GREATSOILS

January 2022

Project Report No. 91140002-07a

Use of organic and biological amendments in horticultural production systems and monitoring for any effects on soil and plant health: Onions

Erika Wedgwood[1], Ruth D'urban-Jackson[1,] Emma Chapelhow[2] and Anne Bhogal[3]

[1]ADAS Boxworth, Cambridgeshire, CB23 4NN

[2]Fera Science Ltd., Sand Hutton, YO14 1LZ

[3]ADAS Gleadthorpe, Meden Vale, Mansfield, NG20 9PD

This is one part of the final report of a 48-month project (Project 7 of the Soil Biology and Soil Health Partnership, completed in 2021) which started in September 2017. The work was funded by AHDB and BBRO for £79,942.

CONTENTS

1. Abstract

As part of the work within the Soil Biology and Soil Health Research and Knowledge Exchange Partnership, this project explored the effects on soil and plant health of amendments in horticultural crop production; particularly their direct or indirect potential suppressive effects on soil-borne pathogens. In all the trials within Project 7 [onion, *Narcissus*, raspberry], organic material was incorporated pre-planting to determine any benefits or otherwise to crop health. In this trial, two types of organic material (cover crop and green compost) were incorporated into the soil to determine if there were any differences in the incidence of Fusarium basal rot in subsequently planted onions, and whether there were any changes in soil health before and after treatment. Molecular diagnostics were used to quantify the presence of *Fusarium oxysporum* in the soil and analytical methods were used for a range of soil physical, chemical and biological properties (Soil Health scorecard).

The trial was set up in a field with a recent history of onion Fusarium basal rot, shown from molecular testing in 2017 to contain 438 pg of *Fusarium oxysporum* per gramme of soil. Strips of cover crop (80% Rye, 15% Vetch and 5% Phacelia) established poorly due to dry conditions at sowing in August 2018 and so would have provided minimal organic matter. There was some organic matter input from cereal volunteers. PAS 100 certified green compost was applied at 21.5 t/ha to half the plots (half with, half without the cover crop) in March 2019 prior to onion planting. Four 1.8 m wide beds were marked out down the field, at right angles to the cover crop strips. Six plots were marked out with each bed, alternating every 11 m between cover crop and no cover crop strips, resulting in treatments: T1 untreated, T2 cover crop, T3 green compost and T4 cover crop and green compost. Onion sets were planted on 1 April 2019, with incorporation of the organic amendments during bed-making.

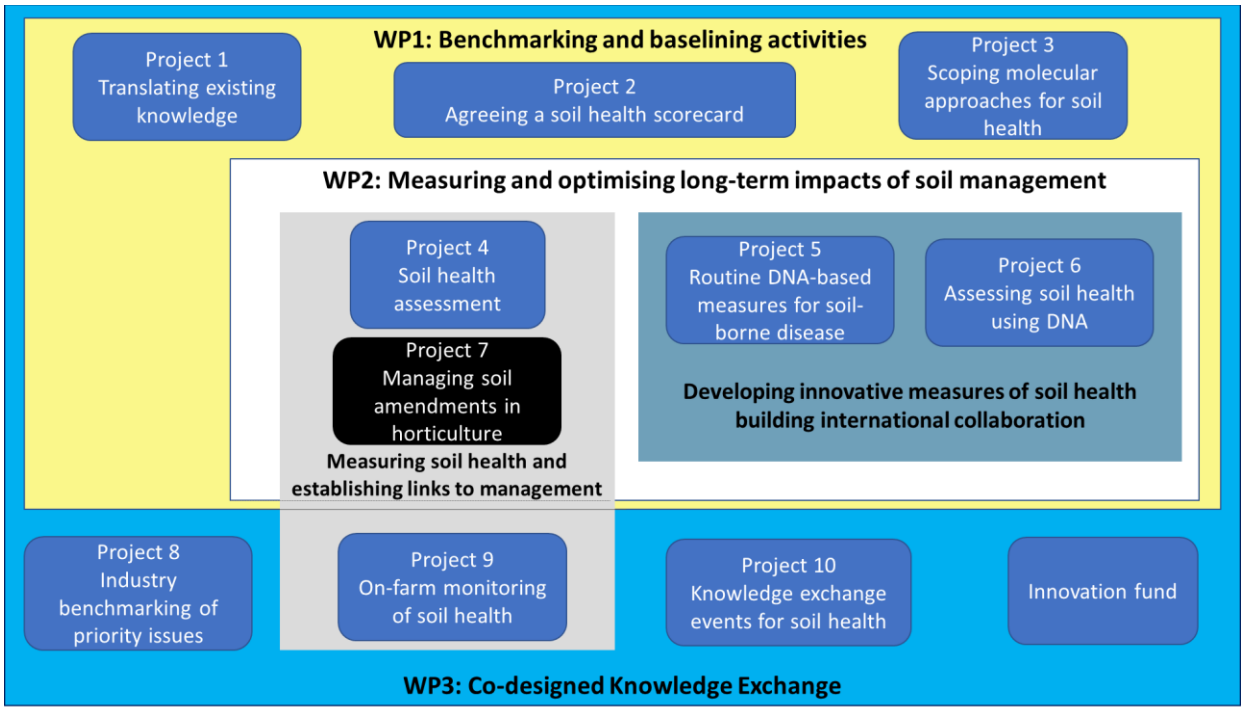
By mid-July 2019 only 1.5% of the onion plants had started to yellow from Fusarium infection of the basal plate via the roots. However, some exceptionally hot weather followed and with rotted roots unable to take up water; by mid-August 2019 foliar symptoms of Fusarium wilt had progressed greatly, with internal bulb browning seen. Only 9.5% of the onions would have been marketable, with no difference between the untreated and any treatments with added organic materials.

There were no treatment differences in the populations of free-living nematodes at assessment in the following winter wheat crop in November 2019. Soil analysis in November 2019 showed no significant treatment difference between the plots that had had poorly established cover crop in 2018 and those without it, or as a result of green compost application. Neither approach to adding organic matter was able to change the overall soil health after one application.

2. Introduction

This project is part of a suite of integrated projects within the Soil Biology and Soil Health Research and Knowledge Exchange Partnership (see Diagram below of how this project fits into the wider organisation of projects). This project (Project 7 of the Soil Biology and Soil Health Partnership – SBSH, together with Project 5) aimed to gain an understanding of any benefits gained from non-chemical inputs in horticultural cropping systems in the management of intractable soil diseases via potential changes to the soil microbial population and other biological, physical, and chemical aspects impacting on soil and crop health. This was focussed on a greater understanding of the effects on soil and plant health of amendments in horticultural crop production; particularly their direct or indirect potential suppressive effects on soil-borne pathogens.

