# PURE

## Pesticide Use-and-risk Reduction in European farming systems with Integrated Pest Management

Grant agreement number: FP7-265865

**Collaborative Project**
**SEVENTH FRAMEWORK PROGRAMME**

# D10.3

# Recommendations on the potential of cultivation methods to achieve pathogen soil suppression in wheat based farming systems

**Due date of deliverable:** M 36

**Actual submission date**: M 37

**Start date of the project**: March 1st, 2011 **Duration**: 48 months

**Workpackage concerned:** WP10

**Concerned workpackage leader: Dr. Graham Begg**

**Organisation name of lead contractor: DLO**

| **Project co-funded by the European Commission within the Seventh Framework Programme (2007 - 2013)** | |
|---|---|
| **Dissemination Level** | |
| **PU** Public | **PU** |
| **PP** Restricted to other programme participants (including the Commission Services) | |
| **RE** Restricted to a group specified by the consortium (including the Commission Services) | |
| **CO** Confidential, only for members of the consortium (including the Commission Services) | |

# Table of Contents



## 1. Summary

A wide range of soil biota as well as disease suppressive properties of the soil were assessed in a field trial with a winter wheat based rotation. The objective was to detect shifts in the communities of different soil biota due to cultural and management strategies. Such information could facilitate the design of management strategies to promote microbe-mediated soil suppressiveness toward soil-borne pathogens.

Two management systems were compared: (1) a control system including ploughing before sowing which is the currently applied system in the region, and (2) an innovative system targeting a better energy ratio, less greenhouse gas emissions, time saving for farmers and reduction of inputs especially nitrogen fertilization and pesticide applications. Both crop rotations consisted of winter wheat, winter oilseed rape, sugar beet and faba bean, but the innovative system had an additional crop linseed and cover crops between the main crops.

Soil management strategies and crop rotation influenced the communities of bacteria, fungi including arbuscular mycorrhizal fungi, and nematodes in soil. Community shifts could be either due to the preceding crop, or due to the management system. Soil suppressiveness differed for 1 out of 3 soil-borne pathogens tested. However, in general the natural soil in the assessed field was quite suppressive against the diseases, and several antagonistic bacteria (*Pseudomonas* and *Lysobacter*) were isolated from the soil samples.

The results showed the influence of management practices on soil biota and soil suppressiveness, indicating the potential of ecological engineering approaches to IPM through habitat manipulation at the field. However, distinct advises on management practices in relation to IPM will depend on the pathogens present in the field and the environmental conditions such as soil type, crop rotation and management. Extensive research is needed to get sufficient knowledge on the relevant soil processes before practical implementation is possible.

## 2. Objectives

The objective of Workpackage 10 is to explore the ecological processes that underpin the response of pests to changes in habitat and design ecological engineering strategies based on the management of habitat to achieve pest suppression. This workpackage will focus on ecological engineering approaches to IPM through habitat manipulation at the field, farm and landscape scales. The lack of understanding of the ecological mechanisms at play creates a bottleneck in our ability to build ecologically based IPM solutions. Workpackage 10 will address these knowledge gaps.

This deliverable from task 10.1 is focussing on soil: the action of soil microbes in suppressing pathogens will be studied at the field scale. Soil is a highly complex habitat, with an exceptional diversity of microbial life. Interactions within the microbial community can be manipulated by rotation, tillage and organic amendment (Garbeva et al., 2003). However, the responses are not yet predictable, as the mechanisms linking these to soil suppression are not fully understood (Janvier et al., 2007). The objective of task 10.1 is to investigate and design management strategies to promote microbial mediated soil suppressiveness toward soil-borne pathogens and measure changes in soil biota due to these management strategies.

Task 10.1 will provide a comprehensive picture of the response of soil biota to the cultural and management aspects of existing and future innovations in **wheat based rotations**.

## 3. Deliverable procedure

An existing long-term field trial was selected in the North of France meeting the following criteria:

- Wheat based rotation,
- Different management practices associated with a rotation based management typical of arable farming systems,
- A proper experimental design with true replicates (randomized block design with 3 replications for each treatment),
- Each crop of the rotation is present each year,
- Few years of history (changes in soil will take several years).

In this field trial two management systems are compared: (1) a control system including ploughing before sowing which is the currently applied system in the region, and (2) an innovative system targeting a better energy ratio, less greenhouse gas emissions, time saving for farmers and reduction of inputs especially nitrogen fertilization and pesticide applications. Both crop rotations consisted of winter wheat, winter oilseed rape, sugar beet and faba bean, but the innovative system had an additional crop linseed and cover crops between the main crops.

The soil was sampled in all winter wheat plots in 2 subsequent years in autumn (start of the crop) as well as in spring. The soil was then sent to the different partners for extensive analysis, focusing on the response of a wide range of biotic components of the soil system to cultural and management practices.
The following assessments were performed:

1) Information about the treatments in the field, performance of the crop, yield, physical and chemical soil parameters.

2) Ability of the soil to suppress different diseases, as well as the presence and quantity of *Lysobacter* spp. (a newly described antagonist known to be present in Dutch soils with suppressiveness against Rhizoctonia).
3) Changes in diversity and community structure of bacteria and fungi, including genes involved in antagonism. Monitoring is based on a set of DNA-based techniques.
4) Changes in diversity and community structure of Arbuscular mycorrhizal fungi (beneficial mutualistic root symbionts) were analysed using morphological and DNA based techniques (NGS Next generation sequencing).
5) The community structure of nematodes, expressed as maturity index, is proposed as indicator for soil quality aspects. Monitoring will be performed by microscopic and DNA based techniques.

These groups were selected as targets providing complimentary measures of soil quality and the general and specific diseases suppressive capacity of soil.

The data on microbial and nematode responses and crop development are analysed to test the effects of cultural and management treatments on soil responses and crop health in accordance with the established experimental design. These assessments were expected to provide a uniquely comprehensive basis contributing to new insights in the disease suppression potential of the tested management strategies and an understanding of the mechanisms responsible for these effects.

## 4. Summary of results

Treatments: the control system was ploughed before sowing in case the previous crop was Winter Oil Seed Rape (WOSR) (2011, 2012) or faba bean (2012), whereas the innovative system had no tillage before sowing. The chemical treatments including herbicides, growth regulators, fungicides and insecticides were somewhat lower in the innovative system in the cropping seasons 2011-2012 and 2012-2013, mainly due to less fungicide applications.

Disease occurrence in the field:
No major symptoms of diseases were seen on the design for all the 3 years. However, Yellow rust and Septoria were present in the cropping season 2011-2012 in the innovative system.

Harvest: Grain yields of winter wheat in the innovative system (no-tillage) were significantly lower for all preceding crops than in the control (ploughed); yield reduction was 15% in both seasons, 2011-2012 and 2012-2013.

Disease suppression:

- The inoculated pathogens produced only little to moderate disease symptoms. Inoculum density was doubled in 2012, but this higher inoculum density did not cause more disease. We suggest that the field soil is already quite suppressive towards the diseases tested. This could be due to the fact that the soil is a heavy clay soil.
- Roots of the test plants were never white. Even in the control without pathogens added, the roots had some light brown discoloration. This was not the case when using another soil from another location where no wheat had been grown for the last 30 years. Therefore, probably all kinds of wheat (minor) pathogens are present in the soil without giving distinct symptoms.

- *Pythium* reduced germination (pre-emergence damping-off) in autumn 2011 and autumn 2012 by 10-20%; no differences were seen due to crop or tillage.
- *Rhizoctonia* caused a distinct decrease in root mass with the autumn 2011 and autumn 2012 samples. There was a significant crop and tillage effect. Disease suppression was significantly higher in ploughed soil compared to no tillage.
- The root mass was clearly reduced by Microdochium, but no differences were seen due to crop or tillage.

Potential antagonistic population *Lysobacter* spp.:

- *Lysobacter* spp. were isolated from the field; species were identified as *L. antibioticus* and *L. gummosus*. These isolates appeared to inhibite *Rhizoctonia solani* on R2A-medium.
- In general, *Lysobacter* populations were lower in spring compared to autumn. This is in line with other results showing that *Lysobacter* has problems with surviving periods with low temperatures (<5°C).
- In general no big differences between treatments occurred, but there was a very remarkable difference in the no-till with WOSR as previous crop: significantly higher numbers of *Lysobacter* occurred in spring than all other treatments. It is not clear which treatment could have induced this higher population numbers which occurred both in spring 2012 and in spring 2013.

