue *etc*.) and medical products (Rankine, 1999; Heimer, 2010).

Countries with large-scale productions of soybean include the USA (~82 million t/year), Brazil (~66 million t/year) and Argentina (~52 million t/year) (FAO, 2013). Around 20% of the soybean imported by Sweden comes from South America (mainly Brazil) (Heimer, 2010). Soybean is grown annually on about 130 million hectares in South America (FAO, 2011a; FAO, 2013). Importing soybean has a negative impact on the environment through *e.g*. deforestation and long transportation routes before arriving in Sweden (Heimer, 2010; JTI, 2012). According to Heimer (2010), 1.2 million hectares of forest disappear in the Amazonas region each year. Gasparri *et al.* (2013) found a strong correlation between soybean expansion and deforestation in Argentina. Aide *et al.* (2013) also concluded that at the beginning of 21^{st} century, extensive areas in South America were converted to agricultural fields to produce crops such as soybean. This conversion was the result of increased global demand for meat.

One solution to the problems caused by soybean cultivation in South America would be to cease soybean importing entirely and instead use other crops that can be grown in Sweden. However, since soybean contains a high amount of protein, it is a suitable crop for use in food and feed production and is therefore difficult to replace. Another solution would be to grow soybean in Sweden. In fact, interest in growing soybean in Sweden has increased, as farmers want to produce their own feed or sell feedstuffs to other farmers (JTI, 2012). Moreover, national production would have a positive economic effect, since Sweden could reduce its import costs and improve its balance of trade.

In Sweden, import of soybean started at the beginning of the 20th century. Since then, there have been crop trials in Sweden with the aim of producing varieties more suitable for the Swedish climate (Håstadius, 1949; Holmberg, 1956). Today, there are some varieties that can withstand the Swedish climate (Holmberg, 1950; Holmberg, 1973). Moreover, it has been demonstrated that adequate yields can be achieved in Sweden, with reported yield ranging between 800-1900 kg/ha (Holmberg, 1950; Holmberg, 1973; Bengtsson & Larsson, 1979; Fogelberg, 2009). This is comparable to the yield in Japan (highest yield ~1700 kg/ha (FAO, 2013), a country with relatively high production of soybean. However, compared with the high-producing countries USA, Brazil and Argentina that can obtain maximum yields of about 2700, 2600 and 2300 kg/ha, respectively, it is obvious that Sweden still has low yields, although approaching those obtained in Argentina (FAO, 2013).

Soybean is unable to fix nitrogen without a symbiosis with the nitrogen-fixing bacterium, *Bradyrhizobium japonicum*. The definition of symbiosis is when two (or more) organisms live together. In this case, the relationship between bacteria and soybean is mutualistic, which means that both species benefit from living together (Madigan *et al.*, 2009; Mrkovacki, 2011).

To ensure effective symbiosis and thereby obtain nitrogen fixation, farmers need to inoculate soybean seed with *B. japonicum* before sowing. Inoculation is simply done by applying *B. japonicum* directly onto the seeds. Inoculation is considered necessary when there is insufficient *B. japonicum* in the soil (Dowdle *et al.*, 1985; Bailey, 1989; Sprent & Sprent, 1990; Catroux *et al.*, 2001; Albareda *et al.*, 2009a; Fogelberg, 2009). According to Albareda *et al.* (2009b), inoculation of soybean seed with *B. japonicum* is common in Europe, since the soils are reported to be lacking in this bacterium (Fogelberg, 2009).

It is currently considered necessary to inoculate soybean seed with *B. japonicum* every time this crop is grown to ensure nitrogen fixation. The question is if annual inoculation is really necessary. There is a possibility that *B. japonicum* can survive in the soil after growth of an inoculated soybean crop. According to Breed *et al.* (1957), *B. japonicum* can be found in soils where soybean has been grown previously. Similarly, Albareda *et al.* (2009b) reported that *B. japonicum* was able to survive in a soil in southern Spain with soybean cultivated three years earlier, although the amount decreased year after year. In one of the soils investigated, re-inoculation was not necessary. Albareda *et al.* (2009a) identified *B. japonicum* in a soil after previous soybean cultivation ten years earlier, but did not indicate whether the amount found was sufficient to inoculate a new soybean crop or not. In another study investigating if *B. japonicum* could survive the winter in prairie soils in Canada, it was found that the *B. japonicum* did not survive the winter (Bailey, 1989).

Aim of the work

Given this background, the aim of the present work was to investigate if *B. japonicum* is able to survive in Swedish soils after a soybean crop. The specific research questions examined were:

1) Can *B. japonicum* survive in soils after soybean cultivation?

2) How long can *B. japonicum* survive in the soil? Is it necessary to re-inoculate when soybean seeds are planted in soils with previous soybean cultivation?

3) How do different soil factors and cultivation measures affect *B. japonicum*? Is there a difference in survival rate of *B. japonicum* in different soil types?

4) Are the inoculation cultures Hi-Stick and E11 suitable as inoculation material? Is there a difference in nitrogen fixation between plants inoculated with Hi-Stick and E11?

Symbiosis between *Bradyrhizobium japonicum* and soybean

When symbiosis occurs between *B. japonicum* and the soybean plant, nodules are produced on the host plant (Breed *et al.*, 1957; Jordan, 1982; Solomin *et al*., 2012). Nodules are structures that are formed when there is a symbiosis between the plant and bacteria. The bacteria cause cell-division in the plant which in turn produces the nodules on the plant (Sprent & Sprent, 1990; Madigan *et al*, 2009; Mrkovacki, 2011). According to Bailey (1989), no nodules are produced in non-inoculated soybean. Therefore, nodule weight is a good indicator of the presence of *B. japonicum* in the soil. The greater the production of nodules, the more *B. japonicum* is present in the soil. Furthermore, according to Solomin *et al*. (2012) the higher the nodule dry weight, the higher the nitrogen fixation. Therefore, the nitrogen content in the plant biomass is also a good indicator of if there is *B. japonicum* in the soil. The more nitrogen fixation, the more dry biomass weight should be achieved, since the plant uses nitrogen for growth. This makes plant biomass weight a good indicator of *B. japonicum* incidence in the soil. According to Mrkovacki (2011), effective nitrogen fixation in soybean results in good plant growth, and therefore developmental growth stages is also a good indicator of the presence of *B. japonicum* in the soil.

Factors affecting *Bradyrhizobium japonicum* and soybean

Bradyrhizobium japonicum is member of a family of bacteria called the Rhizobiaceae (Rhizobia). *Bradyrhizobium japonicum* seems to be relatively tolerant to low pH compared with other species of Rhizobia, but cannot handle extremely high pH levels. According to a study by Graham & Parker (1964), *B. japonicum* cannot grow at pH>8.5. There are some isolates of *B.*

japonicum that are more tolerant to low pH (~4.2-4.6) than others (Taylor *et al*., 1991; Indrasumunar *et al*., 2012). Some cultures are able to grow at pH 3.5, although the growth is sparse. Graham & Parker (1964) also showed that the optimal pH for *B. japonicum* growth is 4.5-8.0.

Increasing the pH seems to promote *B. japonicum* growth, including cultures tolerant to acidic conditions (Graham & Parker, 1964). By just increasing the pH from 4.2 to just 4.6, the growth can be improved for some cultures (Taylor *et al*., 1991). Moreover, Cooper *et al*. (1985) observed better growth of *B. japonicum* at pH 6.7 than at pH 4.5 and also obtained significantly greater production of nodules at the higher pH level. However, low pH has a low inhibitory effect on the nodulation process itself.

In a study by Albareda *et al.* (2009b), *B. japonicum* was found to gain a benefit from low pH. The same study investigated if *B. japonicum* could survive in a field with a soybean crop grown three years earlier. Two soils were used; one with pH 8 with a loam texture and the other with pH 6.6 with a sandy-loam texture. In the slightly acidic soil, the amount of *B. japonicum* was higher than in the alkaline soil. The authors concluded that there might be enough with bacteria in the acidic soil to meet the symbiotic requirements of the next soybean crop. The soil texture could also have affected the *B. japonicum*, as the texture was lighter in the acidic field than in the alkaline field. Based on these results, *B. japonicum* seems to prefer lighter soil.

Nutrients affect the growth of both *B. japonicum* and soybean. Although soybean fixes nitrogen, the plant (like most other crops) needs nitrogen to start growing (Gan *et al*., 2003). In a study by Gan *et al.* (2002), lack of a nitrogen supply lowered plant biomass and nitrogen accumulation in soybean seedlings grown in the greenhouse. When nitrate was applied at the transplanting or V2 stage of the crop (see Table 1), plant growth and nodule production were stimulated. Moreover, giving a starting nitrogen dose of 25 kg/ha in the field proved to have a positive effect on nodule production and nitrogen fixation (Gan *et al*., 2002). After this starting dose of 25 kg/ha, positive effects were obtained with applying nitrogen during later stages as well. In studies by Gan *et al.* (2002, 2003), applying 50 kg nitrogen/ha during stage R1 or R5 of the crop was shown to have a positive impact on nitrogen fixation, plant biomass and seed yield. For example, in the study by Gan *et al.* (2003), a topdressing of 50 kg nitrogen/ha at the V2 stage of the crop increased plant biomass, nodule dry weight, total accumulation of nitrogen in the plant and seed yield.

However, applying more nitrogen during growth of the soybean plant could also have negative impacts. In the study by Gan *et al.* (2003), topdressing nitrogen at R3 or R5 did not significantly increase plant biomass, total accumulation of nitrogen in the plant or seed yield. Moreover, according to Gan *et al.* (2002), topdressing 50 or 75 kg nitrogen/ha during V4 stage gave lower dry weight of nodules during the R1 stage of the crop.

There are some reports that applying nitrate can suppress the production of nodules. In a study by Sodek & Silva (1996), increasing nitrate from 0 to 15 mM was shown to decrease the weight of the nodules by about 87.2%. Moreover, according to Albareda *et al.* (2009b), applying 50 kg nitrogen/ha at R1 stage also decreased nodule production.

As a conclusion, applying nitrogen could have negative or positive effects on the soybean plant growth and nodule production. The effect depends on the amount of N and during what growth stage of the plant it is applied.

The growth of both *B. japonicum* and the soybean plant is also affected by phosphorus. Some cultures of the *B. japonicum* seem to grow slowly when there are low amounts of phosphorus (Cassman *et al*., 1981). Other cultures are more tolerant to phosphorus deficiency than others (Indrasumunar *et al.*, 2012). The growth of the soybean plant is also negatively affected by phosphorus deficiency, which in turn affects the nodulation process negatively (Bordeleau & Prévost 1994). The growth of the plants improves when there is sufficient phosphorus present (Indrasumunar *et al.*, 2012). According to Singleton *et al.* (1985) and Israel (1987, 1993), increasing the amount of phosphorus in the soil increases the amount and weight of soybean nodules. Moreover, Cassman *et al.* (1981) reported that *B. japonicum* is positively affected by increased amount of phosphorus, since by increasing the phosphorus concentration from $4x10^{-7}$ to $6x10^{-4}$) 410^{-7} M (mol/dm^3), the generation time of *B. japonicum* was increased by a factor of 0.1-2.4.

Too high concentrations of phosphorus have also been shown to have a negative effect on nodule production. In a study by Tsvetkova & Georgie (2003), increasing the phosphorus concentration from 1 to 3 mM decreased nodule production. A concentration of 0.1 mM also decreased the weight of nodules, but still gave a higher weight of nodules than the concentration of 3 mM (Tsvetkova & Georgie, 2003).