Diagram to show how Project 7 (shown in black) fits within the integrated project delivery of the Soil Biology and Soil Health Research and Knowledge Exchange Partnership.



Inter-related objectives in Project 7 aimed to gain a better understanding of the soil biology and key soil health metrics that should be recorded by growers in order to be able to manage soils to be good for plant health and development:

1. To identify three fields with a history of fungal and/or oomycete soil-borne diseases and quantify the presence of up to six intractable soil pathogens by qPCR.
2. To carry out physical, chemical and biological assessments of the field soils in tandem with sampling for molecular assay and seek to determine any relationship between these.
3. To record changes in the soil microbiome following the use of soil amendments and determine any relationship between the microbial population composition and levels of disease in the crop.

Work on soil-borne diseases was carried out as part of the wider Project 7 within the Soil Biology Soil Health programme, each of the three crops studied (onion, *Narcissus* and raspberry) have been covered in separate reports. Soilborne plant pathogens are among the most important limiting factors for UK horticultural crop production and build up with repeat cropping of susceptible hosts, often surviving between crops using resting spores. In all three crops, organic material was incorporated pre-planting to determine any benefits or otherwise to crop health. In onion, reported here, two different sources of organic matter were utilised before planting; an autumn sown cover crop and from spring applied green compost. The other two crops examined were *Narcissus* (where a mycorrhizal product was applied at planting; report 91140002-07b) and raspberry (where a plant protection product containing a beneficial fungus was applied; report 91140002-07c).

Soil amendments in horticulture can be used to increase soil organic matter, nitrogen and other important minerals and nutrients for crop production, as well as improve the soil microbial diversity. This in turn can potentially lead to improved crop establishment, rooting and yield, with plants potentially more resilient to pest and disease. Some organic amendments such as composts and crop residues also have potential to make soils suppressive to specific pathogens. Although soil features have been associated with disease suppression, these are variable, interacting and complex; they include physical, chemical and biological components (Gamliel *et al*., 2000; Hoitink & Boehm, 1999; Noble & Coventry, 2005; Bonanomi *et al*., 2007; 2010; O'Neill, 2010). Cover crops can improve crop yield, environmental quality and improve soil physical, chemical and biological properties. In addition to enhancing organic matter, they can increase nutrient release, suppress weeds, and control pests. The species selected, their termination stage and termination method all have a bearing on these benefits (Adetunji *et al*., 2020).

Fusarium basal rot (caused mainly in the UK by soil-borne *Fusarium oxysporum* f. sp*. cepae*) leads to loss of onions in field and in storage. Fusarium basal rot management of *Allium* spp. using organic soil amendment was found to have potential for the reduction of this disease using a range of plant materials, although across the worldwide experiments reviewed the results could be inconsistent (Le, Audenaert and Haesaert, 2021).

3. Materials and methods

3.1. Treatment application and cover crop and onion planting

3.1.1. Site history

A site was selected, with a grower-reported history of Fusarium basal rot in the onion crops grown in it (field named Claypits, Bedfordshire). The field had not had an onion crop for three years (**Table 1**). Fusarium basal rot was reported by the grower to have occurred previously. The field was also being used in 2019 for another onion trial (by John Clarkson, University of Warwick) and the grower reported that *F. oxysporum* f. sp. *cepae* had been confirmed in the soil of this adjacent trial using molecular diagnostics.

Table 1: Cropping history of Claypits field between 2012 and 2020.

Sown / planted	**Cropping year**	**Crop**
Spring	2012 - 2013	Spring Barley
Spring	2013 - 2014	Potatoes
Autumn	2014 - 2015	Winter wheat
Summer	2015 - 2016	Onions
Autumn	2016 - 2017	Winter wheat
Autumn	2017 - 2018	Winter wheat (2nd) to August 2018
Summer	2018 - 2019	Trial area: Cover crop of Rye, Vetch & Phacelia sown August 2018 in three 11 m wide strips with 11 m unsown between strips
Spring	2019 - 2019	Trial area: Brown Onions March – August 2019 Rest of field: Quinoa
Autumn	2019 - 2020	Winter wheat

3.1.2. Treatments and plot layout

The treatments were an autumn sown cover crop, with and without a following spring application of green compost. The remaining plots were left without any organic matter incorporation. Onion sets were planted in Spring 2019, directly after the green compost addition. There were six replicate blocks of four treatments (**Table 2** & **Figure 1**).

Table 2: Organic matter treatments incorporated before onion planting in April 2019 on Claypits field.

Trt. Number	Treatment
1	Untreated
2	Cover Crop sown in August 2018
3	Green Compost applied in March 2019
4	Cover Crop and Green Compost

