Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Department of Soil and Environment

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– a comparison between Swedish fields with and without a recent history of common bean cultivation

Christina Hultman

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Christina Hultman

Supervisor: Georg Carlsson, Department of Biosystems and Technology, SLU
Assistant supervisor: Anna Mårtensson, Department of Soil and Environment, SLU
Examiner: Björn Lindahl, Department of Soil and Environment, SLU

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Abstract

Legumes such as common bean, *Phaseolus vulgaris* L. are climate-smart protein sources which can be part of sustainable agriculture and eating habits to increase the resilience in our food system. Common bean cultivation in Sweden is expanding to new and larger areas where common bean has not been grown before, and it is currently unclear whether farmers should be recommend to inoculate with efficient rhizobia when sowing common bean on fields where the crop has not been grown before.

To evaluate the need for inoculation, this study has examined root nodulation of common bean plants cultivated on fields with and without a recent history of common bean cultivation. Farmer's fields on the two Swedish islands Öland and Gotland were used for the investigations and a farmer survey was conducted to collect information about the fields. Soil pH, root nodules and plant biomass were measured at pod fill and pods as well as residual plant biomass were measured at full maturity.

There was a clear difference in presence of root nodules between the two types of fields. Both root nodule abundance and proportion of active root nodules per plant with root nodules were significantly higher on fields with a recent history of common bean cultivation than on fields where common bean was not cultivated for at least 20 years. However, neither aboveground biomass weight at pod fill, nor pod weight at maturity were significantly correlated with number of root nodules or proportion of active root nodule.

These results emphasize the importance of giving recommendations to inoculate common bean seeds with the right species of rhizobium bacteria before sowing, when cultivating common bean on a field for the first time. An additional recommendation might be to decrease the amount of nitrogen fertilization of common bean, since common bean plants grew well even with a very low abundance of root nodules and where many of them were inactive, indicating that soils contained enough plant-available nitrogen for plant growth without help of nitrogen fixation. However, further investigations are needed before to give firm recommendations on reduced nitrogen fertilization of common bean.

Keywords: sustainability, protein-source, legume-based food, nitrogen fixation, root nodules

Sammanfattning

Baljväxter såsom trädgårdsböna, *Phaseolus vulgaris* L. är klimatsmarta proteinkällor som kan vara en del av ett hållbart jordbruk och hållbara matvanor. Den svenska odlingen av trädgårdsböna har ökat under senare år, vilket innebär att nya arealer, där grödan inte odlats tidigare, tas i anspråk för odling av trädgårdsböna. I dagsläget förekommer olika rekommendationer angående inokulering av trädgårdsböna, och det finns osäkerhet hos lantbrukare om de bör inokulera trädgårdsböna när den odlas på nya arealer.

För att utvärdera behovet av inokulering har denna studie undersökt förekomst av rotknölar på plantor av trädgårdsböna som odlats på fält med och utan tidigare odling av trädgårdsböna, för att utvärdera behovet av inokulering. Fält hos lantbrukare på de två Svenska öarna Öland och Gotland användes för undersökningarna och lantbrukarna intervjuades för att få information om fälten. Jordens pH-värde, förekomst av rotknölar på plantorna samt växtbiomassa undersöktes vid baljsättning, och baljor samt resterande växtbiomassa undersöktes vid mognad.

Det var stor skillnad i rotknölsutveckling mellan de två typerna av fält. Både rotknölsförekomst och andel aktiva rotknölar per planta med rotknölar var signifikant högre på fält med tidigare odling av trädgårdsböna än på fält där trädgårdsböna inte odlats på minst 20 år. Dock var varken vikt av växtbiomassa vid baljsättning eller baljvikt vid mognad signifikant korrelerade med rotknölsförekomst eller andel aktiva rotknölar.

Dessa resultat betonar vikten av att ge rekommendationen att inokulera utsäde till trädgårdsböna med rätt art av rhizobium bakterier då trädgårdsböna odlas för första gången på ett fält. En ytterligare rekommendation skulle kunna vara att minska på mängden gödsel till trädgårdsböna, eftersom att plantor av trädgårdsböna växte väl även med en mycket liten rotknölsförekomst och där många rotknölar var inaktiva, vilket indikerar att jordarna innehöll tillräckliga mängder växttillgängligt kväve för en god tillväxt utan hjälp av kvävefixering. Detta behöver dock utforskas mer för att kunna ge säkrare rekommendationer angående reducerad kvävegödsling till trädgårdsböna.

Nyckelord: hållbarhet, proteinkälla, baljväxtbaserad mat, kvävefixering, rotknölar

Populärvetenskaplig sammanfattning

- Kan det vara värt att inokulera utsäde till trädgårdsböna?

Odlingen av trädgårdsböna, såsom brun böna har under senare år expanderat till arealer där denna sorts böna inte tidigare odlats i Sverige. Bruna bönor har odlats i Sverige i flera hundra år och framförallt på Öland, där utbudet av trädgårdsbönor utvidgats till att nu utgöra flera sorter såsom kidneyböna, borlottiböna, svart- och vit böna med mera. Under säsongen 2017 utökades odlingen av bruna bönor till Gotland med drygt 100 hektar. På dessa fält hade trädgårdsbönor inte odlats på minst 20 år innan säsongen 2017, men trots detta var det ingen utav lantbrukarna som inokulerade utsädet innan sådd.

Inokulering med rätt sorts kvävefixerande bakterie för trädgårdsböna kan bidra till större kvävetillförsel till plantan, tack vare den symbios som uppstår mellan bakterier och trädgårdsböna. Bakterierna formar rotknölar på bönplantans rötter och kan därigenom fixera luftens kväve och förse växten med kväve i utbyte mot energi. Därmed kan gödsling av bönplantorna minskas, vilket skulle vara både en miljömässig och en ekonomisk vinst. Kvävefixering tack vare symbios med marklevande bakterier är något som sker hos alla typer av odlade baljväxter, allt ifrån klöver och lusern till ärter och bönor, om rätt sorts kvävefixerande bakterie finns i marken eller blivit inokulerad i utsädet.

Det är inte helt klart huruvida rätt sorts kvävefixerande bakterier finns naturligt i svenska åkermarker eller ej och därmed är det inte heller klarlagt hur betydelsefull en eventuell inokulering av utsäde till trädgårdsböna skulle vara. För att ta reda på mer om inokuleringsbehov av trädgårdsböna undersöktes rotknölar från plantor av trädgårdsbönan brun böna som odlats på fält med och utan tidigare odling av trädgårdsböna. Fält hos lantbrukare på Öland och Gotland användes för undersökningarna och lantbrukarna intervjuades för att få information om fälten. Jordens pH-värde, rotknölar på plantorna samt växtbiomassa undersöktes vid baljsättning, och baljor samt resterande växtbiomassa undersöktes vid mognad.

Den här studien har visat att rotknölsförekomst och andel aktiva rotknölar är signifikant större hos plantor av brun böna på fält där trädgårdsbönor odlats regelbundet i växtföljden de senaste åren, än på fält där trädgårdsböna odlades för första gången på minst 20 år. Däremot påverkades varken vikt av växtbiomassa vid baljsättning eller baljvikt vid mognad av en högre rotknölsförekomst eller en större andel aktiva rotknölar, vilket tyder på att kväve troligtvis inte var den främsta tillväxtbegränsande faktorn.

Dessa resultat betonar vikten av att ge rekommendationer att inokulera utsäde till trädgårdsböna med rätt sorts kvävefixerande bakterier före sådd, vid odling av trädgårdsböna för första gången på ett fält. En ytterligare rekommendation skulle kunna vara att minska på mängden gödsel till trädgårdsböna, eftersom att plantorna av trädgårdsböna växte väl även med en mycket låg rotknölsförekomst och där många rotknölar var inaktiva, vilket indikerar att jordarna innehöll tillräckliga mängder växttillgängligt kväve för god tillväxt utan kvävefixering. Resultaten är således betydelsefulla för en fortsatt expansion av framgångsrik trädgårdsbönodling i Sverige, samtidigt som de visar på att mer forskning behövs, kring effektiv inokulering samt behov av kvävegödsling, för att maximera miljövinsten tack vare trädgårdsbönans förmåga till kväveförsörjning via symbiotisk kvävefixering.

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1 Introduction

1.1 Agriculture and sustainable development

Agriculture contributes to climate change at the same time as climate change affects agriculture (European Environment Agency 2015). Agriculture and other food-production systems need to become more sustainable and resilient in order to deliver enough food and other goods in a changed climate, and to a growing world population (European Environment Agency 2015). Resilience is a systems ability to absorb disturbance, recover from it and maintain the same structure, function and feedbacks afterwards (Walker et al. 2004).

Most scientists today agree that climate change is ongoing and that human activities are the main drivers behind it. Different and independent scientific analyses of the climate system and changes in greenhouse gas concentration in the atmosphere for example, show the evidence (IPCC 2013).

Rockström et al. (2009) have defined nine planetary boundaries below which humanity is expected to live and operate safely. It is expected that if we live within this safe operating space without exceeding any of the boundaries, our planet Earth will stay in the relatively stable and resilient state of Holocene (Rockström et al. 2009). On the other hand, if we live beyond these boundaries and exploit the planet and its resources too intensively, which we already do at many points today, we risk to push our planet out of its stable state and then face unpredictable weather events and climatic changes (Rockström et al. 2009). These unpredictable changes have already started to take place since we entered a new era in conjunction with the industrialization around year 1800. The era is called Anthropocene because we humans have become the greatest geological force on Earth (Crutzen 2002; Steffen et al. 2007).

In 1987 the United Nations formulated in their report “Our Common Future” the need for sustainable development, to ensure humanity’s needs for today as well as our needs for tomorrow and for future generations. This implies, for the human population to live in accordance with the ecosystems and the biosphere as a whole. The report acknowledged that sustainable development depend on many different aspects which all are connected and cannot be treated one by one. All aspects such as biodiversity, population, food security, energy and economy have to be included in the work for sustainable development (United Nations 1987). According to the report it is a challenge where sacrifices and choices have to be made, and political incentives has a big role to play (United Nations 1987).

In September 2015 the United Nations (2015) published 17 Sustainable Development Goals which include many different aspects of sustainable development. The aim is to reach the goals by 2030. These goals all together aim to end poverty, to ensure prosperity and to solve the climate crises in order to keep a healthy planet for all people on Earth. Thus including all 3 dimensions of sustainable development: economic, social and environmental (United Nations 2015). These 3 dimensions are also included in the model: “the safe and just space for humanity”, by Oxfam (2012). It illustrates how economic, social and environmental aspects play a role for a sustainable living. “The safe and just space for humanity” is a model shaped like a doughnut and represents the room in which humanity can live socially fair and environmentally safe. If human activities extend the environmental ceiling, defined by the planetary boundaries, formulated by Rockström et al. (2009) it will lead to degradation of the environment. If human life standards are below the social foundation on the other hand, human life will be scanty (Oxfam 2012).

Human’s farm land is occupying more land than ever of the planet. In many places of the world, intensive agricultural systems with high environmental impact, produces many goods, but an increased fraction goes to non-human food such as animal feed or for biofuels (Foley et al. 2011). At the same time as one out of nine people today are undernourished in our world (United Nations 2015).

Agricultural systems need to be transformed to be environmentally sustainable at the same time as they produce enough food for our growing world population (Foley et al. 2011). Analysis show that different approaches have to be applied simultaneously to meet this goal (Foley et al. 2011). We have to halt expansion of agriculture, close the yield gaps on the least productive land, use resources more efficiently in agriculture, as well as increase food delivery by changing our diets to a more vegetable based, rather than an animal based diet. Another central aspect is to reduce waste (Foley et al. 2011; Godfray et al. 2010). Foley et al. (2011) mean that the priority is to secure the supply and access to enough food and nutrients for our world population. Thereafter the change of our food systems should be addressed to radically decrease greenhouse gas emissions, halt biodiversity losses and stop water pollution from agricultural chemicals.

1.2 Roles of legumes in sustainable agriculture

Leguminous plants are suitable for a sustainable food system for many reasons. From an environmental point of view: Legumes form symbiosis with rhizobium bacteria in the soil to fix nitrogen from the atmosphere, which decreases the need for energy consuming and costly chemical fertilizers (Berrada & Fikri-Benbrahim 2014; Granstedt 2012; Nemecek et al. 2008). The symbiosis contribute to improved productivity for subsequent crops and preserved soil fertility (Berrada & Fikri-Benbrahim 2014). Legumes allow reduced tillage and can contribute with diversity to a cereal dominant cropping system, which contributes to decrease weed- and pathogen pressure (Nemecek et al. 2008). A sustainable food system also include the eaters, which means that it has to satisfy human need for nutrients, at the same time as it functions in an environmentally sustainable way. Legumes is a cheap and resource efficient protein source compared to animal protein, thus include the economic as well as social and health aspects of sustainability (Röös et al. 2016).