How *B. japonicum* is affected by phosphorous seems to differ between the cultures of this bacterium. It seems though that deficiency of phosphorous inhibit the growth of the bacteria. Also, the plant growth, and thereby nodulation production, is also negatively affected by deficiency of phosphorous. But too much phosphorous could also decrease the nodule production.

Increasing the potassium concentration has been shown to increase plant dry matter and nodule weight. For example, in a study by Premaratne & Oertli (1994), increasing the potassium concentration from 1.0 mM to 20.0 mM increased root and shoot dry weight by about 115% and 125%, respectively. The nodule weight was also increased, by about 153% (Premaratne & Oertli, 1994).

Materials and methods

Soil sampling and field management history

Soil samples were collected in 2014 from fields on the following farms: Edsberg Gård (N 59°22.391', E 13°15.022', Värmland), Berga Gård (N

59°11.433', E 14°52.910', Närke), Munktorp Prästgård (N 59°32.301', E 16°8.604', Västmanland) and Sjöö Gods (N 59°42.616', E 17°30.293', Uppland). Information about the history of cultivation in the field was obtained through interviews with the farmers. Questions asked during these interviews are shown in Appendix 1. At Edsberg Gård and Berga Gård, soil samples were collected from fields where a soybean crop had been grown in the previous year (2013). At Munktorp Prästgård, samples were collected from two fields where soybean had been grown during 2011 and 2013, respectively. At Sjöö Gods, soil samples were collected from three fields where soybean had been grown in 2011, 2012 and 2013, respectively. Soil samples were also collected from a field at the farm Sjöö Gods with no previous history of soybean cropping.

The soil samples were taken from the topsoil (0-20 cm depth). A total of 20-30 samples of 1-2 L soil (each sample consisting of 5-6 subsamples of 0.5-3 L) were collected in each field. The samples were collected diagonally of an area of the field; in Västmanland 2000 m^2, Värmland 500 m^2, Närke 500 m^2 and Uppland 100 m^2.

Experimental design

I. Survival rate of *B. japonicum* in soil with soybean grown previously in 2011, 2012 or 2013:

Soil samples from two fields located in Västmanland with different soybean cultivation history were compared. The year of soybean cultivation in those fields was 2011 and 2013. In addition, soils from three fields located in Uppland were compared with each other. The year of soybean cultivation in those fields was 2011, 2012 and 2013, respectively.

II. Survival rate of *B. japonicum* depending on soil characteristics and field site:

Soils collected from fields with different soil characteristics in which a soybean crop had been grown in the previous year (2013) were compared. These fields were located in Värmland, Närke, Västmanland and Uppland.

III. Effect of re-inoculation in soil with previous soybean cultivation:

Soil from a field in Uppland in which soybean was grown in 2011 was used for studying the effect of re-inoculation in soil with a previous soybean crop. Inoculated soybean crops were compared with non-inoculated soybean. Two different strains, Hi-Stick (obtained from Becker Underwood) and a single *B. japonicum* culture E11, were used as inoculants and their effectiveness as an inoculation culture to soybean was compared.

IV. Effect of inoculation in soil with no previous history of soybean cultivation:

Soil was collected from a field in Uppland which had no previous history of soybean cultivation. The inoculants used were Hi-Stick and a single *B.*

japonicum culture E11, and the strain's suitability as an inoculation culture to soybean was assessed.

Growing conditions

The greenhouse studies started in November 2013 and finished in January 2014. In all experimental cultivations, there were seven pots of each soil. All soils were mixed with pumice stone in order to avoid compact soils, since soybean does not grow well in heavy, packed soils (Fogelberg, 2009). Each pot had about 1.3-1.4 L soil and 0.6-0.7 L pumice stone. Five soybean seeds were planted in each pot. The cultivar used was Moravians, which originates from Czech Republic. This cultivar is medium early and has a germination rate of 75-80%. Conditions set for the soybeans were 20^{o}C, light intensity of 250-350 μmol/m^{3}/s, duration of light 17 hours/day and humidity 60%. No nutrients were applied to the plants. Underground watering started six days after sowing, and comprised ten seconds seven times per day. Manual watering was performed when necessary. Moreover, plant screening was performed when necessary, which was done when the plants needed more space to grow. In each pot, there were between 1 and 3 plants. After about two months of growth, the plants were harvested. During these months, the growth process was documented. The results are shown in Appendix 2.

Since nodule dry weight, plant dry biomass weight and nitrogen content in plant biomass are good indicators of if *B. japonicum* is present in the soil, these data were gathered during harvest. Moreover, the stage of the crop just before harvest was estimated according to Table 1. It was postulated that there is a correlation between these parameters and the content of *B. japonicum* in soil. The roots were washed and separated from the nodules. Plant biomass and nodules were dried at 105°C for a couple of hours. Nodule and total dry biomass weight were measured by a balance that weighed the material to an accuracy of two decimals. The soil samples from each field were analysed for pH value and content of nitrogen, phosphorus, potassium and carbon. These soil characteristics are considered to be constant over a couple of years. The plant material was also analysed to determine the nitrogen content in plant biomass.

Statistical analysis

For each experiment, mean values of each treatment were calculated and compared with each other using the two-sample t-test. The following hypotheses were tested:

H_0: There is no difference between the mean values
H_1: There is a difference between the mean values

Probability (P) values to test hypothesis H_0 were calculated using the Minitab program. If the differences between means were found to be significant ($P<0.05$), the Hypothesis H_0 was rejected, meaning that there was a difference between the mean values for different treatments/soils (Olsson *et al.,* 2005).

Some data values were deleted since these differed considerably from the other values in one soil/treatment and were considered to be outliers. This was done to obtain better mean values. These outlying values could have been due to weighing errors or different rates of plant growth.

All P-values, mean values and standard errors are presented in full in Tables A1-A5 in Appendix 3 and are summarised in diagrams in the Results section. Standard error is a measure of how well the mean value estimates the population's mean value, meaning how much the mean value varies (Olsson *et al.*, 2005). If the standard error in each treatment/soil overlaps, there is no significant difference between the mean values.

In some diagrams, a trend line is included to show how soil factors affect the soybean plant. In these diagrams, the coefficient of determination (R^2) is also shown for each trend line. This coefficient is used to determine if there is a correlation between the x- and y-values and has a value between 0-1. There is little or no correlation between the soil factor and soybean plant for R^2 values around 0, while R^2 values close to 1 indicate a strong correlation between soil factor and soybean plant (Olsson *et al.*, 2005).

Soybean growth stages

The growth stages of soybean are listed and described in Table 1. The growth stages are divided into two main parts: Vegetative phase (V) and reproductive phase (R). During the vegetative phase, the amount of nodes indicates the crop's growth stage (Agri-Growth, 2000).

Table 1: Soybean stages and their descriptions (Agri-Growth, 2000).

Stage of the crop	Description
VE	Emergence of the crop
VC	First leaf node unfolded
V1	First node apparent
V2	Second node apparent
V3	Third node apparent
V(n)	N^{th} node apparent
R1	Start of flowering (there is one flower open)
R2	Full flowering, *i.e.* a flower with one of two uppermost nodes is open on the main stem
R3	Pods start to develop
R4	Pod production finished
R5	Seed production starts in the pods
R6	Pods filled with seeds that are still green in colour
R7	Maturation starts (plant starts to senesce)
R8	Maturation ends, *i.e.* 95% of the pods have reached mature pod colour

Results

Results from statistical analysis

I. Survival rate of *B. japonicum* in soil with soybean grown previously in 2011, 2012 or 2013:

The soil from Västmanland in which soybean had been grown previously in 2011 and 2013 still contained *B. japonicum*, since all plants in each treatment produced nodules (see Table A1 in Appendix 3). However, there were no significant differences between years in terms of nodule weight (P=0.29) plant biomass weight (P=0.30), nitrogen content in plant biomass (P=0.092) and stage of the crop (P-value not determined). This indicates that there was a similar amount of surviving *B. japonicum* in both soils.

As shown in Table A2 (see Appendix 3), all plants grown in soils from Uppland (with soybean grown previously in 2011, 2012 and 2013) produced nodules, which indicated presence of *B. japonicum*. There were no significant differences between the soils in terms of nitrogen content in plant biomass and stage of the crop. However, there were significant differences as regards nodule weight and plant biomass weight (Figure 1a, 1b). The highest plant biomass weight and nodule weight were obtained in soil with soybeans grown previously in 2011 and the lowest values were obtained in soil with soybean grown previously in 2013. This indicates that there was a higher survival rate of *B. japonicum* in the soil with soybeans grown previously on 2011.

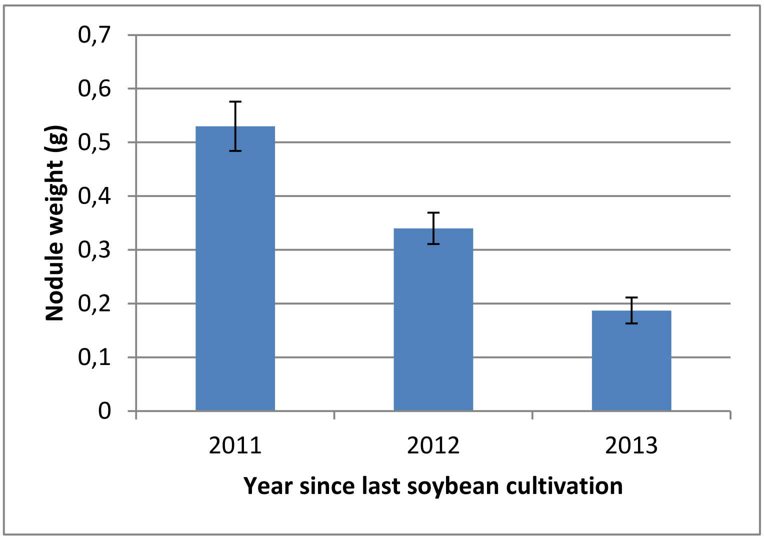

Figure 1a

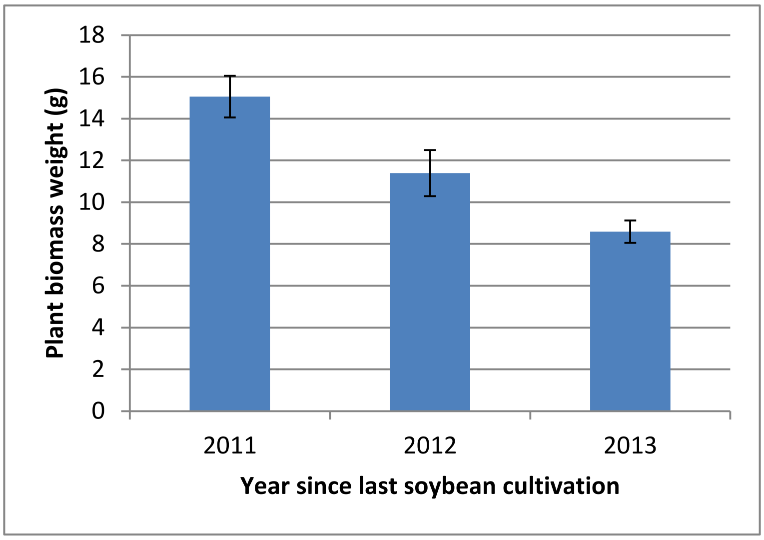

Figure 1b

Figure 1: (a) Nodule weight and (b) plant biomass weight of soybean grown in soil collected from three fields in Uppland in which soybean had been grown previously in 2011, 2012 and 2013, respectively. Mean value and standard error.