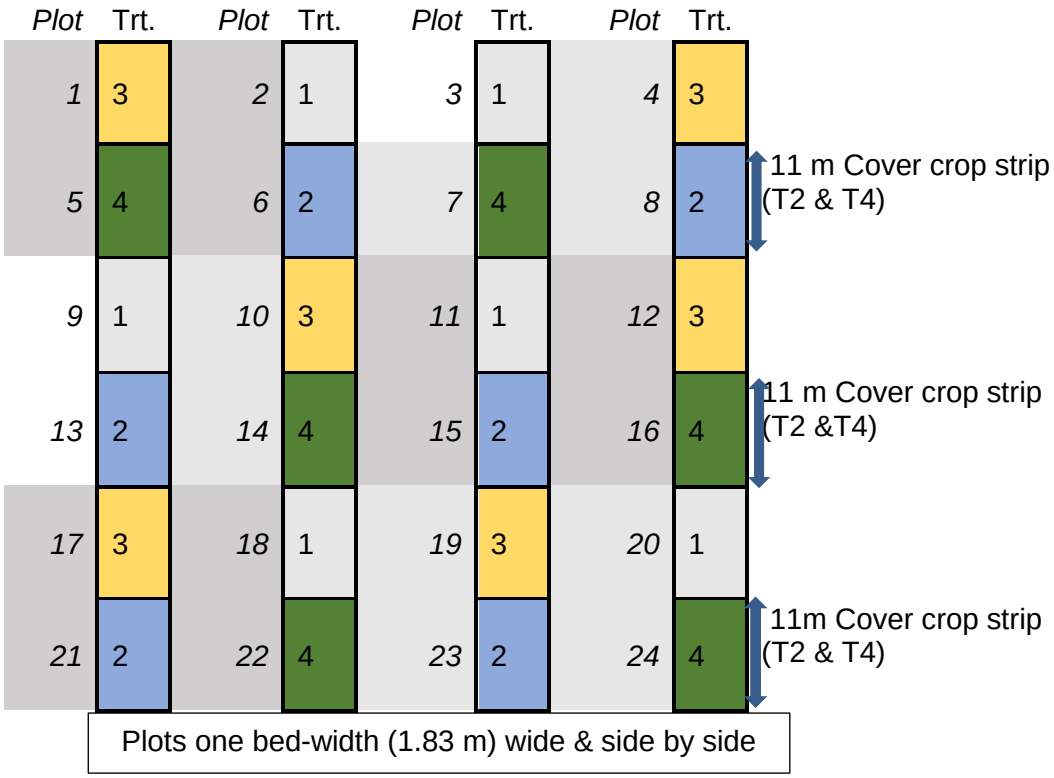


Figure 1: Layout of alternating cover crop strips in cereal stubble Autumn 2018, followed by incorporation of green compost to half the plots in Spring 2019 before onion planting. Plots 1 to 4 were at the southern end of the field, with both the previous cereal crop and the subsequent onion rows running from south to north, while the cover crop strips ran east to west.

3.1.3. Organic material addition to the soil before onion planting

Cover crop

On 31 August 2018, directly after wheat harvest (a second-wheat in the rotation), three 11 m drill-width strips of cover crop were direct drilled (using discs) into the stubble with a commercial cover crop seed mix at 35 kg/ha. Each drilled strip was commenced and finished the equivalent of a bed-width either side of the plot area to ensure full coverage in the plot area. Each strip was spaced one drill-width apart so that there were alternating strips with and without the cover crop (**Figure 1**). The cover crop selected was a mixture of 80% Rye cv. Turbogreen, 15% Vetch cv. Kwarta and 5% Phacelia cv. Stala sold as “EnviroSeeds Autumn DM”, from Boston Seeds, Lincolnshire. This was selected because it was suitable for early autumn sowing with high dry matter production. Phacelia is capable of putting on a large amount of growth in a short period of time and the three different rooting structures have been reported to have the capability of improving soil structure.

In Spring 2019, flailing off the cover crop top growth and then using a systemic herbicide (glyphosate), in order to kill any Phacelia and Vetch not already killed by frost, plus the Rye, was

considered (as standard practice) before bed formation for onion planting. However, this treatment of the cover crop strips was found not to be needed due to the absence of significant cover.

Green compost

A second source of organic matter was sourced from GreenWorld (King's Lynn), this was good quality green compost, PAS 100 certified, and graded at 0-30 mm (**Figure 2**). This supplier charged a little more for their product than elsewhere, but by restricting the feedstock sources (i.e., not taking in domestic green bin and street-sweeping waste) they had negligible plastic contamination and also took out as much as they could of non-organic waste before putting the material through the shredder. An analysis of the material was obtained in advance to guide the rate of application, and a sample of the batch used was also sent for nutrient analysis. Analysis included dry matter, organic carbon, organic matter, N, P, K, Mg, S, Ca and pH.

The green compost comprised a high content of dry matter, the majority of the medium being made up of shredded tree and bush branches (**Table 3**).

Table 3: Dry matter % and nutrients calculated for fresh weight (FW) and % organic carbon content of dry matter (DM) for green compost applied as two of the treatments (with and without cover-crop) to Claypits field on 7 March 2019.

Property	**Green Compost**
Dry matter (%)	76.8
Total N (kg/t FW)	12.5
Readily available N (kg/t FW)	0.69
P_2O_5 (kg/t FW)	5.79
K_2O (kg/t FW)	8.6
MgO (kg/t FW)	3.2
SO_3 (kg/t FW)	4.28
Organic C % DM	14.2

On 7 March 2019, weighed amounts of the compost was applied by shovel to half the plots marked out within replicate blocks; half with (T4) and half without (T3) having had the cover crop (**Figure 2**). A quarter of the plots were thus left untreated (T1) and another quarter only had the cover crop (T2) (**Table 2** & **Figure 1**).

The material contained 8 kg of total Nitrogen per fresh tonne. Working to a maximum application of 250 kg/ha of total N, the rate 30 t/ha was set, with 60 kg to be applied to each 20 m^2 plot (to give a treated length of 11 m). On delivery, the 1 tonne dumpy bag filled completely was found to only hold enough to apply 43 kg of compost per plot (or 21.5 t/ha). This reduced amount of compost was

spread across the designated plot areas leaving out a 0.4 m strip along each side of the plot area and 1 m at either end, giving a length of 9 m per plot for crop assessments. Some movement of the compost within the whole plot area was anticipated to occur with the cultivations for bed preparation.

The green compost was added onto plots so that replicates of treatments were arranged as square blocks so that each replicate block included two plots from the 11 m wide cover crop strip and two plots above them not sown with cover crop (**Figure 1**). There was no discard between strips.

a) Green compost as delivered b) Weighing out the green compost prior to spreading

Figure 2: Green compost that was applied to half the stubble without cover and half of the cover crop plots on 7 March 2019, note the size of wood fragments and no visible plastic contamination.

3.1.4. Onion planting

Prior to planting, a sample of 30 onion sets were cut open and incubated in moist conditions, and this determined that they were healthy and free of *F. oxysporum*.

Four 1.83 m wide beds were cultivated and formed side by side at right angle to the 11 m cover crop strips. This resulted in a trials area of 7.32 m x 66 m. Two further bed-widths were left as discard without and onion crop either side of the treatment plots and a further discard was left at the top and tail of the treatment beds. The machine passes for bed-making, which incorporated both the cover crop and the green compost, were done just before onion set planting.

The brown onion sets cv. Rumba were planted on 1 April 2019, at the grower's standard density in five rows per bed to an approximate 150 mm depth. About 550 onions established per 11 m row. Subsequent N:P:K fertiliser and foliar fungicides, and herbicides were applied as standard. The surrounding crop was quinoa.

3.2. Meteorological records

Meteorological data was obtained for the period of the trial from “METMAKER” and mean daily air temperature calculated. The rainfall data for each day related to total in the period from 09:00 to 09:00 the next day.