New Legume Foods (2017) is a 4 years Swedish research and innovation project, which started in January 2017 and includes collaboration between scientists, food industry, regional councils and farmers. The purpose is to develop climate-smart and protein rich food products based on different varieties of domestic legumes. Legumes suitable for cultivation in the Nordic climate and which can be grown in cropping systems supported by ecosystem services and depend less on fossil fuel (New Legume Foods 2017). The project also aims to look at the effect of different process techniques on the nutrient quality of new legume based products. Thus the project assesses all 3 dimensions of sustainability: economic, environmental and social, in the development of New Legume Foods. My master thesis project is part of this bigger project and focuses on nodulation and biomass growth of common bean, *Phaseolus vulgaris* L. Therefore it will contribute to investigate how a suitable and already well established legume in Sweden, can be supported by ecosystem services for an efficient nitrogen fixation. Findings in this study will contribute to a knowledgebase for successful cultivation of common bean on fields without earlier common bean cultivation and thus enable further expansion of common bean cultivation in Sweden.

1.2.1 Legumes' nitrogen fixation

The symbiosis between some species of nitrogen fixing bacteria and legumes began during the Cenozoic era, about 65.5 million years ago (Sadava et al. 2012). This resulted in the first "green revolution", when the amount of available nitrogen in the terrestrial system increased radically (Sadava et al. 2012). All living organisms require nitrogen to form amino acids to build up proteins, thus nitrogen fixation is essential for life (Sadava et al. 2012). The symbiosis between nitrogen fixing bacteria and legumes initiates by the release of various chemical signals of the legumes roots whereon rhizobia attracts to the root zone and release Nod factors which further initiate the formation of a root nodule. Once the rhizobia has entered the root cells they differentiate into bacteroids which fix nitrogen (Sadava et al. 2012). Until present, 49 rhizobial species have been identified, that is both nitrogen-fixing and legume-nodulating species of bacteria (Weir 2016).

The legume family *Fabaceae* includes 19 700 species, which are spread all over the world (Berrada & Fikri-Benbrahim 2014). Different legume species form symbiosis with different rhizobia strains (Berrada & Fikri-Benbrahim 2014). For the symbiosis to take place the right bacterial species for the cultivated legume has to be present in the soil, either naturally accumulated through repeated cultivation of the specific legume species, or through inoculation of the seeds (Vargas et al. 2003). Inoculation means that an effective rhizobium bacteria is added to the seeds before sowing or during planting. The purpose is to ensure that the right rhizobium species exist in the soil, for an effective symbiosis to occur between the legume and the rhizobium bacteria (Loynachan, n.d.). Inoculation of the seeds for the first year of cultivation, has shown significant increase in nodulation and grain yield on common bean (Vargas et al. 2003). Additionally, Vargas et al. (2003) found almost no effect from inoculation of common bean seeds, cultivated on fields with sufficient amount of indigenous bean rhizobium bacteria from repeated bean cultivation. These results suggest that inoculation is important for the first year of cultivation of common bean. However, the need for inoculation when cultivating a legume for the first time in a field might differ between different species of legumes.

Results from Swedish farmers who cultivated different types of legumes (clover, peas and vetches) with and without inoculation, during some years before 1945, show on average 20 % yield increases after inoculation with rhizobia. Positive effects of inoculation were found both on soils where the same type of legume had been cultivated some years earlier and on soils where the legume had not been cultivated before (Bjälfve 1945). In addition, numbers of nodules as well as size of the nodules increased with inoculation (Bjälfve 1945). Cultures of bacteria for inoculation increased in Swedish agriculture around 1945 and there seems to have been consensus that inoculation of any kind of legume is important for protein yield and total yield (Bjälfve 1945).

Nitrogen fixation of nodulated legumes is negatively affected by environmental factors such as increased soil acidity and other stresses such as drought and light- or potassium deficiency (Schubert 1995). Additionally a high amount of plant available nitrogen in the soil, due to high mineralization or application of nitrogen fertilizer, also decreases the amount of biologically fixed nitrogen (Da Silva et al. 1993) as well as number of root nodules and their dry weight (Vargas et al. 2003).

Plentiful of root nodules normally gives a high rate of nitrogen fixation, however few root nodules can be compensated by a higher weight per root nodule (Evans et al. 1990). Root nodule size can have an effect on nitrogen fixation activity according to results found on peanuts, where medium sized (1.5− 2.0 mm in diameter) root nodules gave the highest rate of nitrogen fixation whereas larger root nodules did not have a significant correlation between nodule size and nitrogen fixation (Tajima et al. 2007). Root nodules that are active in their nitrogen fixation are red inside because of the presence of leghemoglobin (Ott et al. 2005). Leghemoglobin is necessary for symbiotic nitrogen fixation within the root nodules, although it is not necessary for plant growth, if an external source of plant available nitrogen is present (Ott et al. 2005). Leghemoglobin is important because of its affinity to oxygen and thereby efficient delivery of oxygen to respiring rhizobium even at low concentrations (Appleby 1984). The oxygen delivery is just enough to support the respiration of rhizobium bacteria and at the same time kept enough low to protect the oxygen-sensitive and nitrogen fixing catalyst (enzyme) nitrogenase from inhibition (Sadava et al. 2012).

1.3 *Phaseolus vulgaris*, common bean

Common bean is a highly variable species with climbing- and bushy cultivars. The pods are harvested and eaten as fresh vegetables or as mature, dry beans (FAO 2017). The wild relative to common bean is probably an indigenous species of South America (FAO 2017). Common bean needs a relatively warm climate and is therefore limited to the very south parts of Sweden and the two islands, Öland and Gotland in south east Sweden (Jordbruksverket 2015). Cultivation of common bean in Sweden started in the 17th century (Jordbruksverket 2015).

Common bean can form symbiosis with several different rhizobial species, including *Rhizobium etli, R. gallicum, Sinorhizobium fredii, R. giardinii and R. leguminosarum bv phaseoli* (Herrera-Cervera et al. 1999). Strains from *R. gallicum bv. gallicum, R. gallicum bv. phaseoli* and *R. giardinii bv. phaseoli* all nodulate and fix nitrogen with common bean (Amarger et al. 1997). López-López et al. (2010) isolated another species, *R. endophyticum* from common bean grown in a laboratory. The systematics and naming of rhizobia nodulating common bean has been revised several times (Berrada & Fikri-Benbrahim 2014).

On fields around Seibersdorf laboratory in Austria, Sessitsch et al. (1997) found that common bean can be well nodulated even without a host plant present for several decades. One of the detected bacteria had the same characteristics as *R. etli* and another bacteria was similar to the species found by Geniaux et al. (1993) in France: *R. gallicum* (Sessitsch et al. 1997). However they suggest that the bacteria strains nodulating common bean in this study most likely came from Mesoamerica with contaminated seeds (Sessitsch et al. 1997; Herrera-Cervera et al. 1999).

A Swedish experiment from 1950 on common bean of the type with brown seeds (the Swedish brown bean), shows the importance of choosing the right bacterial strain for inoculation. The experiment was set up on a nitrogen poor, sterile sand where phosphate and potassium chloride was added. Brown bean seeds were inoculated with one effective- and one ineffective bacterial strain isolated from common bean as well as one effective bacterial strain from faba bean (*Vicia faba* L.) also shown to be effective on pea (*Pisum sativaum* L.). Inoculation with the effective bacterial strain from common bean resulted in the richest nodulation whereas the ineffective common bean strain as well as the effective faba bean strain both resulted in few and small nodules. The control without inoculation did not nodulate (Bjälfve

1961). In 1952 another experiment was performed, which showed that the ineffective bacterial strain of common bean was effective to nodulate and fix nitrogen on clover (*Trifolium* spp.*)*. Accordingly the efficiency of different bacterial strains differ much from one host species to another. One bacterial strain can be very effective both to nodulate and to fix nitrogen on for instance faba bean, but rather ineffective when inoculated on brown bean seeds. These experiments show that bacterial strains isolated from one genus can be more effective in nodulating another genus and vice versa (Bjälfve 1961).

The same author meant that inoculation always is necessary if a legume of the same genus has not been cultivated on the same area of land before (Bjälfve 1950). However, today the recommendations about inoculation on Swedish soils are less consistent. Fogelberg (2008) mean that inoculation is necessary when growing common bean on a soil where common bean has not been cultivated before, whereas Hushållningssällskapet (2013) mean that it is not necessary because the bacteria exist naturally in Swedish soils for kidney bean, a type of common bean.

1.4 Why is this study of interest?

Cultivation of common bean in Sweden is expanding to new and larger areas where common bean has not been grown before and today's recommendations about inoculation of common bean in Sweden differ between different reports. This study includes many samples of both categories of fields, with and without regular cultivation of common bean. Hopefully this study can contribute to reinforce the knowledge base to support national recommendations and guidelines about inoculation of common bean.

1.5 Aim and research question

The aim of this study is to investigate whether the presence of active nodules on common bean roots differ between soils with regular common bean cultivation (at least twice during the last 15 years) and soils where common bean has not been cultivated for at least 20 years. Farmers' fields were used for sampling and measurements to answer the following research questions: Is there a difference in nodulation of common bean roots between soils with and without a regular cultivation of common bean? Do root nodules form on roots of common bean, if cultivated on soils where common beans have not been present in the crop rotation for at least 20 years? And if yes, are they active?

2 Materials and methods

2.1 Study site

This study comprised interviews with farmers and field studies on farmers' fields on the two Swedish islands Öland and Gotland (Figure 1). The two islands are situated in the Baltic Sea, on the southeast coast of Sweden's main land. Both Öland and Gotland are characterized of a lime bedrock, which contain at least 50 % carbonate minerals, mainly calcium carbonate (SGU 2009). The soil pH is therefore generally from around 6.9 and higher (SGU 2009). The soil type is mostly clay moraines on both islands (SGU 2009). According to the Köppen climate classification system the islands are situated in the warm temperate climatic zone with deciduous forest (SMHI 2017).

The field work comprised two sampling occasions. First sampling at pod fill during ten days from July 18th to July 26th, 2017 and second sampling during one week at full maturity, from August 28th to September 3rd, 2017. The fields included in the study were used by the farmers for growing the brown bean type of common bean during the season of 2017. The selection of specific fields was based on the history of cultivating common bean. Ten fields where common bean had not been cultivated for at least 20 years were selected on 4 farms. Three of those farms were situated on Gotland and one on Öland. Ten other fields where common bean has been cultivated regularly, at least twice during the last 15 years were selected on 3 farms, all situated on Öland. Ideally the two categories of fields should have been more equally distributed on the two islands, or all fields concentrated on one island. Unfortunately it was not possible to find fields with a regular common bean cultivation on Gotland or to find more than two fields with first common bean cultivation on Öland, therefore the distribution of the two categories of fields was skewed.

Figure 1. Map of southern Sweden, showing the geographical location of the islands Öland and Gotland. (Source: © Lantmäteriet).

2.2 Sampling at pod fill

The first field sampling was performed in the end of July, when the brown bean plants were in the development stage from full flowering: 65, to the stage where 50 % of pods have reached final length: 75 (Meier 2001). A car was used for the transport to- and around on the islands to the different fields. On each field, 3 sampling sites were chosen along a transect that was placed to cover most of the visually apparent variation within the field, *e.g.* from the highest to the lowest point (Figure 2). None of the 3 sampling sites were closer than 30 meters to the edge of the field.

One collected soil sample per sampling site was taken to determine soil pH. Each collected soil sample included soil from 10 soil cores of about 20 cm depth and 3 cm in diameter, taken in a circle of about 4 m in diameter around the center of the sampling site (Figure 2). A radius was made by using a 2 m yardstick from the center of the sampling site out in ten different directions to drill each of the 10 soil cores.

All ten soil cores were mixed in a bucket and 0.25 liter of the mixed soil was collected in a paper box. Each paper box was marked according to a code that gave each sample a unique ID, with a letter for the field and numbers for the 3 sampling sites within that field (*e.g.* A1, A2 and A3 for the 3 sampling sites on the first field). The bucket was emptied by hand between the sampling sites. Since 20 fields were included in the study, a total of 60 soil samples were thus collected. The paper boxes with soil samples were dried in 80 °C for 24-48 hours and stored until September.