II. Survival rate of *B. japonicum* depending on soil characteristics and field site:

Soils from Värmland, Närke, Uppland and Västmanland contained *B. japonicum*, since all soybean plants produced nodules (see Table A3 in

Appendix 3). However, there were significant differences between all soils (except Värmland and Närke, which had rather equal results) as regards nodule weight, plant biomass weight and nitrogen content in plant biomass (Figure 2a-2c). These parameters were significantly higher in soils from Värmland and Närke, which indicates a higher survival rate of *B. japonicum* in these soils compared with Uppland and Närke. Comparing Västmanland with Uppland, the nodule weight and total biomass weight were lower for Uppland soil, but it had a higher content of nitrogen in plant biomass (Figure 2a-2c). This indicates a higher presence of *B. japonicum* in the soil collected from Västmanland.

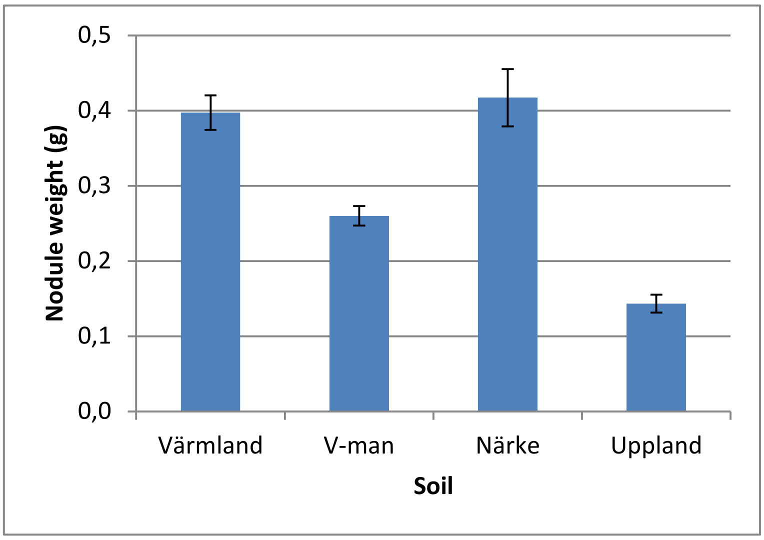

Figure 2a

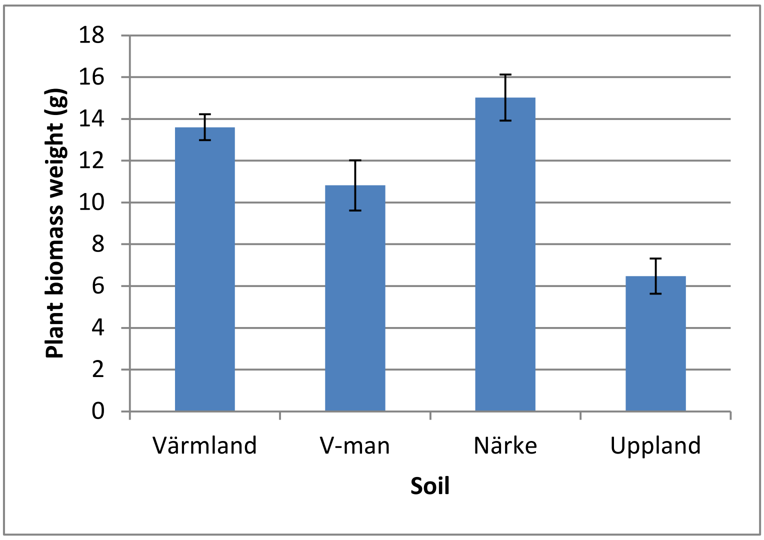

Figure 2b

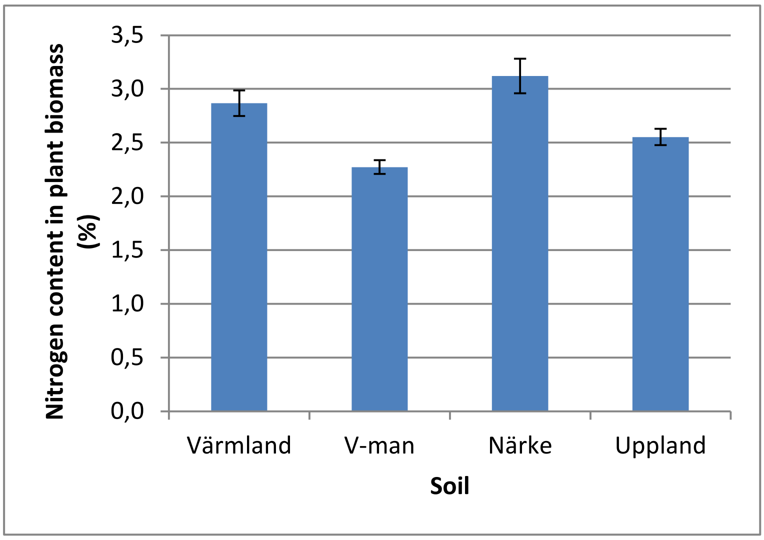

Figure 2c

Figure 2: (a) Nodule weight, (b) plant biomass weight and (c) nitrogen content of soybean grown in soils collected from fields in Värmland, V-man, Närke and Uppland in which soybean had been grown previously in 2013. Mean value and standard error.

III. Effect of re-inoculation in soil with previous soybean cultivation:

There were no significant differences between the uninoculated and inoculated soybean regarding stage of the crop, biomass weight, nodule weight and nitrogen content in plant biomass (see Table A4 in Appendix 3). However, according to the statistical analysis, there was a significant difference between the cultures used, with E11 giving a higher weight of nodules than Hi-Stick (Figure 3).

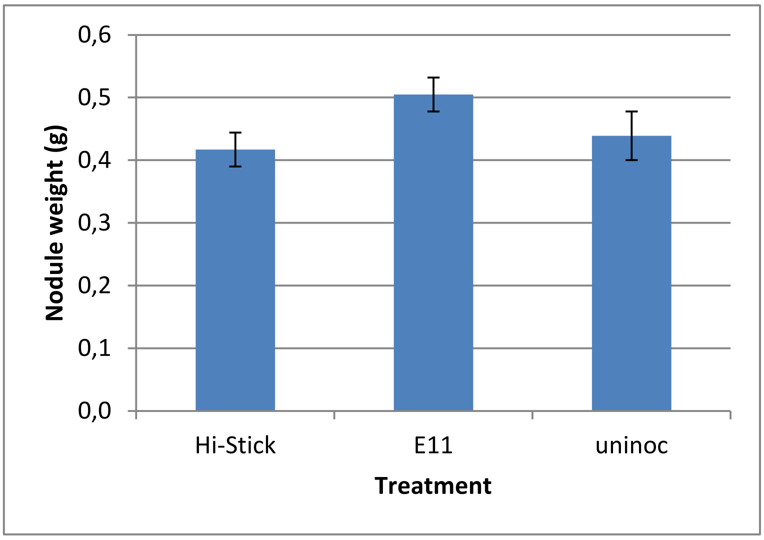

Figure 3: Effect of inoculation on nodule weight of soybean grown in soil collected from a field in Uppland in which soybean had been grown previously in 2013. Presented as mean value and standard error.

IV. Effect of inoculation in soil with no previous history of soybean cultivation:

In each treatment, nodules were produced (Table A5 in Appendix 3). There were significant differences between the different treatments in terms of nodule weight, plant biomass weight and nitrogen content in the biomass (Figure 4a-4c). However, there were no significant differences between the culture E11 and uninoculated treatment as regards nodule and plant biomass weight.

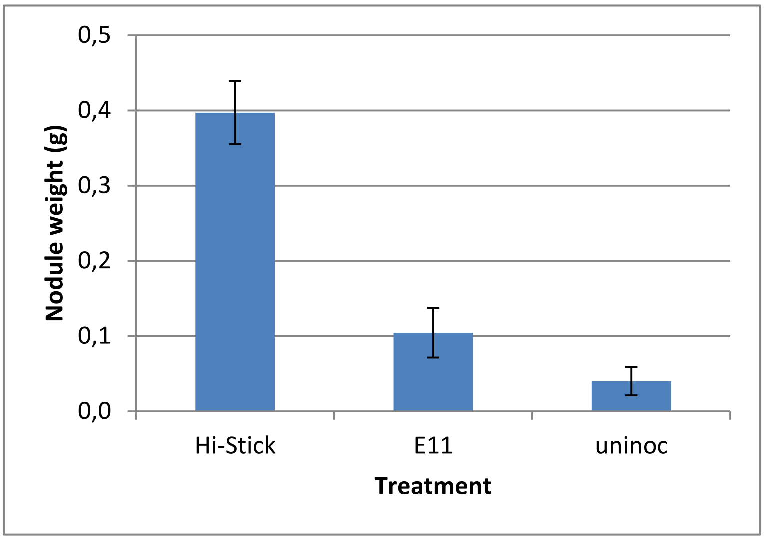

Figure 4a

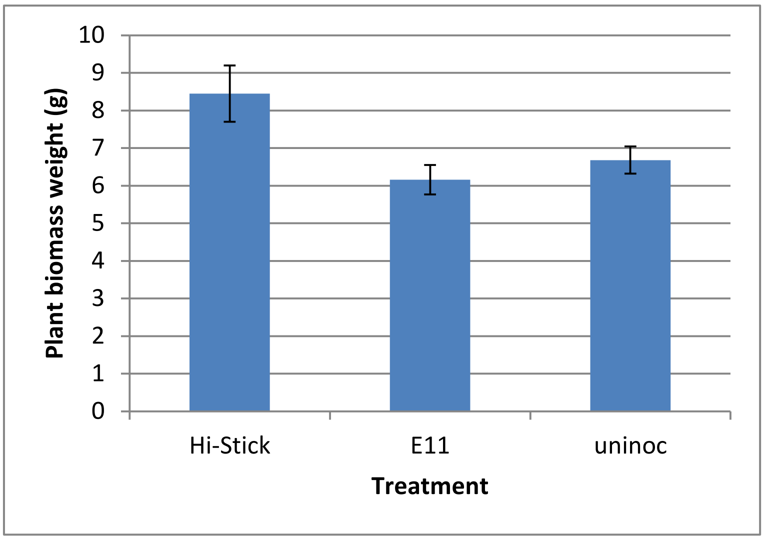

Figure 4b

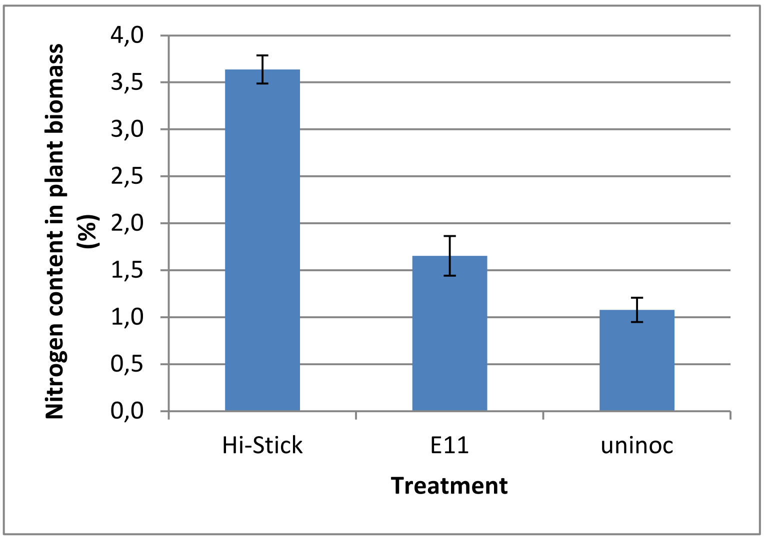

Figure 4c

Figure 4: Effect of inoculation on (a) nodule weight, (b) plant biomass weight and (c) nitrogen content of soybean grown in soil collected from a field in which soybean had not been grown previously. Presented as mean and standard error.

