

Project title: Vining peas: The effect of soil phosphate levels on rhizobial populations

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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

The application of starter fertilisers to peas at drilling did not consistently improve yields. Fertiliser applications did not impact on rhizobial populations in soil.

Background

Pea yields have reached a plateau in many areas over recent years and one option to try and boost yields is the application of starter fertilisers. Legume crops have the ability to fix nitrogen in nodules which are formed on their roots by colonisation of rhizobia. Starter fertilisers contain phosphorus which is important for root development, nodule formation and nitrogen fixation by rhizobia. Nitrogen fixation not only delivers nitrogen to the pea crop but also increases soil nitrogen contents for subsequent crops. Some starter fertilisers, however, contain nitrogen which can be damaging to nodule formation with negative impacts on the pea crop and soil nitrogen contents. Thus, it is important to maintain soil conditions that sustain healthy rhizobial populations in soil. The project therefore investigated whether applications of starter fertilisers increased pea yields and whether they had an effect on rhizobial populations in soil.

Summary

Field experiments were established over three growing seasons in 2014, 2015 and 2016. In each year, three field experiments were carried out in an early, mid and late drilled pea crop. Each experiment consisted of seven treatments – an untreated control, application of Primary P (a starter fertiliser that contains 40% P and 10% N) or Microstar (a starter fertiliser that contains 45% P but does not contain N) at three rates of 7.5 kg/ha, 10 kg/ha (commercially recommended rate) and 12.5 kg/ha. Each plot was approximately two hectares in size and was treated, other than the application of starter fertiliser, as the surrounding commercial crop. The effects of fertiliser application on shoot mass at first pod and on yield were measured. Shoot mass was measured of 20 randomly selected pea plants. Unreplicated yield data was gathered in the first two years and four sub-plots per treatment in the 2016 season. The application of starter fertiliser did not influence shoot mass in any of the seasons (Figures I, II and III) and yields were not affected (Figure IV). Yield data for each individual plot are listed in Appendix 1.

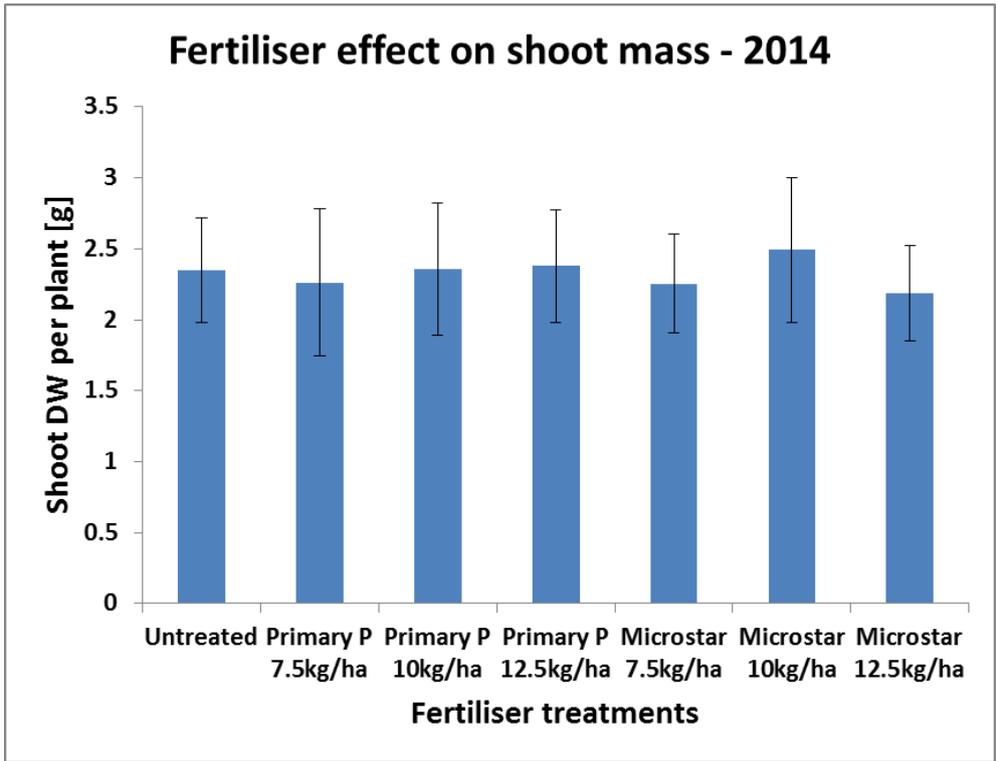


Figure I. Shoot mass of plants collected from the different field trials in 2014. Field plots had been drilled early, mid or late during the season. Mean values (n = 12) and standard error.

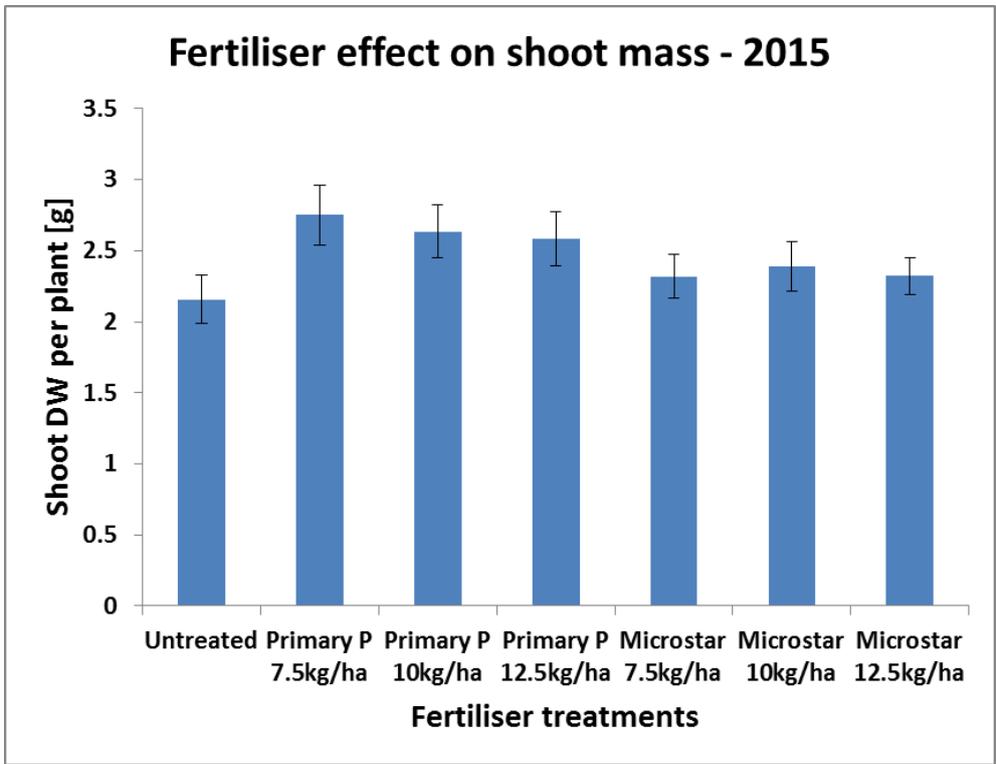


Figure II. Shoot mass of plants collected from the different field trials in 2015. Field plots had been drilled early, mid or late during the season. Mean values (n = 12) and standard error.

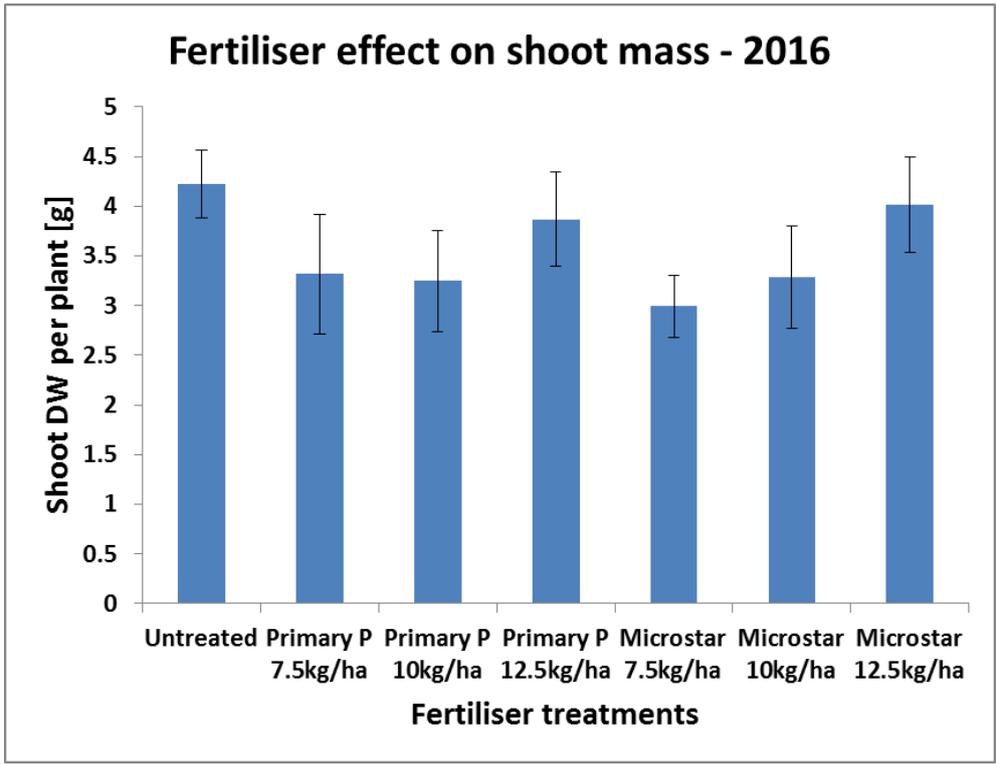


Figure III. Shoot mass of plants collected from the different field trials in 2016. Field plots had been drilled early, mid or late during the season. Mean values (n = 12) and standard error.

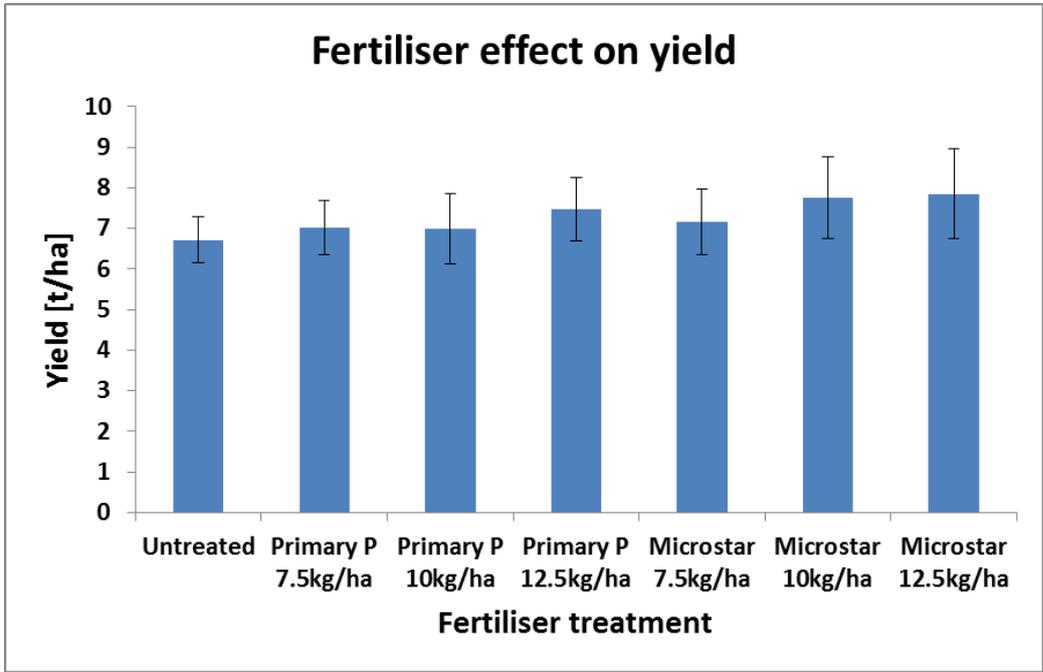


Figure 13. Yields recorded from plots that had received different fertiliser treatments. Field plots had been established in 2014, 2015 and 2016, and had been drilled early, mid or late during each season. Mean values (n = 14) and standard error.