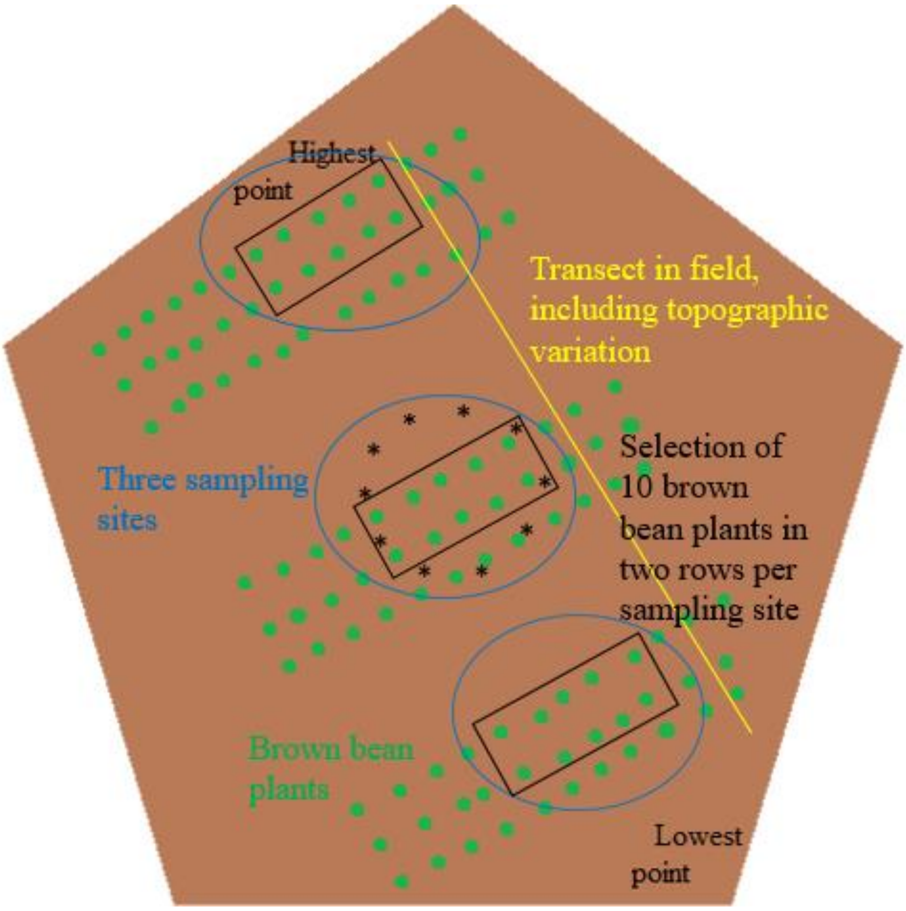

Figure 2. Schematic illustration of the sampling at pod fill. Sampling sites (blue circles) were distributed along a transect (yellow line) which included most of the topographic variation within the field. Ten soil cores in a circle of about 4 meters in diameter around the center of each sampling site were collected (black asterisks). Ten brown bean samples per sampling site were collected (green dots inside the black rectangles). (Illustration: Christina Hultman).

Within each sampling site, roots of 10 individual plants were sampled by digging out each plant from the soil with a pitchfork. Five bean plants in one row and another 5 bean plants in the row beside were selected. A total of 30 brown bean plants per field were thus dug out of the soil for further investigations (Figure 2), which made a total of 600 plant samples.

The sampling sites were marked with a flag about 2 meters from the sampling center, and each site was marked on a map, in order to find approximately the same places for the second sampling at full maturity in case the flags were removed.

A farmer survey was conducted to collect information about soil type, pH, plant-available K and P, crop rotation for the last 5 years, fertilization/manuring for the last 5 years and pre-history of common bean cultivation in the studied fields. The information was collected in one table per field (Appendix 1), which was filled by the investigator together with the farmer during a telephone interview or physical meeting on the farm.

2.2.1 Presence of root nodules

The roots of each of the 10 brown bean plants per sampling site were investigated to see if they had root nodules or not. The plants with root nodules were categorized further if they had less or more than 5 root nodules. For each plant, 10 representative root nodules with a diameter above 2 mm, or all nodules (with a diameter above 2 mm) if less than 10 were present, were cut in half with a knife on a wooden cutting board to see the internal color (white/pale/same as the root vs pink/red/darker than the root) as an indication of nodule activity (Figure 3). All information about nodulation per individual plant at each sampling site was noted in a form (Appendix 2), which also contained a comment about nodule size.

Figure 3. Photo of a brown bean plant, with an arrow pointing at root nodules and a small photo at the top displaying a root nodule cut in half with a reddish/pinkish internal color. An illustration of a scissor indicate the thickening on the stem where the aboveground biomass was cut off. (Photo: Christina Hultman).

2.2.2 Biomass production

On each of the 10 brown bean plants per sampling site, above ground biomass was separated from roots by cutting at the stem thickening in the transition between hypocotyl and epicotyl with a scissor (*i.e.* the position of cotyledons before they fall off) (Figure 3). The above ground biomass of each plant was then put into a paper bag which was marked with the plant ID number (*e.g.* A10-A39 for the 30 samples on the 3 sampling sites on the first field). The samples were dried at in 80° C for 24-48 hours at the research stations Torslunda on Öland and Hallfreda on Gotland, where they were stored until September when they were transported to Ultuna, Uppsala, for further drying and weighing.

2.3 Sampling at full maturity

The second sampling was performed in late August – early September, when the brown bean plants were approaching full maturity. The investigator went back to the same fields with flags where the first samplings were made (Figure 2). Three sampling sites per field were used this time as well, which were placed about 2 meters from the center of the sites for the first sampling. In 2 sampling sites of one field, the flags that were placed at the first sampling had been accidentally removed, and in these cases the positions indicated on the map were used to identify the approximate locations of the first sampling sites. Within each sampling site the above-ground biomass of brown bean plants along 1 meter in 2 parallel rows was collected, equal to 1 square meter. The measurements was made with a 1 meter long stick.

2.3.1 Pod- and biomass yield at maturity

The residual above ground biomass of each individual brown bean plant along the 2 meters within each sampling site was collected by cutting each plant at the thickening of the stem in the transition between hypocotyl and epicotyl with a secateurs. The pods from all plants along these 2 meters per sampling site were removed and put into a paper bag and the residual aboveground biomass were then put into a larger paper bag. The paper bags were marked with the same codes as for soil samples. Three bags of pods and 3 bags of residual aboveground biomass were thus collected in each field, which means a total of 60 bags of each content. These samples were dried in 80° C for 24-48 hours at the research stations Torslunda on Öland and Hallfreda on Gotland before transport to Ultuna, Uppsala for further drying and weighing.

2.4 Sample processing and analyses

All samples of aboveground biomass, pods and soil were transported to Ultuna, Uppsala, after the second sampling in beginning of September. All samples were dried again in 80°C until they were completely dry. Thereafter all samples were weighed one by one on the same scale (Mettler PM4600 Delta Range) and the weights were noted in gram with 2 decimals in an excel form. The average weight of 3 paper bags of the same size was subtracted from the measured weight including paper bags.

The soil samples were milled by hand and sieved through a 2 mm mesh sieve to keep only the fine soil fraction for pH-determination. Five ml fine soil from each sample was taken out with a spoon and put into a plastic vial, where 25 ml deionized water was added before shaking them vigorously for 15 minutes with a shaking machine. All samples rested for about 18 hours before shaking them again thoroughly for 30 seconds, before another resting period of 10 minutes to 2 hours before measuring the pH with a calibrated pH-meter (PHM 93 REFERENCE PH METER). The pH for the 60 soil samples were noted in an excel form.

Mean proportion of active root nodules per plant that had root nodules was calculated by first summing up all individual proportions of active root nodules per plant within each sampling site, and then dividing this sum by the amount of plants that had root nodules within the sampling site.

2.5 Databases used

For the literature research databases such as PubMed, Google Scholar and Google were used. Terms for the research were: “Sustainable development”, “climate change agriculture”, “legumes as source of protein”, “review rhizobia”, “nitrogen fixation legumes”, “common bean nodulation”, “root nodule size and nitrogen fixation” and “*Rhizobium* and *Phaseolus vulgaris*” etc.

2.6 Statistical analysis

All statistical tests were conducted in the program Minitab 18. The statistical tests used to process the data were: General Linear Model ANOVA and Mann Whitney’s test. Data from these tests and additional descriptive statistics were used to make figures and tables in Microsoft Excel 2013.

The choice to collect ten plants per sampling site was made to get a representative group of samples per sampling site, but the ten individual plants should be considered as pseudo-replicates (i.e. these observations are not independent). The GLM ANOVA was used to take into consideration the pseudo replication of plants within a sampling site as well as plants belonging to the same field. This was made by using average values per sampling site as responses (60 data points) and history of common bean cultivation as well as field-ID as explaining factors, where field-ID was nested in history of common bean cultivation. Average values were used for all measurements except for pH, pod weight and residual aboveground biomass where there was only one collected sample per sampling site, thus all of these measurements were used in the GLM ANOVA. Average values per field were used in order to avoid pseudo replication in the Mann Whitney’s test.

3 Results

3.1 Description of the fields

There were variations in several field properties between the investigated fields (Appendix 1). Different mineral fertilizers and manure were used in different quantities. Three fields received manure during the season (A, B and L) and one field received nothing during the season, but manure during the previous season (K), whereas the remaining 16 fields received mineral fertilizer during the season. Crop rotations also differed between farms, and included crops such as different cereals, onion, rape seed, peas, potato, ley and common bean. Only two of the fields had ley in the crop rotation during the last 5 years. Field K had ley for the last four years before common bean and field L had onion as pre-crop and ley for three years before that. Soil type differed from exclusively sandy soil without stones, to more compact and/or stony soils with more or less clay and humus.

The 7 farms were located at different places on Öland and Gotland (Figure 4). Two of the fields (K and L) where common bean was cultivated for the first time since at least 20 years, were located on Öland, whereas the remaining fields without history of common bean cultivation were located on Gotland. All fields with regular common bean cultivation (at least twice during the last 15 years) were located on Öland.

Figure 4. Map of Öland and Gotland, showing the geographical location of the 7 farms, numbered from 1-7. Letters represent the different fields in the study, where fields A-J have a regular common bean cultivation whereas on fields K-T common bean was cultivated this season for the first time since at least 20 years. (Source: © Lantmäteriet).

3.1.1 Soil pH

There were only small visual differences in average soil pH between fields (Figure 5) and all pH-values were around neutral pH. However, a General Linear Model ANOVA of soil pH per sampling site showed that soil pH differed significantly between fields (P-value = 0.000, F-value = 4.63) and between the two categories of fields; fields with a regular common bean cultivation (pH=6.8) and fields where common bean was cultivated for the first time (pH=7.1), (P-value = 0.043, F-value = 4.72). A completing Mann Whitney's test of average soil pH per field also shows that pH was significantly lower on fields with a regular common bean cultivation than on fields with first cultivation (P-value = 0.045, W-value = 78.00).

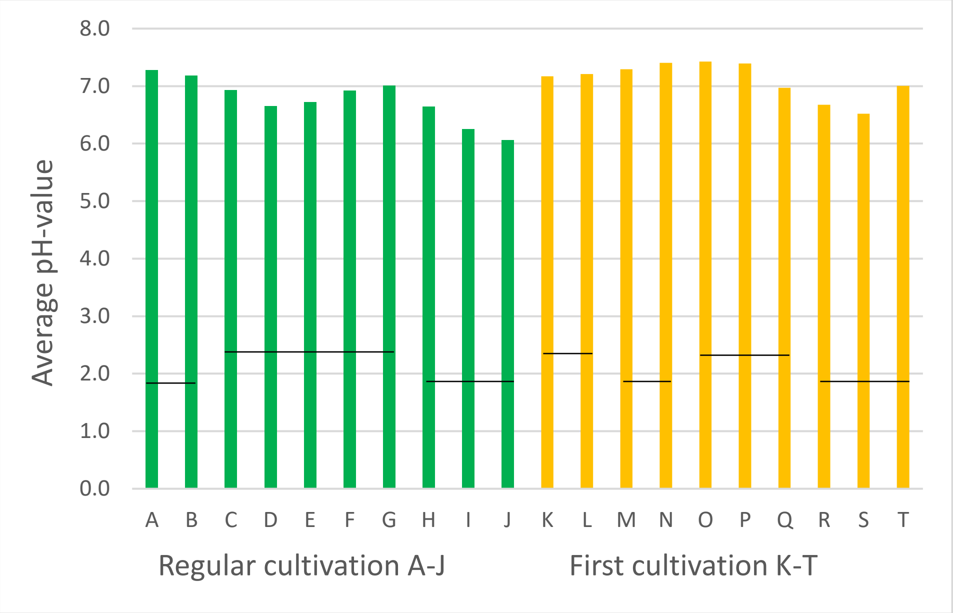

Figure 5. Average pH-value per field. Each bar represents the mean value of 3 pH-measurements. Fields with regular bean cultivation in green (A-J) and fields where beans were cultivated for the first time in yellow (K-T). Bars linked with a line belong to the same farm.