3.3. Soil sampling

Pre-trial soil assessment

The field was sampled on 7 December 2017 to determine the presence of onion pathogens and free-living nematodes (FLN). Nematode species were extracted, identified and counted by ADAS, but not repeated after trial establishment for treatment records.

Both *Sclerotium cepivorum* and *Fusarium oxysporum* and bacterial and fungal presence in general were quantified by Fera using qPCR.

After cover-crop establishment soil assessments

On 4 December 2018, a representative sample of topsoil (0-15 cm depth) was taken from both the cover crop and the stubble treatment areas and sent for nutrient analysis.

Soil samples from each of 24 areas of 1.83 m x 11 m, that were marked out in the stubble and destined to become the treatment plot locations, were also taken on 4 December by Fera as part of Project 5 for quantitative molecular assay (qPCR) for soil-borne pathogens and overall bacterial and fungal populations (Elphinstone *et al.,* 2022; report 91140002-05). For qPCR analysis, DNA was extracted from 10 g sub samples of thoroughly mixed soil using the DNeasy PowerMax Soil Kit (Qiagen, Netherlands) according to the manufacturer’s instructions. qPCR was performed using *Fusarium oxysporum*, *Sclerotium cepivorum*, bacterial 16S rRNA and fungal 18SrRNA assays to quantify populations of specific pathogens, total bacteria and total fungi. For metabarcoding analysis, DNA was extracted using the full DNeasy Powersoil kit (Qiagen, Netherlands) procedure on 10 g subsamples of thoroughly mixed soil from each sample. Metabarcoding of bacterial 16S rRNA and fungal 18S rRNA barcodes was performed. The *F. oxysporum* detected was generic i.e., the *formae speciales* causing basal rot of onion (*F. oxysporum* f.sp*. cepae)* was anticipated to be quantified alongside other host-specific formae speciales in the species complex and potentially any non-pathogenic *F. oxysporum* isolates present.

Pre-onion harvest soil assessments

A further set of samples were taken for qPCR only, just before the onion harvest in August 2019. The same quantification techniques were used as were done in December (before onion planting in March 2019) in the plots with and without cover crop.

Post-onion harvest soil assessments

On 18 November 2019, once autumn wheat had established in the field, topsoil (0-15 cm) samples were taken for nutrient and FLN analysis from the positions of the six strips in November 2018, with and without the cover crop (whether or not they received green compost in spring 2019). The samples were sent to external laboratories (NRM and Hillcourt) for the determination of pH, extractable Phosphate (P), Potassium (K) and Magnesium (Mg), organic matter (loss on ignition and Dumas methodologies), total Nitrogen (N), respiration (CO_2-burst) and potentially mineralisable Nitrogen (PMN). Four pits for VESS and earthworms were dug to give an assessment across the trial area.

3.4. Onion crop establishment and Fusarium yellowing assessment

Disease assessment on the onions commenced on 16 July 2019 when wilting, which was likely to have been as a result of *F. oxysporum* infection, started to be seen. Initially a random sample of 50 plants per plot from the central three rows were examined and the number yellowing counted. As the proportion was found to be low, another count was done to record the total number of plants per plot with the foliar yellowing typical of early stage Fusarium basal rot.

3.5. Onion Fusarium wilt assessment at harvest

Onion bulb marketability was assessed on 13 August 2019. One hundred onions were inspected per plot. These were taken from within three 0.5 m lengths of the inner three crop rows, each length containing 30 to 35 onions. These were classed as marketable when no foliar disease/wilt symptoms were present. Throughout the assessment, a range of onions were pulled up and bulbs cut open to look for the typical internal basal discoloration caused by *F. oxysporum*, to confirm that the foliar yellowing and wilting symptoms being recorded correlated to visual internal infection of the onion base.

4. Results

4.1. Cover crop establishment

After the cover crop was drilled on 31 August 2018 the weather was very dry and by 4 December 2018 some areas had only about 1% coverage (**Figure 3**). The Vetch and Phacelia plants were much smaller than seen on another field on the farm drilled a fortnight earlier. All the plots had well established tillering cereal volunteer growth in the stubble and there was no obvious increase in monocotyledonous plant coverage from the Rye component of the cover crop. Consequently, by 4 December the three 11 m wide bands of cover crop that had been drilled 11 m apart at right-angles to the cereal crop tractor tramlines were indistinct (**Figure 4**). Cover crop growth did not improve over the winter and with losses to frost it was unnecessary to flail it or treat the strips with systemic herbicide. The whole area was sprayed off prior to bed preparation for the onions which were planted with the rows in the same direction, within the same wheelings, as the previous cereal crop.

Figure 3: Poor growth of cover crop Vetch and Phacelia on 4 December 2018 three months after drilling strips across the field into cereal stubble for two of the treatments. Viewed from the side to show plant height, and from above to show plant distribution. Claypits field.

a) Looking south from the eastern flank of the trial area (near plot 21) in December 2018. (Trial area extends to the right of the picture).

b) Looking north from the eastern flank of the trial area (near plot 1) in December 2018. (Trial area extends to the left of the picture).

Figure 4: Claypits Field showing mainly cereal volunteer growth on the trial area and barely visible 11 m plot length cover cropping sown at right angles to the stubble alternating with unsown strips.

4.2. Onion crop establishment & wilting

By the 9 May 2019, the onion sets planted on 1 April had produced foliage relatively evenly across the four beds of plots (**Figure 5**).

Figure 5: Onion growth (5 rows/plot) on 9 May 2019, five weeks after planting in Claypits field.

4.2.1. Foliar yellowing of onions by 16 July 2019

By 16 July 2019, mid-way through the growing season, very few plants had started showing foliar yellowing symptoms as a result of Fusarium basal rot. The random sample of 50 plants per plot from within the centre three rows contained from zero to three symptomatic plants and there were no significant (P>0.05) treatment differences, with a mean 1.5% of plants affected (**Table 4**).

Table 4: The proportion of plants yellow within 50 plants from the centre three rows and the number yellow across each of the plots (of around 2250 plants) on 16 July 2019, at Claypits.

Fusarium 16 July 2019	Treatments						15 df
	Untreated	Cover crop	Green compost	Cover & Compost	Overall mean	L.s.d.	F value
% of plants yellowing (out of 50 / plot)	0.33	2.00	1.67	2.00	1.50	2.217	0.356
Total number of plants yellowing / plot	6.7	14.8	7.5	8.0	9.2	7.75	0.138

The count of all yellowing plants in the plot, including the outer rows, also showed no significant difference between the treatments (P>0.05). Across whole plots of assessed length of 9m about 2250 onions (50 per metre of row) there was only on average nine plants showing yellowing, so on this assessment less than 1% of plants per plot were showing symptoms of Fusarium infection. The data for the individual plots was very variable and so, although the mean number of yellowing plants for the cover crop (T2) appears high, two T2 plots had few plants affected.