Healthy rhizobial populations are of importance in pea crops because nodulation delivers nitrogen to the crop. Application of nitrogen fertilisers can reduce nodulation and the project investigated whether low amounts of nitrogen applied to the pea crop would negatively impact rhizobial populations in soils. In order to investigate the size of rhizobial populations in soils their potential to form nodules on pea roots was investigated in a glasshouse experiment. Pea seedlings were inoculated with soils collected from the field experiments and after four weeks the number of nodules formed on the pea roots was counted. The higher the number of nodules per plant the larger is the rhizobial community in the soil which has the potential to colonise pea plants in the field. None of the fertiliser treatments had any effect on rhizobial populations demonstrating that low amounts of nitrogen applied to pea crops is not damaging to rhizobia (Figure V).

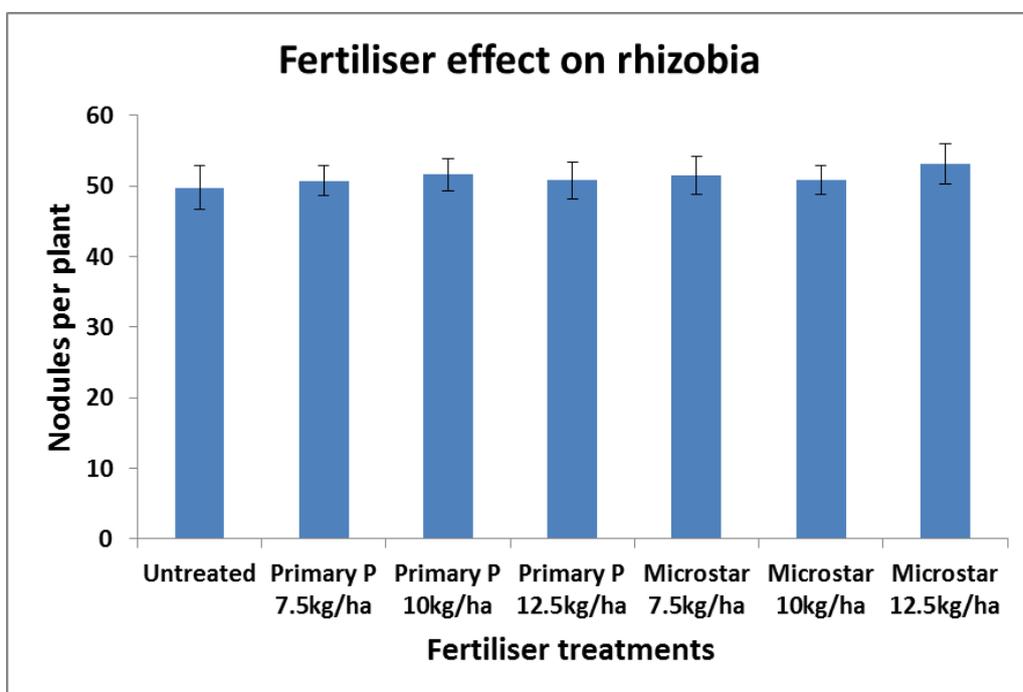


Figure V. Nodules per plant representing rhizobial population sizes at first pod. Fields plots had been established in 2014, 2015 and 2016. Field plots had been drilled early, mid or late during the season. Mean values (n = 108) and standard error.

Financial Benefits

Overall, no reliable yield improvements were seen due to fertiliser application. However, starter fertilisers are not very costly at approximately £25/ha. The average price for vining peas is around £345/t and a pea yield increase of greater than 73 kg/ha will therefore result in an economic benefit for pea growers.

Action Points

The project has shown that the application of starter fertilisers with low amounts of nitrogen does not reduce rhizobial populations in soils. The application of starter fertilisers does therefore not seem to be disadvantageous to pea crops. Although yields were not consistently improved by the application of starter fertilisers a benefit might be seen in situations when plant establishment and initial growth are slow.

SCIENCE SECTION

Introduction

Vining peas rely on the symbiotic relationship with rhizobia that provide nitrogen to the plant which is vital for plant growth and achieving optimum yield. A result of this relationship is a crop without a need for artificial nitrogen applications which increases soil nitrogen content for the subsequent crop. In many areas pea yields have reached a plateau and one suggested solution to increase yield is the use of starter fertilisers. Phosphorus promotes root growth and is essential for root nodulation to occur. Applying starter fertiliser containing phosphorus to peas theoretically has the potential to increase yield and help maintain healthy populations of rhizobia in soils. The more rhizobia there are in the soil, the greater the potential for root infection, nodulation and the amount of nitrogen available to the pea will be optimised. A potential issue with application of starter fertilisers to peas is that some contain nitrogen. Nitrogen is potentially damaging to the nitrogen fixation potential in the pea crop.

The project investigated the influence of phosphorus starter fertilisers on pea yields and the effects of these fertilisers (with and without nitrogen) on rhizobial populations in soils. Field trials were established in three years with three sites each year. As an add on to project FV 380 (Critical P) it was investigated whether there was a correlation between varying P indices and rhizobial population size.

Materials and methods

Three field trials were established in each year (2014, 2015 and 2016) using early, mid and late commercial vining pea varieties (Table 1). Three rates of two different starter fertilisers were applied to each crop at drilling (Table 2) along with an unfertilised control. Starter fertiliser Primary P (10% N) and Microstar (0% N), were both applied at 7.5 kg/ha, 10 kg/ha and 12.5 kg/ha (Table 3). In 2014, the late drilled crop received 4 kg/ha, 7 kg/ha and 8.5 kg/ha of Microstar. In order to show results from all three seasons and field sites in each year, labelling will be kept as 7.5 kg/ha, 10 kg/ha and 12.5 kg/ha. Each field plot was approximately two hectares in size and other than the application of starter fertiliser was treated, the same as the surrounding commercial crop.

Table 1. Field trials 2014, 2015 and 2016. Location of field trials, drilling dates and varieties used.

Name	Location	Field	Drilling date	Variety
Early	G. H. Emmerson	TF2843	28/03/2014	Novella
Mid	J Ward and Sons	TF3634	09/04/2014	Geneva
Late	R. G. Farms	TF3147	07/05/2014	Kenobi
Early	Blossom Hall Farms	BH061	31/03/2015	Novella
Mid	Ward Farming	JW038	10/04/2015	Geneva
Late	G.H. Emerson	GE132	04/05/2015	Serge
Early	R. Hardy Vegetables Ltd	JN080	01/04/2016	Savannah
Mid	AE Cheer Ltd	AC087	11/04/2016	Waverex
Late	R. Pocklington Ltd	RP158	23/05/2016	Serge

Table 2. Rates of starter fertilisers Primary P and Microstar applied at drilling.

Fertiliser	Application	Rates
Primary P	7.5 kg/ha; 10 kg/ha; 12.5 kg/ha	75%; 100%; 125% recommended rate
Microstar	7.5 kg/ha; 10 kg/ha; 12.5 kg/ha	75%; 100%; 125% recommended rate

Table 3. Composition of starter fertilisers Primary P and Microstar.

Fertiliser	Composition
Primary P	40% phosphorus, 11% sulphur oxide, 10% nitrogen, 2% manganese, 2% zinc
Microstar	45% phosphorus pentoxide, 3% magnesium oxide, 0.5% copper, 0.5% manganese

Soil and plant sampling

Soil samples were taken to assess numbers of rhizobia in soils, for P, K, Mg analysis and to measure nitrogen compounds. Nitrogen analysis was carried out because a fertiliser containing nitrogen was used in a Nitrate Vulnerable Zone. Samples were tested by Hill Court Farm Ltd. Soil samples for assessing rhizobia numbers were taken before drilling and at first pod. Soil samples for nutrient analyses were taken before drilling and after harvest. For rhizobial and P, K, Mg measurements soils were sampled in a W shape across each plot. For nitrogen analysis soil cores were taken to a depth of 90 cm. In 2016, soil samples for P, K, Mg analysis were taken at first pod and soil cores for nitrogen analysis could only be taken from the field with the late drilled crop. In 2014, twenty plants per plot were sampled at first pod to measure shoot dry weights (DW). In 2015 and 2016, twenty plants per plot were sampled at first pod to measure shoot DW as well as to visually score nodulation on plant roots. In 2015, nodulation was scored on a scale from 1 to 5 with 1 being low and 5 being high. In 2016, nodules per plant were counted. In 2014 and 2015, yields were measured per plot at harvest. In 2016, four sub-plots (2*4 m) were harvested in each treated plot to get replicated yields. In 2014, the early drilled crop and in 2016, the late drilled crop were affected by foot rot. In 2016, the mid drilled crop was badly affected by bird damage, showed poor establishment and was not harvested.

Assessment of rhizobial populations in soils

1. Most Probable Number (MPN)

The Most Probable Number (MPN) method is described in the Handbook for Rhizobia by Somasegaran and Hoben (1994) and is used to calculate viable rhizobia per gram of substrate. The method uses a ten-fold dilution series of soil suspensions to inoculate seedlings grown under sterile conditions. Nodules per plant are counted and viable numbers of rhizobia calculated using tables published in the Handbook for Rhizobia. Peas or vetch were grown in boiling tubes containing 20 ml of nitrogen free nutrient agar (Broughton and Dilworth, 1971) or a sterile 1:1 vermiculite/perlite mixture, respectively, inoculated with soil dilutions, grown at 15°C with 16 h light for 5 weeks and assessed for nodulation.

2. Pot tests to assess rhizobia numbers in soils

Peas were grown in pots (5 plants per pot) filled with a sterile 1:1 vermiculite/perlite mixture fertilised with a nitrogen free nutrient solution (Broughton and Dilworth, 1971). One week after plant establishment, soil slurries containing 2 g of soil per 20 ml sterile

distilled water (SDW) were added. All treatments were carried out in triplicate. Four weeks after inoculation, nodules per plant were counted.

Results

Field experiments – plant performance and yield

In all three years, shoot DWs of pea plants at first pod were measured to get an indication of plant performance. In 2015 and 2016, nodulation in the field was also assessed.

In all three seasons, time of drilling significantly influenced shoot DW but effects differed in each season probably driven by differing weather and soil conditions (Figures 1, 2 and 3). In 2014 and 2015, shoot DWs were highest in mid drilled crops in 2016 early drilled crops performed best. Nodulation in the field varied only slightly in 2015 but was significantly lower in mid drilled crops than in early or late drilled crops (Figure 4). In 2016, differences in nodulation between field sites were greater with highest number of nodules on early drilled pea plants (Figure 5).

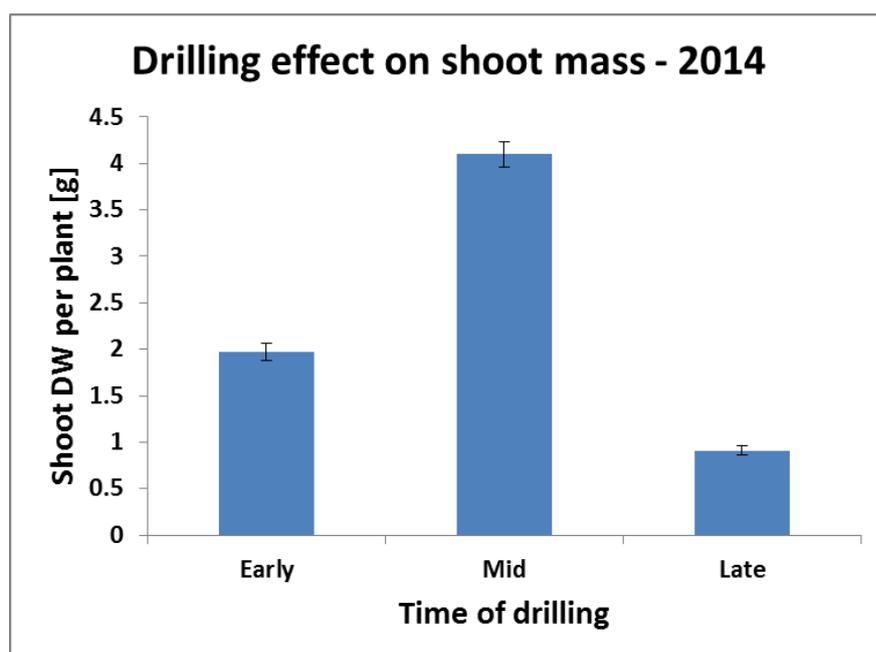


Figure 1. Shoot DW of plants collected from the different field trials in 2014. Field plots had been left untreated or had received three rates (7.5 kg/ha, 10 kg/ha, 12.5 kg/ha) of starter fertilisers with nitrogen (Primary P) and without nitrogen (Microstar). Data show mean values ($n = 28$) and standard error (ANOVA, $F_{2,84} = 276.46$, $p < 0.001$).