Microbial communities

- Fingerprints were made of fungal and bacterial communities and of different bacterial groups using molecular methods (PCR-DGGE).
- Strong crop effects in the rhizosphere were measured.
- There is evidence for a lasting effect of the crop on the bulk soil communities in winter.
- There is only a weak evidence for a tillage effect:
    - Some rhizosphere bands seemed to be specific for tillage
    - Soil bacterial fingerprints after oilseed rape had separate clusters for tillage and no tillage.
    - Fungal fingerprints from soil after sugar beet had separate clusters for tillage and no tillage
- Total number of colony-forming units (CFU) and antagonistic isolates did not give evidence for crop or tillage effects.

Microflora – antagonists

Functional significance of bacterial communities – presence of genes *phlD*, *phz*, *prnD* and *pltC:*

- In general, for *phlD*, *prnD* and *phz* genes, the effect of the type of sample was observed; the populations containing these genes were more enriched in wheat rhizospheres than in bulk soil.
- Tillage and crop rotation did not affect the abundance of populations containing these functional genes, but minor and transient effect on the abundance of *Pseudomonas* populations containing *phlD* was observed.

Arbuscular mycorrhizal fungal communities

- Winter wheat root fragments showed a low mycorrhization level with a mean value of 1-2%. Although presents in low densities, mycorrhiza were clearly present inside the roots , i.e. intercellular and intracellular hyphae and arbuscules; in the bulk soil also spores typical for AM fungi were observed.

- The soil AMF community was dominated by Glomeraceae followed by Diversisporaceae and Gigasporaceae families. Acaulosporaceae and Claroideoglomeraceae as well as members belonging to Archaeosporaceae, Ambisporaceae and Paraglomeraceae were rarely present.
- Besides *Glomus*, a large number of environmental and unclassified Glomeraceae were also found in arable soils. This phenomenon indicates that a phylogenetically diverse not yet identified Glomeraceae community may inhabit the high-input managed agro-ecosystem.
- The ploughed treatments favoured taxa belonging to Glomeraceae and Acaulosporaceae.
- The reduced tillage treatments favoured taxa belonging to Gigasporaceae, Diversisporaceae and Claroideoglomeraceae.
- Winter wheat with WOSR as preceding crop incremented the percentage of taxa belonging to Glomeraceae. No visible changes at family level for both faba bean and sugar beet when used as preceding crops.

Nematodes:

- Overall, the results indicate that the preceding crop has a stronger selection pressure on the nematode community than system treatment (i.e., ploughed x no-tillage).
- The fungivorous nematodes occurred in higher densities in the ploughed than in the no-tillage system.
- Examples of a strong effect of the preceding crop are:
    - With the bacterivorous genus *Eucephalobus*, numbers in WOSR as preceding crop were, in general, higher than those in faba bean which in turn were higher than in sugar beet as pre-crop.
    - Although found in small amounts, the omnivorous family of Dorylaimoidea were significantly higher in WOSR than in sugar beet in two out of 4 samples.
    - Among the plant parasites, the genus *Pratylenchus* was pre-crop-dependent, with amounts in WOSR as preceding crop generally higher than in sugar beet.
- Examples of a strong effect of due to the management systems (ploughing x no-till):
    - the number of omnivorous nematodes tended to be higher in the ploughed treatments. This largely also resulted in comparable differences between the systems in the maturity index. Higher amounts of omnivorous nematodes or a higher maturity index have been regarded as a positive soil quality attribute. This would indicate that the ploughed system certainly does not have a lower soil quality than the innovative (no-tillage) system.

## 5. Conclusions

The extensive assessment of field soil samples from a winter wheat based rotation in autumn and spring during 2 seasons showed the influence of management practices on soil biota and soil suppressiveness, indicating the potential of ecological engineering approaches to IPM through habitat manipulation at the field.

Soil suppressiveness was changed for 1 out of 3 soil-borne pathogens tested. Suppressiveness against *Rhizoctonia solani* was influenced by the management system: less disease symptoms occurred after inoculation of the pathogen into the samples of ploughed compared to reduced tillage treatments. Samples of all treatments were equally suppressive against *Pythium* and *Microdochium*. In general, the natural soil in the assessed field is proposed to be quite suppressive against the tested diseases, since inoculation with the pathogens did not result in

high disease levels. Several antagonistic bacteria (*Pseudomonas* and *Lysobacter*) were isolated from the soil samples.

Soil management strategies and crop rotation influenced the communities of bacteria, fungi including arbuscular mycorrhizal fungi, and nematodes in soil. Community shifts occurred either due to the preceding crop, or due to the management system (see summary in the table below).

Arbuscular Mycorrhizal fungal communities were influenced by the preceding crop as well as the management system. The nematode communities were in general more influenced due to the preceding crop. However, the number of omnivorous nematodes tended to be influenced by the management system with higher numbers in the ploughed treatments.

The results from the evaluated field showed that the innovative system with reduced tillage had lower grain yields, was less suppressive against Rhizoctonia, and had less omnivorous nematodes which is regarded as a positive soil quality attribute.

Relevance for the future:
In Europe, several long-term experiments aiming to improve soil quality and reduce damage caused by soil-borne pathogens through management practices, addition of organic matter, alternative crop rotations and green manure crops are carried out. Effects are detected, but there is a lack of understanding of the ecological mechanisms; i.e. soil and the interaction with its inhabitants is extremely complex, as well as invisible. Extensive research on the relevant soil processes in different soils and with different pathogens and crops in relation to management practices should be performed to allow distinct advises on management practices in relation to IPM of soil-borne pathogens.

Table: Summary of shifts in different parameters:

| **Parameter** | **Management system** | **Preceding crop** |
|---|---|---|
| Grain yield | Control > Innovative | No effect |
| *Pythium* suppression | No effect | No effect |
| *Rhizoctonia* suppression | Control > Innovative | Unclear |
| *Microdochium* suppression | No effect | No effect |
| *Lysobacter* population | All equal, except increase in spring in the innovative system with previous crop WOSR | |
| Antibiotic genes in bacterial populations | No effect | No effect |
| Bacterial populations | Some shifts | Shifts |
| Fungal populations | Some shifts | Shifts |
| Arbuscular mycorrhizal fungi | Shifts | Shifts |
| Glomeraceae and Acaulosporaceae | Control > Innovative | |
| Gigasporaceae, Diversisporaceae and Claroideoglomeraceae. | Control < Innovative | |
| Fungivorous nematodes | Control > Innovative | |
| Omnivorous nematodes | Control > Innovative | |
| Bacterivorous nematode genus *Eucephalobus* | | Higher after WOSR |
| Omnivorous nematode genus Dorylaimoidea | | Higher after WOSR |
| Plant parasitic nematode *Pratylenchus* | | Higher after WOSR |

# 6. Annex I - Field trial - Agronomic Information

Xavier PINOCHET, Patrick DEVAUX, Stéphane SCHRYVE, Nathalie LANDE (CETIOM)

**Field**

The experimental design is a comparison of 2 cropping systems with all the crops present each year. One is the classical one, the control ("Raisonné", RAI) where fields are ploughed, and the other the innovative system (INNO) where tillage is reduced. There are 3 replications of 12 modalities.

Aim: to use less energy, less ploughing

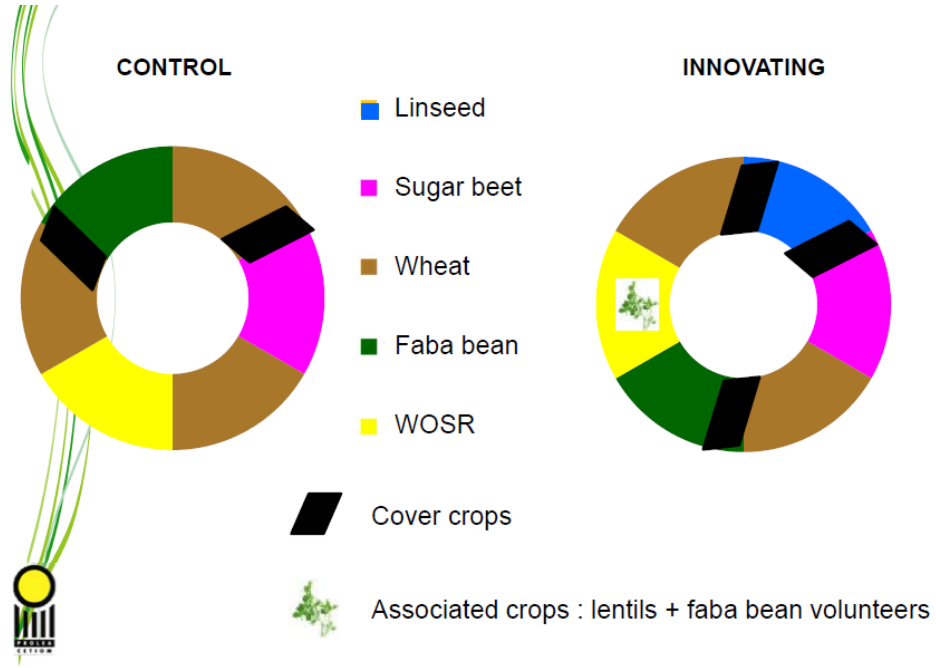
**Crop history**

2009-10 is the previous crop (winter wheat) for the all field. The experimental design started for sowings of autumn 2010. Each year, all the crops of the rotation are present. Rotations for both systems are the following:

**"Raisonné"** (RAI): WW1=>(cover crop: B. juncea) sugarbeet=>WW2=>WOSR=> WW3=>(cover crop: phacelie) Fababean

"**Innovative"** (INNO): (cover crop: oat +Vesce) Linseed=>(cover crop: trifolium + raphanus sativa) Sugarbeet=>WW1=> (cover crop: Phacelie) Fababean => WOSR (associated with gelive legumes) =>WW2

The "raisonné" rotation has 3 times winter wheat , and the "innovative" rotation twice.




| 2009-10 | Winter wheat (WW) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2010-11 | Faba bean | WW1 | Sugar beet | WW2 | WORS | WW3 | WW2 | Lin-seed | Sugar beet | WW1 | Faba bean | WOSR |
| 2011-12 | WW1 | Sugar beet | WW2 | WOSR | WW3 | Faba bean | Lin-seed | Sugar beet | WW1 | Faba bean | WOSR | WW2 |
| 2012-13 | Sugar beet | WW2 | WOSR | WW3 | Faba bean | WW1 | Sugar beet | WW1 | Faba bean | WOSR | WW2 | Lin-seed |

**Soil sampling and analysis**

Soil samples are taken in November and April during 2 seasons (2011-12 and 2012-13). Supplementary samples were collected in June 2011. Soil sampling was done only on winter wheat plots.

For bulk soil, 10 to 15 samples of 50-100 grams (0-20 cm) along a W were collected per elementary plot with an earth drill ("Tarière" in French). Half of the Ws were kept separately.

For rhizosphere soil, twice 5 consecutive plants were taken.

Samples were sent to the other partners of WP10.1.

Soil analysis were done each year on sugar beet plots after the harvest of the previous crop (Winter Wheat for RAI rotation and linseed for INNO rotation) and before sowing of the cover crop => 2 soil analysis per year.

The following table summarizes the main soil parameters:

| | Initial analysis Sept 2010 | RAI Sept 2012 | INNO Sept 2012 | RAI Sept 2013 | INNO Sept 2013 |
|---|---|---|---|---|---|
| Clay % | 14.3 | | | | |
| Total Loam % | 68.5 | | | | |
| Total sand% | 7.1 | | | | |
| $CaCO_3$ % | 7.7 | 0.7 | 10.2 | <0.1 | 11.2 |
| Organic Matter % | 2.4 | 2.3 | 2.5 | 1.9 | 2.5 |
| Organic carbone % | 1.39 | 1.35 | 1.47 | 1.13 | 1.46 |
| Cationic exchange capacity meq/100g | 11.6 | 10.6 | 11.8 | | |
| pH $H_2O$ | 8.3 | 8.2 | 8.5 | 8.2 | 8.1 |
| pH KCl | 7.7 | | | 7.7 | 7.6 |
| $P_2O_5$ Olsen mg/kg | 78 | 72 | 68 | 62 | 62 |
| $K_2O$ mg/kg | 234 | 242 | 269 | 234 | 281 |
| CaO mg/kg | 10470 | 7356 | 11463 | 7095 | 13115 |
| MgO mg/kg | 90 | 84 | 81 | 95 | 100 |

**Climatic conditions**

The average rain per year on the last 20 years is 726 mm, and the average ETP (potential evapo-transpiration is 724 mm).

2010-11 is characterized by a particular dry autumn, winter and spring with at spring a drought that could be compared with spring 1976.

In 2011-12 we had a dry autumn and February, with a total closed to the 20 years average value. The second half of February was particularly cold.

**Climat de l'année :** pluviosité PV, ETP, température (moyenne journalière) Tj : **Station Cambrai-Epinoy**
*Mettre en gras les valeurs exceptionnelles*

| mois | 8 | 9 | 10 | 11 | 12 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PV 2010-2011 | 106,7 | 73,3 | 39 | 87,3 | 47,4 | 40 | 24,2 | 10,2 | 30,6 | 5,2 | 55,8 | 65,5 |
| ETP 2010-2011 | 98,3 | 65,5 | 38,1 | 17,5 | 6,37 | 11,67 | 15,16 | 47,34 | 91,88 | 124,18 | 117,74 | 112,36 |
| Tj 2010-2011 | 17,81 | 14,81 | 10,93 | 6,09 | 0,95 | 3,95 | 5,53 | 7,61 | 13,6 | 14,3 | 16,68 | 16,32 |

**Climat de l'année :** pluviosité PV, ETP, température (moyenne journalière) Tj : **Station Cambrai-Epinoy**
*Mettre en gras les valeurs exceptionnelles*

| mois | 8 | 9 | 10 | 11 | 12 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PV 2011-2012 | 106,70 | 44,10 | 32,30 | 14,80 | 132,20 | 48,20 | 14,00 | 35,90 | 64,40 | 22,70 | 79,60 | 123,90 |
| ETP 2011-2012 | 100,37 | 72,89 | 39,83 | 12,61 | 16,95 | 16,36 | 16,89 | 47,24 | 57,84 | 94,26 | 94,34 | 112,05 |
| Tj 2011-2012 | 17,81 | 16,82 | 12,81 | 8,61 | 6,45 | 5,25 | 0,92 | 9,03 | 8,31 | 13,79 | 15,50 | 17,20 |

| **Month** 2012-2013 | **9** | **10** | **11** | **12** | **01** | **02** | **03** | **04** | **05** | **06** | **07** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **rain** | 19.5 | 75 | 51.5 | 94.5 | 25.2 | 30.5 | 22 | 21 | 89.5 | 70 | 13 |
| **T° average** | 14.3 | 11.2 | 6.7 | 5.1 | 2.1 | 1.5 | 3.3 | 9.0 | 10.8 | 15.4 | 19.6 |

**Wheat Agricultural practices**

Calendar for the 3 years:

| Dates | RAI WW1-2-3 | INNO WW1-2 |
|---|---|---|
| 30/08/2010 | stubble cultivation | stubble cultivation |
| 20/10/2010 | **Ploughing + sowing** | **Rotative + Sowing with Sulky** |
| 20/10/2010 | Sowing: cv BAROK 250 plants /m2 | Sowing: cv BAROK 250 plants /m2 |
| 01/03/2011 | Ammo 27 200kg/ha 54N | Ammo 27 200kg/ha 54N |
| 28/03/2011 | Ammo 27 315kg/ha 85N | Ammo 27 315kg/ha 85N |
| 28/03/2011 | Herbicide: Aloès 220 gr/ha + Nikos 0,07 l/ha + Vegelux 1l/ha | Herbicide: Aloès 220 gr/ha + Nikos 0,07 l/ha + Vegelux 1l/ha |
| 13/04/2011 | Growth regulator C5 Sun 2 l/ha | Growth regulator C5 Sun 2 l/ha |
| 27/04/2011 | Ammo 27 200kg/ha 54N | Ammo 27 200kg/ha 54N |
| 25/05/2011 | Fungicide Piano 0,7 l/ha + Sticman 0,1 l/ha | Fungicide Piano 0,7 l/ha + Sticman 0,1 l/ha |
| 15/06/2011 | Insecticide Karaté Express 0,125 kg/ha | Insecticide Karaté Express 0,125 kg/ha |
| 01/08/2011 | Harvest : 9.4 t/ha | Harvest : 8.6t |