No significant differences (P>0.05) were shown between the replicate blocks for either the 50 plant or the whole plot assessment. A single factor analysis and a cover crop x green compost factorial were carried out for both of the yellowing assessments to determine whether treatments either with or without the cover crop or with or without the green compost differed significantly, or whether there was any interaction but nothing significant was found.

4.2.2. Fusarium basal rot symptoms

By mid-August the bulbs were fully formed and the foliage had fallen over but was not showing natural senescence, most leaves still being green. Fusarium basal rot had progressed rapidly, with the majority of the onion field showing foliar leaf tip yellowing. Plants were examined in detail from a range of yellowing severities and cut open to assess basal discoloration (**Figure 6**). In many instances, seemingly healthy onions had very slightly yellowing tips that could be mistaken for natural

senescence, but when the onion was pulled out, one or two roots were stained purple, and when the bulb was cut open, the basal plate was becoming stained rather than a healthy white colour. Even a small length of tip yellowing produced an obvious browning towards the base plate (**Figure 6c**). Badly affected onions had only dry brown leaves (**Figure 6a & b**).

a) b) c)

Figure 6: Onion with dead leaves with inside rotting (a and b). Slight basal discoloration inside an onion (c), making it unmarketable, from a plant with only leaf tip yellowing. 13 August 2019

On severely infected onions, many roots had a purple stained colouration and when pulling the bulbs out of the ground these roots usually snapped off (**Figure 7**).

a) Leaf yellowing. b) Fusarium root purpling.

Figure 7: Severe Fusarium basal rot in onion, with typical leaf yellowing and purple roots. At harvest of the trial on 13 August 2019, Claypits field.

A sample of onions were taken back to the ADAS laboratory, where they were cleaned, and incubated at ambient temperature to confirm the presence of *Fusarium oxysporum* (**Figure 8**). Mycelium and spores typical of *F. oxysporum* developed on the basal plates and isolations were made and sent to Fera for species confirmation using PCR. *Fusarium oxysporum* was confirmed, although it was not possible with the molecular assay used to confirm that it was *F. oxysporum* f. sp. *cepae.*

a) b) c)

Figure 8: Incubation of onions that had just started showing yellowing leaf tips (early symptoms), indicative of Fusarium basal rot. Day 0 (a), Day 5 (b) and a close-up photo (c) of white *Fusarium oxysporum* mycelium growing out from the onion basal plug. Sampled 13 August 2019.

4.2.3. Onion marketability assessment

On 13 August 2019, one hundred onions per plot were assessed for marketability. Basal rot symptoms were developing rapidly, showing leaf tip yellowing and internal rotting of bulbs. Marketability was based on the absence of foliar yellowing that could be attributed to the effect of *F. oxysporum* root and basal plate infection (**Figure 9**). After the initial check-sampling of a range of leaf symptoms against bulb internal symptoms, unless there was doubt about the cause of leaf yellowing, onions were not uprooted and cut open to check the basal plate for the absence of staining to determine marketability. Commercially, any onion showing any symptoms of basal rot would be rejected.

Figure 9: Assessing leaf yellowing caused by Fusarium basal rot of the onions. 13 August 2019.

During the marketability assessment, the majority of plants had clear foliar symptoms, where early symptoms showed as yellowing leaf tips, starting with older leaves. Onion bulbs were either deemed as going to be marketable or unmarketable, depending on whether their leaves were symptomatic or not. As the pathogen infects plants at different stages of growth, the size of the onion did not relate to the progression of the disease. Bulb weights were not assessed.

The majority of bulbs in all plots were unmarketable, ranging from zero to only 23 bulbs marketable out of the hundred per plot assessed. Further symptoms were likely to develop as the crop dried for brown onion harvest. With such a high incidence of visibly infected bulbs the whole crop would be rejected; the grower determined the crop was not worth harvesting so the onions were left in the field, and the subsequent cereal was direct drilled into the beds (without cultivations).

No significant treatment differences were found ($P>0.05$), with a mean 9.5% of the bulbs marketable at the time of the assessment (**Table 5**). No significant differences ($P>0.05$) were shown between the replicate blocks.

Table 5: The proportion of marketable bulbs per treatment out of 100 per plot (six replicates) assessed for foliar yellowing on 13 August 2019. Claypits field.

Yield measure 13 August	Treatments					15 df	
	Untreated	Cover crop	Green compost	Cover & Compost	Overall mean	L.s.d.	F value
% of onions marketable (out of 100 / plot)	11.67	9.00	8.00	9.33	9.50	5.843	0.601

4.3. Pathogens, nematodes, bacteria and fungi in soil samples

Greater detail on the molecular analyses that were carried out to determine pathogens, bacteria and fungi in the soil is available within the Project 5 report (91140002-05).

Pre-cover crop soil sampling

The soil sampled from Claypits field in December 2017 was confirmed to be heavily infested by *Fusarium oxysporum*, but the *formae speciales* (which relate to the specific host) were not able to be determined with the method used. No *Sclerotium cepivorum* (the fungus causing white rot) was detected (**Table 6**). *Verticillium* spp. microsclerotia were not checked for, as these pathogens do not cause disease in onions.

Table 6: Molecular (qPCR) results from soil sampled on 7 December 2017 from Claypits field during a second winter wheat crop. Onions had preceded the wheats.

Sclerotium cepivorum (pg/g)	*Fusarium oxysporum* (pg/g)	Bacteria U16S (Ct)	Fungi FQ (Ct)
0	438.6	13.68	20.66

Ct (cycle threshold) the number of cycles required for the fluorescent signal to exceed background levels in real time PCR assays. The lower the Ct level the greater the amount of target nucleic acid in the sample. A positive reaction would have a Ct of 36 or less (Anon, undated).

Free living nematodes were quantified by extraction and visual assessment from a proportion of the soil sampled from a wheat crop growing in Claypits on 7 December 2017. No *Ditylenchus* sp. (Stem lesion nematodes), *Longidorus* sp. (Needle nematodes), *Xiphinema* sp. (Dagger nematodes), *Trichodorus* sp. (Stubby root nematodes), *Meloidogyne* sp. (Root knot nematodes), or *Heterodera* sp. juveniles (Potato cyst nematodes) were present. Per one litre of soil there were 525 *Tylenchorynchus* sp. (Stunt nematodes) and 250 *Pratylenchus* sp. (Root lesion nematodes) with a soil dry weight of 395.1 g / 200 ml. However, thresholds for onion losses are only acknowledged by the ADAS sample laboratories for stem, stubby root, and needle nematodes, which can be virus vectors, and so any presence would have been of significance to the crop.