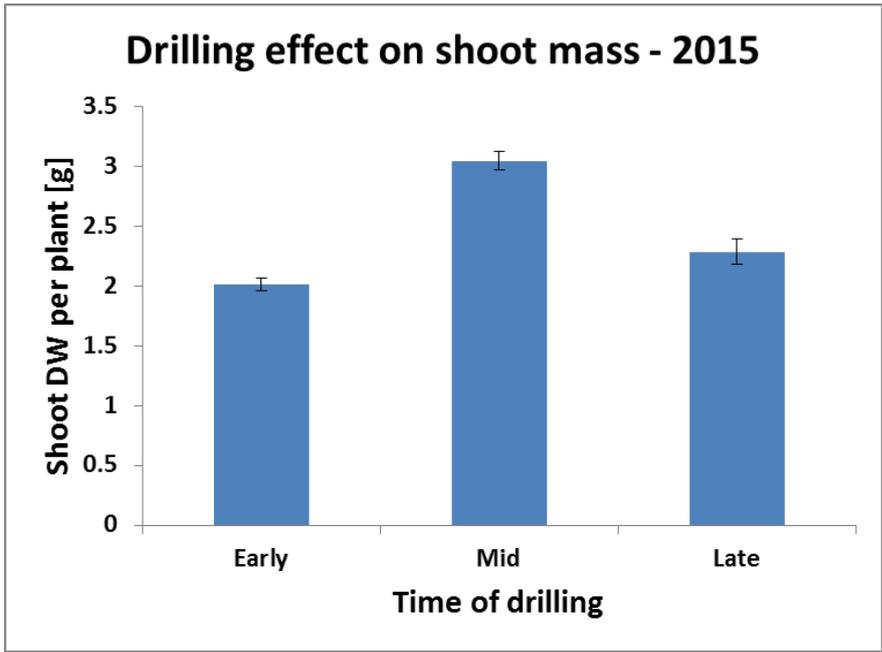


Figure 2. Shoot DW of plants collected from the different field trials in 2015. Field plots had been left untreated or had received three rates (7.5 kg/ha, 10 kg/ha, 12.5 kg/ha) of starter fertilisers with nitrogen (Primary P) and without nitrogen (Microstar). Data show mean values (n = 28) and standard error (ANOVA, $F_{2,84} = 42.95$, $p < 0.001$).

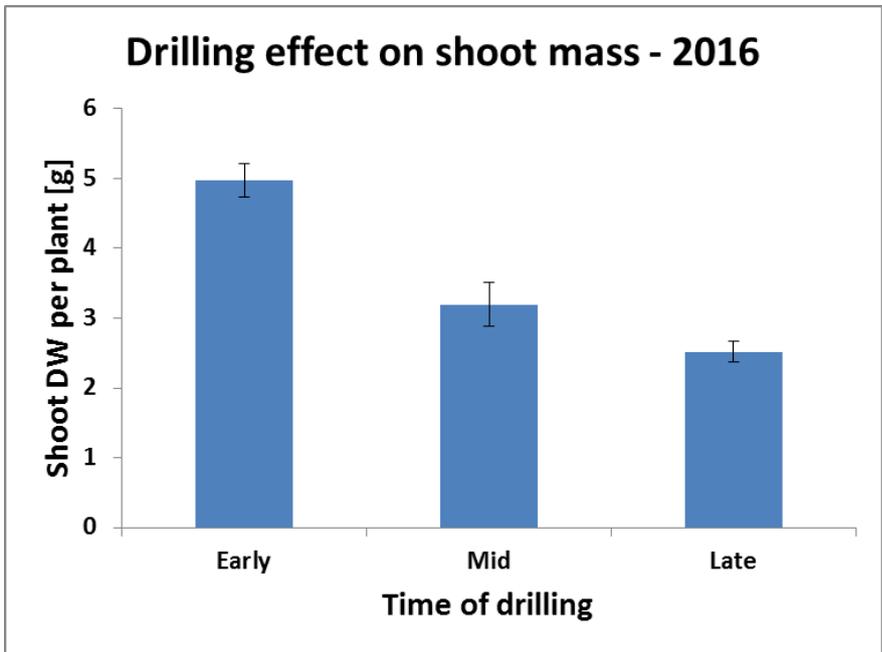


Figure 3. Shoot DW of plants collected from the different field trials in 2016. Field plots had been left untreated or had received three rates (7.5 kg/ha, 10 kg/ha, 12.5 kg/ha) of starter fertilisers with nitrogen (Primary P) and without nitrogen (Microstar). Data show mean values (n = 28) and standard error (ANOVA, $F_{2,84} = 27.8$, $p < 0.001$).

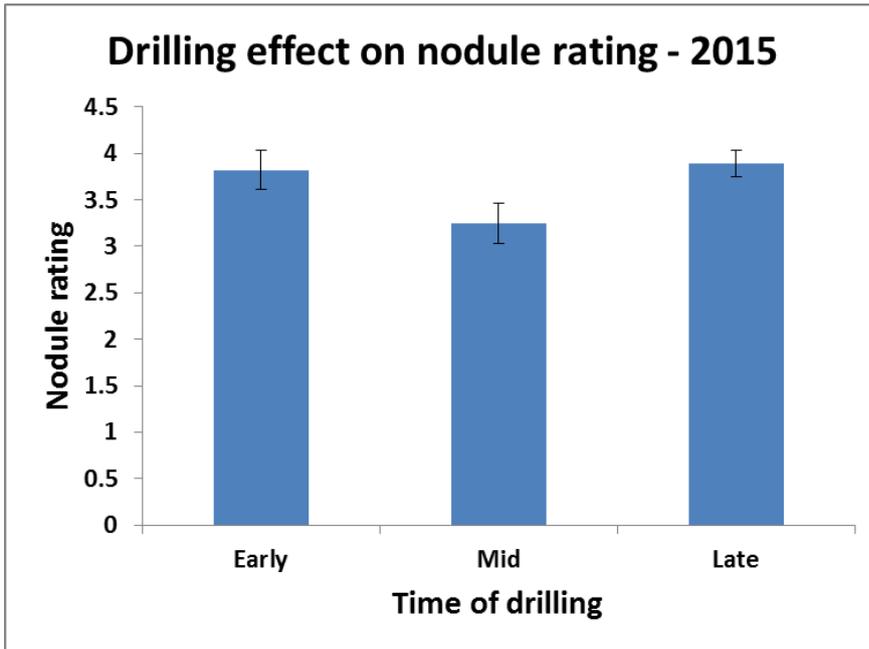


Figure 4. Nodule rating of plants collected from the different field trials in 2015. Field plots had been left untreated or had received three rates (7.5 kg/ha, 10 kg/ha, 12.5 kg/ha) of starter fertilisers with nitrogen (Primary P) and without nitrogen (Microstar). Data show mean values (n = 28) and standard error (ANOVA, $F_{2,84} = 3.35$, $p = 0.04$).

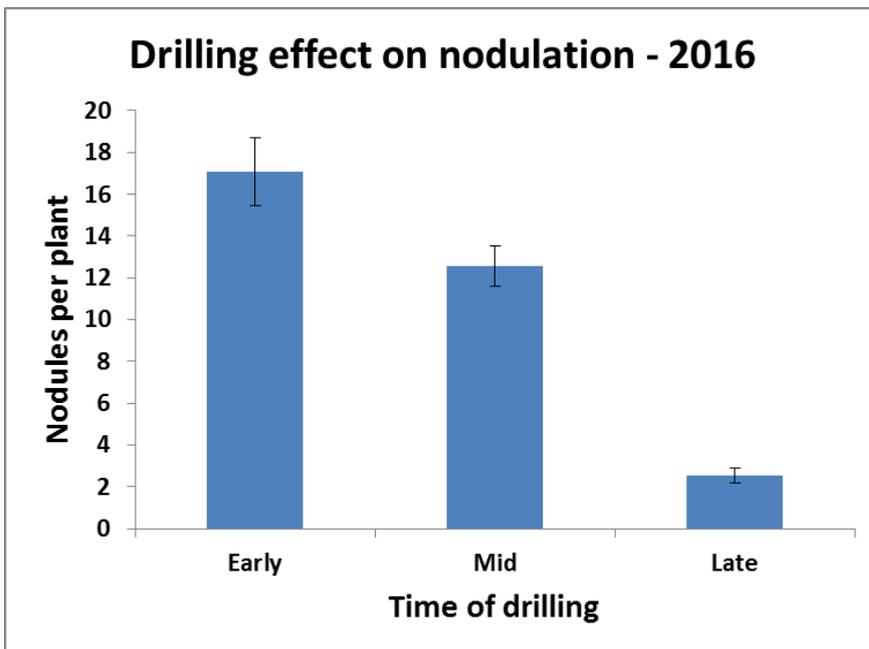


Figure 5. Nodule per plant of plants collected from the different field trials in 2016. Field plots had been left untreated or had received three rates (7.5 kg/ha, 10 kg/ha, 12.5 kg/ha) of starter fertilisers with nitrogen (Primary P) and without nitrogen (Microstar). Data show mean values (n = 28) and standard error (ANOVA, $F_{2,84} = 44.65$, $p < 0.001$).

Fertiliser applications did not have an effect on shoot DW in any of the three seasons (Figures 6, 7 and 8). Neither did fertilisers affect nodulation in the field (Figure 9 and 10).

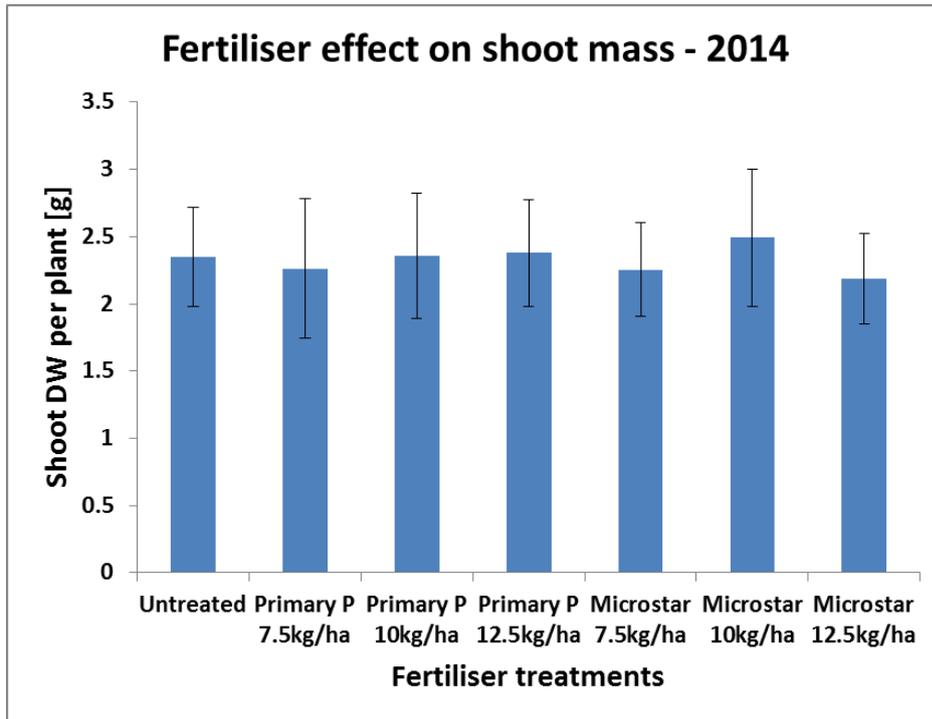


Figure 6. Shoot DW of plants collected from the different field trials in 2014. Field plots had been drilled early, mid or late during the season. Data show mean values (n = 12) and standard error.