3.2 Sampling at pod fill

3.2.1 Presence of root nodules

Descriptive statistics of presence of root nodules can be overviewed in Table 1. Approximately 5 times more plants grown on fields with a regular bean cultivation had root nodules, than on fields where common beans were cultivated for the first time since at least 20 years. As a consequence, much more root nodules on fields with a regular bean cultivation were cut/investigated and most of them were active, compared to the number of root nodules from fields where beans were cultivated for the first time.

Table 1. *Quantitative overview of plants investigated for presence of root nodules, and nodules investigated for internal color as an indication of nodule activity.*

	Total number of investigated plants	Number of plants with root nodules	Number of investigated/cut nodules	Number of active root nodules
Regular cultivation	300	293	2180	2085
First cultivation	300	58	114	48

A GLM ANOVA showed that the proportion of investigated plants that had root nodules per sampling site differed significantly between the two categories of fields; fields with a regular common bean cultivation had a significantly higher proportion of plants that had root nodules than fields with first bean cultivation (P-value = 0.000, F-value = 101.08). A complementing Mann Whitney's test of proportion of the 30 investigated plants per field that had root nodules also showed a significant difference between the two categories of fields (P-value = 0.000, W-value = 155.00). On fields with a regular common bean cultivation almost all investigated plants had root nodules, whereas on fields where common beans were cultivated for the first time almost no plants had root nodules, except on field K (Figure 6).

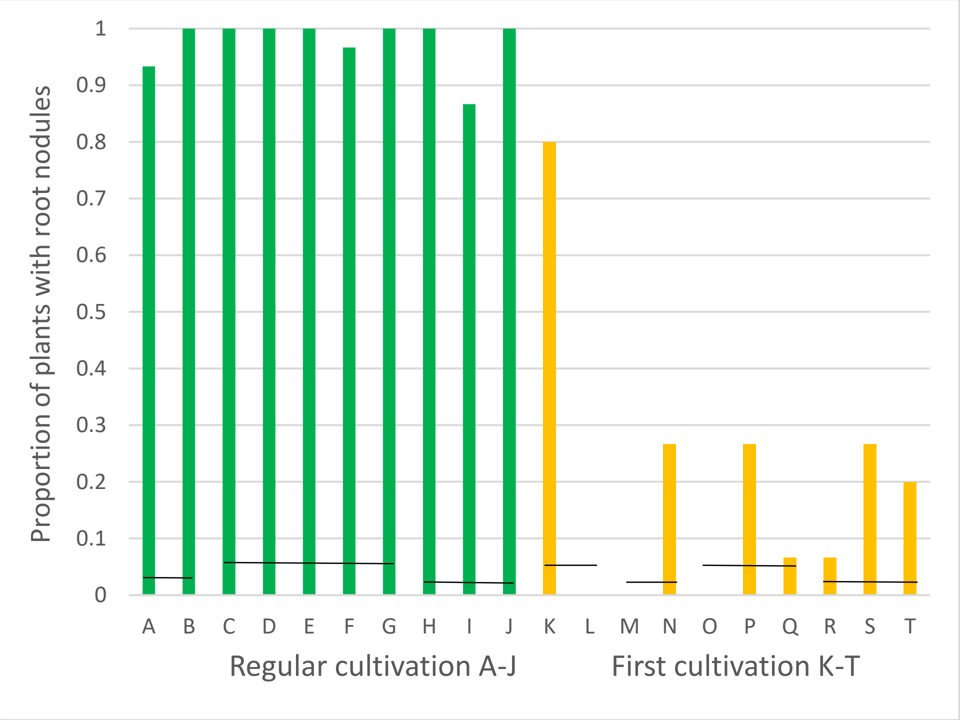

Figure 6. Proportion of plants with root nodules per field. Fields with regular bean cultivation in green (A-J) and fields where beans were cultivated for the first time in yellow (K-T). Bars linked with a line belong to the same farm.

Additionally, most of the root nodules on common bean plants cultivated on fields with regular common bean cultivation were active, whereas few root nodules were active on common bean plants cultivated on fields for the first time (Tables 1 and 2). All sampling sites on fields with regular bean cultivation had plants with root nodules whereas about half of the sampling sites, on fields with cultivation of beans for the first time, had plants with root nodules (Table 2). Field observations also showed that fields with regular bean cultivation generally had bigger root nodules.

A GLM ANOVA of proportion of active root nodules per plant that had root nodules, per sampling site where at least one plant had root nodules, showed that there were significantly more active root nodules per plant that had root nodules on fields with a regular common bean cultivation than on fields where common beans were cultivated for the first time (P-value = 0.000, F-value = 50.95). On fields with regular bean cultivation almost all investigated plants had root nodules (Table 1 and Figure 6) and most of their root nodules were active (Table 1, Table 2 and Figure 7), whereas on fields where beans were cultivated for the first time since at least 20 years, few plants had root nodules and only a third of them were active.

Table 2. *Descriptive statistics of number of sampling sites per field type, regular- or first bean cultivation that had plants with root nodules and mean proportion of active root nodules per plant that had root nodules.*

Sample	Number of sampling sites where at least one of the sampled plants (in total 30) had root nodules	Mean proportion of active root nodules per plant	SE Mean
Regular cultivation	30	0.9	0.03
First cultivation	16	0.3	0.08

A GLM ANOVA showed that both field-ID (P-value = 0.000, F-value = 4.83) and history of common bean cultivation (P-value = 0.000, F-value = 101.08) had significant effect on proportion of plants that had root nodules per sampling site (Figure 6). Additionally, the proportion of active root nodules per plant that had root nodules, per sampling site where at least one plant had root nodules (Table 2), differed significantly between the two categories of fields (P-value = 0.000, F-value = 50.95), but did not differ significantly between single fields (P-value = 0.247, F-value = 1.33). F-values indicate that the correlation between history of common bean cultivation and proportion of plants that had root nodules as well as proportion of active root nodules per plant with root nodules were stronger than the effect of variations between fields.

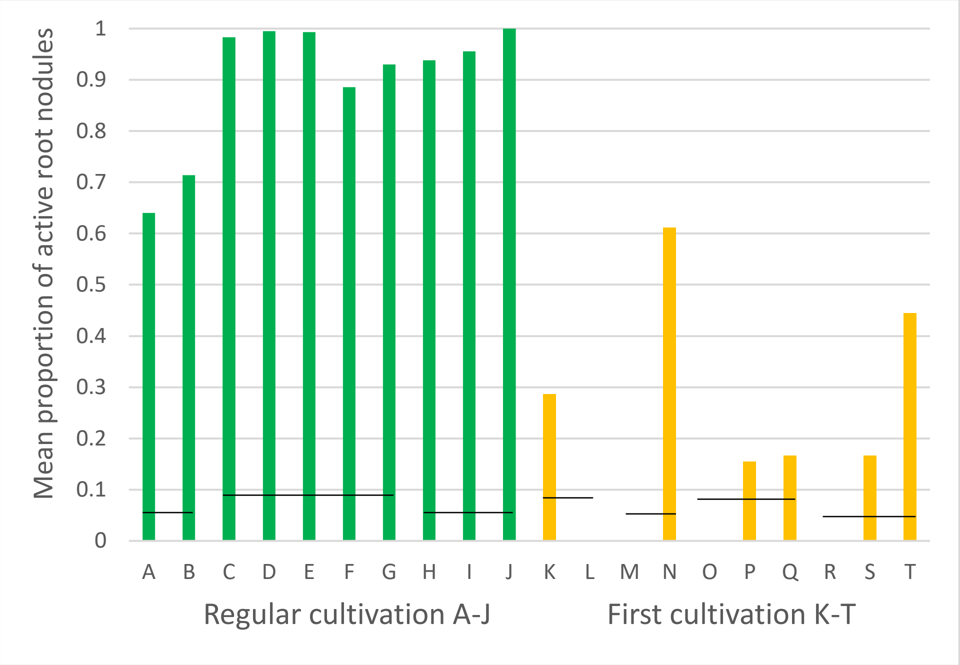

Figure 7. Proportion of active root nodules per plant with root nodules per field. Fields with regular bean cultivation in green (A-J) and fields where beans were cultivated for the first time in yellow (K-T). Bars linked with a line belong to the same farm.

3.2.2 Biomass production

There were large variations between fields in average aboveground plant biomass at pod fill (Figure 8). Average aboveground biomass weights were 9.55 grams (regular cultivation) and 7.39 grams (first cultivation) for the two categories of fields respectively. GLM ANOVA of average aboveground biomass per sampling site showed no significant difference between the two categories of fields (P-value = 0.129, F-value = 2.54), however there was a significant difference between the different fields (P-value = 0.000, F-value = 7.26). On the other hand GLM ANOVA showed no significant correlation between neither proportion of plants that had root nodules per sampling site (P-value = 0.886, F-value = 0.02), nor with proportion of active root nodules per sampling site where at least one plant had root nodules (P-value = 0.321, F-value = 1.02) and average aboveground biomass per sampling site.

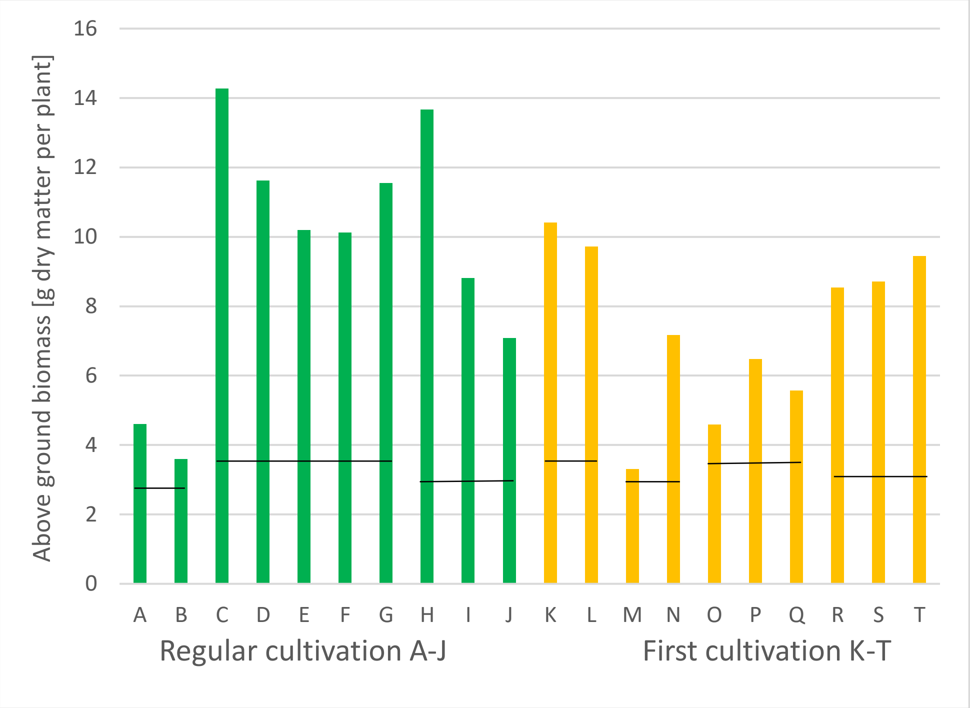

Figure 8. Average weight of aboveground biomass per plant per field. Each bar represents the mean value of 30 plant samples. Fields with regular bean cultivation in green (A-J) and fields where beans were cultivated for the first time in yellow (K-T). Bars linked with a line belong to the same farm.

3.2.3 Influence of soil pH

Results from GLM ANOVA of soil pH per sampling site, showed no significant correlation between neither proportion of plants with root nodules (P-value = 0.479, F-value = 0.51), nor with proportion of active root nodules per plant that had root nodules per sampling site where at least one plant had root nodules (P-value = 0.960, F-value = 0.00). Additionally, there was no significant correlation between soil pH and average aboveground biomass per sampling site (P-value = 0.010, F-value = 7.44).

3.3 Sampling at full maturity

3.3.1 Pod- and residual biomass yield at maturity

Average pod weight per square meter per field is shown in Figure 9. Results from a GLM ANOVA of pod weight in a square meter per sampling site showed that field-ID had a significant effect on pod weight (P-value = 0.001, F-value = 3.26) whereas history of bean cultivation did not have a significant effect on pod weight (P-value = 0.681, F-value = 0.17). Thus pod weight varied more depending on field-ID than on history of bean cultivation. Additional GLM ANOVA showed no significant correlation between neither proportion of plants with root nodules per sampling site and pod weight, nor between proportion of active root nodules (per plant that had root nodules per sampling site where at least one investigated plant had root nodules) and pod weight (P-values = 0.883 and 0.278, F-values = 0.02 and 1.22). On the other hand, there was a significant, positive correlation between average aboveground biomass weight per sampling site at pod fill and pod weight at maturity (P-value = 0.005, F-value = 9.04).