Within cover-crop, pre-onion, soil sampling

The samples taken from each of the 24 plots (half with, half without cover crop) on 4 December 2018 were analysed for microbial content using qPCR. *F. oxysporum* was detected at relatively similar levels of between 0.143 and 1.550 fg DNA per kg soil in all plots of the trial prior to planting onion, following two winter wheat crops since a basal rot infected onion crop. These levels of DNA thus appeared to be sufficient to cause a mean 90.5% of bulbs to show symptoms of foliar wilting attributed to basal rot, without significant difference in levels between treatments. No correlation was observed between *F. oxysporum* DNA levels detected in the individual plot's soil before onion planting and the disease incidence (ranging between 77% and 100% of onions affected) recorded in the onions of same plots at harvest ($R^2 = 0.0066$).

Pre-planting (of the onion crop) quantification of total fungal DNA in the plots showed 18SrRNA levels ranging from 0.7242 to 2.176 pg DNA per kg soil, except for two spikes from different treatments; plot 5 (recording 7.303 pg DNA per kg soil) and plot 23 (4.8 pg DNA per kg soil).

Within onion crop, pre-harvest soil sampling

By August 2019, the amount of target *F. oxysporum* DNA detected in the soil by qPCR had increased between 2- and 9-fold during the onion cropping season. The lowest level was 1.493 fg DNA per kg and an outlier plot had 8.516 fg DNA per kg of soil. However, all but five of the 24 plots had below 5 fg DNA per kg of soil. The higher ranging plots belonged to each of the four treatments, and there was no other obvious correlation of particular levels with treatments. No pattern was apparent in the position of higher or lower DNA containing plots in the field.

Unfortunately, a qPCR assay for specific detection of *F. oxysporum* f. sp. *cepae* did not become available within the life of Project 5 and it was not therefore possible to measure the exact population dynamics of this onion pathogen so it's level in the soil may have been over-estimated due to presence of other *F. oxysporum* variants.

Within-crop quantification of total fungal DNA in each of the 24 plots showed levels above those detected pre-planting, except for in plot 5 (cover crop plus compost) and plot 12 (compost). 18SrRNA levels ranged principally from 1.611 to 3.963 pg DNA per kg of soil. Plot 12 was lower than this at 0.527 pg DNA per kg soil. A high value was again seen for plot 23 (cover crop) with 6.230 pg DNA per kg soil. There was no obvious pattern of particular fungal levels for plots with or without earlier cover cropping or having received green compost.

Comparison of the relative abundances of amplicon sequence variants (ASVs) identified by metabarcoding of bacterial 16S rRNA barcodes were made as part of Project 5 (report 91140002-05). The same five species comprised around 25% of the bacterial species in each sample, with

around 50 other species comprising the other 75%. Within the different taxa identified at species level, no significant differences were found between duplicate soil samples from the 24 plots, indicating a uniform diversity of bacteria across the trial site.

Post-onion trial soil sampling

The topsoil samples taken for free-living nematode extraction on 18 November 2019, (after the onion marketability assessment and when winter wheat had again been sown), showed a greater number of *Tylenchorynchus* sp. / *Helicotylenchus* sp. across one half the trial area than the other, with a distinct increase as samples were taken further from the southern end (moving down the field from plots 1 to 4) (**Table 7**). The trend was not apparently affected by the alternating strips of poorly established cover crop (T2 and T4) and no cover crop (T1 and T3) that had been present from September 2018 to March 2019. Each of the strips had green compost on T3 and T4 plots so no comparison can be made for this treatment alone. The strips had fewer *Pratylenchus* sp. than the 250/L counted two years earlier in the field. However, both *Trichodorus* and *Meloidogyne* spp. were now recorded in this later sample, with no obvious pattern to the densities recorded across the trial area (**Table 7**).

Table 7: Free-living nematode species counted per litre of soil taken from each of the 11 m plot lengths combined across each strip with cover crop (shaded green) and without it sown before the onions. Claypits field,18 November 2019 in winter wheat crop following onions.

		Number of each nematode species / L soil							
Posit-ion	Treat-ments	Trichodorus	Tylencho-rynchus	Heterodera	Pratylenchus	Longidorus	Xiphinema	Ditylenchus	Meloidogyne
Strip 1-4	T1 & T3	25	125	0	50	0	0	0	50
Strip 5-8	T2 & T4	25	125	0	25	0	0	0	0
Strip 9-10	T1 & T3	50	275	0	125	0	0	0	50
Strip 13-16	T2 & T4	50	500	0	100	0	0	0	150
Strip 17-20	T1 & T3	25	1025	0	50	0	0	0	0
Strip 21-24	T2 & T4	25	2325	0	50	0	0	0	125

4.4. Soil sample results

Pre-onion trial soil sampling for soil health measures

Samples were taken on 4 December 2018 from where cover crop was growing (thinly) and where it had not been sown. There was no replication, but the soil organic matter was 2.6 without the cover crop and 2.9 with it (Appendix **Table 9**).

Post-onion trial soil sampling and soil health scorecard

By November 2019, although the cover-crop establishment in August 2018 had been sparse and patchy and subsequent growth (height and spread) of the Vetch and Phacelia was smaller than seen elsewhere on the same farm, a range of measurements were numerically higher in the strips that had a cover crop compared to those which did not (**Table 8**). However, no significant differences were shown by ANOVA comparing with and without the cover crop (2 df), although the differences in extractable P and SOM were significant at P<0.1. The sampling pattern adopted (to obtain soil from plots in the three strips which had had a cover crop, and the three strips which had not had a cover crop, irrespective of whether or not green compost had subsequently been added) meant that the effect of compost addition could not be determined. Details for individual strips are given in the Appendix **Table 10**. VESS and earthworm counts were only undertaken on a site basis, with the VESS score indicating a good soil structure, whereas earthworm numbers were depleted.

Table 8 : Soil health scorecard from 18 November 2019 for Claypits field after the onion crop had been replaced by winter wheat, based on a medium soil type (21% clay) in a low rainfall region and P values close to significance at P <0.01 following analysis of variance of means (2 d.f.).