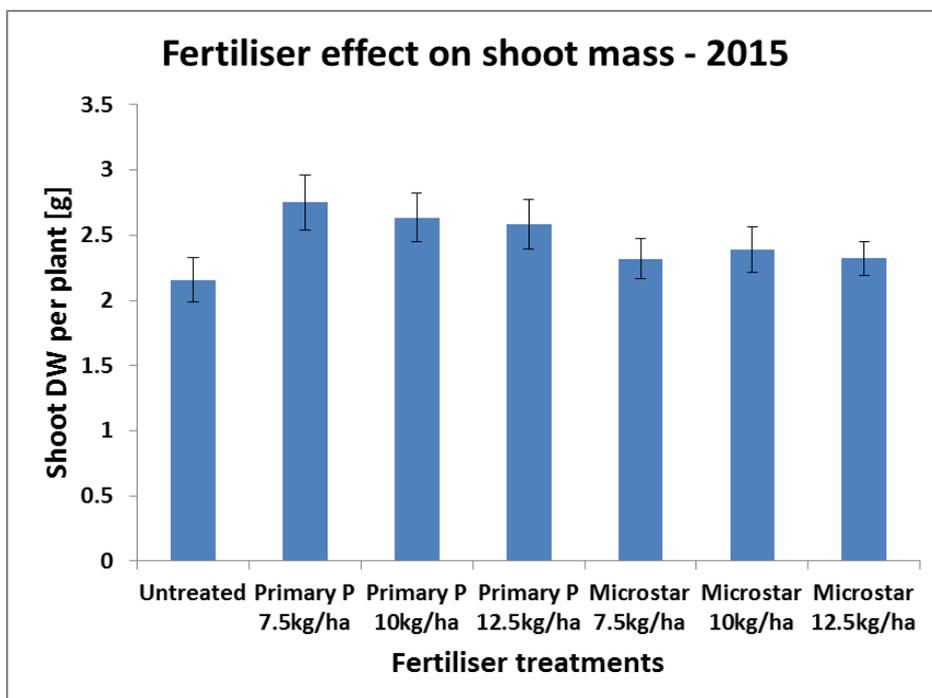


Figure 7. Shoot DW of plants collected from the different field trials in 2015. Field plots had been drilled early, mid or late during the season. Data show mean values (n = 12) and standard error.

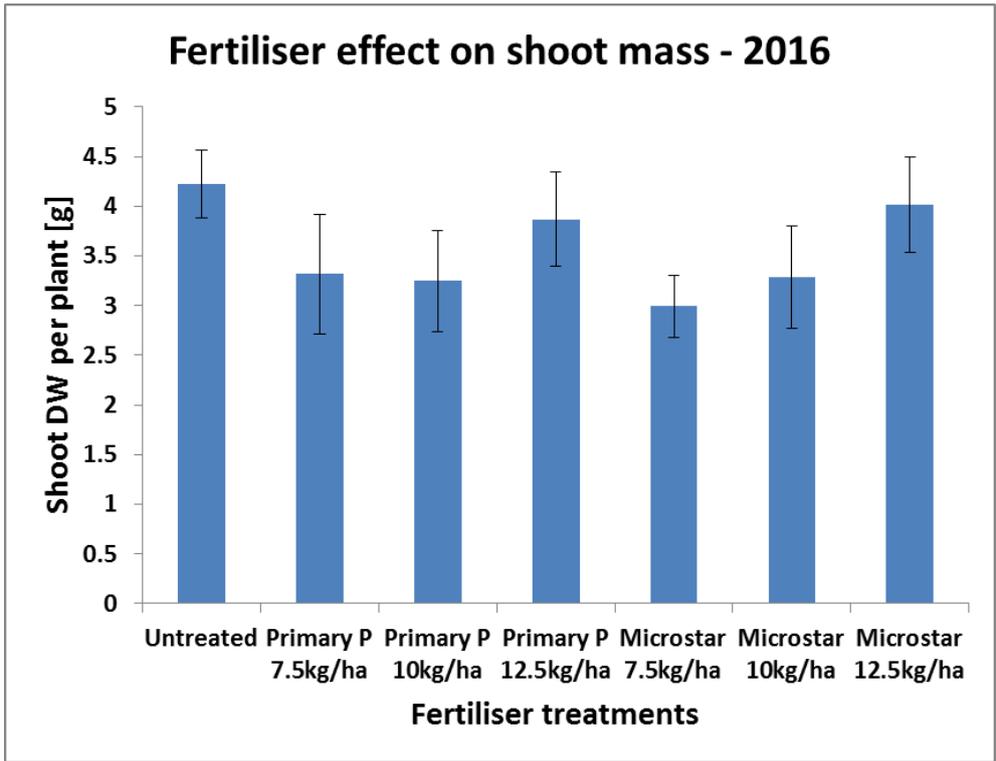


Figure 8. Shoot DW of plants collected from the different field trials in 2016. Field plots had been drilled early, mid or late during the season. Data show mean values (n = 12) and standard error.

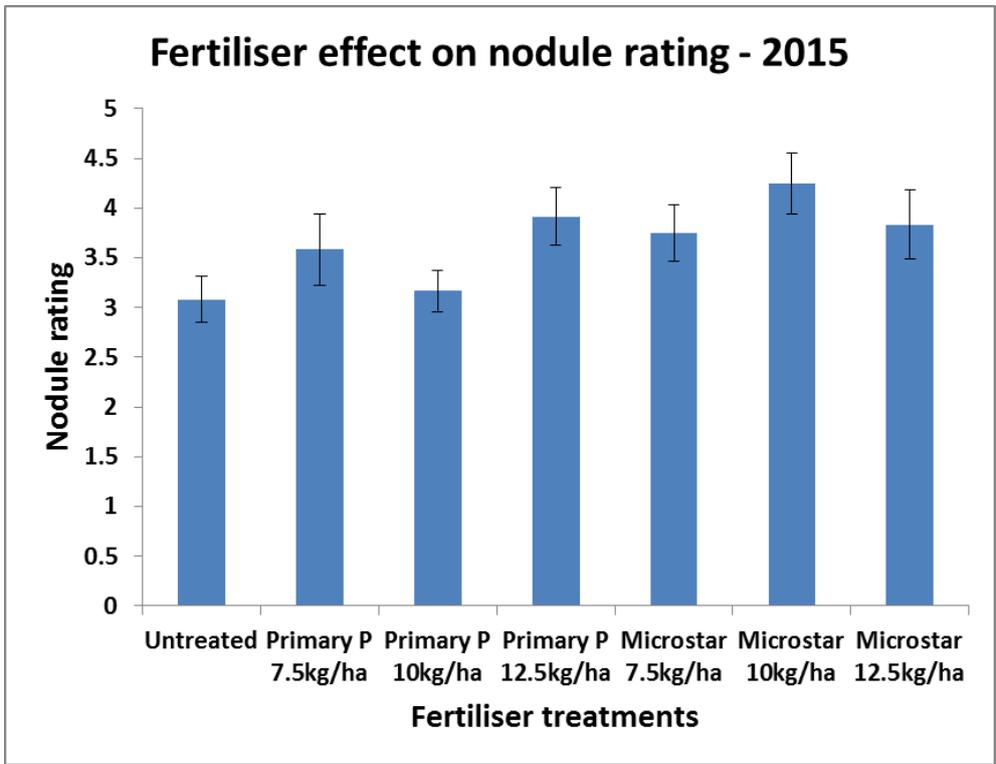


Figure 9. Nodule rating of plants collected from the different field trials in 2015. Field plots had been drilled early, mid or late during the season. Data show mean values (n = 12) and standard error.

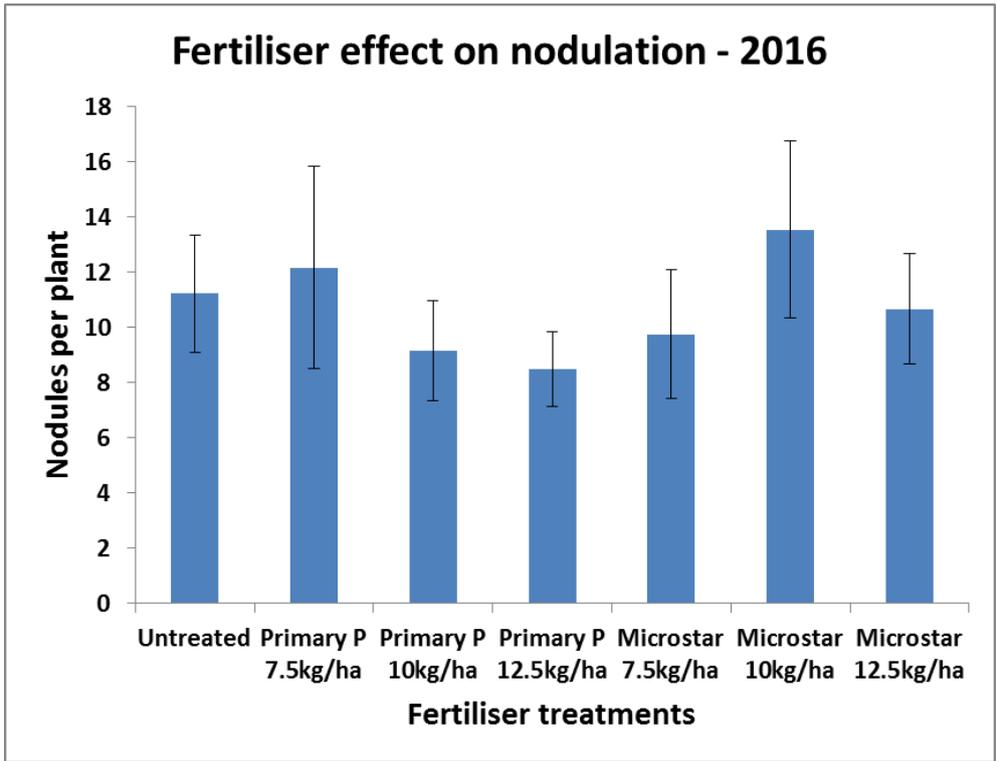


Figure 10. Nodules per plant of plants collected from the different field trials in 2016. Field plots had been drilled early, mid or late during the season. Data show mean values (n = 12) and standard error.

Yields were affected by season with highest yields obtained in 2015 (Figure 11) and drilling time with highest yields obtained in mid drilled crops (Figure 12). Fertiliser application, however, did not affect yields when assessed over three seasons and three different field sites in each year (Figure 13). Individual yields per year and field site are listed in Appendix 1.

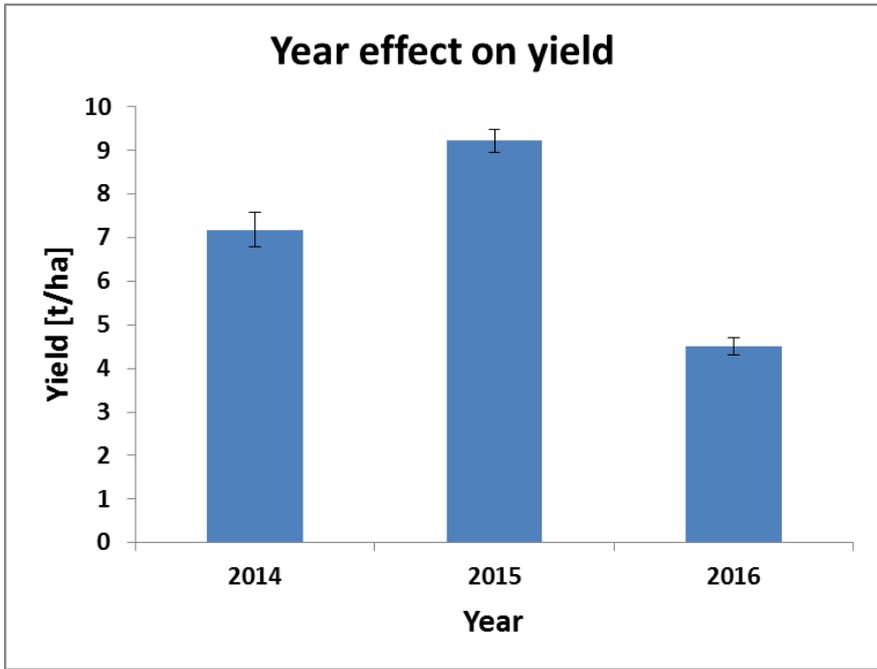


Figure 11. Yields recorded from the different field trials. Field plots had been drilled early, mid or late during each season. Field plots had been left untreated or had received three rates (7.5 kg/ha, 10 kg/ha, 12.5 kg/ha) of starter fertilisers with nitrogen (Primary P) and without nitrogen (Microstar). Data show mean values ($n = 21$) and standard error (ANOVA, $F_{2,56} = 50.09$, $p < 0.001$).