2010-11: No major difference except the soil tillage, with or without ploughing. No diseases seen on wheat plots. Low plant density and a growth regulator application because BAROK is susceptible to lodge. One fungicide to protect the upper leaves from Septoria. 0.8 t of yield

difference (less diseases from ploughing the stubble, less competition with weeds ? (late herbicide application with sulfuron-methyl))

| Dates | RAI WW1 after faba bean | RAI WW2 after sugar beet | RAI WW3 after WOSR | INNO WW1 after sugar beet | INNO WW2 after WOSR |
|---|---|---|---|---|---|
| 20/08/2011 | | | stubble cultivation | | |
| 31/08/2011 | stubble cultivation | | | | stubble cultivation |
| 30/09/2011 | | | ploughing | | |
| 14/10/2011 | | | | | Round up |
| 25/10/2011 | Sowing | | sowing | | |
| 02/11/2011 | | dechaumage | | | |
| 02/11/2011 | | sowing | | sowing | sowing |
| 14/03/2012 | Ammo 27 260 kg/ha 70u | Ammo 27 260 kg/ha 70u | Ammo 27 260kg/ha 70u | Ammo 27 260 kg/ha 70u | Ammo 27 260 kg/ha 70u |
| 16/03/2012 | Herbicide: Aloès 220 gr/ha + Nikos 0,07 l/ha + Actirob 1l/ha | Herbicide: Aloès 220 gr/ha + Nikos 0,07 l/ha + Actirob 1l/ha | Herbicide: Aloès 220 gr/ha + Nikos 0,07 l/ha + Actirob 1l/ha | Herbicide:Aloès 220gr/ha + Nikos 0,07 l/ha + Actirob 1l/ha | Herbicide: Aloès 220 gr/ha + Nikos 0,07 l/ha + Actirob 1l/ha |
| 12/04/2012 | Ammo 27 230 kg/ha 62u | Ammo 27 185 kg/ha 50u | Ammo 27 185 kg/ha 50u | Ammo 27 305 kg/ha 82u | Ammo 27 285 kg/ha 77u |
| 13/04/2012 | Growth regulator C5 Sun 2 l/ha | Growth regulator C5 Sun 2 l/ha | Growth regulator C5 Sun 2 l/ha | | |
| 15/05/2012 | | Ammo 27 150 kg/ha 40u | Ammo 27 150 kg/ha 40u | | |
| 23/05/2012 | Fungicide Adexar 1 l/ha | Fungicide Adexar 1 l/ha | Fungicide Adexar 1 l/ha | | |
| 16/06/2012 | Fungicide Piano 0,7 l/ha | Fungicide Piano 0,7 l/ha | Fungicide Piano 0,7 l/ha | | |
| 17/08/2012 | Harvest: 7.9t | Harvest: 7.7t | Harvest: 8.0t | Harvest : 6.8 t | Harvest : 6.6 t |

2011-12: More differences of practices among modalities: date of sowing, ploughing or not, plant growth regulation, nitrogen, and diseases protections. INNO modalities were relatively risky without any fungicide. Yield differences between RAI and INNO are bigger and partially due to fungal pathogens yellow rust and Septoria on INNO plots ( but no detailed notations).

| Dates | RAI WW1 after faba bean | RAI WW2 after sugar beet | RAI WW3 after WOSR | INNO WW1 after sugar beet | INNO WW2 after WOSR |
|---|---|---|---|---|---|
| 28/08/2012 | | | stubble cultivation | | |
| 30/08/2012 | | | | | stubble cultivation |

| | | | | | |
|---|---|---|---|---|---|
| 04/09/2012 | stubble cultivation | | | | |
| 22/10/2012 | ploughing | | ploughing | | Round up |
| 22/10/2012 | sowing | | sowing | | |
| 26/10/2012 | | | | | sowing |
| 29/10/2012 | | stubble cultivation | | | |
| 29/10/2012 | | sowing | | sowing | |
| 07/03/2013 | N Fertilization ammo27 185 kg/ha 50N | N Fertilization ammo27 185 kg/ha 50N | N Fertilization ammo27 185 kg/ha 50N | N Fertilization ammo27 185 kg/ha 50N | N Fertilization ammo27 185 kg/ha 50N |
| 19/04/2013 | N Fertilization ammo27 300 kg/ha 80N | N Fertilization ammo27 300 kg/ha 80N | N Fertilization ammo27 300 kg/ha 80N | N Fertilization ammo27 300 kg/ha 80N | N Fertilization ammo27 300 kg/ha 80N |
| 25/04/2013 | Herbicide Aloes 200g/ha + nikos 0.07l/ha+Actirob 1l | Herbicide Aloes 200g/ha + nikos 0.07l/ha+Actirob 1l | Herbicide Aloes 200g/ha + nikos 0.07l/ha+Actirob 1l | Herbicide Abak 250g/ha +Actirob 1l | Herbicide Abak 250g/ha +Actirob 1l |
| 06/05/2013 | Fungicide Pixel 1.2l/ha + Growth regulator C5 sun 2l/ha | Fungicide Pixel 1.2l/ha + Growth regulator C5 sun 2l/ha | Fungicide Pixel 1.2l/ha + Growth regulator C5 sun 2l/ha | Growth regulator C5 sun 2l/ha | Growth regulator C5 sun 2l/ha |
| 29/05/2013 | N Fertilization ammo27 166 kg/ha 45N | N Fertilization ammo27 166 kg/ha 45N | N Fertilization ammo27 166 kg/ha 45N | N Fertilization ammo27 166 kg/ha 45N | N Fertilization ammo27 166 kg/ha 45N |
| 04/06/2013 | Fungicide tenax 0.6l/ha + acanto 0.25 l/ha + herbicide Effigo 2l/ha | Fungicide tenax 0.6l/ha + acanto 0.25 l/ha + herbicide Effigo 2l/ha | Fungicide tenax 0.6l/ha + acanto 0.25 l/ha + herbicide Effigo 2l/ha | Fungicide prosaro 0.7 l/ha + herbicide Effigo 2l/ha | Fungicide prosaro 0.7 l/ha + herbicide Effigo 2l/ha |
| 18/06/2013 | Fungicide kestrel 0.7l/ha | Fungicide kestrel 0.7l/ha | Fungicide kestrel 0.7l/ha | | |
| 14/08/2013 | Harvest :9.7t | Harvest : 9.9t | Harvest : 10.1t | Harvest : 8.2t | Harvest : 8.5t |

2012-13: Grain yield differences are increasing compared to the previous years. Nevertheless no visible diseases were detected, and contrary to the previous year a single fungicide application has been done on INNO plots, versus three applications on RAI plots. Absence of visible symptoms does not mean absence. No molecular detection or quantification from the plants was done.

**Agrochemicals (quantity of active ingredient /ha)**

- Aloès (bayer) : mésosulfuron-methyl sodium + iodosulfuron-methyl sodium : 6.6 g/ha for each
- Nikos (dow): florasulame 3.5 g/ha
- C 5SUN: chlormequat chlorure 920 g /ha for 2 liters

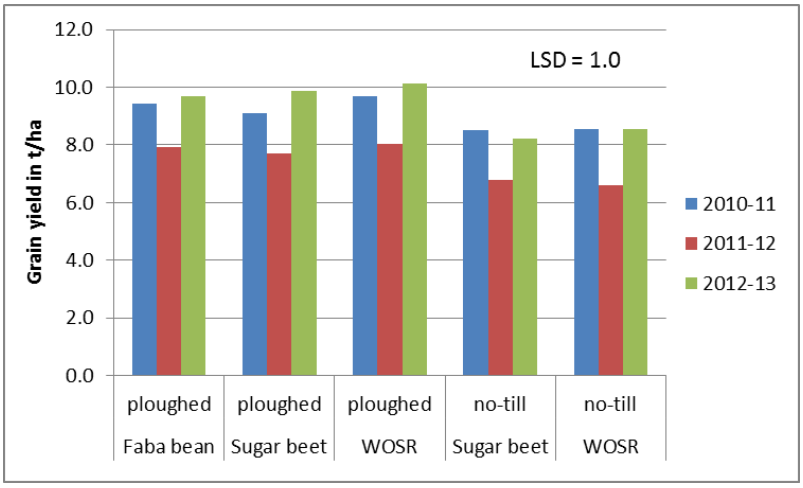
- Adexar (basf): fluxapyroxad + epoxiconazole 2 x 62.5 g /liter
- Piano (bayer) : prothyoconazole + tebuconazole 2 x 125 g /liter => 2 x 87.5 g/ha
- Karaté express (Syngenta) : lambda-cyhalothrine 6.25 g / ha
- Abak (dow): pyroxsulame + cloquintocet-mexyl 37 g /ha
- Pixel (syngenta): cyproconazole + chlorothalonil: 40g et 375 g /liter => 48 et 450 g/ha
- Tenax (BASF): fluxapyroxad + epoxiconazole: 62.5 +62.5 g/liter => 75 g/ha (37.5 x2)
- Acanto (dow): picoxystrobine 250g/l => 62.5 g/ha
- Effigo (dow): clopyralid + 2-4mcpa 35+350 g /l => 770 g/ha
- Kestrel (bayer): prothioconazole + tebuconazole 160+80 g/l => 168 g/ha
- Prosaro(bayer): prothioconazole + tebuconazole 125 +125 g/l => 175 g/ha