Attribute	No cover crop in 2018	Cover crop in 2018	ANOVA P value	ANOVA L.s.d. of means
pH	6.4	6.6	n.sig.	-
Ext P (mg/l)	33.8	35.7	0.057	2.008
Ext K (mg/l)	243.3	263.0	n.sig.	-
Ext Mg (mg/l)	107.3	111.4	n.sig.	-
SOM (% LOI)	1.9	2.1	0.074	0.284
VESS score (limiting layer)	1.5		n.a.	-
PMN (mg/kg)	20.6	24.5	n.sig	-
CO_2-C (mg/kg)	68.3	81.3	n.sig	-
Earthworms (No./pit)	2		n.a.	-

Mean results colour-coded according to the soil health scorecard protocol:
Red = Investigate, Amber = review, and Green = continue rotational monitoring

4.5. Weather

Throughout the period from cover crop establishment in 2018 to onion harvest a year later, conditions differed from the seasonal averages, with both temperature peaks and extremes of rainfall (**Figure 10**). The early winter of 2018 was mild, followed by a dry January. In 2019 a warm and particularly dry period in April followed onion planting. An exceptional amount of rain fell on 10 June 2019 which would have puddled on the clay soil and facilitated Fusarium spore dispersal in the soil and spore survival on germination. The 25 July 2019 was exceptionally hot, which would have increased the water stress of plants with reduced root area due to Fusarium infection leading the high incidence of leaf yellowing and necrosis recorded on 13 August 2019.

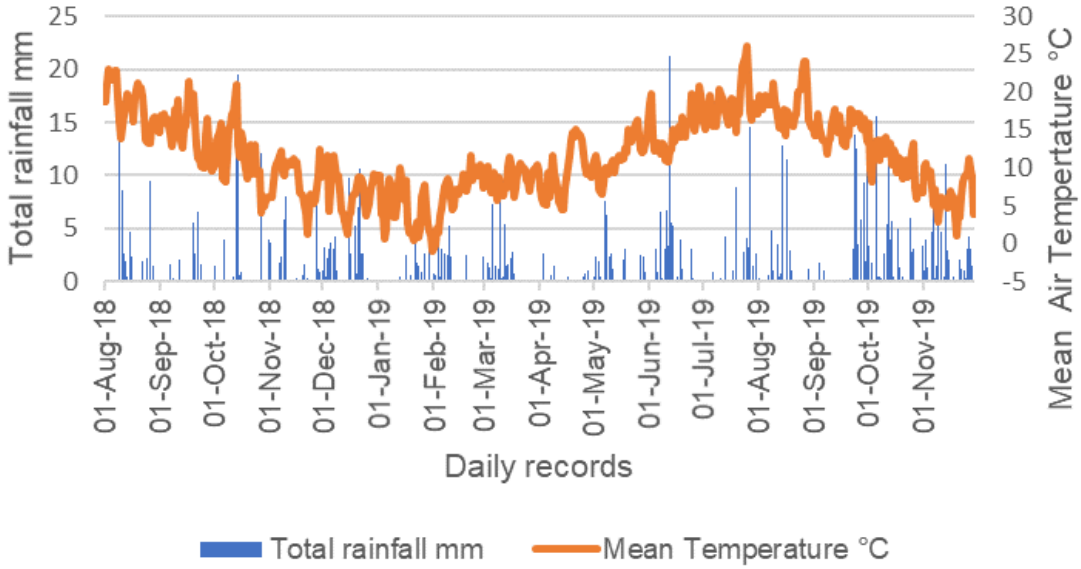


Figure 10 : Mean daily air temperature and the total depth of rain per day (1 August 2018 to 29 November 2019) in the period between cover-crop drilling and the topsoil sampling after the onion harvest assessment and with the subsequent cereal crop growing. Data for Claypits Field obtained from “METMAKER”.

5. Discussion

The species mix selected for the cover crop included a high proportion of Rye which would normally give a tall stand able to withstand winter frosts. Phacelia also usually quickly produces a dense foliage, although it often succumbs to frost, as does Vetch. Each of the three species have different rooting structures which can potentially help improve soil structure. However, the window for establishing an autumn cover crop after winter cereal harvest is narrow. In this project it was likely that moisture in the soil allowed seed germination but the subsequent fortnight without rain in early September led to a low survival rate. Had the Rye grown, then an extra farm operation of flailing in Spring might have been needed. The cost of the seed, the species chosen and need for extra machinery operations needs to be considered if cover cropping is to be used to increase soil organic matter. In the current trial, the ground cover of cereal volunteers provided some organic matter input after spraying-off in Spring.

The addition of organic matter, either from the cover crop or the green compost, was not anticipated to be able to be sufficient to improve crop vigour enough to resist infestation by *F. oxysporum* as this is a very aggressive disease which by attacking the basal plate of the onion bulb kills both foliage and roots. A recent review of Fusarium basal rot in *Allium* spp. concluded that although many studies have shown a great potential of compost amendments and organic matter in reduction of the disease, the effectiveness of this technique is inconsistent and unpredictable (Le, Audenaerte & Haesaert, 2021). The quantity of compost allowed is limited in practice because of the nitrogen content (with 250 kg of N/ha able to be applied) and so this can only give a thin covering of the soil surface. Over a number of years of repeat compost application (with consideration of final soil nitrogen levels) this should increase the moisture holding capacity of the soil. The plants might then be able to bulk up quickly and become ready for harvest thereby "escaping" from the effects of the pathogen, provided the level of invasion from *F. oxysporum* resting spores in the soil had been reduced by a suitable rotation without onions.

In AHDB Project CP 196 (Clarkson *et al.,* 202) qPCR detection of *F. oxysporum* f.sp. *cepae* across four soil types was only consistent for inoculum levels ≥1 x 10^4 colony forming units/g of soil. However, onion basal rot disease development only occurred at inoculum levels of ≥1 x 10^5 cfu /g of soil for this experiment at least and so it was suggested that the qPCR would be able to predict disease in a pre-planting test. Amplicon sequencing to quantify *F. oxysporum* and other microbe species in soil was also used in Project CP 196: when *F. oxysporum* f.sp. *cepae* was introduced into different soil types, the pathogen was successfully detected using different gene targets including ITS (to genus level), TEF (to species level) and OG4952 (to F. oxysporum f.sp. level) with a similar detection threshold as for the qPCR (≥1x10^4 cfu g soil) across all the four soils. In the current project it was not possible to relate the initial *F. oxysporum* DNA levels detected in the soil to the level of disease seen later in the plots as there was little difference in basal rot incidence with most onions affected. The qPCR assay for specific detection of *F. oxysporum* f. sp. *cepae* developed within Project CP 196 should be used in any future work to measure the exact population dynamics of the onion pathogen, to avoid over estimation due to presence of other *F. oxysporum* variants.