Soil chemical characteristics

Table 2: pH, C, N, P and K content in the soils used in greenhouse experiments. The year indicates when soybean was grown.

Soil	pH	Tot-C %	Tot-N %	C/N	P-AL (mg/100g)	K-AL (mg/100g)	HCl-P (mg/100g)	HCl-K (mg/100g)
V-man 2011	5.55	2.34	0.18	13.4	3.50	15.3	54.6	363.6
V-man 2013	5.55	2.65	0.22	12.2	5.20	15.4	64.6	414.6
Uppl. 2011	6.68	1.87	0.14	13.8	23.1	27.1	93.6	599.1
Uppl. 2012	5.78	2.79	0.26	10.7	5.40	17.6	68.7	477.3
Uppl. 2013	6.31	1.79	0.13	13.9	5.20	24.4	54.1	576.3

Värm 2013	5.78	1.66	0.05	34.1	7.10	3.50	62.2	76.3
Närk 2013	5.32	3.81	0.29	13.1	6.40	5.00	94.8	92.2
00	8.03	1.92	0.10	19.9	14.9	25.8	73.1	671.8

Interview with farmers – responses

The questions used in the interview can be found in Appendix 1.

Table 3: Cultivation measures in fields in Uppland

	Fields			
	2011	2012	2013	00
Crop rotation	2010: Winter wheat 2011: Soybean 2012: Winter wheat	2011: Winter oilseed rape 2012: Soybean 2013: Spring wheat	2012: Winter wheat 2013: Soybean	2013: Spring barley & winter oilseed rape
Tillage management	Autumn tillage with field cultivator, harrowing 1-2 times before sowing of soybean, mechanical weed control	Autumn tillage with field cultivator, harrowing 1-2 times before sowing of soybean, mechanical weed control	Autumn tillage with field cultivator, harrowing 1-2 times before sowing of soybean, mechanical weed control	Autumn tillage with field cultivator, harrowing 1-2 times before sowing of soybean, mechanical weed control
Cultivar	Bohemia	Merlin, Bohemia, Anuschka	Bohemia	-
Conventional/organic	Conventional	Conventional	Conventional	Conventional
Area (ha)	6	14	2	-
Yield (kg/ha)	1000	Harv. not possible	500	-
Inoculation culture	Hi-Stick	Hi-Stick	Hi-Stick	Hi-Stick
Soil texture	Clay loam	Clay loam	Clay loam	Clay loam

Table 4: Cultivation measures in fields in Västmanland, Närke and Värmland

	Fields			
	2011, V-man	2013, V-man	2013, Närk	2013, Värm
Crop rotation	2010: Spring wheat 2011: Soybean 2012: Spring wheat	2012: Oat 2013: Soybean	2012: Oat 2013: Soybean	2012: Spring barley 2013: Soybean
Tillage management	Autumn ploughing, harrowing 2 times before sowing, used 25 tons burnt animal manure	Autumn ploughing, harrowing 2 times before sowing.	Autumn ploughing, harrowing 2 times before sowing, used 15 tons poultry manure.	Autumn ploughing, harrowing before sowing, used 600 kg/ha biofer 10:3:1, mechanical weed control with a harrow
Cultivar	Bohemia	Bohemia	Bohemia	Bohemia
Conventional/organic	Organic	Organic	Organic	Organic
Area (ha)	1	1.5	0.5	0.6
Yield (kg/ha)	1500	300 (low yield caused by animals)	600	667-833

Inoculation culture	Fogelberg special	Fogelberg special	Fogelberg special	Fogelberg special
Soil texture	Light clay	Clay loam	Fine sand light clay	Fine sandy soil

Discussion

***Survival rate and sustainability of* B. japonicum**

There was some *B. japonicum* present in all soils, since the plants in every treatment produced nodules, (which is a good indicator of an occurring symbiosis). In addition, plants with no inoculation treatment and grown in soil with no previous history of soybean cultivation produced nodules. There would not have been any production of nodules since there should not be *B. japonicum* present, according to *e.g*. Solomin *et al*. (2012). Rather, the explanation for the results observed could be accidental cross-contamination of these plants when sowing the seeds or accidental transfer of inoculum through the underground watering during the greenhouse experiment from pots with inoculation treatment to un-inoculated pots.

In the experiment with soil from Västmanland where soybeans were cultivated 2011 and 2013,both soils both soils had equal amounts of *B. japonicum*, since there were no significant differences regarding the parameters measured. In the same experiment, the results obtained for soybean grown in soils from Uppland (with soybeans grown previously in 2011, 2012 and 2013, respectively) indicated higher presence of *B. japonicum* in the soil with soybean grown in 2011. The soil with soybean grown in 2013 had the lowest presence of *B. japonicum*, as indicated by the lowest nodule and plant biomass weight. Thus the results show that *B. japonicum* is able to survive for at least two years without decreasing in amount. However, as previously written, the possible cross-contamination of bacteria between the plants could have affected the results as well.

Assuming that the bacteria were able to survive in soil in two years, the results from this experiment contradict Albareda *et al.* (2009b), who reported that the amount of *B. japonicum* decreases in soil year after year. Moreover, Bailey (1989) found that *B. japonicum* is not able to survive the harsh winter in Canada, but the results from the present investigation on soils with soybean previously grown in 2011 and 2012 showed presence of *B. japonicum*, which indicates that it had survived the Swedish winter. The reason of why the results differed between the experiments could be explained by differing weather factors e.g. colder winters in Canada that could have had a negative effect on the bacteria. The accidental cross-contamination in this report's experiment could also be another explanation of why bacteria were found in the soils.

***Soil factors affecting survival rate of* B. japonicum**

Soil factors have had conflicting effects on *B. japonicum* in previous studies. In terms of pH value in soil, *B. japonicum* is favoured by higher pH according to Graham & Parker (1964) and Taylor *et al*. (1991), whereas Albareda *et al.* (2009b) found a better survival rate of *B. japonicum* in acidic soil. The chemical analysis of soils in the present study (Table 2) showed that the pH was equal in both soils collected from fields in Västmanland (with soybean grown previously in 2011 and 2013, respectively). Thus the soil pH cannot have affected the survival rate of *B. japonicum*, since the amount appeared to be the same in both soils. In the soil from three fields in Uppland (with soybean grown previously in 2011, 2012 and 2013, respectively), the highest pH was obtained in the soil with soybean grown in 2011, which also had the highest survival rate of *B. japonicum*. These results agree with those of Graham & Parker (1964) and Taylor *et al.* (1991). However, the pH was lower in the soil with soybean grown previously in 2012 than in that with soybean grown in 2013. The soil with soybean grown in 2013 had the lowest survival rate, which means that the higher pH did not favour *B. japonicum* in this soil. These results agree with Albareda *et al.* (2009b). In the experiment investigating the effect of soil characteristics and field site (Värmland, Uppland, Närke and Västmanland) on the bacterium, the pH was highest in Uppland and lowest in Närke (Table 2). It seems that lower pH was favourable in terms of higher survival rate of *B. japonicum* in the soil collected from Närke, which does agree with Albareda *et al.* (2009b).

As shown in Figure 5a, the nodule and plant biomass weight increased with increased pH in Uppland soil. However, R^2 was about 0.22 for both trend lines, indicating that there was no strong correlation between nodule and plant biomass weight and soil pH. In Uppland, Värmland, Närke and Västmanland soil (Figure 5b), nodule and plant biomass decreased with increased pH. This time, the correlation was stronger (R^2 = 0.6-0.74) between nodule and plant biomass weight to pH. There was also a decrease in nitrogen content with increased pH in those soils (Figure 5c). However, this correlation was low (R^2 = 0.1354).

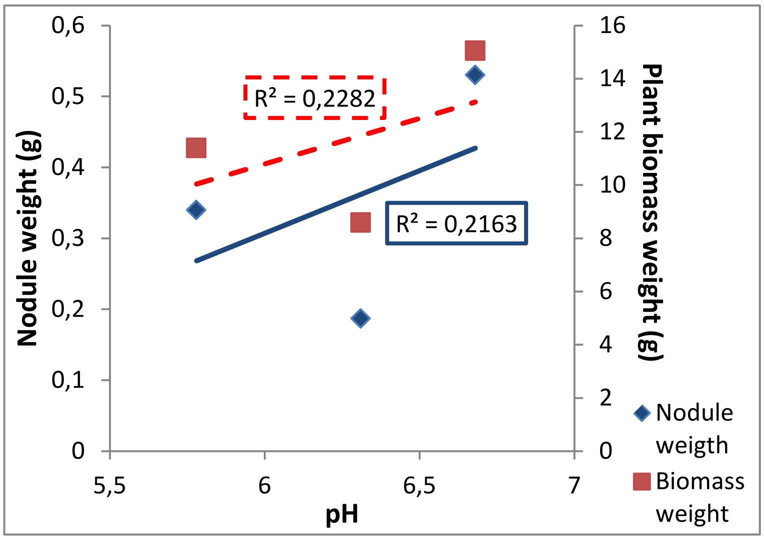

Figure 5a

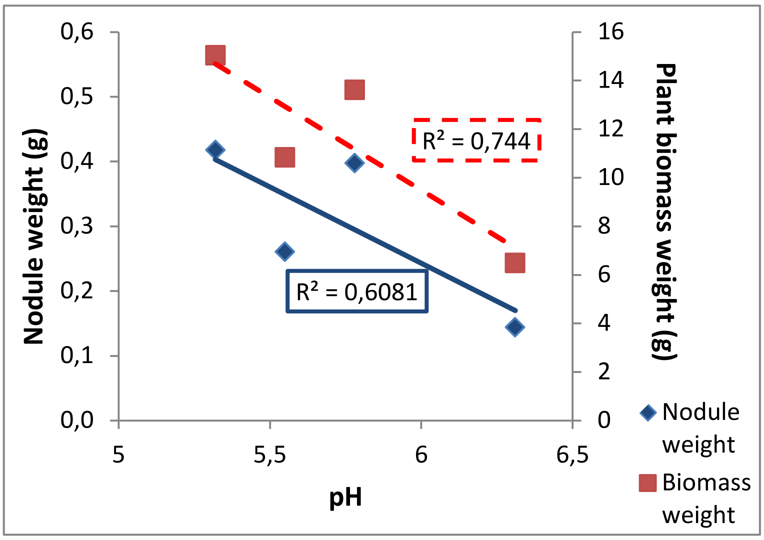

Figure 5b

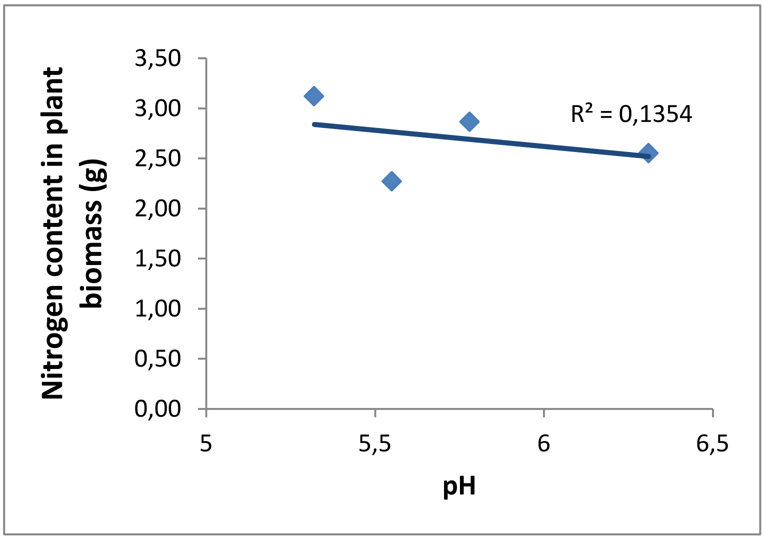

Figure 5c

Figure 5: Effect of soil pH on nodule dry weight and plant dry biomass of soybean grown in (a) soil collected from three fields in Uppland in which soybean had been grown in 2011, 2012 and 2013, respectively and (b) soil collected from fields in Uppland, Värmland, Närke and Västmanland in which soybean had been grown previously in 2013. (c) Effect of soil pH on nitrogen content of soybean grown in soil collected from fields in Uppland, Värmland, Närke and Västmanland in which soybean had been grown previously in 2013.