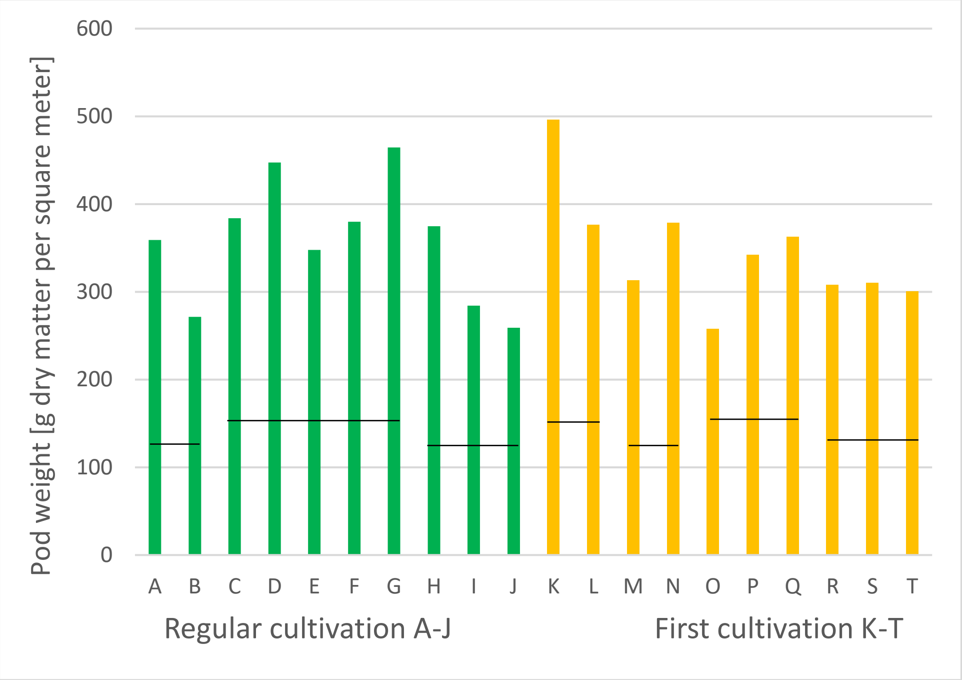

Figure 9. Average pod weight per square meter at each field. Fields with regular bean cultivation in green (A-J) and fields where beans were cultivated for the first time in yellow (K-T). Bars linked with a line belong to the same farm.

There were large variations between fields in average residual aboveground biomass per square meter (Figure 10). There were also variations between the two categories of fields. Fields with a regular common bean cultivation had in general less average residual aboveground biomass per square meter than fields where common bean was cultivated for the first time had. Results from a GLM ANOVA of residual aboveground biomass in a square meter per sampling site showed that history of common bean cultivation (P-value = 0.000, F-value = 22.30) as well as field-ID (P-value = 0.002, F-value = 3.08) had significant influence on residual aboveground biomass weight.

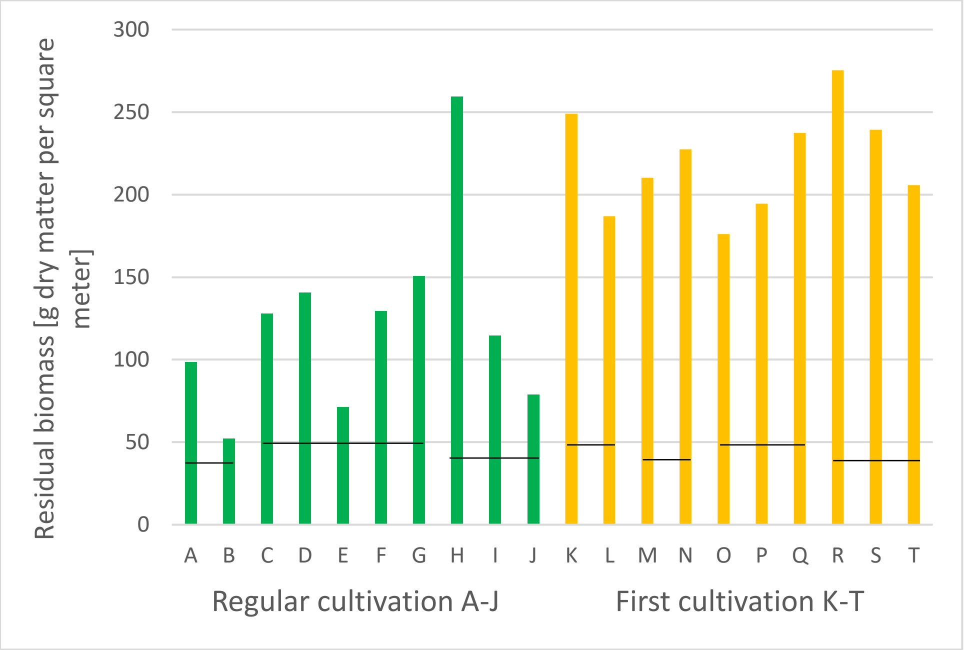

Figure 10. Average aboveground residual biomass weight per square meter per field. Fields with regular bean cultivation in green (A-J) and fields where beans were cultivated for the first time in yellow (K-T). Bars linked with a line belong to the same farm.

Average pod weight per square meter was slightly lower on fields with regular bean cultivation than on fields with first bean cultivation (Table 3). Average residual aboveground biomass per square meter was almost doubled on fields with first common bean cultivation compared to fields with regular bean cultivation.

Table 3. *Showing average pod- and residual aboveground biomass weight of the two types of fields.*

	Average pod weight [g]	Average residual aboveground biomass weight [g]
Regular cultivation	342	122
First cultivation	352	220

3.3.2 Influence of soil pH

GLM ANOVA showed that soil pH per sampling site did not correlate significantly with pod weight per square meter per sampling site (P-value = 0.449, F-value = 0.58) nor with average residual aboveground biomass weight per square meter per sampling site (P-value = 0.821, F-value = 0.05).

4 Discussion

4.1 Sampling at pod fill

4.1.1 Presence of root nodules

The results found in this study gave a clear answer to all three research questions. On fields with regular bean cultivation almost all investigated plants had root nodules and most of their root nodules were active, whereas on fields where beans were cultivated for the first time since at least 20 years, very few plants had root nodules and only a third of them were active. Thus there was a difference in root nodule abundance between fields with and without a recent history of common bean cultivation, indicating that the right type of rhizobial bacteria does not exist, or only in very small amount, in soils without a pre-history of common bean cultivation.

These findings are contradictory to what is written in the report by Hushållningssällskapet (2013), where it is stated that inoculation is not necessary when growing kidney bean (another type of common bean) on fields where common bean has not been cultivated before. However, the recommendations to not inoculate kidney bean in this report should be carefully interpreted since they are not based on a scientific study. On the other hand it is interesting to mention since none of the farmers who cultivated common bean for the first time in this study chose to inoculate. According to my results, only few- and sometimes no investigated plants on fields without a pre-history of common bean cultivation had root nodules. Thus, the comparatively high biomass production and yield of these plants were probably a result of a sufficient amount of plant available nitrogen in the soil.

These results also differ from results in a study by Sessitsch et al. (1997) who found that common bean can be well nodulated even without a hostplant present for several decades. On the other hand some of the common bean plants in this study did form root nodules on fields where common bean has not been cultivated in the crop sequence for at least 20 years, and some of them were active. This result indicate that the right rhizobial species might exist in sufficient amounts in certain fields although common bean has not been cultivated since at least 20 years. This could be explained in the same way as Sessitsch et al. (1997) explained their results, namely that the seeds might have been contaminated with the right rhizobial strain. However it does not explain why field K had many plants with root nodules whereas field L, belonging to the same farm, had no plants with root nodules. Another interesting aspect of differences in root nodulation between fields with first bean cultivation is that field K had many plants with root nodules, but only few of them were active (Figure 6 and 7), whereas field N had few plants with root nodules and many of them were active. Thus indicating that plants on both of these fields might have had low nitrogen fixation anyway, but of different reasons; inactive root nodules on field K and few root nodules on field N.

On the other hand the results are consistent with Bjälfve (1950) and Fogelberg (2008) who meant that inoculation is necessary if a legume of the same genus has not been cultivated on the same area of land before, to ensure nitrogen fixation when the right rhizobial species to form symbiosis with common bean normally is missing.

History of common bean cultivation had significant correlation with presence of root nodules as well as with proportion of active root nodules per plant with root nodules. Additionally, proportion of plants with root nodules differed significantly between single fields. Proportion of active root nodules per sampling site where at least one plant had root nodules did not differ significantly between fields, though there were large visible differences when looking at Figure 7. An explanation for this non-significant result could be that only 16 out of 30 sampling sites on fields with first common bean cultivation had at least one plant with root nodules, which decreased the dataset for the fields with the largest visible variation between fields. Although variations between fields had a weaker correlation with these two measurements, and especially with proportion of active root nodules, it is important to consider this aspect since data differed considerably between fields. This is not surprising since the study was carried out in farmers' fields, thus farm- and field locations differed as well as soil types and properties, crop rotations (Appendix 1) and other management methods which all contribute to differences in the development of the crop, its roots and its interactions with soil microbes etc. It is interesting that even if field-ID had a significant effect on root nodule abundance, the effect of history of common bean cultivation or not, was considerably stronger for both measurements (when looking at the F-values of history of common bean cultivation; 101.08 and 50.95 compared to field-ID; 4.83 and 1.33), which showed evidence for its important effect on root nodulation.

Additionally root nodules of common bean plants on fields with regular bean cultivation were larger than root nodules on fields with first bean cultivation, according to my field observations. In a study on peanuts, Tajima et al. (2007) found a significant correlation between nitrogen fixation and nodule size up to medium-sized nodules (1.5-2.0 mm in diameter), while nitrogen fixation was almost constant for nodules larger than 2 mm. Additionally they found color differences inside root nodules depending on size: small root nodules were white, medium-sized nodules were red and large nodules were greenish inside, which indicate that medium-sized nodules had higher concentration of leghemoglobin and thus a more effective nitrogen fixation (Tajima et al. 2007). I observed larger root nodules in general on the common bean plants (small: less than 2 mm in diameter, medium: 2-3 mm and large: more than 3 mm) than observed on peanut in the study by Tajima et al. (2007). In addition, medium–sized and large root nodules were almost always red in my investigations, indicating that they were active in their nitrogen fixation.

4.1.2 Biomass production

Aboveground biomass weight at pod fill differed significantly between fields, indicating that field variations as a consequence of different locations of fields, different soil types, different crop rotations, different management practices etc. (Appendix 1) have an influence. However, the aboveground biomass weight did not differ significantly between the two categories of fields. There were no significant correlation between neither proportion of plants with root nodules and biomass weight, nor between proportion of active root nodules per sampling site where at least one plant had root nodules and biomass weight. Thus indicating that root nodulation and their nitrogen fixation activity was not influencing biomass production namely, which further suggest that there might have been enough plant-available nitrogen in the soil for plant growth without help from nitrogen fixation.

4.1.3 Influence of pH

There were small differences in average soil pH between individual fields and also between the two types of fields, although average soil pH was significantly lower on fields with a regular common bean cultivation than on fields where beans were cultivated for the first time. However, there were no significant correlations between soil pH and any of the other measurements (proportion of plants with root nodules, proportion of active root nodules, aboveground biomass weight at pod fill, pod weight or residual aboveground biomass weight at maturity). Thus indicating that soil pH did not have an important influence of any of the measurements. Since all pH-values were around neutral, and thus no soils were acid, there were probably other factors such as nutrition, water, light availability (Schubert 1995) and other soil properties that affected root nodulation, rather than soil acidity.

4.2 Sampling at full maturity

4.2.1 Pod- and residual biomass yield at maturity

Variations between fields had a significant effect on pod weight and on residual aboveground biomass weight, whereas history of bean cultivation did not have a significant effect on pod weight. On the other hand average residual aboveground biomass weight was only about half as high on fields with regular common bean cultivation compared to fields where beans were cultivated for the first time. These results could be partly explained by my observations in the fields, where common bean plants in fields with a regular common bean cultivation, in general were more mature and ready for harvest at the time I visited the fields (drier pods and defoliated plants), compared to common bean plants in fields where common bean was cultivated for the first time (Figure 11). This could have affected the results of pod weight as well as of residual aboveground biomass since a greater proportion of plants on fields, where common bean was cultivated for the first time, had big, fleshy pods and more leaves on the plants at harvest than bean plants had on the other type of field, with a regular bean cultivation. The observed differences in crop development may have affected the weight of both categories so that both pod weight and residual aboveground biomass weight of plants on fields with first cultivation were overestimated in study. However, it is likely that the difference in crop development stage had a larger effect on residual aboveground biomass than on pod weight, because of the large differences in the amount of leaves remaining on the plants at the time of the second harvest.