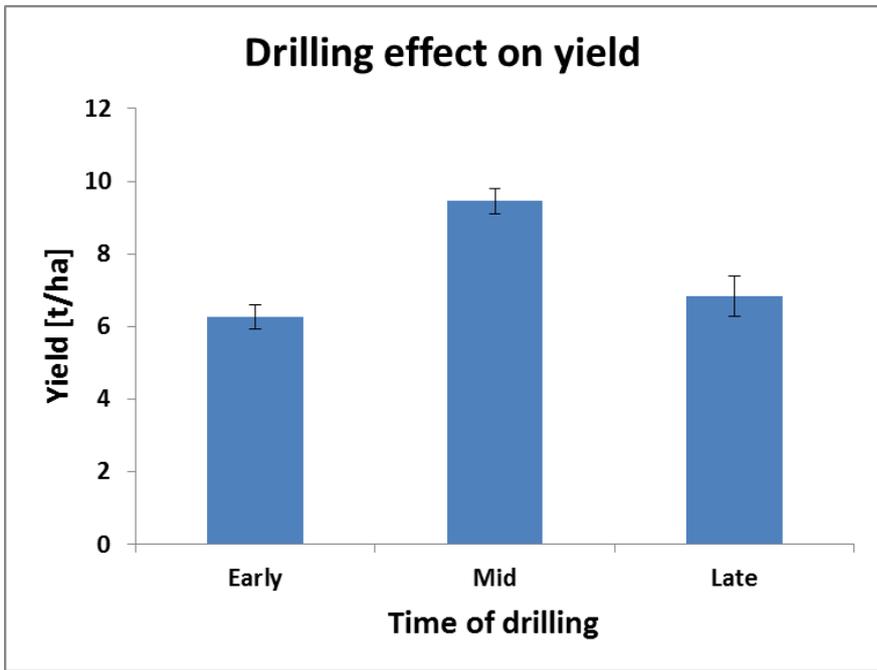


Figure 12. Yields recorded from the different drill timings. Field plots had been established in 2014, 2015 and 2016. Field plots had been left untreated or had received three rates (7.5 kg/ha, 10 kg/ha, 12.5 kg/ha) of starter fertilisers with nitrogen (Primary P) and without nitrogen (Microstar). Data show mean values ($n = 21$) and standard error (ANOVA, $F_{2,56} = 12.42$, $p < 0.001$).

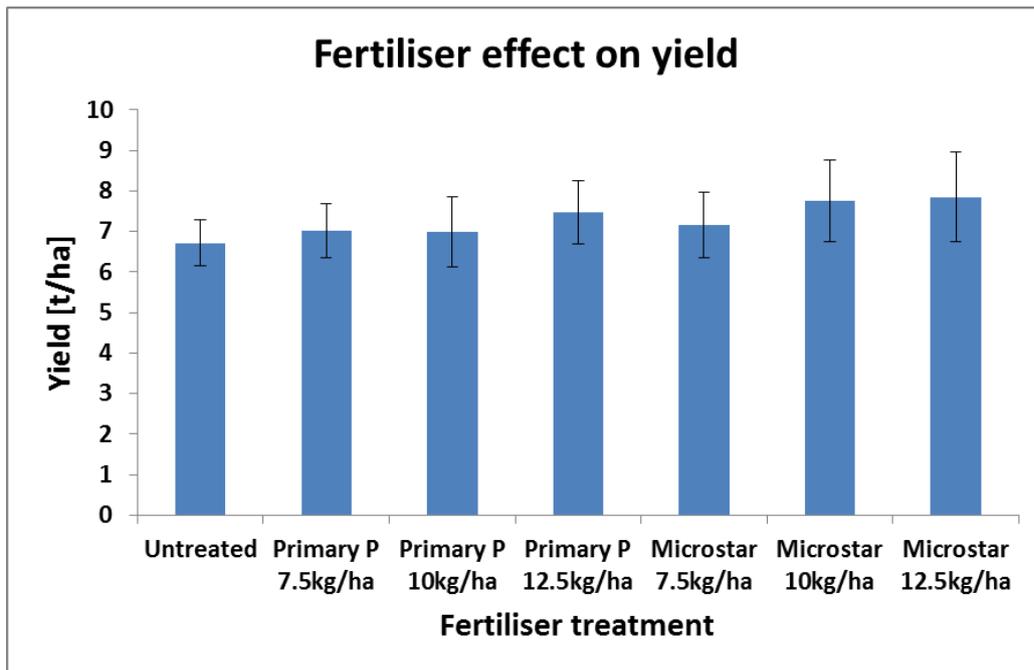


Figure 13. Yields recorded from plots that had received different fertiliser treatments. Field plots had been established in 2014, 2015 and 2016, and had been drilled early, mid or late during each season. Data show mean values (n = 14) and standard error.

Assessment of rhizobial populations in soils

The MPN method using pea seedlings to determine numbers of viable rhizobia in soil was unsuccessful. As described in the annual report from January 2016, the failure of the MPN method was most likely due to two reasons. Firstly, the use of agar as growing medium stopped free movement of rhizobia through the medium and also restricted oxygen flow which is vital for nodulation to occur. Secondly, the size of the tubes used to grow the peas was too small and restricted plant growth. Plant health was not optimal which reduced likelihood of successful nodulation. It was therefore decided to replace the MPN method with a pot test in which peas are grown in a sterile 1:1 vermiculite/perlite mixture inoculated with soil slurries and nodules per plants are counted. In order to verify the new pot test, vetch seedlings were used in the MPN test and results compared to the pot test. Vetch seedlings were grown in a sterile 1:1 vermiculite/perlite mixture to allow free movement of the rhizobial bacteria and to allow for unrestricted oxygen flow. Vetch seedlings are much smaller than pea seedling and could grow freely within the tube (Figure 14). Seedlings were inoculated with soils collected from untreated plots from each of the three field sites in 2015. The same soil was used to inoculate vetch and pea plants grown in pots (Figure 15). Vetch seedling were successfully nodulated in the MPN test (Figure 16) but failed to grow in the pot test most likely due to higher sensitivity to drying out of the growth medium. Peas, however, grew very well in the pot tests and were successfully nodulated (Figure 17). Results obtained in the MPN method

and the pot test using vetch and pea, respectively, were highly correlated (Figure 18) thereby demonstrating the validity of the pot test.



Figure 14. Vetch plants growing in boiling tubes (MPN method).



Figure 15: Pea plants growing in pots.



Figure 16: Nodules on vetch roots (MPN method).



Figure 17. Nodules on pea roots (pot test).

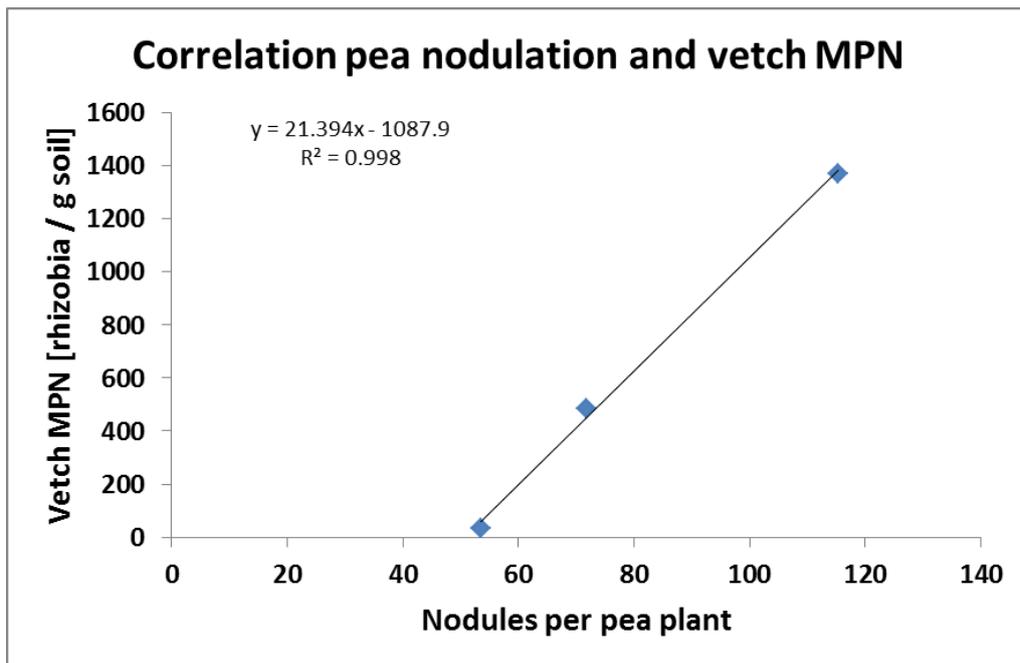


Figure 18. Relationship between size of rhizobial population assessed by the MPN method using vetch and by the pot test using peas. Plants had been inoculated with soils from untreated plots from each field site in 2015. Data show mean values (n = 4).

Field experiments - influence of starter fertilisers on rhizobial populations

In each year and from each field site soil samples were taken prior to planting and from each plot at first pod to assess size of the rhizobial populations. Rhizobial communities pre planting were stable in each field (Figure 19) but drilling time had an effect with late drilled crops having lower overall population sizes at first pod (Figure 20). For each fertiliser treatment, 3 replicated pots were assessed giving a total of 108 pots being assessed per fertiliser treatment. The application of starter fertilisers either with or without nitrogen had any impact on the size of rhizobial populations (Figure 21).

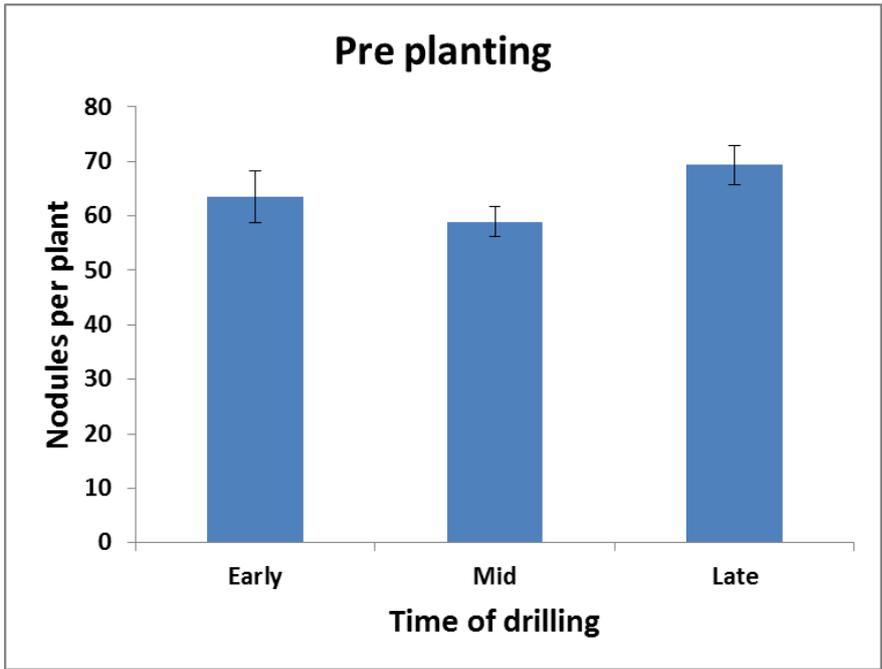


Figure 19. Nodules per plant representing rhizobial population sizes prior to planting peas. Fields plots had been tested in 2014, 2015 and 2016. Data show mean values (n = 18) and standard error.