There are no previous reports of how carbon and nitrogen in soil affect *B. japonicum*. In the experiment investigating survival rate of *B. japonicum* in soil from Västmanland with soybeans previously cultivated 2011 and 2013 the highest amount of carbon and nitrogen was found in the field with soybeans grown 2013 . However, since there was a similar amount of *B. japonicum* in both soils, the amount of carbon and nitrogen apparently had no effect on the survival rate of *B. japonicum* (Table A1 in appendix 3). In figure 6a and 6b (with soil from Uppland) the R^2 value was very small, which indicates that there was a small correlation between soil nitrogen and carbon content to nodule and plant biomass weight of soybeans. In soils from Värmland, Uppland, Närke and Västmanland with soybean grown previously in 2013 (Figure 6c-6f), the R^2 values were higher. However, there was still a weak correlation between nodule weight, plant biomass weight and nitrogen content of soybean, and soil nitrogen and carbon.

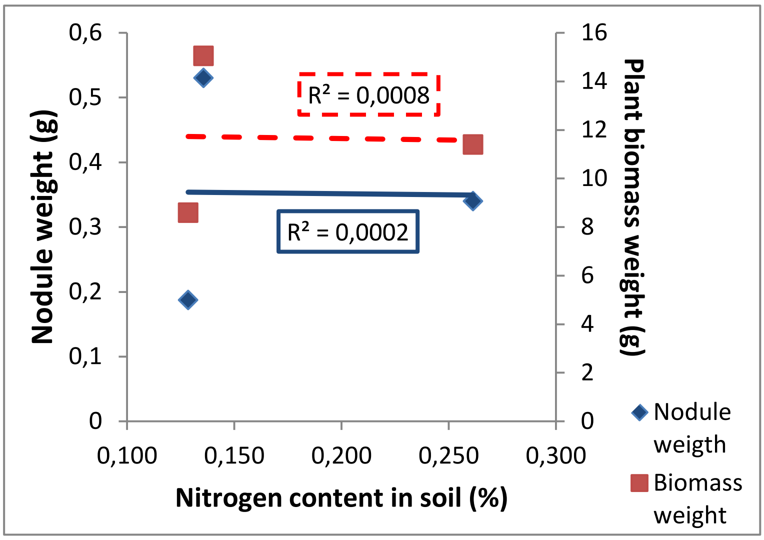

Figure 6a

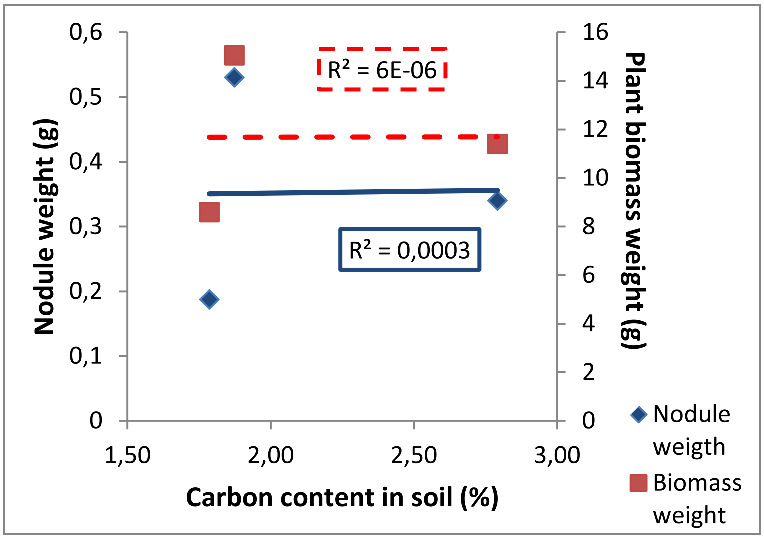

Figure 6b

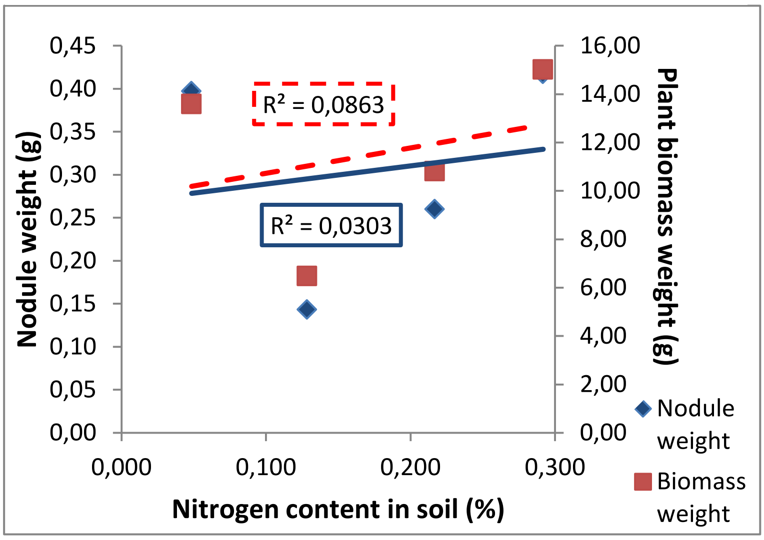

Figure 6c

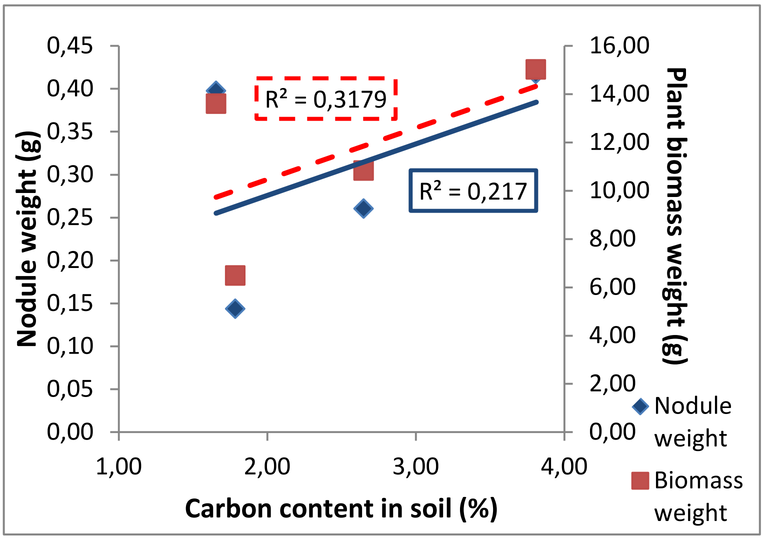

Figure 6d

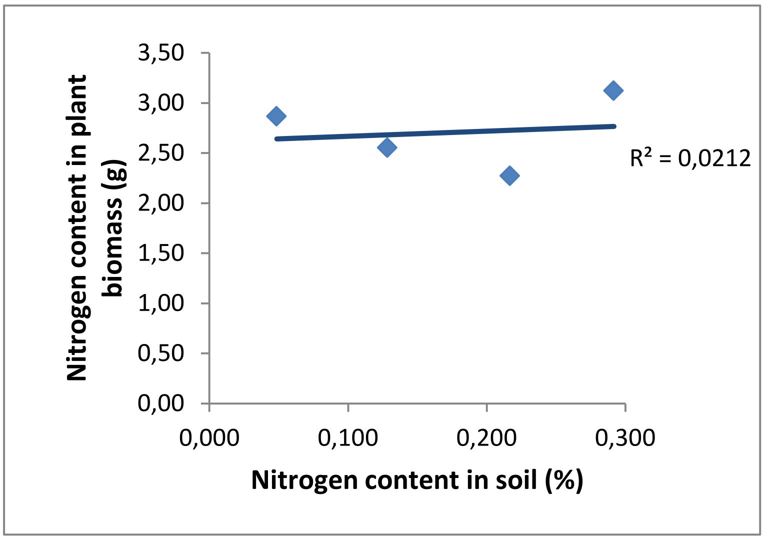

Figure 6e

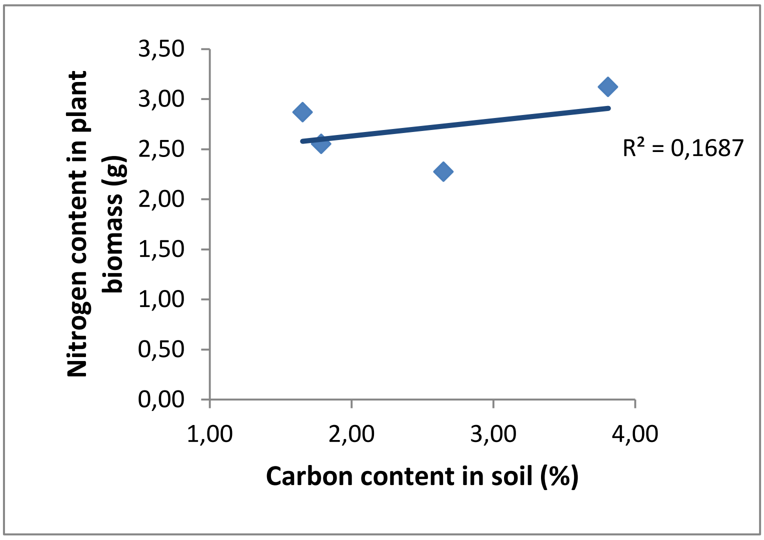

Figure 6f

Figure 6: Effect of soil nitrogen and carbon on nodule dry weight, plant biomass weight and nitrogen content of soybean. Diagrams 6a and 6b shows results obtained for soybean grown in soil from three fields in Uppland in which soybean had been grown previously in 2011, 2012 and 2013, respectively. Diagrams 6c-6f show the corresponding results for soybean grown in soil from Värmland, Uppland, Närke and Västmanland in which soybean had been grown previously in 2013

According to Cassman *et al.* (1981), Singleton *et al.* (1985), Israel (1987; 1993), Bordeleau & Prévost (1994) and Premaratne & Oertli (1994), higher soil phosphorus concentration promotes growth of *B. japonicum* and soybean plants. According to results obtained from soybean grown in soils collected from Västmanland with a previous soybean crop grown in 2011 and 2013 (Table A1 in Appendix 3), there was a higher amount of phosphorus in the soil with soybeans grown in 2013 (see Table 2). However, this higher amount of phosphorus did not favour *B. japonicum* in this soil, since the survival rate was equal in both soils. The results obtained for soils from Uppland with soybean grown previously in 2011, 2012 and 2013 (Table 2) showed a higher phosphorus content (both plant-available and sediment-bound) in the field with soybeans grown in 2011. Since there was a higher survival rate in 2011 than in 2012 and 2013, these results agree with those of Cassman *et al.* (1981), Singleton *et al.* (1985), Israel (1987; 1993), Bordeleau & Prévost 1994 and Premaratne & Oertli (1994). The amount of phosphorus was lowest in the soil with soybean grown previously in 2013, which seems to agree with previous studies. The soils from Värmland and Närke (which had the highest survival rate of *B. japonicum*) had a higher amount of plant-available phosphorus than the Uppland and Västmanland soils. There was a strong correlation ($R^2 \approx 0.81$-0.99) between nodule and plant biomass weight and phosphorus amount in Uppland soil (Figure 7a, 7b). The other soils showed similar results, but with a lower correlation ($R^2 \approx 0.49$-0.77) (Figures 7c-7f). Based on these results, increased amount of phosphorus in soil increases the survival rate of *B. japonicum*.