Figure 11. Two photos of fields at the second sampling, illustrating the differences in maturity. The photo to the left is representative for how most of the fields with a regular common bean cultivation looked like and the photo to the right is representative for how most of the fields with first common bean cultivation looked like. (Photo: Christina Hultman).

A possible explanation for maturity differences between the two categories of fields is that farmers who cultivated common bean for the first time probably were less experienced in the cultivation of common bean, which could have contributed to suboptimal establishment and management on fields with first cultivation of common bean.

According to additional yield information from the farmers, most of the fields where few plants had root nodules and where few of the root nodules were active (fields with first common bean cultivation) also gave relatively high yields (Appendix 3). Thus indicating that there was enough plant-available nitrogen in the soils for plant growth even without help of nitrogen fixation.

Aboveground biomass weight at pod fill was positively correlated with pod weight at maturity, indicating that establishment and growth of the common bean plants earlier in the season had importance for pod development.

4.3 Implications of the results

These results are important for common bean cultivation in Sweden and especially for the expansion of common bean cultivation to areas where common bean has not been cultivated before. According to the findings it would be appropriate to give the recommendation to inoculate common bean seeds with the right species of rhizobium bacteria before sowing, when cultivating common bean on a field for the first time.

The fact that common bean plants grew well even with a very low abundance of root nodules and where many of them were inactive indicates that the amount of plant-available nitrogen in the soil was not limiting plant growth. As a consequence, an additional recommendation from this study could be to decrease the amount of nitrogen fertilization of common bean, and instead make sure that the crop relies on nitrogen fixation. However, this interpretation is based on indirect observations, and further research about plant nitrogen acquisition under different nitrogen fertilization strategies is needed before firm recommendations on reduced nitrogen fertilization can be made.

4.4 Methodological aspects

I used the parametric test General Linear Model ANOVA for all statistical data analysis and almost always the residuals were normally distributed. Only for soil pH and for proportion of plants that had root nodules the residuals were not normally distributed and the GLM ANOVA test was complemented by a Mann Whitney’s test to support the significant results.

The GLM ANOVA test was conducted on 60 data points for all measurements except for proportion of active root nodules. The data set contained different numbers of observations in the two groups for proportion of active root nodules per sampling site, depending on how many sampling sites that had plants with root nodules. All sampling sites (30) on fields with regular bean cultivation had plants with root nodules whereas only about half (16) of the sampling sites on fields with cultivation of beans for the first time had plants with root nodules (Table 2). The two Mann Whitney’s test were conducted on average values per field. Thus average pH-value per field and proportion of the investigated 30 plants per field that had root nodules where used to avoid pseudo replication in this test.

4.5 Limitations of the study and other factors affecting the results

Despite meticulous preparations before going out in the field I realized that I missed some training looking at root nodules beforehand, to get some experience on how to distinguish between the two categories of active and inactive root nodules. It was difficult to see the color inside very small root nodules and also to distinguish between very light beige/pink color and the same color as the root. Thus some of the root nodules were perhaps categorized in the wrong way. An active root nodule could have been categorized as a non-active and vice versa. However, in most cases where the root nodules were at least 2-3 mm in diameter I was sure of the color. During the work with root nodule investigations in the fields I got continuous training on how to distinguish between active and inactive root nodules. Another factor that could have affected the categorization of the color inside the root nodules was the red toned sun glasses that I used in the first 2-3 fields because of strong sun light, until I realized that it could have had an additional misleading effect of the root nodule categorization.

Depending on the soil type it was more or less difficult to dig out the root system of the bean plants intact. In some places I suspect that parts of the root system including root nodules were left in the soil, which could have affected my results. However these difficulties to dig up intact roots are not expected to cause any major bias in the results, since there was a clear difference both in root nodule abundance and in proportion of active root nodules per plant that had root nodules, between the two types of fields.

Field K is a bit of an outlier since many of the plants I investigated in this field had many root nodules compared to other fields of the same category, where common bean has not been cultivated before. However, most of the root nodules found in this field were tiny and about half of them were inactive. When looking at the data there are no obvious explanation for this. Two special things about this field compared to most of the fields with first common bean cultivation were that it was located on Öland and had ley as pre-crop for the previous four years. Only one other field was located on Öland and had ley in the crop rotation and it is field L which belong to the same farm. Field L had onion as pre-crop but ley was on the field for the three years before that. Since the two fields belong to the same farm and most likely received similar management, but one field had remarkably many root nodules (K) and one had not a single root nodule (L), it is thus difficult to explain why.

Aboveground biomass weight at pod fill and pod weight at maturity did not vary much between the two types of fields, with and without a recent cultivation of common bean, despite big differences in root nodule abundance and abundance of active root nodules. Thus indicating that nitrogen was not a limiting factor for plant growth and -development in this study, which further suggest that there was probably a large amount of plant available nitrogen in the soil as a result of fertilization/manuring (Appendix 1).

Since this study was carried out on real farmers' fields there were many factors that differed between the fields, such as management methods, irrigation practices and time for mechanical weed control etc. (Appendix 1). An additional aspect is that farmers who cultivated common bean for the first time probably were less experienced in the cultivation of common bean, which could have contributed to suboptimal establishment and management on fields with first cultivation of common bean.

Time of sowing was likely different between fields, as well as the time I visited the fields at the two different sampling occasions differed, which could have affected differences in the results of *e.g.* biomass growth and maturity. However, the specific timing of sowing and sampling were not taken into consideration in this study, besides the aim to try to conduct the two respective sampling occasions as effective as possible to diminish the time differences between the first sampled field and the last sampled field per sampling occasion.

Another important aspect of using farmers' fields for the investigations was the geographical distribution of the fields. It was not possible to select fields with an equal distribution of the two different categories of fields on each island. Thus there was a skewed distribution of the two categories of fields, so that most of the fields with first common bean cultivation were situated on Gotland and all fields with a regular common bean cultivation were situated on Öland. This could have affected results such as biomass growth and time for maturity etc. because of climatic differences between the two islands. However, the most important results in this study, those about root nodule abundance and abundance of active root nodules were probably not affected of this geographical aspect.

5 Conclusions

5.1 Principal findings and their impacts

The findings in this study clearly indicate that the soil in fields without recent history of common bean cultivation do not contain sufficient amounts of rhizobia for efficient nodulation and active nitrogen fixation. These results emphasize the importance of giving recommendations to inoculate common bean seeds with the right species of rhizobium bacteria before sowing, to ensure root nodulation and nitrogen fixation when cultivating common bean on a field for the first time. An additional recommendation could be to decrease the amount of nitrogen fertilization of common bean, since common bean plants grew well even with a very low abundance of root nodules and where many of them were inactive, indicating that soils contained enough plant-available nitrogen for growth even without help of nitrogen fixation. However, this interpretation is based on indirect observations and further investigations on nitrogen fixation of common bean plants under different nitrogen fertilization strategies are needed before to give firm recommendations on reduced nitrogen fertilization for common bean. These findings are especially valuable since the study was carried out on real farmers' fields, and therefor are representative for practices in farmers' real life. Hopefully this study can contribute to reinforce the knowledge-base to support national recommendations and guidelines about inoculation of common bean. As a consequence, the findings can contribute to a successful common bean cultivation on fields without earlier common bean cultivation and thus enable further expansion of common bean cultivation in Sweden.

5.2 Further research

A succeeding study, of the fields with first common bean cultivation, would be interesting to evaluate if root nodule abundance increases with continuous common bean cultivation. In addition it would be interesting to conduct a study under more controlled circumstances, where all 20 different soils were included, but transported to the same place and managed in the same manner to decrease the influence of different external factors. In such a study it would be easier to evaluate which factors that affect the root nodule abundance as well as economic yield (yield of mature beans). Such a study could include more data of amount of plant available nitrogen in the soils and/or include different manuring strategies etc. Further research should also investigate the effects of inoculation, to evaluate choice of rhizobium species/genotypes and inoculation methods for successful establishment of the nitrogen-fixing symbiosis in fields where common bean is cultivated for the first time.

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Appendix 1. Information on farms and fields used in the study

Farm: Kolstad, Borgholm, Öland, Sweden.
Fields: A and B
Brown beans cultivated in 2009 and 2011 on field A and B.

Figure A1. Map showing the location of the 3 sampling sites of field A and field B respectively.

Background information about the fields:

Field A	**Year**	**Fertilization /ha**	**Crop rotation**
Common bean yield 2017: 1000 kg/ha (about ½ of the field was not possible to harvest) Coordinates: 56.883129, 16.735414 10 hectares (of a bigger field of 25 hectares) Soil type: A light and stony soil. pH: 8.1-8.3 (mostly 8.3), I measured 7.2-7.3 K-AL: mostly IV, some parts III P-AL: V	2017	25 ton cow manure	Brown beans (Karin)
	2016	500 kg NS 27-4	Winter wheat
	2015	20 ton pig manure 550 kg NS 27-4	Spring wheat
	2014	475 kg NS 27-4	Winter wheat
	2013	> 150 kg NS 27-4 and PK. According to the recommendations for onion.	Yellow onion

Field B	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean yield 2017: 500 kg/ ha Coordinates: 56.872379, 16.683536 10 hectares Soil type: Light, slightly compact and stony soil. pH: 7.1-8.3, I measured 6.5-7.5 K-AL: III-IV P-AL: mostly IV	2017	25 ton pig manure	Brown beans (Karin)
	2016	20 ton pig manure 500 kg NS 27-4	Winter wheat
	2015	30 ton pig manure 500 kg NS 27-4	Winter rape seed
	2014	25 ton pig manure 400 kg NS 27-4	Winter barley
	2013	25 ton pig manure 400 kg NS 27-4	Spring wheat

Farm: Övra Västerstad, Mörbylånga, Öland, Sweden
Fields: C, D, E, F and G.
Brown beans cultivated once every fourth year on these fields.

Figure A2. Map showing the location of the 3 sampling sites of field C, D, E, F and G respectively.

Background information about the fields:

Field C	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean yield 2017: 2600 kg/ha (avg. C, D, E, F & G) Coordinates: 56.430167, 16.410144 6.4 hectares Soil type: pH: I measured 6.6-7.1 K-AL: - P-AL: -	2017	300 kg ProMagna 11-5-18	Brown beans (Katja)
	2016	200 kg Axan 27-4 100 kg PK 11-21 350 kg Axan 27-4	Winter wheat
	2015	200 kg Axan 27-4 300 kg Axan 27-4 95 kg P 20 120 kg Axan 27-4	Winter wheat
	2014	350 kg ProMagna 11-5-18 350 kg ProMagna 11-5-18 300 kg Lime saltpeter granules	Red onion
	2013	300 kg ProMagna 11-5-18	Brown beans (Katja)

Field D	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean yield 2017: 2600 kg/ha (avg. C, D, E, F & G) Coordinates: 56.434812, 16.412641 5.4 hectares Soil type: Light sand pH: I measured 6.5-7.0 K-AL: - P-AL: -	2017	300 kg ProMagna 11-5-18	Brown beans (Katja)
	2016	200 kg Axan 27-4 200 kg PK 11-21 350 kg Axan 27-4	Winter wheat
	2015	200 kg Axan 27-4 300 kg Axan 27-4 160 kg Potassium salt 120 kg P 20	Winter wheat

		120 kg Axan 27-4	
	2014	350 kg ProMagna 11-5-18 350 kgProMagna 11-5-18 300 kg Lime saltpeter granules	Yellow onion
	2013	300 kg ProMagna 11-5-18	Brown beans (Katja)

Field E	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean yield 2017: 2600 kg/ha (avg. C, D, E, F & G) Coordinates: 56.433790, 16.417774 3.6 hectares Soil type: Light sand pH: I measured 6.6-6.8 K-AL: - P-AL: -	2017	300 kg ProMagna 11-5-18	Brown beans (Katja)
	2016	200 kg Axan 27-4 100 kg PK 11-21 300 kg Axan 27-4	Winter wheat
	2015	200 kg Axan 27-4 300 kg Axan 27-4 120 kg P 20	Winter wheat
	2014	350 kg ProMagna 11-5-18 350 kg ProMagna 11-5-18 300 kg Lime saltpeter granules	Yellow onion
	2013	300 kg ProMagna 11-5-18	Brown beans (Katja)

Field F	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean yield 2017: 2600 kg/ha (avg. C, D, E, F & G) Coordinates: 56.432296, 16.427129 2.9 hectares Soil type: F1: Compact with rocks, F2: Compact and granulated sand. pH: I measured 6.5-7.3 K-AL: - P-AL: -	2017	300 kg ProMagna 11-5-18	Brown beans (Katja)
	2016	350 kg ProMagna 11-5-18 350 kg ProMagna 11-5-18 350 kg Lime saltpeter granules	Red onion
	2015	80 kg Potassium salt	Peas (Clara)
	2014	250 kg Axan 27-4 100 kg PK 11-21 477 kg Lime saltpeter granules	Winter wheat
	2013	300 kg ProMagna 11-5-18	Brown beans (Signe)

Field G	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean yield 2017: 2600 kg/ha (avg. C, D, E, F & G) Coordinates: 56.431242, 16.421010 2.4 hectares Soil type: Light. pH: I measured 6.8-7.2 K-AL: - P-AL: -	2017	300 kg ProMagna 11-5-18	Brown beans (Katja)
	2016	350 kg ProMagna 11-5-18 350 kg ProMagna 11-5-18 300 kg Lime saltpeter granules	Red onion
	2015	200 kg Axan 27-4 300 kg Axan 27-4 150 kg P 20	Winter wheat
	2014	450 kg NPK 21-3-10-4	Barley
	2013	300 kg ProMagna 11-5-18	Brown beans (Katja)

Farm: Bjärby, Mörbylånga, Öland, Sweden
Fields: H, I and J.
Brown beans cultivated once every sixth year on these fields.