**Plant diseases and TFI (treatment frequency index)**
No major symptoms were seen on the design for all the 3 years.

| | Herb TFI | Growth reg TFI | Fung TFI | Insect TFI | **Total TFI** |
|---|---|---|---|---|---|
| 2010-11 RAI | 0.9 | 1 | 0.7 | 1 | **3.6** |
| 2010-11 INNO | 0.9 | 1 | 0.7 | 1 | **3.6** |
| 2011-12 RAI | 0.9 | 1 | 1.2 | 0 | **3.1** |
| 2011-12 INNO | 0.9 | 0 | 0 | 0 | **0.9** |
| 2012-13 RAI | 1.9 | 1 | 1.85 | 0 | **4.75** |
| 2012-13 INNO | 2 | 1 | 0.7 | 0 | **3.7** |

**Grain Yields:**

| Tons /ha | RAI WW1 | RAI WW2 | RAI WW3 | INNO WW1 | INNO WW2 |
|---|---|---|---|---|---|
| 2010-11 | 9.4 | 9.1 | 9.7 | 8.5 | 8.6 |
| 2011-12 | 7.9 | 7.7 | 8.0 | 6.8 | 6.6 |
| 2012-13 | 9.7 | 9.9 | 10.1 | 8.2 | 8.5 |




There are significant differences only between “Raisonnés” and “Innovatives”

# 7. Annex II – Disease suppression and antagonistic Lysobacter spp.

Joeke Postma & Mirjam Schilder (DLO)

**Method**

Soil sampling
Five treatments – 3 plots – 2 subsamples – 4 sampling times.

Bioassays with wheat pathogens
Isolates of different wheat pathogens were selected within different phyla (see table).

- Pythium ultimum : missing, stunted and poorly tillered plants, more destructive in wet soil
- Rhizoctonia solani : bare patch; root rot, stunted plants, occasionally seedlings are killed
- Gaeumannomyces graminis var. tritici : take-all; disease of roots, crown and basal stem, blackened roots if soil moisture is insufficient,
- Microdochium nivale (Fusarium nivale) : Pink snow mold, Fusarium patch; poor establishment of new crop, seedling blight, reduction of seedling emergence, at low temperature.

(2010, compendium of wheat diseases)

All pathogens were tested for pathogenicity before the experiments in 2011 and 2012. Gaeumannomyces did not give symptoms in 2011 and was substituted by Microdochium nivale in 2012. Both belong to the phylum Ascomycota.

| **Pathogen species** | **isolate** | **Phylum** | **experiment** | **Source/reference** |
|---|---|---|---|---|
| Pythium ultimum | | Heterokontophyta | 2011 , 2012 | |
| Rhizoctonia solani | AG 8/45 | Basidiomycota | 2011 , 2012 | (Hanse, IRS) |
| Gaeumannomyces graminis var. tritici | R3-1Ha | Ascomycota | 2011 | (Raaijmakers, WUR) |
| Microdochium nivale | 34 | Ascomycota | 2012 | Hudec & Muchova 2010 |

Inoculum production – on sterilized wheat kernels.
Plastic pots with 105 gram soil (soil was 2.5 cm high, diameter 9 cm)
Inoculum with 5 kernals at 5 locations at the bottom of the pot 2011; 10 kernels in 2012.
Wheat cultivar: Barok (sensitive for the selected pathogens); untreated seeds
25 seeds per pot. Seeds 1.5 cm below surface.
Water 3 times a week, N fertilizer when needed
Greenhouse climate: 20/18 °C day/night; 16 h light
Microdochium nivale at 15 °C in a climate room – score after 8 weeks

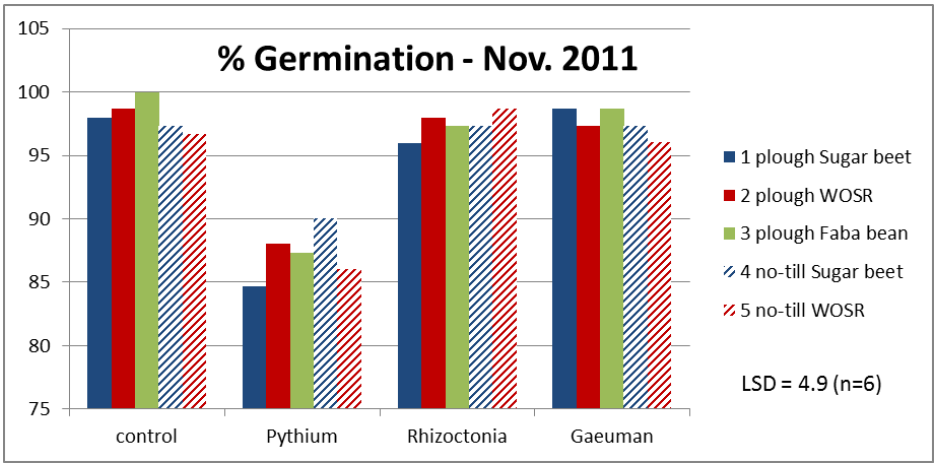
Score of symptoms:

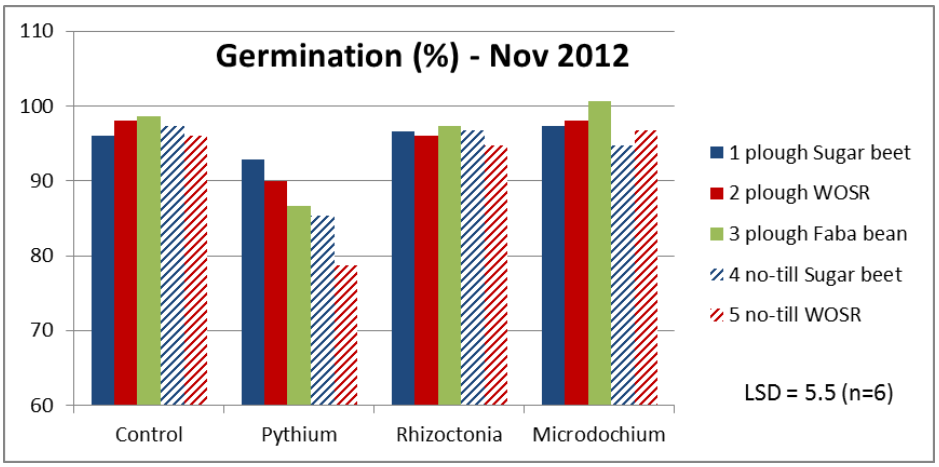
- germination after 2 weeks
- root rot – with index 0-3 after 6 weeks
- root mass – with index 0-3 after 6 weeks

**Results**

Germination:

- Pythium reduced germination (pre-emergence damping-off) in 2011 and 2012 with 10-20%.
- However, there is no consistent difference between the treatments in 2011 and 2012. Thus, no influence of crop or tillage is seen with the reduction of germination due to Pythium.
- All other pathogens did not reduce germination



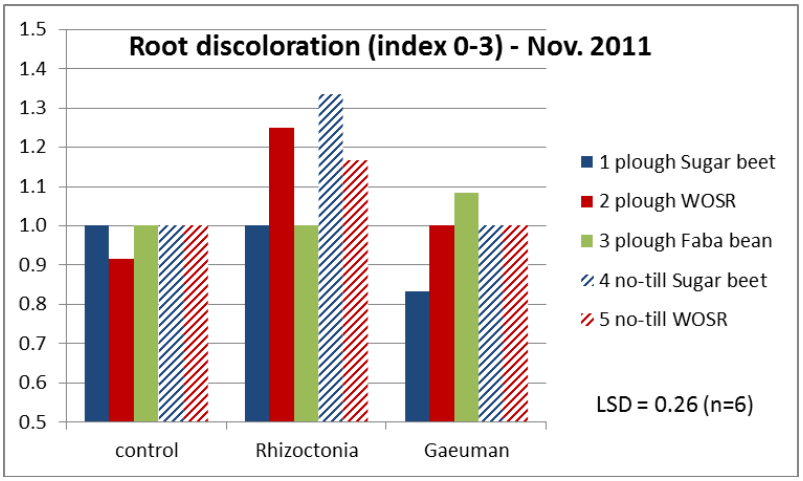

Root discoloration :