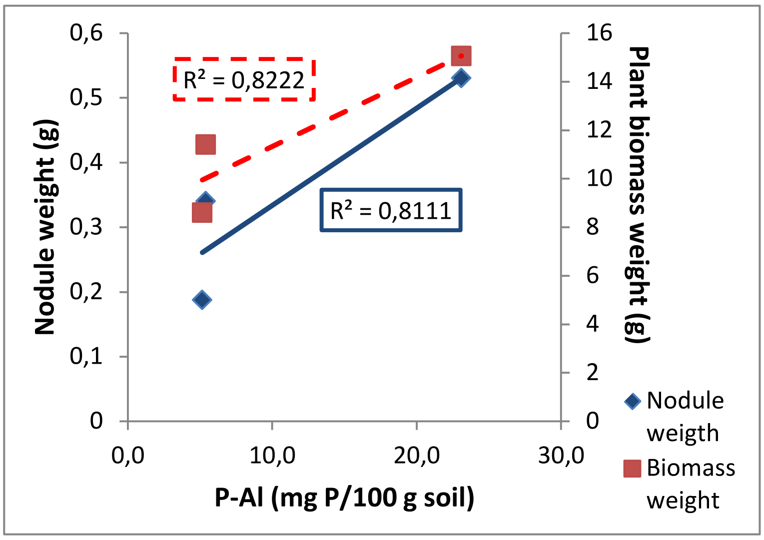

Figure 7a

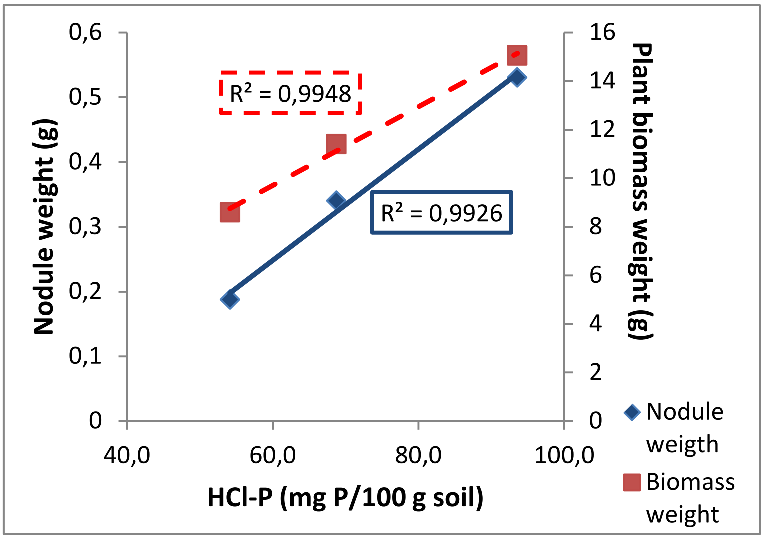

Figure 7b

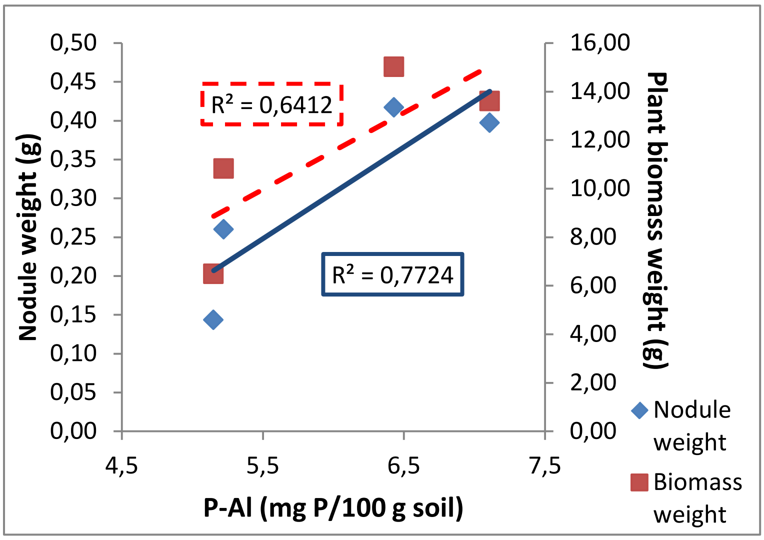

Figure 7c

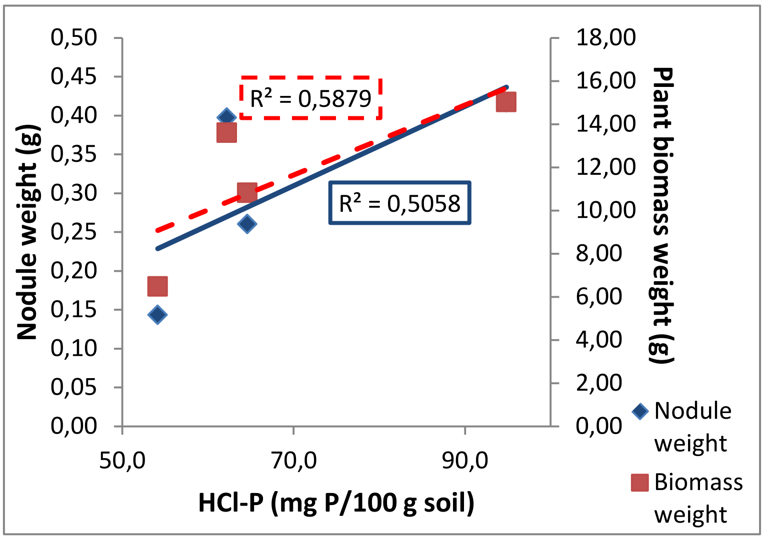

Figure 7d

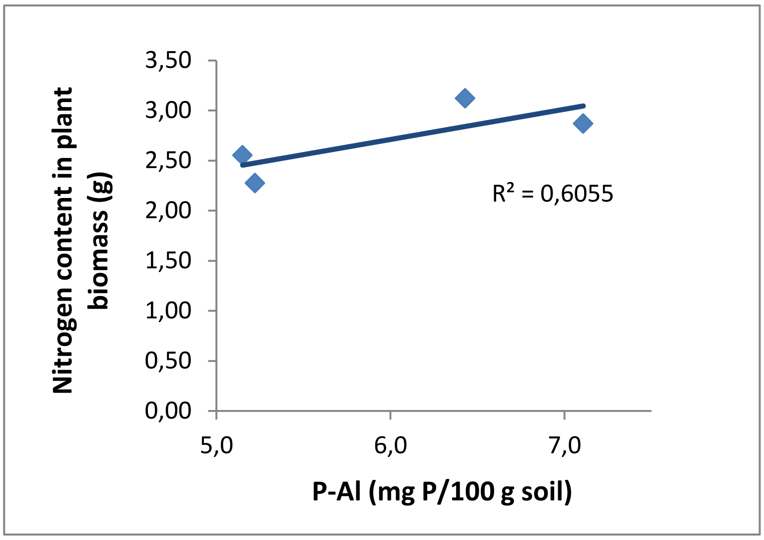

Figure 7e

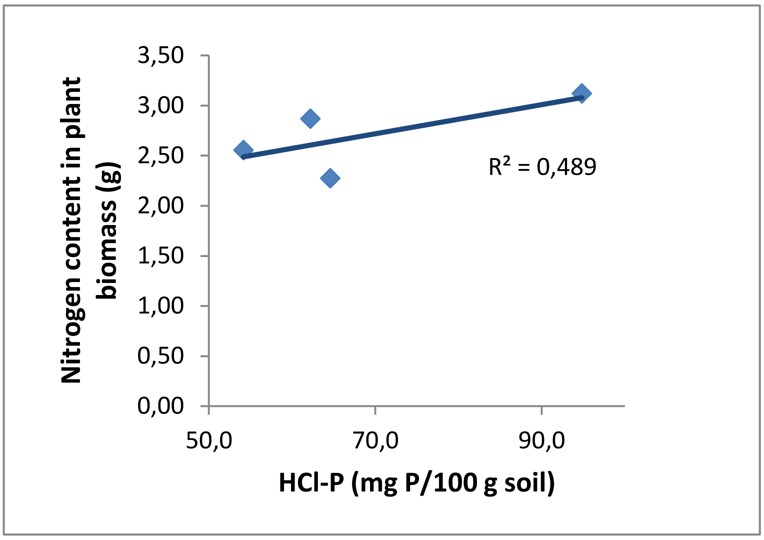

Figure 7f

Figure 7: Effect of soil-available and sediment-bound phosphorus status on nodule weight, plant biomass weight and nitrogen content of soybean. Diagrams 7a-7b shows the results obtained for soybean grown in soil collected from fields in Uppland (in which soybean had been grown previously in 2011, 2012 and 2013). Diagrams 7c-7f shows the corresponding results for soybean

grown in soil from Uppland, Värmland, Västmanland and Närke in which soybean had been grown previously in 2013.