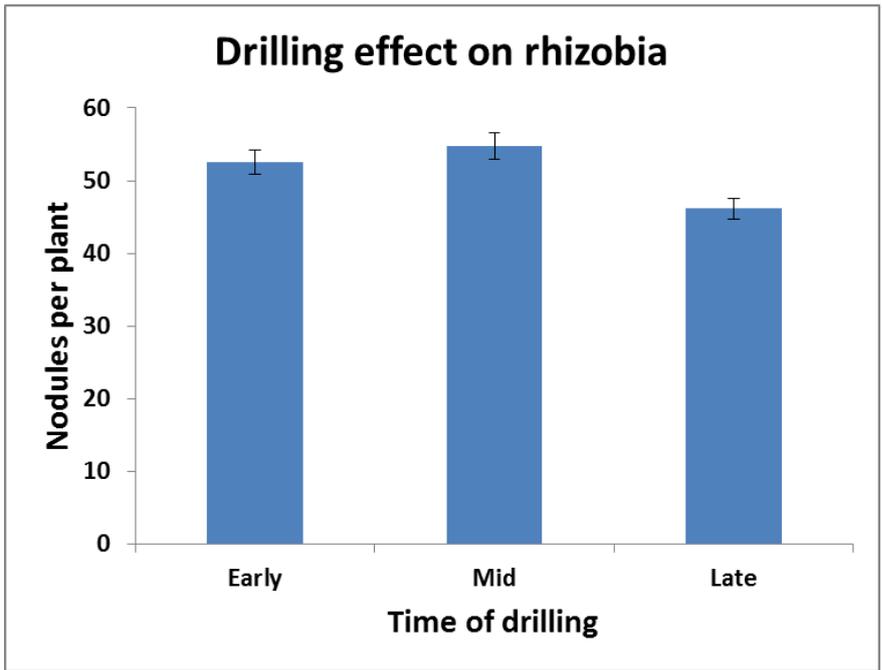


Figure 20. Nodules per plant representing rhizobial population sizes at first pod. Fields plots had been established in 2014, 2015 and 2016. Field plots had been left untreated or had received three rates (7.5 kg/ha, 10 kg/ha, 12.5 kg/ha) of starter fertilisers with nitrogen (Primary P) and without nitrogen (Microstar). Data show mean values (n = 256) and standard error (ANOVA, $F_{2,756} = 7.48$, $p < 0.001$).

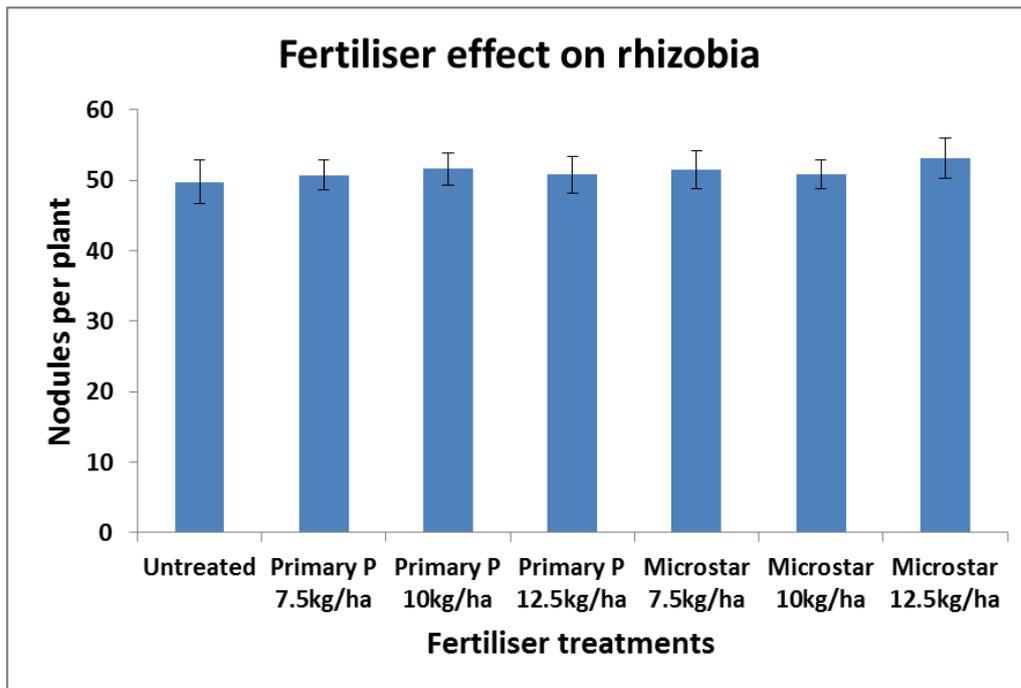


Figure 21. Nodules per plant representing rhizobial population sizes at first pod. Fields plots had been established in 2014, 2015 and 2016. Field plots had been drilled early, mid or late during the season. Data show mean values (n = 108) and standard error.

Critical P - influence of differing P contents on rhizobial populations

In project FV 380, soil from field sites with differing levels of phosphorus ranging from 10 to 100 mg P per litre had been collected. Soil samples had been taken in 2013 (Kirby) and 2014 (Brocklesby and Norwich). Rhizobial population sizes did not correlate with the availability of P in the soil (Figure 22).

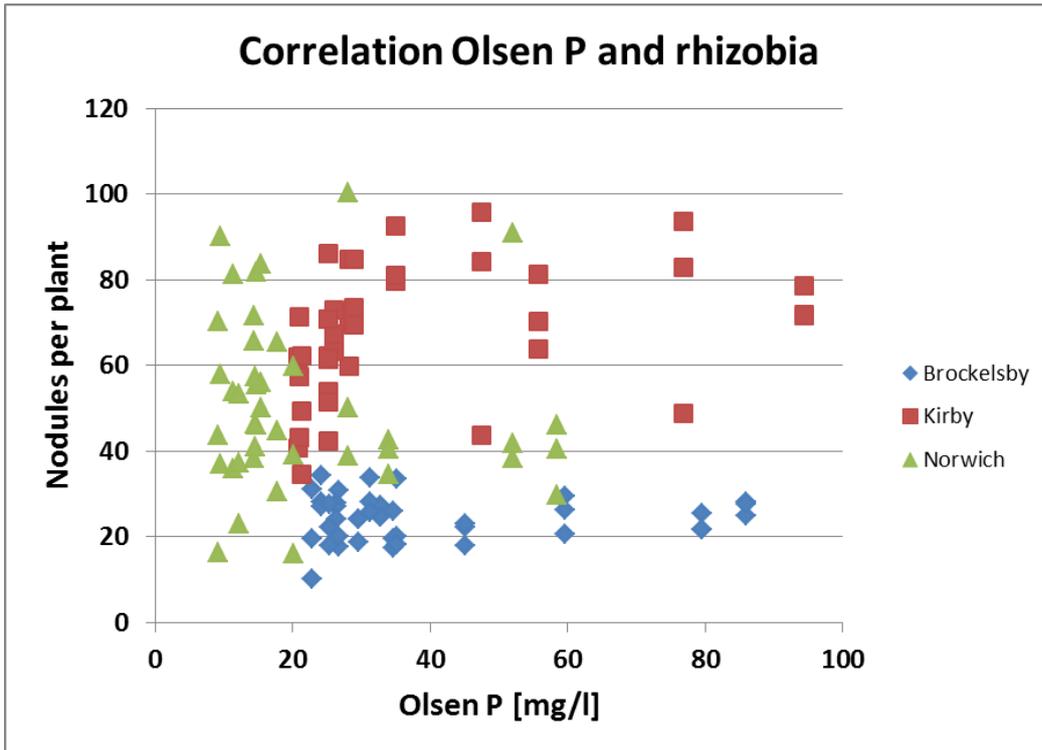


Figure 22. Nodules per plant representing rhizobial population sizes. Fields plots had been established in 2013 and 2014 as part of project FV 380. Soils differed in P concentration [Olsen P]. Data show mean values (n = 42) and standard error.

Soil nutrition

Nutrient data (potassium, phosphorus and magnesium) and pH were measured before drilling and after harvest (Appendix 2). Soil type, nitrate, ammonium, available nitrogen, potentially mineralisable nitrogen (PMN), organic matter (OM) and total nitrogen (tN) were measured before drilling and after harvest in untreated plots and plots that had received Primary P (Appendix 3).

Discussion

The project investigated whether the application of starter fertilisers can improve pea yields. Yield benefits were highly season and field site dependent. When analysed over three years with three field experiments per year no yield improvement could be shown. Neither did the application of starter fertilisers improve plant mass at first pod. Time of drilling always influenced both yield and plant mass but effects were year dependent. The different pea varieties used for the different drilling times and variation in weather conditions will have been responsible for the observed differences. Yields were highest with the mid drilled crop in 2015. These results heavily influenced overall mid-season yield data.

The main aim of the project was to investigate whether application of starter fertilisers impacted upon rhizobial populations in soils. In order to assess rhizobial populations pea seedlings grown in pots were inoculated with field collected soils and numbers of nodules were counted. Nodule numbers counted using the pot method correlated with results obtained using the MPN method (Somasegaran and Hoben, 1994). This demonstrated that the pot test is a valid and reliable method to assess rhizobial population sizes for pea crops. In addition to the pot test, nodulation in the field was also assessed. Both assessments showed that the application of starter fertilisers did not influence rhizobial populations. Quantities of P and N applied to the pea crop were low. Although it had been shown that P can be beneficial for nodule numbers and nodule effectiveness at an application rate of 60 kg/ha (Achakzai, 2007) and a meta-analysis revealed that generally plant-bacteria partnerships benefit from the availability of P (Shantz et al., 2016) no positive impacts were seen here. Furthermore, rhizobial population sizes were not correlated with the differing P levels indicated by an Olsen P analysis showing that small changes in P availability did not impact on the effectiveness of nodulation. Amounts of N applied in this work were also very small with a maximum of 1.25 kg N / ha applied. Nitrogen applications have been shown to reduce rhizobial numbers but only when concentrations are high. In pea crops, 25 kg N / ha and 40 kg N / ha have been shown to reduce nodule number (Achakzai, 2007; Clayton et al., 2004) and 30 kg N / ha affected nodulation in lentils (Bowness et al., 2016). Overall, the project demonstrated that the application of starter fertilisers to pea crops did not reduce the health of soil rhizobial communities.

Conclusions

- Application of starter fertilisers to peas did not consistently improve yields.
- Application of starter fertilisers did not impact on rhizobial population sizes – P did not stimulate rhizobial numbers and low nitrogen application did not suppress them.
- Varying levels of P in soil did not impact on rhizobial population sizes.