Figure A3. Map showing the location of the 3 sampling sites of field H, I and J respectively.

Background information about the fields:

Field H	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean yield 2017: ca 2500 kg/ha Coordinates: 56.449643, 16.410850 Ca 4.5 hectares (half of a field) Soil type: Light sandy soil. pH: 6.6, 6.7, 7.8, I measured 5.8-7.1 K-AL: II-III P-AL: V	2017	200 kg ProMagna 11-5-18	Brown beans (Karin)
	2016	105-110 kg N	Winter wheat
	2015	-	Barley
	2014	12 ton poultry manure 100 kg N 100 kg K	Yellow onion
	2013	105-110 kg N	Winter wheat

Field I	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean yield 2017: ca 2000 kg/ha Coordinates: 56.450040, 16.415650 2.94 hectares Soil type: Light sand. pH: 6.9, I measured 5.9-6.5 K-AL: III P-AL: IVa	2017	200 kg ProMagna 11-5-18	Brown beans (Karin)
	2016	12 ton poultry manure 70 kg N	Potatoes
	2015	-	Rye
	2014	12 ton poultry manure 110 kg N	Winter rape seed
	2013	105-110 kg N	Winter wheat

Field J	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean yield 2017: ca 2000 kg/ha Coordinates: 56.442467, 16.415724 4.38 hectares Soil type: Sand. pH: 6.6-7.5 (6.9) I measured 6.0-6.1 K-AL: III P-AL: IVb -V (sampling site 3 - sampling site 1)	2017	200 kg ProMagna 11-5-18	Brown beans (Karin)
	2016	105-110 kg N	Winter wheat
	2015	12 ton poultry manure 110 kg N	Potatoes
	2014	80 kg N	Winter wheat
	2013	10-12 ton poultry manure 70 kg N	Yellow onion

Farm: Skärlöv, Mörbylånga, Öland, Sweden
Fields: K and L.
Brown beans have not been cultivated for at least 20 years on these fields.

Figure A4. Map showing the location of the 3 sampling sites of field K and L respectively.

Background information about the fields:

Field K	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean yield 2017: 2400 kg/ha Coordinates: 56.429184, 16.563376 12.7 hectares Soil type: Sandy soil with a gradient to more clay in sampling site 3. pH: 7.9, I measured 7.0-7.3 K-AL: III P-AL: III	2017	-	Brown beans
	2016	360 kg NS 27-4 120 kg N34 35 ton cow manure	Ley
	2015	360 kg NS 27-4 150 kg N34 15 ton cow manure (summer) 25 ton cow manure (autumn)	Ley
	2014	360 kg NS 27-4 150 kg N34 15 cow manure (summer) 25 ton cow manure (autumn)	Ley
	2013	300 kg NS 27-4 150 kg N34 20 ton cow manure (autumn)	Ley

Field L	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean harvest was not possible Coordinates: 56.419969, 16.570293 5 hectares Soil type: Much stones, clayey sand. pH: 7.9, I measured 7.1-7.3 K-AL: IV P-AL: III	2017	20 ton cow manure (spring)	Brown beans
	2016	1130 kg NPK 200 kg Potassium- and magnesium sulphate 25-6-18 200 kg Lime saltpeter	Onion
	2015	360 kg NS 27-4 330 kg N 34 15 cow manure (summer)	Ley
	2014	360 kg NS 27-4 120 kg N34 15 ton cow manure (summer) 25 ton cow manure (autumn)	Ley
	2013	300 kg NS 27-4 110 kg N34 15 ton cow manure (summer) 25 ton cow manure (autumn)	Ley

Farm: Levide Pejnarve, Hemse, Gotland, Sweden
Fields: M and N.
Brown beans have not been cultivated for at least 20 years on these fields.

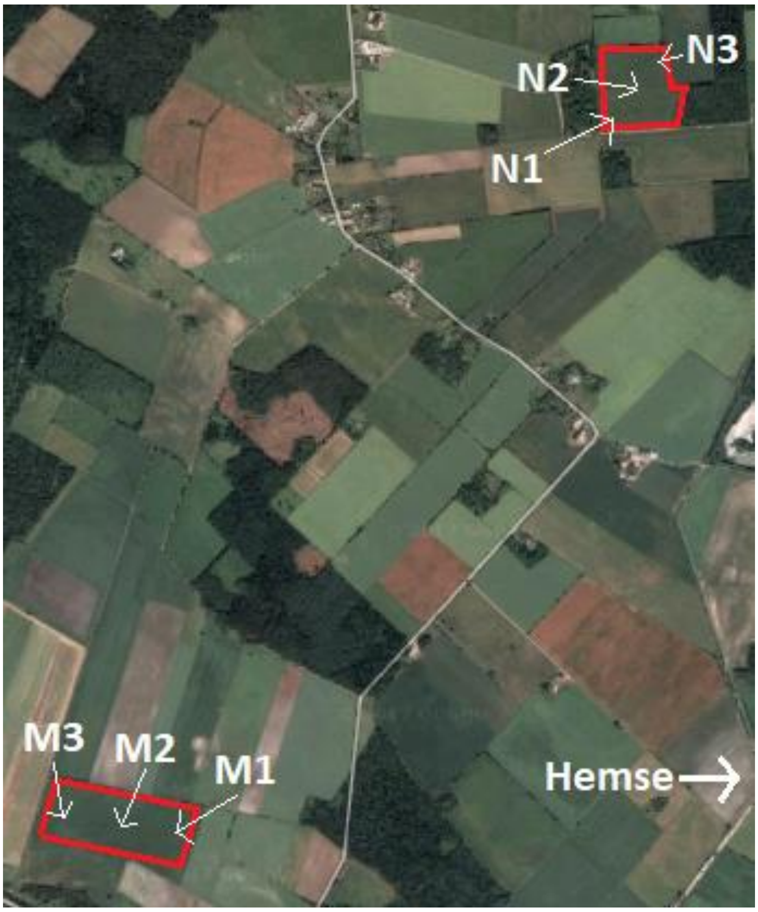

Figure A5. Map showing the location of the 3 sampling sites of field M and N respectively.

Background information about the fields:

Field M	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean yield 2017: 1950 kg/ha Coordinates: 57.246161, 18.247621 9.57 hectares Soil type: Humus rich clay loam Comment: The whole field had small pods and thin plants pH: 8.1, I measured 7.2-7.4 K-AL: III (M3) up to IV (M1). P-AL: III	2017	220 kg NPK 11-5-18 including micro nutrients	Brown beans (Katja)
	2016	350 kg Yara Mila, NPK 23-3-7	Barley
	2015	375 kg NPKS 27-3-3-3	Spring wheat
	2014	500 kg NPK 23-3-7 Yara Mila 60 kg MAP, NP 12-23	Rape seed
	2013	365 kg Yara Mila, NPK 23-3-7	Barley

Field N	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean yield 2017: 2000 kg/ha Coordinates: 57.268359, 18.276548 7.18 hectares Soil type: Clayey sand with some humus Comment: Tall but this plants with small pods pH: 8.0, I measured 7.4 K-AL: III P-AL: IV	2017	220 kg NPK 11-5-18 including micro nutrients	Brown beans (Katja)
	2016	400 kg NPK 24-4-5 Yara Mila	Barley
	2015	500 kg NPKS 27-3-3-3	Winter wheat
	2014	210 kg Axan N27-4 550 kg NPK 23-3-7 Yara Mila	Winter rape seed
	2013	350 kg NPKS 27-3-3-3	Barley

Farm: Fardhem Myre, Hemse, Gotland, Sweden
Fields: O, P and Q.
Brown beans have not been cultivated for at least 20 years on these fields.

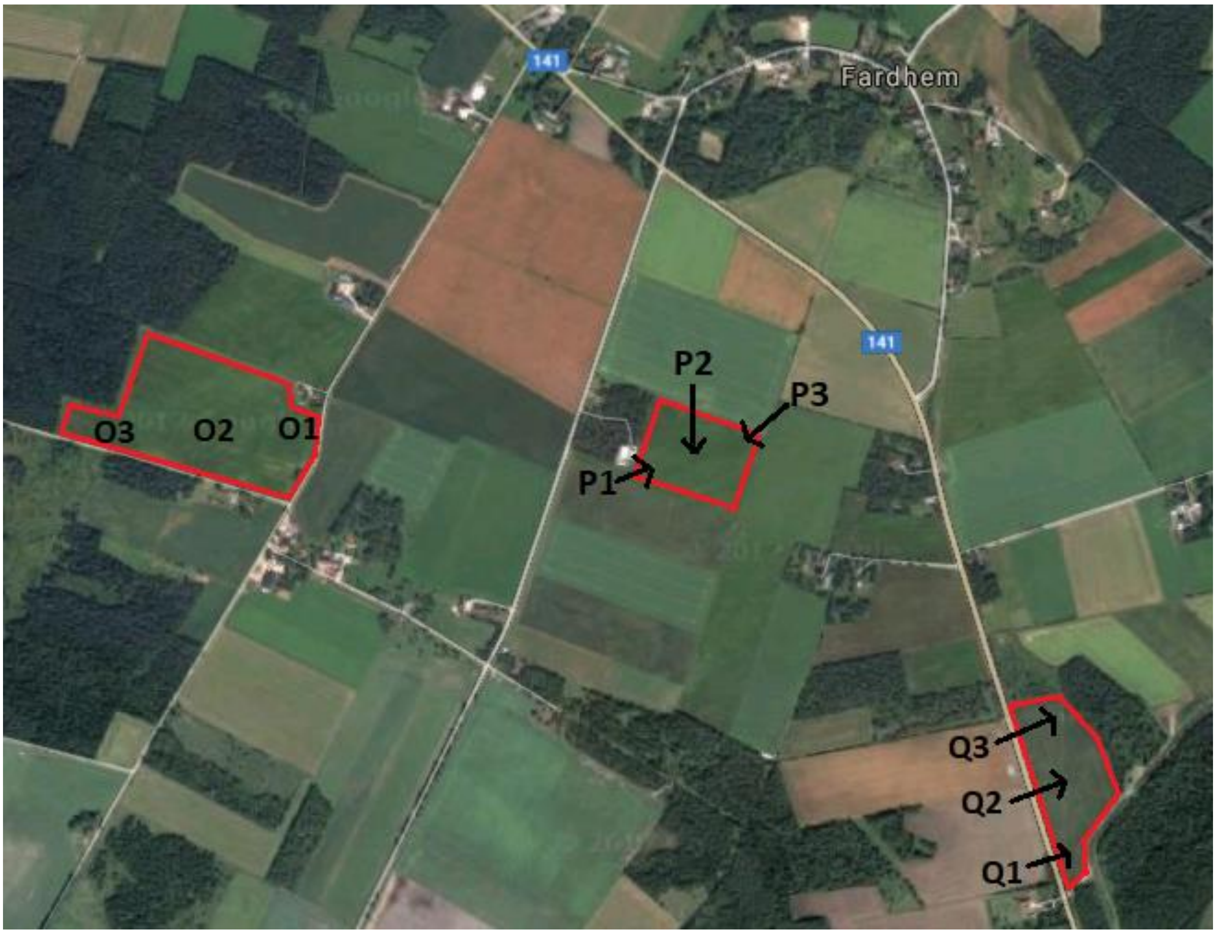

Figure A6. Map showing the location of the 3 sampling sites of field O, P and Q respectively.