The paper by Premaratne & Oertli (1994) is the only one that has previously examined how potassium affects production of plant biomass and nodules. They found that increased potassium concentration increased plant dry matter and nodule weight. In the experiment investigating survival rate of *B. japonicum* in soil from Västmanland with soybeans previously cultivated 2011 and 2013, the survival rate was equal in both soils. The higher amount of potassium in the field with soybean grown previously in 2013 (Table 2) did not favour *B. japonicum*. In the In the same experiment with soil from Uppland, the amount of potassium was higher in that in which soybean had been grown previously in 2011, and the experimental soybean in that soil also had higher plant biomass and nodule weight than in the other fields, with soybean grown previously in 2012 and 2013. Comparing the fields with soybean grown previously in 2012 and 2013, the amount of potassium was lower in the former soil, and it had a higher survival rate of *B. japonicum*, which means that the higher amount of potassium did not favour *B. japonicum*. In the experiment investigating how soil characteristics and field site affects the bacterium, the amount of potassium was much higher in soil from Västmanland and Uppland (which had a lower *B. japonicum* survival rate) than in soil from Värmland and Närke. The correlation between amount of potassium in soil to nodule and plant biomass weight was weak for Uppland soil (Figure 8a, 8b), meaning that potassium did not have a great effect on nodule and plant biomass weight of soybeans grown in that soil. Looking at Figure 8c and 8d, there was a strong correlation (R^2 =0.92-0.98) for soils from Uppland, Värmland, Närke and Västmanland. This means that the amount of potassium had a large effect on the nodule and plant biomass weight. There was also a strong correlation between nitrogen content in plant biomass and amount of potassium in those soils (Figure 8e and 8f). Overall, in the soils from Uppland, Värmland, Västmanland and Närke (Figure 8c-8f), a higher amount of potassium had a negative effect on nodule weight, biomass plant weight and nitrogen content in plant biomass. This again means that *B. japonicum* was negatively affected by higher amounts of potassium.

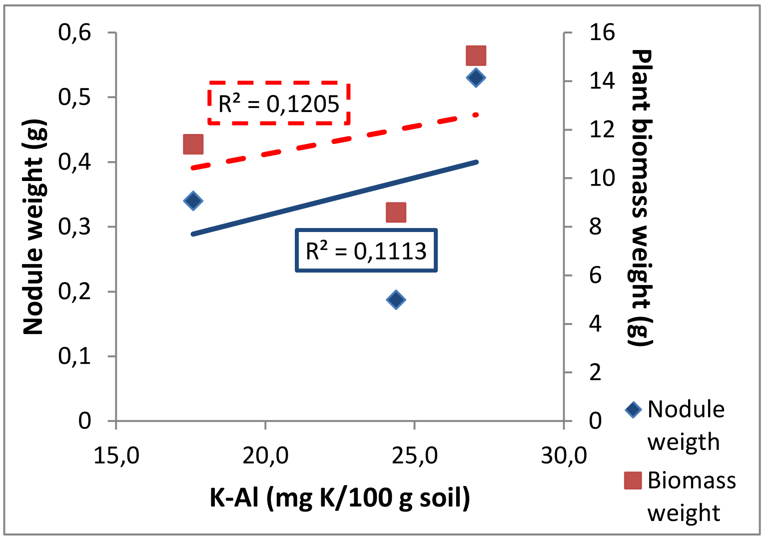

Figure 8a

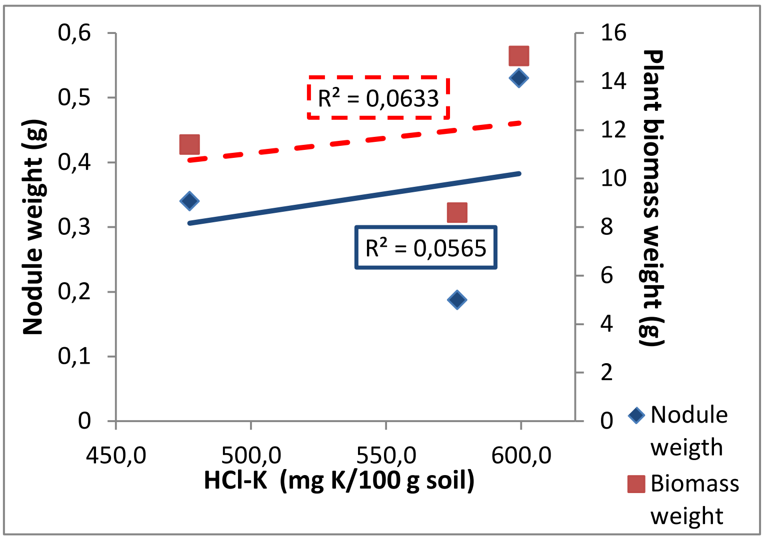

Figure 8b

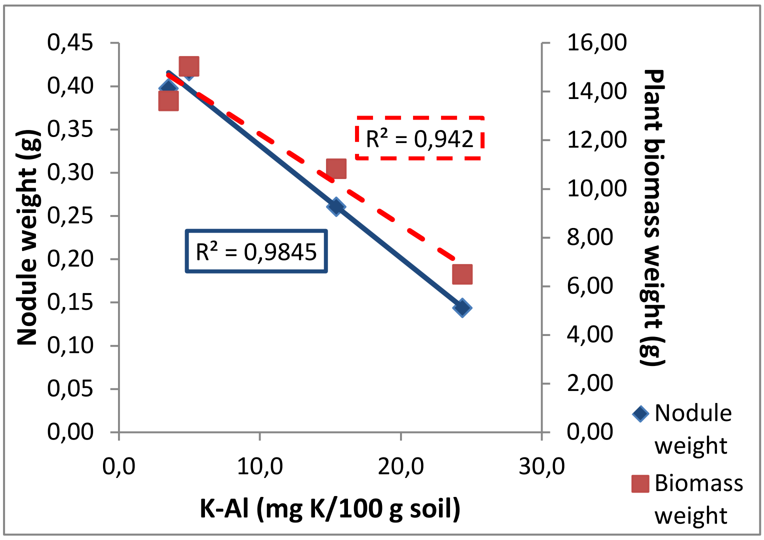

Figure 8c

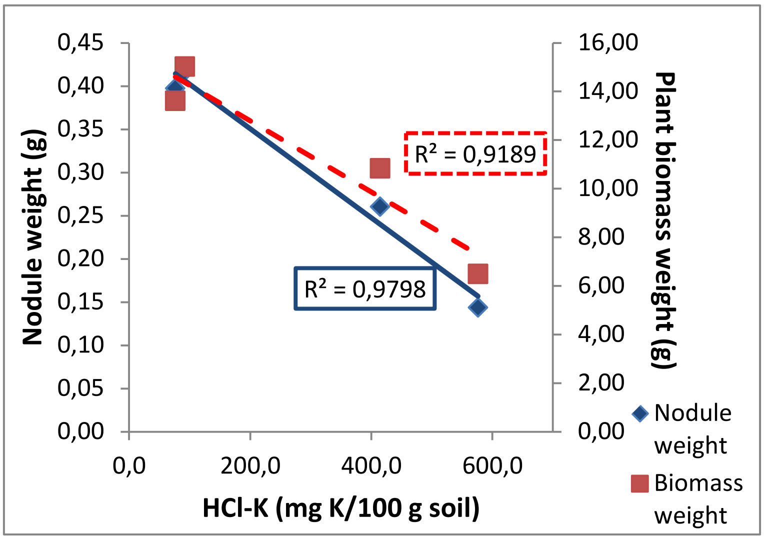

Figure 8d

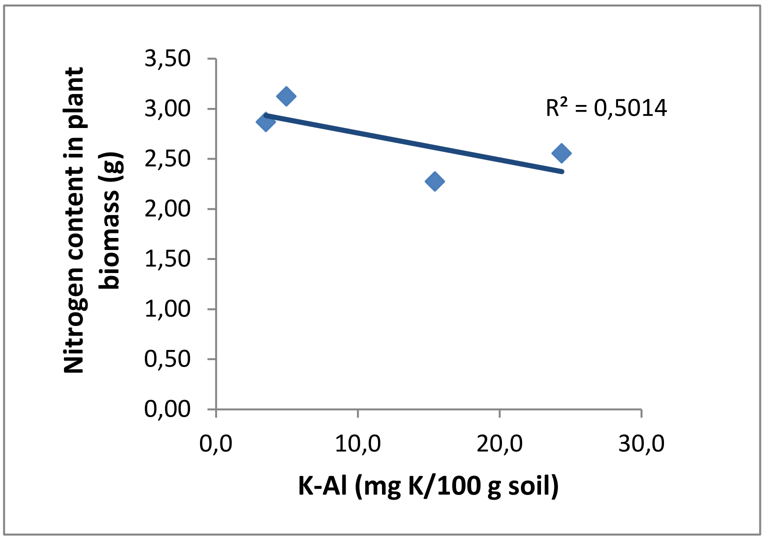

Figure 8e

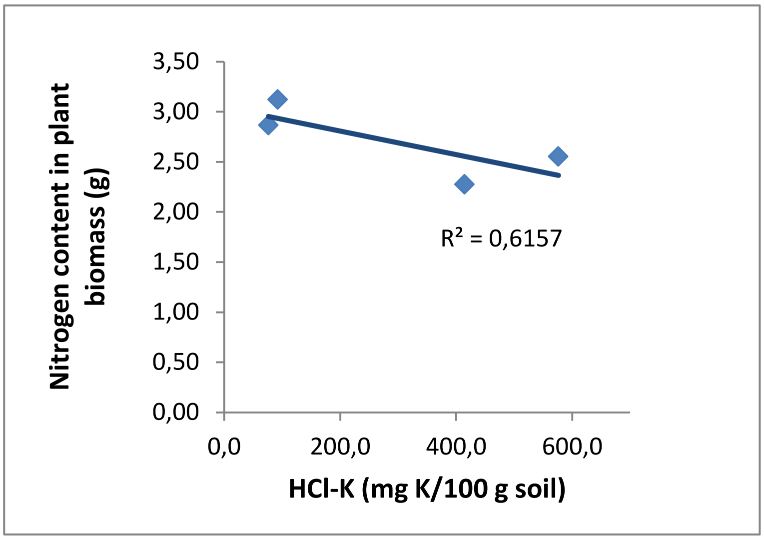

Figure 8f

Figure 8: Effect of soil-available and sediment-bound potassium status on nodule weight, plant biomass weight and nitrogen content of soybean. Diagrams 8a-8b shows the results obtained for soybean grown in soil collected from fields in Uppland (in which soybean had been grown previously in 2011, 2012 and 2013). Diagrams 8c-8f shows the corresponding results for soybean

grown in soil from Uppland, Värmland, Västmanland and Närke in which soybean had been grown previously in 2013.

The experiment investigating the effect of soil characteristics and field site on the bacterium showed how soil texture affects *B. japonicum*. It was found that the soils from Värmland and Närke (which had a higher survival rate of *B. japonicum*) were lighter in texture than soils from Uppland and Västmanland (Table 4). These results agree with Albareda *et al.* (2009b) who reported a better survival rate of *B. japonicum* in lighter soil rather than in loamy textured soil.

Soil measures affecting survival rate of B. japonicum

According to Gan *et al.* (2002) applying nitrogen stimulates nodule production, nitrogen fixation and biomass growth in plant. In the present study, manure was applied in the field in Västmanland with soybean grown previously in 2011, but not in the field with soybean grown in 2013 (Table 4). In this case, application of manure did not favour *B. japonicum*, since the results from the experiment investigating survival rate of *B. japonicum* in soil (from Uppland and Västmanland) with soybeans previously cultivated 2011, 2012 and 2013 showed an equal amount of *B. japonicum* in all soils. However, no manure was used in the fields from Uppland and Västmanland but was applied to the fields from Värmland and Närke (Tables 3 and 4). This could explain the higher production of nodules, nitrogen content and biomass weight in plants cultivated in soils from Värmland and Närke.

It was difficult to determine whether other soil management practices and pre-crop had an effect on the survival rate of *B. japonicum* or not.