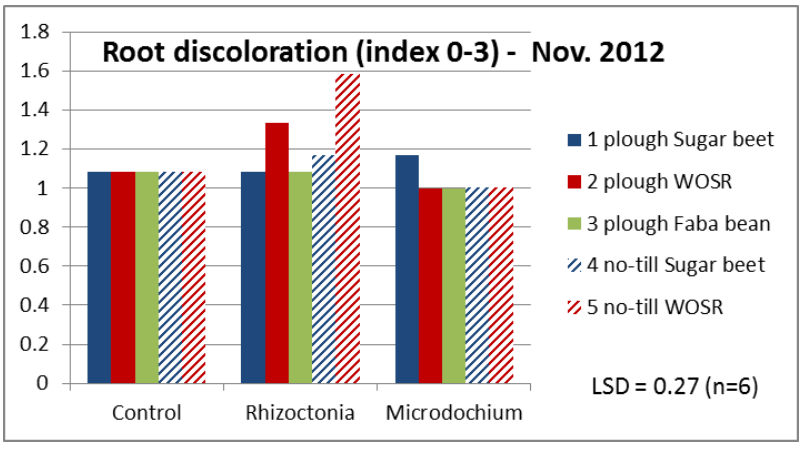
- Roots were never white. Also in the control, without pathogens added, the roots had some light brown discoloration. Wheat roots were white when grown in a clay soil that was never used for wheat tillage during the last 30 years (Zwaagdijk, NL). Sterilized soil ?? Probably all kinds of wheat pathogens are present in the soil without giving distinct symptoms. But no differences between the soil/rotation treatments are present.
- Discoloration by Pythium is not presented in the figures since roots were scored after 2 weeks, whereas the control was scored after 6 weeks.
- Microdochium (2012) and Geaumannomyces (2011): both showed no significant additional discoloration compared to the control.
- Rhizoctonia: discoloration of roots exceeded the control in several of the soil treatments in 2011 and 2012. Trend: more brown roots after WOSR in 2011 and 2012. Also No-till

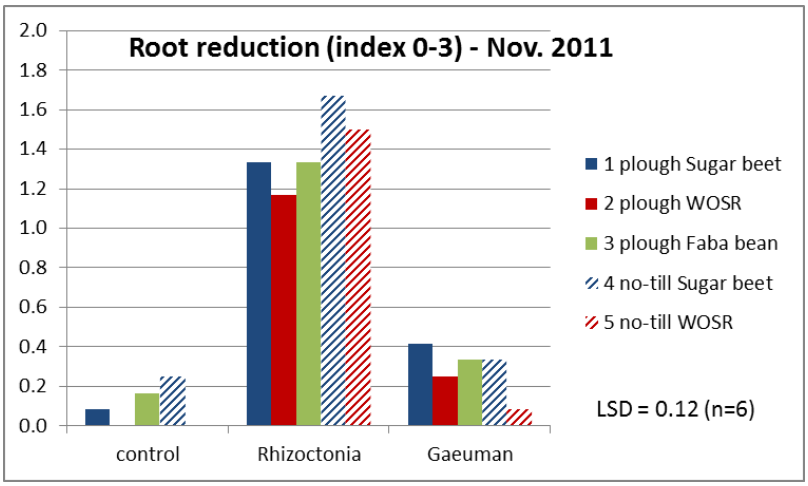
sugar beet had more brown roots, but only in 2011. Statistical analysis of Rhizoctonia bioassay with factors year-tillage-crop did not show significant effects.

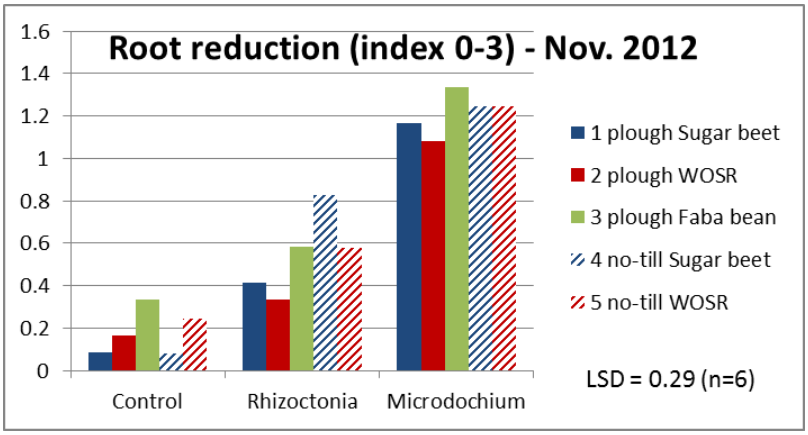
Root mass:

- Distinct decrease in root mass with Rhizoctonia was present in 2011 and 2012. More or less the same trend between treatments in 2011 and 2012: root reduction most clear in treatment no till and sugar beet as previous crop. Least reduction after ploughing with WOSR.
- Statistical analysis of Rhizoctonia bioassay with 3-factorial ANOVA with the factors year-tillage-crop showed significant differences in root reduction: plough < no till
- Gaeumannomyces (2011) caused no distinct root reduction
- Microdochium (2012) caused reduction of roots. But no significant differences were present between the treatments.
- Inoculum density was doubled in 2012: 5 kernels in 2011, 10 kernels in 2012. This higher inoculum density did not cause more disease.






**Lysobacter spp.**

Presence of Lysobacter populations in soil og Cambrai region

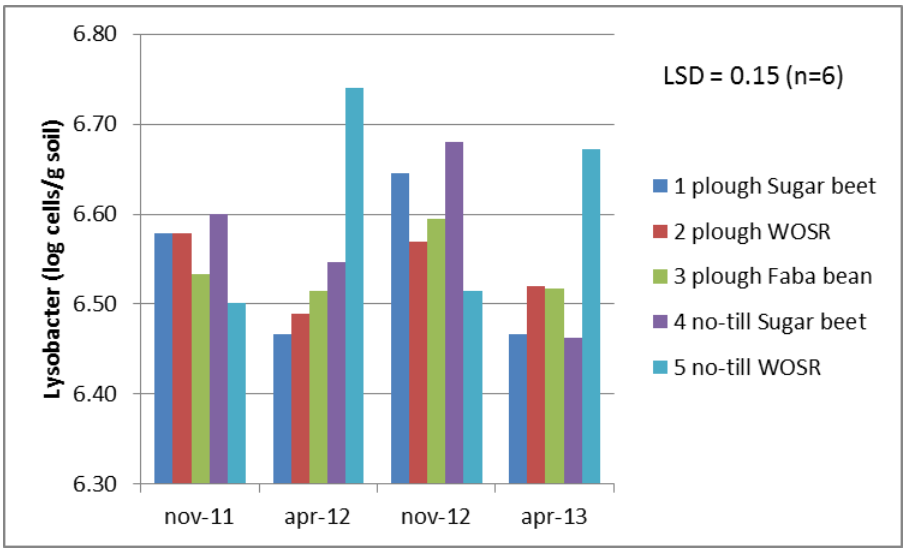
Soil of two test samples in July 2011was incubated with and without 0.3% yeast. After 4 weeks three samples of 0.5 g were taken from the bags and subjected to DNA extraction DNA of Lysobacter spp. was quantified with a TaqMan PCR method (Postma, et al 2011). Increase of Lysobacter populations due to incubation with 0.3% yeast was significant: populations were 3.5 and 2.2 times higher after incubation with yeast. This is the increase we normally find with this dosage of yeast. It proves that Lysobacter is present and can be activated/multiplied in the soil.

Isolation

Soil enriched with yeast was plated on several agar media consisting of R2A with anti-bacterial and anti-fungal compounds. Thirteen bacteria were selected and grown on R2A to obtain pure cultures. After sequencing (1300 bp), 6 isolates appeared to be Lysobacter spp. (5 L. antibioticus, 1 L. gummosus (>97% similarity)), 2 isolates were most similar to Pseudomonas and 5 isolates were lost or not pure.
All Lysobacter isolates inhibited Rhizoctonia solani on R2A-medium.

Population dynamics of Lysobacter

Soil samples of 5 treatments in 3 replicates were supplied by CETIOM at 4 data: Nov. 2011, April 2012, Nov. 2012, April 2013. Of each sample, 2 subsamples were taken for extraction of DNA (in total 120 samples). Lysobacter spp. was quantified with a TaqMan PCR method (Postma et al 2011). Results are presented in the figure below.