Within Project CP 196, amplicon sequencing showed the four soils tested could be distinguished based on the structure, diversity and identity of components of the bacterial, fungal and *Fusarium* spp. communities. However, as their basal rot development was generally similar, then no specific components of these differing microbial communities could be associated with basal rot suppression. Within the current SBSH Project, at least 50 different bacterial taxa were detected by metabarcoding of bacterial 16S rRNA barcodes in the soil of the onion crop, with each plot presenting a different "pattern" of relative abundance of each species, but much more work will be needed to determine any relevance of this within the soil community, preferably where the treatments used are shown to have an effect on plant health. Further research is needed to determine if improvements to soil

health that may lead to changes in the soil biological community (bacteria, fungi and invertebrates) can lead to a reduction of Fusarium in the soil by outcompeting and / or consuming it.

A secondary, but important issue, concerning encouraging uptake by farmers and growers of green compost is the problem of contamination with non-biodegradable waste. This is particularly a problem where green waste intake includes council street sweepings and household green bins as well as waste from arboriculture. The only way to remove plastics is by hand-sorting which is near impossible with regular deliveries into composting mounds, and the shredding machines for the wood scatter fragmented rubbish through the heaps. Sites have to pay to get rid of mixed-plastic waste. Here we managed this risk by using compost of size grade 0 – 30 mm from a composting site that actively restricts intake to manage contaminants and had good quality control of the product.

6. References

Adetunji, A., Ncube, B., Mulidzi, R., & Lewu, F. (2020). Management impact and benefit of cover crops on soil quality: A review. Soil and Tillage Research 204, 104717. https://doi.org/10.1016/j.still.2020.104717

Anon (undated). Wisconsin Veterinary Diagnostic Laboratory. University of Wisconsin-Madison https://www.wvdl.wisc.edu/wp-content/uploads/2018/05/What-does-CTmeanfinahandoutlJanuary2014.pdf

Bonanomi, G., Antignani, V., Pane, C. & Scala, F. (2007). Suppression of soilborne fungal diseases with organic amendments. Journal of Plant Pathology 89, 311-324.

Bonanomi, G., Antignani, V., Capodilupo, M. & Scala, F. (2010). Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. Soil Biology and Biochemistry 42, 136-144.

Clarkson J., Jackson, A, Bates, H., Bennett, J., Deakin G., Armitage, A (2020). Defining relationships between *Fusarium oxysporum* inoculum level, quantitative molecular diagnostics, microbial community composition and basal rot development in different soils to enable disease prediction in bulb onions. AHDB Project CP 196.

Elphinstone, J., Chapelhow, E., Vicente, J., Wedgwood, E. & Bhogal, A. (2022). Development and demonstration of robust DNA-based measures for routine monitoring of soil borne disease and identification of best management practices to minimise disease risk. Project Report No. 91140002-05. Great Soils. AHDB BBRO.

Gamliel, A., Austerweil, M. & Kritzman, G. (2000). Soil amendments: a non-chemical approach to the management of soilborne pests. Crop Protection 19, 847-853.

Hoitink, H.A.J. & Boehm, M.J. (1999). Biocontrol within the context of soil microbial communities: a soil dependent phenomenon. Annual Review of Phytopathology 37, 427-466.

Le, D., Audenaert, K., & Haesaert, G. (2021). Fusarium basal rot: profile of an increasingly important disease in Allium spp. Tropical Plant Pathology 46, 241-253. https://doi.org/10.1007/s40858-021-00421-9

Noble, R. & Coventry, E. (2005). Suppression of soil-borne plant diseases with compost: a review. Biocontrol Science & Technology 15, 3-20.

O'Neill, T.M. (2010). A summary report on useful information on soil disinfestation gathered for UK growers from the XIIth International Symposium on Chemical and Non-chemical Soil and Substrate Disinfestation. Horticultural Development Company. Technical report, 27 pp.

7. Appendix

Table 9 : Soil samples taken from areas with and without cover crop on 4 December 2018 in Claypits field after the cover crop was drilled in August 2018, before onion planting in April 2019.

Treatment	Texture	% clay	pH	Ext P mg/L	Ext K mg/L	Ext Mg mg/L	SOM %LOI	$CaCO_3$ %	CO_2-C mg/kg
no cover	Sandy clay loam	19	6.3	31	192	113	2.60	<1	76
cover crop	Sandy clay loam	19	6.5	37	183	114	2.90	<1	105

Appendix

Table 10: Soil analysis of samples combined across four plots within strips that were either with or without cover crop on18 November 2019. Information was used to create a soil health scorecard (Table 8).

Plots	Treat-ment No.	Treat-ment	Text ure	% sand	% silt	% clay	pH	Ext P mg/L	Ext K mg/L	Ext Mg mg/L	Ext. Na mg/L	Ext. Ca mg/L	SOM % LOI	Total N %	Ca CO_3 %	SOC % (Dum)	SOM % - calc	CO_2-C mg/kg	PMN mg/kg
1,2, 3,4	T1, T3	no cover	Clay Loam	50	30	20	6.4	34.8	252	81	22.0	881	1.41	0.094	<1	1.1	1.9	66	20.6
5,6, 7,8	T2,T4	cover crop	Clay Loam	45	34	21	6.3	37.4	254	79	20.5	746	1.45	0.093	<1	1.1	2.0	86	25.4
9,10, 11,12,	T1, T3	no cover	Clay Loam	44	35	21	6.6	33.8	261	114	26.8	1071	1.44	0.098	<1	1.1	1.8	53	23.8
13,14, 15,16	T2,T4	cover crop	Clay Loam	50	31	19	6.6	34.8	278	110	24.3	1102	1.35	0.108	<1	1.2	2.1	79	24.3
17,18, 19,20,	T1, T3	no cover	Clay Loam	42	35	23	6.3	32.8	217	127	27.6	1240	1.81	0.105	<1	1.1	1.9	86	17.
21,22, 23,24	T2,T4	cover crop	Clay Loam	41	34	25	6.8	34.8	257	145	30.7	1480	1.66	0.112	<1	1.2	2.1	79	23.7