Background information about the fields:

Field O	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean yield 2017: ca 1000 kg/ha (1/2 of the field was not possible to harvest) Coordinates: 57.257116, 18.315051 13.73 hectares Soil type: Sandy soil with few stones. Perhaps some clay. Comment: Weeds (fat-hen and rape seed), have received manure sometimes. pH: I measured 7.4 K-AL: III (10) P-AL: IVb (15)	2017	200 kg Pro Magna 11-5-18	Brown beans (Katja)
	2016	450 kg NS 30-7 500 kg Axan N 27-4	Winter wheat
	2015	375 kg NPK 21-3-10-4	Barley
	2014	400 kg Axan N 27-4	Winter barley
	2013	300 kg NPK 18-4-14 500 kg Axan N 27-4	Rape seed

Field P	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean yield 2017: ca 1700 kg/ha Coordinates: 57.256259, 18.334261 6.07 hectares Soil type: Sandy soil with few stones. Some clay. Comment: Manure application pH: I measured 7.4 K-AL: - P-AL: -	2017	200 kg Pro Magna 11-5-18	Brown beans (Katja)
	2016	150 kg PK 11-21 650 kg Axan N 27-4 200 kg Lime saltpeter granules	Spring wheat
	2015	-	Barley
	2014	-	Winter wheat
	2013	-	Peas (Clara)

Field Q	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean harvest was not possible. Coordinates: 57.249506, 18.348691 6.32 hectares Soil type: Sandy soil with some stones. Perhaps some clay. Comment: Thin plants and small pods. Some weeds (fat-hen and rape seed). pH: I measured 6.8-7.1 K-AL: - P-AL: -	2017	200 kg Pro Magna 11-5-18	Brown beans (Katja)
	2016	450 kg NS 30-7 100 kg PK 11-21 500 kg Axan N 27-4	Winter wheat
	2015	550 kg Axan N 27-4	Winter wheat
	2014	-	Spring barley
	2013	-	-

Farm: Linde Myrungs Hemse, Gotland, Sweden
Fields: R, S and T.
Brown beans have not been cultivated for at least 20 years on these fields.

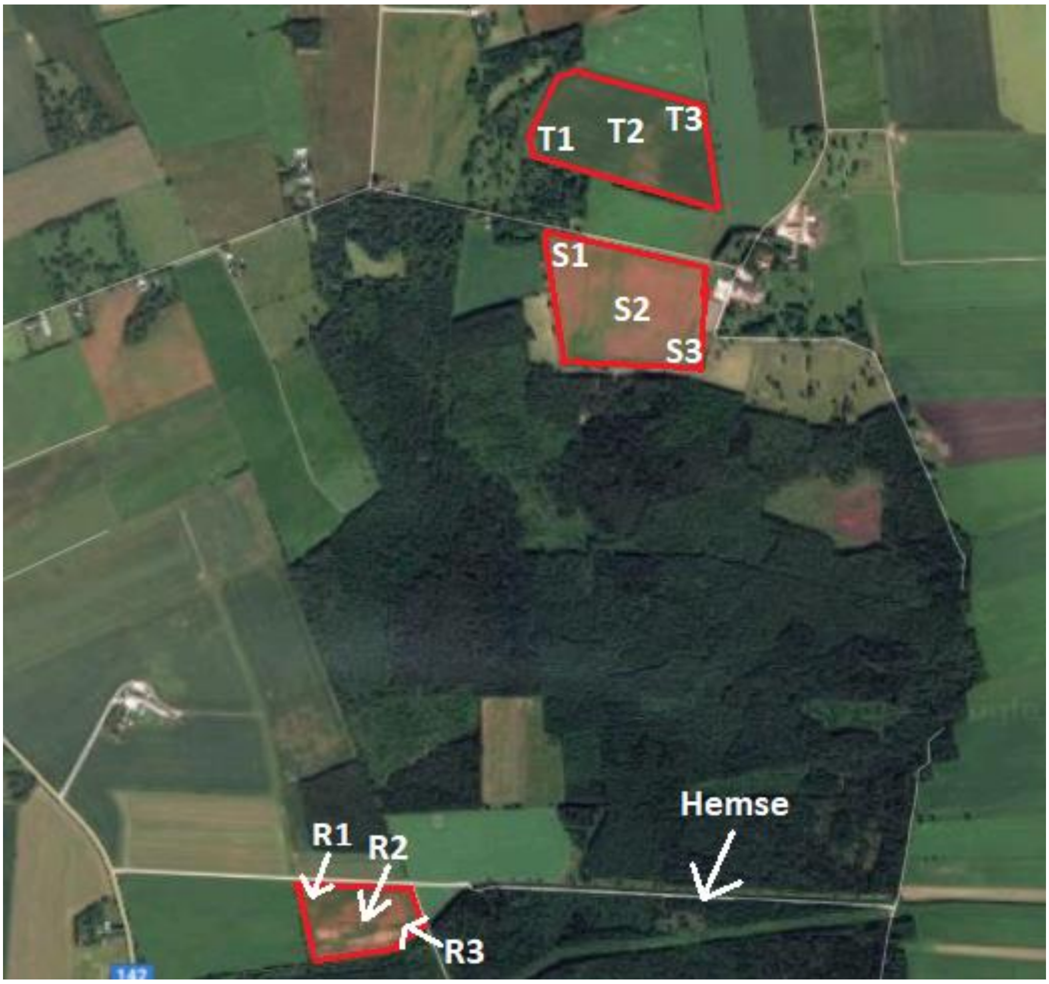

Figure A7. Map showing the location of the 3 sampling sites of field R, S and T respectively.

Background information about the fields:

Field R	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean yield 2017: 2140 kg/ha (avg. R & S) Coordinates: 57.261898, 18.405253 3.67 hectares Soil type: Light sandy soil Comment: Robust plants and only few weeds pH: 7.3, I measured 6.4-7.2 K-AL: II P-AL: V	2017	100 kg NPK 11-5-18 including micro nutrients 100 kg Potassium chloride	Brown beans (Katja)
	2016	650 kg Axan N27-4 240 kg Axan N27-4 20 ton pig manure in autumn (6 % dry matter)	Winter rape seed
	2015	250 kg Axan N27-4 25 ton pig manure in spring (6 % dry matter) + 25 ton pig manure in automne (6 % dry matter)	Winter barley
	2014	500 kg NPK Yara Mila	Rye-wheat

	2013	20 ton pig manure in automne (6 % dry matter) 200 kg Axan N27-4	Barley

Field S	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean yield 2017: 2140 kg/ha (avg. R & S) Coordinates: 57.273790, 18.415404 9.27 hectares Soil type: Sand. Comment: Robust plants, quite big pods compared to the other 2 farms on Gotland pH: 6.9, I measured 6.5-6.6 K-AL: II P-AL: IV a	2017	100 kg NPK 11-5-18 including micro nutrients 100 kg Potassium chloride	Brown beans (Katja)
	2016	20 ton pig manure in automne (6 % dry matter) 500 kg Axan N27-4	Winter rape seed
	2015	20 ton pig manure in spring (6 % dry matter) + 25 ton pig manure in automne (6 % dry matter) 250 kg Axan N27-4	Winter barley
	2014	20 ton pig manure (6 % dry matter) 350 kg Axan N27-4	Rye-wheat
	2013	20 ton pig manure in spring (6 % dry matter) 200 kg Axan N27-4	Barley

Field T	**Year**	**Fertilization/ha**	**Crop rotation**
Common bean harvest was not possible Coordinates: 57.277245, 18.414951 9.65 hectares Soil type: Sandy soil, perhaps some clay Comment: Plenty of weeds, especially in the middle pH: 7.9, I measured 6.9-7.0 K-AL: II P-AL: IV b	2017	100 kg NPK 11-5-18 including micro nutrients 100 kg Potassium chloride	Brown beans (Katja)
	2016	22 ton pig manure (6 % dry matter) 530 kg Axan N27-4	Winter wheat
	2015	45-50 ton pig manure (6 % dry matter) 400-500 kg Axan N27-4	Winter wheat
	2014 Field divided in 2.	20 ton pig manure (6 % dry matter) 100 kg Axan N27-4 (27 N)	Barley
		100 kg Potassium salt	Peas
	2013	20 ton pig manure (9 % dry matter) 450 kg Axan N27-4	Winter wheat

Appendix 2. Form filed in by the investigator in the fields, here is an example for the first field

	Sampling area	**Bean plant ID**	**Plant appearance** (S, M, L) P if pods	**Root nodules?** S-Small M- Medium L -Large	**<5< root nodules**	**Amount of active/amount of cut nodules**
A	A1	A10				
A	A1	A11				
A	A1	A12				
A	A1	A13				
A	A1	A14				
A	A1	A15				
A	A1	A16				
A	A1	A17				
A	A1	A18				
A	A1	A19				
A	A2	A20				
A	A2	A21				
A	A2	A22				
A	A2	A23				
A	A2	A24				
A	A2	A25				
A	A2	A26				
A	A2	A27				
A	A2	A28				
A	A2	A29				
A	A3	A30				
A	A3	A31				
A	A3	A32				
A	A3	A33				
A	A3	A34				
A	A3	A35				
A	A3	A36				
A	A3	A37				
A	A3	A38				
A	A3	A39				

Appendix 3. Figure displaying the additional yield information from the farmers.

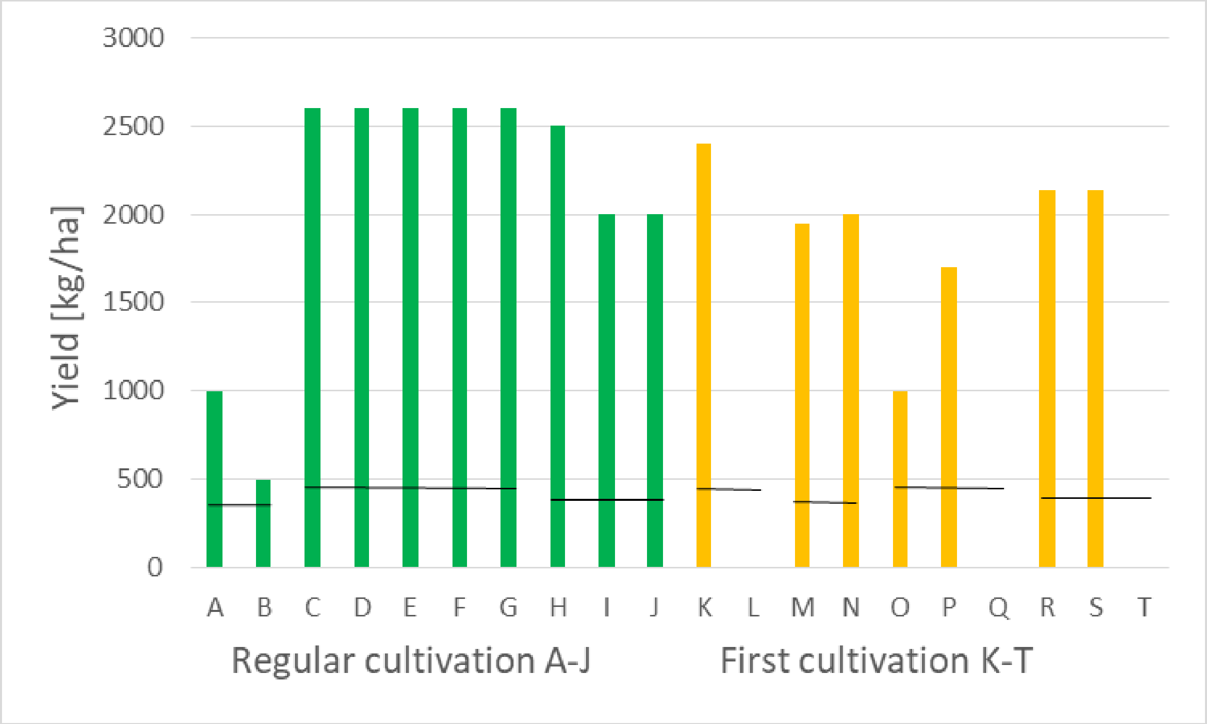

Figure A8. Farmers’ approximate yield per field. Fields with a regular common bean cultivation in green (A-J) and fields where beans were cultivated for the first time in yellow (K-T). Bars linked with a line belong to the same farm.