**GENERAL DISCUSSION / CONCLUSIONS:**

Disease suppression:

- Disease pressure by the inoculated pathogens was in general too low. Inoculum density was doubled in 2012: 5 kernels in 2011, 10 kernels in 2012. This higher inoculum density did not cause more disease. This probably means that the soil is quite suppressive towards the diseases tested (heavy clay soil!!)
- Roots were never white. Also in the control, without pathogens added, the roots had some light brown discoloration, which was not the case when another soil without wheat tillage during the last 30 years was used. Probably all kinds of wheat pathogens are present in the soil without giving distinct symptoms.
- Pythium reduced germination (pre-emergence damping-off) in 2011 and 2012 with 10-20%. But no differences were present due to crop or tillage.
- Rhizoctonia caused a distinct decrease in root mass with in 2011 and 2012. Differences were significant due to crop and tillage. Root reduction was significantly less in ploughed soil compared to no tillage (3-factorial ANOVA).
- Few symptoms of Eyespot were present in the control of treatment 3.1 (Faba bean – ploughed - no pathogens added) in 2012.

Lysobacter spp.:

- Lysobacter spp. were isolated from the field; species were identified with 16S as L. antibioticus and L. gummosus.
- These isolates inhibited Rhizoctonia solani on R2A-medium.
- Lysobacter populations were enhanced by adding yeast to the soil, showing that the DNA-detected populations were including living bacteria.
- In general Lysobacter populations are lower in spring compared to autumn. This is in line with other results showing that Lysobacter has problems with surviving low temperatures (5°C).
- In general no big differences between treatments occurred, but there is a very REMARKABLE difference in the no-till with WOSR as previous crop: significantly higher numbers of Lysobacter in spring than all other treatments!! And relatively low in autumn.

# 8. Annex III – Microbial populations and bacterial antagonists

Kornelia Smalla (JKI)

JKI group focused on the hypothesis that different treatments (crop rotation and tillage and no tillage) will influence the composition of the bacterial and fungal communities which in turn might influence suppressiveness towards different fungal pathogens. To address this question total community (TC) DNA was directly extracted after a harsh lysis from three composite samples per treatment. Total bacteria and different bacterial taxa (Alpha- and Betaproteobacteria, Actinobacteria, Pseudomonas, Streptomyces) were analyzed by amplifying 16S rRNA gene fragments from TC DNA and subsequent analysis of the PCR products by denaturing gradient gel electrophoresis (DGGE). Treatment effects on the fungal communities were analyzed based on ITS fragments PCR amplified from TC DNA and subsequent DGGE. The comparison of bacterial, Pseudomonas, actinobacterial, alpha- and betaproteobacterial fingerprints provided information on the variability of bacterial communities within and between treatments.
Samples in June 2011 and May 2012 bulk soil and rhizosphere samples were taken, while in the November 2012 only bulk soil samples were obtained.

Results of DNA analysis

The analysis of the June samples showed that for all groups analyzed the rhizosphere fingerprints clustered separately from the bulk soil samples. The rhizosphere communities with few exceptions clustered separately for wheat and oilseed rape. Less pronounced was the clustering according to tillage and no tillage. Soil samples did show only distinct communities for oilseed rape under no tillage. The fungal DGGE fingerprints displayed a higher variability in the rhizosphere compared to the bulk soil. The fungal rhizosphere fingerprints of wheat and oilseed rape formed distinct clusters which shared only low similarities (45%).
The analysis of the bulk soil samples from the November samples indicated that despite of the rather short time the field experiment is running effects of the crop could be detected in particular for fungal communities. Only the bacterial fingerprints of bulk soil from winter wheat (WW) after winter oilseed rape (WOSR) plots formed a distinct cluster with separate clusters for tillage and no tillage. Interestingly, the fungal fingerprints of the bulk soil showed distinct crop dependent patterns with soil fingerprints from faba beans sharing only about 50% similarity.
The analysis of the May samples showed that for the different bacterial taxa (Alpha- and Betaproteobacteria, Actinobacteria, and Streptomyces) analyzed the wheat rhizosphere fingerprints clustered separately from the bulk soil samples. The wheat rhizosphere communities did not cluster according to the treatments (previous crop/ tillage or no tillage) while the soil communities with few exceptions clustered separately for WW grown after WOSR. The WW rhizosphere fungal fingerprints clustered together with bulk soil fingerprints when WW was grown after WOSR with a separate clustering according to the treatment. Remarkably the rhizosphere fungal fingerprints of WW grown after sugar beet or faba bean were highly distinct from the bulk soil cluster. Only the fungal fingerprints of the WW rhizosphere grown after WOSR which was contained in the soil cluster showed treatment (tillage *vs.* no tillage) dependent subclusters. Overall a stronger effect of the previous crop was found for fungal communities in the WW rhizosphere. In contrast the previous crop had little effect on the bacterial community composition in the rhizosphere. Except for WOSR little effects of tillage *vs*. no tillage were seen.

Screening Pseudomonas isolates for in vitro antagonistic activity

To investigate whether the previous crop and the treatment influenced the antagonistic potential in the soils and to link these with the bacterial community analysis bulk soil samples (Nov 2011, May 2012) and WW rhizosphere samples 2012 were plated onto King´B medium. 100 colonies per sample type were picked and screened for in-vitro antagonistic activity towards Fusarium graminearum (Fg) and Gaeumannomyces graminis (Gg). The abundance of antagonistic Pseudomonas isolates from rhizosphere (Fg:61; Gg 95) was higher than for bulk soil (Fg:44 Gg:51) and sugar beet as a previous crop had the highest number of antagonistic isolates. The molecular characterization of the Pseudomonas antagonists is in progress.

# 9. Annex IV - Arbuscular Mycorrhizal Fungi (AMF)

Erica LUMINI, Stefano GHIGNONE, Andrea BERRUTI, Valeria BIANCIOTTO (CNR)

The complexity of AMF community was estimated by morphological and molecular analysis in five different experimental sub-plots characterized by different crop rotations and managements (ploughed, or no-tilled). AMF in plant roots and soils were detected by sequencing the nuclear SSU rRNA gene fragment using either cloning followed by Sanger sequencing or 454-sequencing. A total of 500000 filtered sequences were processed and 374 OTUs corresponding to 88 AMF phylogroups (virtual taxa, VTX, Opik et al. 2013) were recorded.

**Material & Methods:**

Winter wheat and soil sampling:
In November 2011 and 2012, and May 2012 and 2013, five soil core samples (5 cm Ø and 20 cm depth) were taken from each of the three replicate fields for the 5 different treatments analyzed. The 5 soil cores samples were mixed to form a composite sample of 50-100 g that was independently packed in ice upon collection and transported to the labs for DNA extraction. The soil samples were sieved (2 mm) to remove fine roots and large organic debris and stored at -80°C. A total of 60 soil samples were analyzed.
During spring sampling, in addition to bulk soil samples, 15-30 randomly chosen winter wheat plants of each plot were excavated with their soil and other material adhering to the roots and send to the partners. Upon the arrival at lab 10 plants from each replicate were washed free of soil and their roots apparatus cutting in small piece of 1–2 cm each and pooled for morphological analyses. The roots were stored at −20°C until used for molecular analyses.

Morphological evaluation of AMF root colonization
Winter wheat roots from the fields were stained with 0.1% cotton blue in lactic acid for about 20 h and then destained four times with lactic acid. One-centimetre root fragments were then mounted onto microscopy slides with lactic acid. At least 60 fragments were observed for each replicate for a total of 1800 root fragments for each of the two year of root sampling.
Both the intensity of the root cortex colonization by AM fungi and the presence of arbuscules were determined, as described by Trouvelot et al. (1986).