Knowledge and Technology Transfer

2014:

Cereals 2014

Grower meeting Bruce Farms

Grower Meeting Holbeach Marsh Pea Growers

PGRO Open day

PGRO Staff Away day

The Pulse Magazine Spring 2014

VAA Meeting

Warwick Crop Centre Seminar

2015:

Agronomy training CCC Agronomy

Agronomy training Hutchinsons (12 meetings)

Agronomy training Saffron Waldon

Cereals 2015

Grower meeting Dengie Crops

Grower meetings Fen Peas Ltd (2 meetings)

Legume panel meetings (2 meeting)

PGRO and Syngenta Roadshows (6 meetings)

PGRO Crop Protection course

PGRO Pulse Open Day Stubton

PGRO Vining pea Open Day Nocton

Terres Inovia and PGRO meeting

2016:

2nd International Legume Society Conference, Portugal (3 day meeting) – Poster and oral presentation (Wiesel et al., 2016)

Agronomy training Bartholomews

Agronomy training Hampshire Arable Systems

Agronomy training Hampshire Crop Management

Agrovista Crop Protection Course

AHDB RB209 meeting

Cereals 2016

CropTech 2016

Grower meeting Bruce Farms (2 meetings)

Grower meeting Dengie Crops

Grower meeting Green Pea Group (2 meetings)

Grower meeting Holbeach Marsh Pea Growers

Grower meeting Robin Appel

Grower meeting Stemgold Peas

Legume panel meeting

PGRO and Syngenta Roadshows (4 meetings)

PGRO Crop Protection courses (2 meetings)

PGRO Vining pea Open Day Nocton

Pulse panel meeting

Scientific workshop on the effects of biotic and abiotic stresses in legumes: The ABSTRESS and BIOTECISOJASUR projects, Argentina (5 day meeting)

The Vegetable Magazine 2016

Visit from Australian Pulse Growers Association

Visit from Canterbury Seeds New Zealand

Visit from students from University of Nottingham

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Appendices

Appendix 1. Yield [t/ha] of each fertilised plot at three different field sites in three subsequent seasons. Field plots had been left untreated or had received three rates (7.5 kg/ha, 10 kg/ha, 12.5 kg/ha) of starter fertilisers with nitrogen (Primary P) and without nitrogen (Microstar). In each year, an early, mid and late drilled crop was included. Field experiments were performed in 2014, 2015 and 2016.

Year	Drilling	Treatment	Yield t/ha	Year	Drilling	Treatment	Yield t/ha
2014	Early	Untreated	5.25	2016	Early	Untreated	4.82
2014	Early	Primary P 7.5kg/ha	5.69	2016	Early	Primary P 7.5kg/ha	5.38
2014	Early	Primary P 10kg/ha	7.15	2016	Early	Primary P 10kg/ha	3.80
2014	Early	Primary P 12.5kg/ha	6.66	2016	Early	Primary P 12.5kg/ha	4.70
2014	Early	Microstar 7.5kg/ha	5.92	2016	Early	Microstar 7.5kg/ha	5.52
2014	Early	Microstar 10kg/ha	4.89	2016	Early	Microstar 10kg/ha	4.99
2014	Early	Microstar 12.5kg/ha	5.07	2016	Early	Microstar 12.5kg/ha	4.86
2014	Mid	Untreated	7.67	2016	Mid	Untreated	*
2014	Mid	Primary P 7.5kg/ha	7.74	2016	Mid	Primary P 7.5kg/ha	*
2014	Mid	Primary P 10kg/ha	9.32	2016	Mid	Primary P 10kg/ha	*
2014	Mid	Primary P 12.5kg/ha	9.41	2016	Mid	Primary P 12.5kg/ha	*
2014	Mid	Microstar 7.5kg/ha	8.76	2016	Mid	Microstar 7.5kg/ha	*
2014	Mid	Microstar 10kg/ha	9.46	2016	Mid	Microstar 10kg/ha	*
2014	Mid	Microstar 12.5kg/ha	11.23	2016	Mid	Microstar 12.5kg/ha	*
2014	Late	Untreated	5.47	2016	Late	Untreated	5.49
2014	Late	Primary P 7.5kg/ha	6.79	2016	Late	Primary P 7.5kg/ha	4.22
2014	Late	Primary P 10kg/ha	5.05	2016	Late	Primary P 10kg/ha	3.78
2014	Late	Primary P 12.5kg/ha	6.51	2016	Late	Primary P 12.5kg/ha	4.53
2014	Late	Microstar 7.5kg/ha	5.94	2016	Late	Microstar 7.5kg/ha	3.42
2014	Late	Microstar 10kg/ha	8.51	2016	Late	Microstar 10kg/ha	4.09
2014	Late	Microstar 12.5kg/ha	8.40	2016	Late	Microstar 12.5kg/ha	3.43
2015	Early	Untreated	7.78				
2015	Early	Primary P 7.5kg/ha	8.18				
2015	Early	Primary P 10kg/ha	8.43				
2015	Early	Primary P 12.5kg/ha	8.46				
2015	Early	Microstar 7.5kg/ha	8.21				
2015	Early	Microstar 10kg/ha	8.00				
2015	Early	Microstar 12.5kg/ha	7.95				
2015	Mid	Untreated	8.30				
2015	Mid	Primary P 7.5kg/ha	8.28				
2015	Mid	Primary P 10kg/ha	9.09				
2015	Mid	Primary P 12.5kg/ha	10.38				
2015	Mid	Microstar 7.5kg/ha	9.56				
2015	Mid	Microstar 10kg/ha	12.16				
2015	Mid	Microstar 12.5kg/ha	10.99				
2015	Late	Untreated	8.90				
2015	Late	Primary P 7.5kg/ha	9.89				
2015	Late	Primary P 10kg/ha	9.25				
2015	Late	Primary P 12.5kg/ha	9.15				
2015	Late	Microstar 7.5kg/ha	9.90				
2015	Late	Microstar 10kg/ha	9.98				
2015	Late	Microstar 12.5kg/ha	10.90				

Appendix 2. pH, phosphorus (P), potassium (K) and magnesium (Mg) measurements. Soil samples were taken before drilling and after harvest. Field plots had been left untreated or had received three rates (7.5 kg/ha, 10 kg/ha, 12.5 kg/ha) of starter fertilisers with nitrogen (Primary P) and without nitrogen (Microstar).

Drilling	Sampling time	Treatment	Year	pH	Phosphorus (P)		Potassium (K)		Magnesium (Mg)	
					mg/l	Index	mg/l	Index	mg/l	Index
Early	Pre drilling	Whole field	2014	7.50	21	2	176	-2	217	4
Early	Post harvest	Untreated	2014	7.81	25	2	308	3	297	5
Early	Post harvest	Primary P 7.5kg/ha	2014	7.71	18	2	317	3	185	4
Early	Post harvest	Primary P 10kg/ha	2014	7.56	15	1	290	3	253	5
Early	Post harvest	Primary P 12.5kg/ha	2014	7.74	24	2	335	3	288	5
Early	Post harvest	Microstar 7.5kg/ha	2014	7.76	21	2	301	3	297	5
Early	Post harvest	Microstar 10kg/ha	2014	7.76	54	4	251	3	219	4
Early	Post harvest	Microstar 12.5kg/ha	2014	7.87	24	2	295	3	207	4
Mid	Pre drilling	Whole field	2014	8.20	24	2	450	4	174	3
Mid	Post harvest	Untreated	2014	8.29	21	2	621	5	176	4
Mid	Post harvest	Primary P 7.5kg/ha	2014	8.22	24	2	430	4	150	3
Mid	Post harvest	Primary P 10kg/ha	2014	8.22	19	2	374	3	139	3
Mid	Post harvest	Primary P 12.5kg/ha	2014	8.25	26	3	504	4	165	3
Mid	Post harvest	Microstar 7.5kg/ha	2014	8.24	30	3	568	4	182	4
Mid	Post harvest	Microstar 10kg/ha	2014	8.09	27	3	509	4	152	3
Mid	Post harvest	Microstar 12.5kg/ha	2014	8.22	33	3	532	4	166	3
Late	Pre drilling	Whole field	2014	7.90	18	2	344	3	283	5
Late	Post harvest	Untreated	2014	7.55	38	3	271	3	176	4
Late	Post harvest	Primary P 7.5kg/ha	2014	7.21	24	2	196	2+	313	5
Late	Post harvest	Primary P 10kg/ha	2014	6.80	30	3	214	2+	262	5
Late	Post harvest	Primary P 12.5kg/ha	2014	7.52	25	2	166	-2	232	4
Late	Post harvest	Microstar 7.5kg/ha	2014	7.49	44	3	244	3	229	4
Late	Post harvest	Microstar 10kg/ha	2014	7.57	107	6	254	3	191	4
Late	Post harvest	Microstar 12.5kg/ha	2014	7.85	34	3	200	2+	180	4

Drilling	Sampling time	Treatment	Year	pH	Phosphorus (P)		Potassium (K)		Magnesium (Mg)	
					mg/l	Index	mg/l	Index	mg/l	Index
Early	Pre drilling	Whole field	2015	7.84	27	3	208	2+	92	2
Early	Post harvest	Untreated	2015	7.89	56	4	433	4	112	3
Early	Post harvest	Primary P 7.5kg/ha	2015	7.78	31	3	266	3	69	2
Early	Post harvest	Primary P 10kg/ha	2015	7.86	39	3	354	3	79	2
Early	Post harvest	Primary P 12.5kg/ha	2015	7.83	36	3	317	3	94	2
Early	Post harvest	Microstar 7.5kg/ha	2015	*	*	*	*	*	*	*
Early	Post harvest	Microstar 10kg/ha	2015	*	*	*	*	*	*	*
Early	Post harvest	Microstar 12.5kg/ha	2015	*	*	*	*	*	*	*
Mid	Pre drilling	Whole field	2015	8.05	18	2	297	3	119	3
Mid	Post harvest	Untreated	2015	8.26	17	2	331	3	134	3
Mid	Post harvest	Primary P 7.5kg/ha	2015	8.20	16	2	404	4	159	3
Mid	Post harvest	Primary P 10kg/ha	2015	8.28	16	2	299	3	112	3
Mid	Post harvest	Primary P 12.5kg/ha	2015	8.28	16	2	314	3	123	3
Mid	Post harvest	Microstar 7.5kg/ha	2015	8.38	16	2	273	3	121	3
Mid	Post harvest	Microstar 10kg/ha	2015	8.07	12	1	408	4	120	3
Mid	Post harvest	Microstar 12.5kg/ha	2015	8.22	12	1	539	4	141	3
Late	Pre drilling	Whole field	2015	7.66	9	0	305	3	218	4
Late	Post harvest	Untreated	2015	8.25	8	0	193	2+	115	3
Late	Post harvest	Primary P 7.5kg/ha	2015	8.03	12	1	235	2+	196	4
Late	Post harvest	Primary P 10kg/ha	2015	7.91	18	2	259	3	155	3
Late	Post harvest	Primary P 12.5kg/ha	2015	8.21	11	1	237	2+	181	4
Late	Post harvest	Microstar 7.5kg/ha	2015	*	*	*	*	*	*	*
Late	Post harvest	Microstar 10kg/ha	2015	*	*	*	*	*	*	*
Late	Post harvest	Microstar 12.5kg/ha	2015	*	*	*	*	*	*	*
Early	Pre drilling	Whole field	2016	7.89	137	6	397	3	81	2
Early	Post harvest	Untreated	2016	7.72	79	5	234	2+	73	2
Early	Post harvest	Primary P 7.5kg/ha	2016	7.81	144	7	434	4	86	2
Early	Post harvest	Primary P 10kg/ha	2016	7.89	112	6	284	3	83	2
Early	Post harvest	Primary P 12.5kg/ha	2016	7.89	90	5	220	2+	73	2
Early	Post harvest	Microstar 7.5kg/ha	2016	7.97	84	5	247	3	80	2
Early	Post harvest	Microstar 10kg/ha	2016	7.94	152	7	439	4	92	2
Early	Post harvest	Microstar 12.5kg/ha	2016	7.89	141	7	374	3	82	2
Mid	Pre drilling	Whole field	2016	7.92	50	4	221	2+	103	3
Mid	Post harvest	Untreated	2016	8.05	72	5	306	3	94	2
Mid	Post harvest	Primary P 7.5kg/ha	2016	8.08	71	5	396	3	97	2
Mid	Post harvest	Primary P 10kg/ha	2016	8.11	54	4	309	3	99	2
Mid	Post harvest	Primary P 12.5kg/ha	2016	8.09	64	4	307	3	97	2
Mid	Post harvest	Microstar 7.5kg/ha	2016	8.10	63	4	312	3	105	3
Mid	Post harvest	Microstar 10kg/ha	2016	8.09	67	4	367	3	101	3
Mid	Post harvest	Microstar 12.5kg/ha	2016	8.00	87	5	272	3	78	2
Late	Pre drilling	Whole field	2016	7.97	13	1	282	3	241	4
Late	Post harvest	Untreated	2016	7.87	22	2	255	3	247	4
Late	Post harvest	Primary P 7.5kg/ha	2016	7.88	11	1	216	2+	278	5
Late	Post harvest	Primary P 10kg/ha	2016	7.71	17	2	262	3	365	6
Late	Post harvest	Primary P 12.5kg/ha	2016	7.49	19	2	243	3	257	5
Late	Post harvest	Microstar 7.5kg/ha	2016	7.73	17	2	310	3	410	6
Late	Post harvest	Microstar 10kg/ha	2016	7.79	13	1	218	2+	308	5
Late	Post harvest	Microstar 12.5kg/ha	2016	7.80	11	1	225	2+	321	5

Appendix 3. Soil type, nitrate, ammonium, available nitrogen, potentially mineralisable nitrogen (PMN), organic matter (OM) and total nitrogen (tN) measurements. Soil samples were taken before drilling and after harvest at all three field sites. Field plots had been left untreated or had received three rates (7.5 kg/ha, 10 kg/ha, 12.5 kg/ha) of starter fertilisers with nitrogen (Primary P).