Other factors affecting the results

The emergence of the soybean crop differed during the greenhouse experiment, which could have affected the results. In the experiment investigating how soil characteristics and field site affects the bacterium, the emergence of the soybeans was estimated visually to start earlier in the soil from Värmland and Närke. Moreover, the growth of the plants in these soils was faster than in the soils from Uppland and Västmanland. This could explain why the nodule and plant biomass weight was higher in Närke and Värmland soils than in Uppland and Västmanland soils. In the experiment investigating survival rate of *B. japonicum* in soil from Uppland with soybeans previously cultivated 2011, 2012 and 2013, emergence started earlier in soil with soybeans grown previously in 2011. This could explain the higher nodule weight and plant biomass weight in soybean grown in this soil than in the soils with soybean grown previously in 2012 and 2013.

As mentioned previously, the amount of nutrients in soil affects the growth of the soybean plant. For example, a starter dose of nitrogen promotes plant growth, which in turn affects nodule production. Soybean plants are probably affected by other parameters as well, such as pH and soil texture. This in turn could have affected the parameters measured in this trial.

Inoculation effect on soybeans

There were no significant differences in terms of the parameters observed between the inoculated and non-inoculated cultures. This means that there is no need for re-inoculation in these soils, since *B. japonicum* is present. Regarding the different cultures (Hi-Stick and E11), there was a significant difference between these showed in experiment investigating the effect of re-inoculation in soil with soybeans previously cultivated. E11 gave a higher weight of nodules (Figure 3), which indicated that E11 was a better inoculation material than Hi-Stick in that experiment. However, in the experiment investigating the effect of re-inoculation in soil with no previous history soybean cultivation, Hi-Stick gave higher weight of nodules, total biomass and nitrogen content of the soybean plants. This indicated that Hi-Stick was a better inoculation material in that experiment.

Conclusions

According to the results from this greenhouse experiment, *B. japonicum* was able to survive in soils from fields in which soybean had been grown previously. The bacteria managed to survive in the soil for two years, which means that the seed of subsequent soybean crops do not need re-inoculation during this period.

Soil pH, nitrogen and carbon content had contradictory effects on the survival rate of *B. japonicum*. However, a starter dose of nitrogen in soil gave better growth of *B. japonicum*. Moreover, a higher amount of phosphorus in soil favoured *B. japonicum*. The amount of potassium in soil had differing effects on *B. japonicum*, but overall a higher amount of potassium seemed to inhibit the bacteria. As regards to soil texture, *B. japonicum* survived better in light soil than in soils with higher clay content. Importantly, plant growth was also affected by soil factors, which in turn could have affected the results. Both Hi-Stick and E11 proved suitable as inoculation cultures, since they both gave the desired result.

To strengthen the conclusions of the amount of *B. japonicum* present in various soils, directly methods could have been used to measure the number of *B. japonicum* in soils, e.g. by a method called *The most probable number technique* (MPN).

One problem remains; as previously written in the report, there could have been accidental cross-contamination of *B. japonicum* between the plants during the experiment when watering the plants. If this is the case, this means that the results obtained from the experiment could have been affected by this and therefore are not representative. This in turn means that the conclusions drawn in this report should be further corroborated.

Future perspective

Further investigations are necessary to obtain more information about the persistence of *B. japonicum* in Swedish soils. It has been found that this bacterium is able to survive for up to ten years in some European soils, but it is not known if the amount surviving can meet the symbiosis needs of the new soybean crop. It would be interesting to investigate if this bacterium is able to survive for a longer period than two years in Swedish soils, as soybean would need to be included in a crop rotation to reduce the risk of pest development. More studies are also needed to confirm the conflicting findings obtained here on the impact of soil factors and cultivation measures on *B. japonicum* survival.

Acknowledgements

I want to express my gratitude to the farmers from Edsberg Gård, Berga Gård, Sjöö Gods and Munktorp Prästgård, who gave me the opportunity to collect soil samples from their fields and also participated to the interview. I also want to thank my supervisors Anna Mårtensson from SLU and Fredrik Fogelberg, who helped me with the execution of this study.

Appendices

Appendix 1: Interviews with farmers

The following questions were used in the interview with farmers:

1) Which pre-crop was used before growing soybean? Which crops were grown after the soybean until 2013?
2) What kind of cultivation measures were used during and after soybean cultivation?
3) Which soybean cultivar was used?
4) Was the soybean cultivation organic or conventional?
5) How large was the area used for soybean cultivation?
6) How high were the achieved yields of soybeans in each field?
7) What kind of inoculation culture was used on the soybeans?
8) What was the soil texture in each field?

Appendix 2: Observations during the cultivation experiments

Date: 2013-11-20
Cultivation of soybeans in greenhouse started.

Date: 2013-11-26
In experiment I, with soil from Västmanland, plants had started to emerge in all pots containing soil in which soybean had been grown previously in 2013. Moreover, plants had emerged from the soil in 3/7 pots containing soil in which soybean had been grown previously in 2011. In soil from Uppland in which soybean had been grown previously in 2011, the emergence had started a little in 4/7 pots. In experiment II, plant emergence had started in all pots with soil from Värmland and Närke. Moreover, the plants in these soils had managed to grow a little; these plants seemed to have the highest biomass when visually compared with the other plants grown in the other soils. Plant emergence had also started in 3/7 pots with soil from Västmanland.

Date: 2013-11-28
In experiment I, plants had started to emerge in 2/7 pots containing of soil from Västmanland in which soybean had been grown previously in 2013. In the Västmanland soil with previous cultivation of soybean in 2011, 5/7 pots had plants that had emerged from the soil. In experiment III plant emergence had started in all inoculation cultures (5/7 pots in each culture) and also in the non-inoculated plants (3/7 pots). In experiment IV, the emergence had started a little in three pots in total.

Date: 2013-12-02
In all pots with soil from Västmanland in experiment I, plants had emerged. In the soil from Uppland, almost all pots with soybean grown previously in 2011 had emerged plants. In a few pots with soybean grown previously in 2012, the plants had started to emerge a little. Emergence had also started a little in some pots containing soil in which soybean had been grown in 2013. In experiment II, the plants grown in soil from Uppland had started to emerge a little in some pots. More plants from experiment III had emerged from the soil. In experiment IV, the emergence was still slow, probably

because of the hard surface formed on the soil. Therefore, these soils were loosened up a little by hand.

Date: 2013-12-03
After loosening up of the soil the day before, more plants had emerged from the soils in experiment IV. Plants had emerged in almost all pots in the greenhouse experiment.

Appendix 3: Results from statistical analysis

I. Survival rate of *B. japonicum* in soil with soybean grown previously in 2011, 2012 or 2013:

Table A1: Symbiotic efficiency of *Bradyrhizobium japonicum* in soils from Västmanland with soybean grown previously in 2011 and 2013, n = 7. Presented as mean value, standard error and p-value.

Year of last soybean cultivation	Symbiotic efficiency (showing mean values for each pot)			
	Stage of the crop	Nodule weight (g)	Plant biomass weight (g)	Nitrogen content in plant biomass (%)
2011	4.86 ± 0	0.24 ± 0.02	7.46 ± 0.51	3.408* ± 0.08
2013	5.00 ± 0	0.19 ± 0.03	8.65 ± 0.95	3.06 ± 0.16
P-value	-	0.29	0.30	0.092

Table A2: Symbiotic efficiency of *Bradyrhizobium japonicum* in soils from Uppland with soybean grown in 2011, 2012 and 2013, n = 7. Presented as mean value, standard error and p-value.

Year of last soybean cultivation	Symbiotic efficiency (showing mean values for each pot)			
	Stage of the crop	Nodule weight (g)	Plant biomass weight (g)	Nitrogen content in plant biomass (%)
2011	5.00 ± 0.00	0.53 ± 0.05	15.1 ± 1.00	2.71 ± 0.12
2012	4.86 ± 0.14	0.34 ± 0.03	11.4 ± 1.10	3.01* ± 0.10
2013	4.71 ± 0.18	0.19 ± 0.02	8.60* ± 0.54	2.81 ± 0.17
P-value, 2011/2012	-	0.01	0.04	0.09
P-value, 2011/2013	-	0.00	0.00	0.66
P-value, 2012/2013	0.55	0.00	0.05	0.34

II. Survival rate of *B. japonicum* depending on soil characteristics and field site:

Table A3: Symbiotic efficiency of *Bradyrhizobium japonicum* in soils from Värmland, Västmanland, Närke and Uppland with soybean grown previously in 2013, n = 7. Presented as mean value, standard error and p-value.

Place	Symbiotic efficiency (showing mean values for each pot)			
	Stage of the crop	Nodule weight (g)	Plant biomass weight (g)	Nitrogen content in plant biomass (%)
Värmland	5.00 ± 0.00	0.40 ± 0.02	13.6* ± 0.62	2.87 ± 0.12
V-man	5.00 ±0.00	0.26 ± 0.01	10.8 ± 1.20	2.27 ± 0.06
Närke	5.00 ± 0.00	0.42 ± 0.04	15.0 ± 1.10	3.12 ± 0.16
Uppland	4.29 ± 0.00	0.14* ± 0.01	6.48* ± 0.84	2.55 ± 0.08
P-value Värm/V-man	-	0.00	0.06	0.002
P-value Värm/Närk	-	0.66	0.31	0.23
P-value Värm/Uppl.	-	0.00	0.00	0.05
P-value V-man/Närk	-	0.01	0.03	0.05
P-value V-man/Uppl.	-	0.00	0.01	0.02
P-value Närk/Uppl.	-	0.00	0.00	0.01

III. Effect of re-inoculation in soil with previous soybean cultivation:

Table A4: Symbiotic efficiency of *Bradyrhizobium japonicum* in soils from Uppland with soybean grown previously in 2011, n = 7. Presented as mean value, standard error and p-value.

Year of last soybean cultivation	Symbiotic efficiency (showing mean values for each pot)			
	Stage of the crop	Nodule weight (g)	Plant biomass weight (g)	Nitrogen content in plant biomass (%)
Uninoc	4.86 ± 0.14	0.44 ± 0.04	15.9* ± 1.40	3.50 ± 0.15
Hi-Stick	4.71 ± 0.18	0.42 ± 0.03	13.6 ± 0.92	3.44 ± 0.18
E11	4.43 ± 0.20	0.51* ± 0.03	13.9* ± 0.64	3.39 ± 0.12
P-value, uninoc/Hi-Stick	0.55	0.66	0.20	0.81
P-value, uninoc/E11	0.11	0.19	0.23	0.59
P-value, Hi-Stick/E11	0.32	0.04	0.81	0.82

IV. Effect of inoculation in soil with no previous history of soybean cultivation:

Table A5: Symbiotic efficiency of *Bradyrhizobium japonicum* in soils from Uppland with soybean not previously grown, n = 7. Presented as mean value, standard error and p-value.

Year of last soybean cultivation	Symbiotic efficiency (showing mean values for each pot)

	Stage of the crop	Nodule weight (g)	Plant biomass weight (g)	Nitrogen content in plant biomass (%)
Uninoc	4.57 ± 0.30	0.04* ± 0.02	6.68 ± 0.36	1.08 ± 0.13
Hi-Stick	3.86 ± 0.40	0.40 ± 0.04	8.45* ± 0.75	3.64 ± 0.15
E11	3.86 ± 0.34	0.10 ± 0.03	6.16 ± 0.39	1.65 ± 0.21
P-value, uninoc/Hi-Stick	0.18	0.00	0.07	0.00
P-value, uninoc/E11	0.14	0.13	0.34	0.05
P-value, Hi-Stick/E11	1.00	0.00	0.03	0.00

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