Drilling	Sampling time	Treatment	Year	Depth	Soil type	Nitrate	Ammonium	Available N	PMN	OM	tN
				(cm)		(kg/ha)	(kg/ha)	(kg/ha)	(kg/ha)	(%)	(%)
Early	Pre drilling	Whole field	2014	0-30	ZCL	16.7	6.1	22.7	113.4	3.18	0.136
				30-60	ZCL	8.8	3.0	11.8			
				60-90	ZCL	10.1	2.3	12.4			
Early	Post harvest	Untreated	2014	0-30	ZCL	18.5	0.0	18.5	60.7	3.09	0.170
				30-60	ZL	21.2	0.0	21.2			
				60-90	ZL	16.4	0.0	16.4			
Early	Post harvest	Primary P 7.5kg/ha	2014	0-30	ZCL	22.9	1.9	24.7	57.8	2.63	0.199
				30-60	ZL	20.7	0.0	20.7			
				60-90	ZL	16.8	0.0	16.8			
Early	Post harvest	Primary P 10kg/ha	2014	0-30	ZCL	20.0	0.3	20.3	70.9	2.66	0.222
				30-60	ZL	22.4	0.0	22.4			
				60-90	ZL	20.4	0.0	20.4			
Early	Post harvest	Primary P 12.5kg/ha	2014	0-30	ZCL	26.6	0.0	26.6	141.8	2.66	0.190
				30-60	ZL	13.3	0.0	13.3			
				60-90	ZL	17.0	0.0	17.0			
Mid	Pre drilling	Whole field	2014	0-30	CL	19.1	10.7	29.7	96.5	3.43	0.147
				30-60	ZCL	20.4	3.2	23.6			
				60-90	ZL	30.1	2.2	32.2			
Mid	Post harvest	Untreated	2014	0-30	ZCL	8.5	6.2	14.7	100.1	2.90	0.153
				30-60	ZL	18.3	0.1	18.4			
				60-90	ZL	28.3	0.0	28.3			
Mid	Post harvest	Primary P 7.5kg/ha	2014	0-30	ZCL	17.5	1.0	18.5	114.7	3.23	0.156
				30-60	ZL	21.6	0.0	21.6			
				60-90	ZL	32.9	0.0	32.9			
Mid	Post harvest	Primary P 10kg/ha	2014	0-30	ZCL	19.5	2.1	21.5	90.2	3.43	0.172
				30-60	ZL	22.1	0.0	22.1			
				60-90	ZL	34.2	0.0	34.2			
Mid	Post harvest	Primary P 12.5kg/ha	2014	0-30	ZCL	21.9	1.8	23.8	78.9	3.42	0.172
				30-60	ZL	34.2	6.2	40.3			
				60-90	ZL	38.3	0.0	38.3			
Late	Pre drilling	Whole field	2014	0-30	ZCL	25.0	8.6	33.6	177.5	3.29	0.162
				30-60	ZCL	26.1	4.4	30.5			
				60-90	ZL	31.4	2.7	34.1			
Late	Post harvest	Untreated	2014	0-30	ZCL	23.3	13.1	36.3	164.1	3.98	0.188
				30-60	ZL	22.8	5.6	28.4			
				60-90	ZL	30.6	62.6	93.1			
Late	Post harvest	Primary P 7.5kg/ha	2014	0-30	ZCL	24.1	7.9	32.0	110.8	3.51	0.185
				30-60	ZL	24.6	0.9	25.5			
				60-90	ZL	28.8	0.0	28.8			
Late	Post harvest	Primary P 10kg/ha	2014	0-30	ZCL	26.5	9.0	35.5	54.0	3.48	0.189
				30-60	ZL	33.9	3.8	37.8			
				60-90	ZL	35.8	0.0	35.8			
Late	Post harvest	Primary P 12.5kg/ha	2014	0-30	ZCL	33.2	13.0	46.2	98.5	3.15	0.167
				30-60	ZL	28.3	0.0	28.3			
				60-90	ZL	31.8	0.0	31.8			

Drilling	Sampling time	Treatment	Year	Depth	Soil type	Nitrate	Ammonium	Available N	PMN	OM	tN
				(cm)		(kg/ha)	(kg/ha)	(kg/ha)	(kg/ha)	(%)	(%)
Early	Pre drilling	Whole field	2015	0-30	ZL	25.2	0.8	25.9	110.8	2.42	0.133
				30-60	ZL	36.9	3.0	39.9			
				60-90	ZL	37.9	2.0	39.9			
Early	Post harvest	Untreated	2015	0-30	ZCL	33.0	2.6	35.6	102.1	3.03	0.166
				30-60	ZCL	41.6	2.6	44.2			
				60-90	ZCL	40.5	1.2	41.8			
Early	Post harvest	Primary P 7.5kg/ha	2015	0-30	ZCL	33.4	1.4	34.8	72.3	2.62	0.145
				30-60	ZL	37.9	0.0	37.9			
				60-90	ZL	43.6	0.0	43.6			
Early	Post harvest	Primary P 10kg/ha	2015	0-30	ZCL	29.5	0.9	30.4	62.1	2.76	0.167
				30-60	ZL	37.0	1.7	38.7			
				60-90	ZL	30.9	0.0	30.9			
Early	Post harvest	Primary P 12.5kg/ha	2015	0-30	ZCL	20.5	1.5	22.0	50.2	2.62	0.122
				30-60	ZCL	28.5	0.0	28.5			
				60-90	ZCL	30.8	0.1	30.9			
Mid	Pre drilling	Whole field	2015	0-30	ZL	24.0	1.3	25.3	114.2	2.61	0.098
				30-60	ZL	43.7	0.0	43.7			
				60-90	ZL	43.9	0.4	44.3			
Mid	Post harvest	Untreated	2015	0-30	ZCL	15.6	3.1	18.6	79.7	3.23	0.136
				30-60	ZL	28.9	1.7	30.6			
				60-90	ZL	41.1	1.9	43.0			
Mid	Post harvest	Primary P 7.5kg/ha	2015	0-30	ZCL	24.0	2.6	26.6	86.7	2.86	0.154
				30-60	ZL	19.9	1.1	21.1			
				60-90	ZL	34.2	1.7	35.9			
Mid	Post harvest	Primary P 10kg/ha	2015	0-30	ZCL	16.1	1.9	17.9	74.6	2.47	0.139
				30-60	ZL	23.4	3.0	26.4			
				60-90	ZL	49.7	6.5	56.2			
Mid	Post harvest	Primary P 12.5kg/ha	2015	0-30	ZCL	25.9	3.6	29.5	67.5	2.38	0.145
				30-60	ZL	31.3	2.3	33.6			
				60-90	ZL	37.7	2.6	40.3			
Late	Pre drilling	Whole field	2015	0-30	ZL	17.6	0.6	18.2	73.5	2.79	0.135
				30-60	ZL	27.3	0.0	27.3			
				60-90	ZCL	14.9	0.0	14.9			
Late	Post harvest	Untreated	2015	0-30	ZCL	13.1	0.6	13.7	40.4	2.94	0.164
				30-60	ZCL	13.1	1.0	14.2			
				60-90	ZCL	14.4	0.0	14.4			
Late	Post harvest	Primary P 7.5kg/ha	2015	0-30	ZCL	16.0	1.6	17.6	63.0	2.82	0.150
				30-60	ZCL	9.7	0.0	9.7			
				60-90	ZCL	6.7	0.0	6.7			
Late	Post harvest	Primary P 10kg/ha	2015	0-30	ZCL	20.0	1.7	21.7	43.2	3.07	0.160
				30-60	ZCL	23.0	1.4	24.4			
				60-90	ZCL	9.9	0.0	9.9			
Late	Post harvest	Primary P 12.5kg/ha	2015	0-30	ZCL	18.7	3.0	21.8	32.6	3.05	0.148
				30-60	ZCL	10.7	0.4	11.1			
				60-90	ZCL	6.3	0.0	6.3			

Drilling	Sampling time	Treatment	Year	Depth (cm)	Soil type	Nitrate (kg/ha)	Ammonium (kg/ha)	Available N (kg/ha)	PMN (kg/ha)	OM (%)	tN (%)
Early	Pre drilling	Whole field	2016	0-30	ZL	62.7	1.7	64.4	127.5	2.53	0.210
				30-60	ZL	246.4	0.0	246.4			
				60-90	ZL	81.5	0.0	81.5			
Early	Post harvest	Untreated	2016	0-30	*	*	*	*	*	*	*
				30-60	*	*	*	*			
				60-90	*	*	*	*			
Early	Post harvest	Primary P 7.5kg/ha	2016	0-30	*	*	*	*	*	*	*
				30-60	*	*	*	*			
				60-90	*	*	*	*			
Early	Post harvest	Primary P 10kg/ha	2016	0-30	*	*	*	*	*	*	*
				30-60	*	*	*	*			
				60-90	*	*	*	*			
Early	Post harvest	Primary P 12.5kg/ha	2016	0-30	*	*	*	*	*	*	*
				30-60	*	*	*	*			
				60-90	*	*	*	*			
Mid	Pre drilling	Whole field	2016	0-30	ZCL	10.7	0.6	11.3	83.4	2.32	0.160
				30-60	ZL	15.7	0.0	15.7			
				60-90	ZL	19.7	0.2	19.9			
Mid	Post harvest	Untreated	2016	0-30	*	*	*	*	*	*	*
				30-60	*	*	*	*			
				60-90	*	*	*	*			
Mid	Post harvest	Primary P 7.5kg/ha	2016	0-30	*	*	*	*	*	*	*
				30-60	*	*	*	*			
				60-90	*	*	*	*			
Mid	Post harvest	Primary P 10kg/ha	2016	0-30	*	*	*	*	*	*	*
				30-60	*	*	*	*			
				60-90	*	*	*	*			
Mid	Post harvest	Primary P 12.5kg/ha	2016	0-30	*	*	*	*	*	*	*
				30-60	*	*	*	*			
				60-90	*	*	*	*			
Late	Pre drilling	Whole field	2016	0-30	ZC	14.1	0.5	14.6	42.8	2.70	0.190
				30-60	ZC	24.6	0.0	24.6			
				60-90	ZL	15.0	0.0	15.0			
Late	Post harvest	Untreated	2016	0-30	ZCL	31.9	6.6	38.5	89.9	3.19	0.160
				30-60	ZCL	46.3	5.3	51.6			
				60-90	ZCL	45.2	2.6	47.8			
Late	Post harvest	Primary P 7.5kg/ha	2016	0-30	ZCL	72.9	9.7	82.6	128.6	2.79	0.147
				30-60	ZCL	74.0	5.3	79.3			
				60-90	ZCL	97.2	3.7	100.8			
Late	Post harvest	Primary P 10kg/ha	2016	0-30	ZCL	49.6	4.0	53.6	46.1	2.98	0.297
				30-60	ZCL	70.2	6.3	76.5			
				60-90	ZCL	54.9	4.1	59.0			
Late	Post harvest	Primary P 12.5kg/ha	2016	0-30	ZCL	43.8	9.6	53.3	90.5	3.29	0.130
				30-60	ZCL	62.1	5.6	67.7			
				60-90	ZCL	54.1	2.8	56.9			