DNA extraction and PCR amplification
Winter wheat pooled root samples (100 mg wet weight) were used in the extraction step (DNeasy Plant Minikit; Quiagen, Hilden, Germany) according to the protocol for frozen samples. DNA extractions were performed from 0.5 g of mixed soil with the FastDNA Kit (MP Biomedicals, LLC, OH, USA) according to a modified protocol as reported by Luis and colleagues (2004). The DNA was resuspended in sterilized water and several dilutions of extracted DNA (1/10, 1/50, 1/250) were prepared. The quality and quantity of DNA samples was assessed through gel electrophoresis of 5 ml subsamples on 1.5% agarose gel and analysis with the ND-1000 Spectrophotometer NanoDropH (Thermo Scientific, Wilmington, Germany). To overcome the difficulties of mixed starting material, species composition of AM fungi in soil and roots was analysed by a nested PCR approach.
Roots were analyzed by cloning and Restriction Fragment Length Polymorphism Analysis (PCR-RFLP) and soils by a metabarcoding approach based on tag-encoded 454

pyrosequencing spanning an hyper-variable regions of the rRNA genes from AM fungal communities. The AML1 and AML2 primers (Lee *et* al. 2008) were used in the first amplification followed by nested amplification with AMADF (Desirò, PhD thesis) and /AMDGR (Lumini et al.2010) . To make sure that the communities were sampled deeply enough; we pooled many PCR products independently amplified from different DNA extractions and dilution for each soil samples

NGS analyses

The 18S 454 datasets were processed with the QIIME (Quantitative Insights Into Microbial Ecology) pipeline. 18S reads were subjected to denoising, quality trimming and chimera removal. During denoising, sequences exhibiting a quality score lower than 25 and length shorter than 250 bp were trimmed and then assigned to different samples based on unique 5-bp barcodes. The cleaned sample files were merged into a single Fasta file (keeping the sample information in each read entry) and subjected to downstream processing. This cleaned dataset was clustered into molecular Operational Taxonomic Units (OTUs) with a 97% identity threshold, by using the USEARCH algorithm, which also implements a native de novo chimera identification and removal feature. Following OTU picking and chimera removal in QIIME, the longest sequence from each cluster was selected as the OTU representative sequence to be used for used for taxonomic identification of the OTU. Taxonomy was assigned to representative sequence for each OTU. AM Fungal OTUs were identified using a customized reference repository, derived from the web-based Maarj*AM* database (Opik et al. 2010), and specially developed to overcome the limitations and biases of currently used fungal AMF databases.

**RESULTS**

AMF colonization of roots

The degree of root colonization by AM fungi was determined microscopically after cotton blue staining. The root fragments analysed showed a low mycorrhization level (M%) with a mean value of 1-2%. Although presents in low concentration the fungus formed inside the roots the typical AMF structures, i.e. intercellular and intracellular hyphae and arbuscules and outside spores (Fig1).

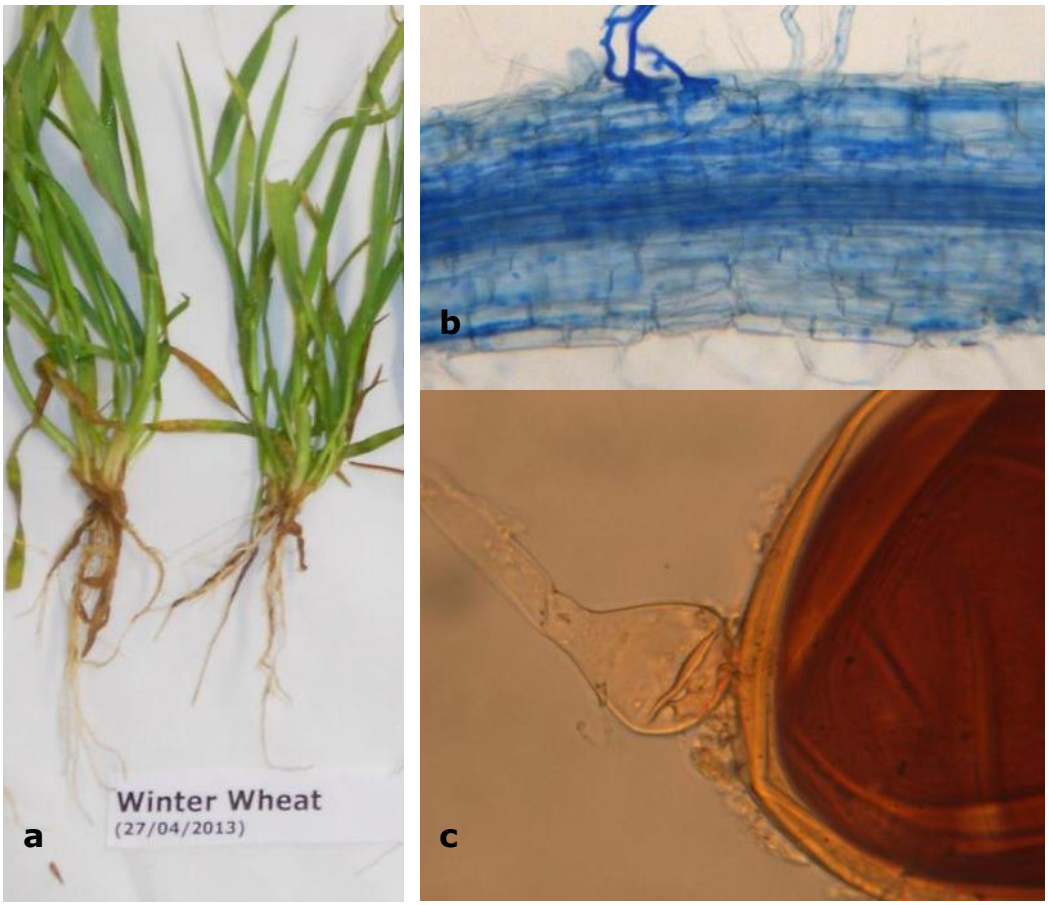



**Fig. 1.** Winter wheat roots (a) stained with Cotton Blue (b). In a fragment of root (b) the tipical colonization structures of the arbuscular mycorrhizal symbiotic fungi are visualized (hyphopodia, inter- and intracellular cellular hyphae). AMF Spores (c) belonging to different species were found in the rhizospheric soil.

AMF biodiversity by NGS

*Overall taxonomic richness*

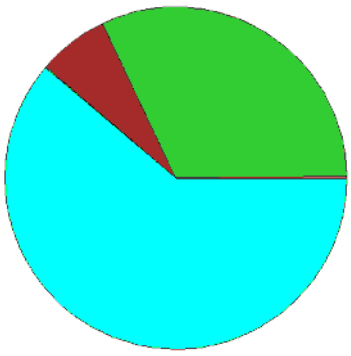
Sequences obtained with the AMADF/AMDGR primer pair with a quality score above 25 and a length over 250 bp were used by QIIME for this investigation, leading to a total of 500 000 filtered sequences and the dominant length distribution is around 390-400bp.

Nested protocols of amplification leading to a very high AM primer specificity: a 99% of sequences belonging to *Glomeromycota* taxa. The overall taxonomic distribution of the 18S sequences obtained in the field trials is shown in Fig. 2.

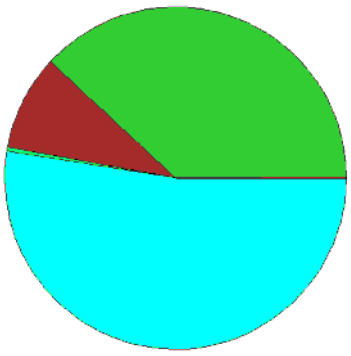
Global soil AMF diversity Fig.2

Soil AMF assemblages at AMF family level in the different field treatments

Management system

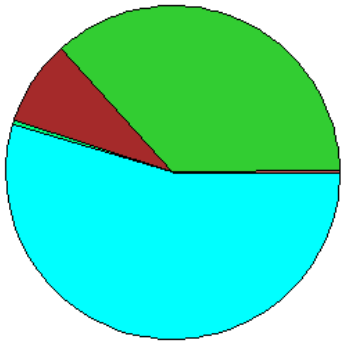


Ploughed Plots Labour

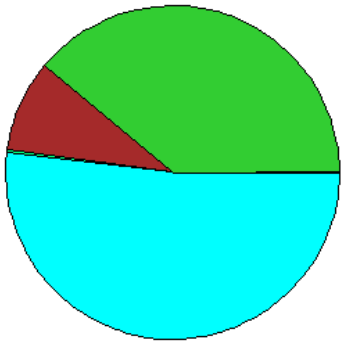


Reduced Tilled Plots TCS

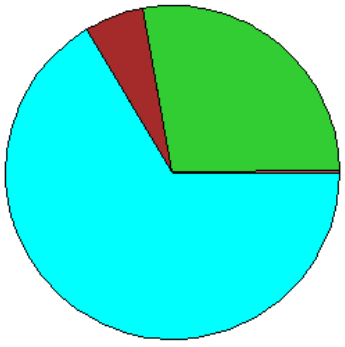
Crop rotations



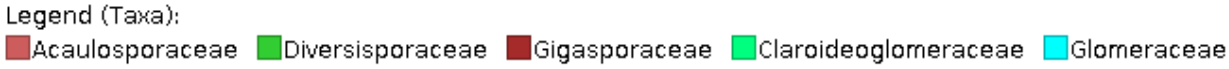
Barley-faba bean-ww



Barley-sugar beet-ww



Barley-WOSR-ww




**Preliminar remarks:**

- Ploughed favoured taxa belonging to Glomeraceae and Acaulosporaceae
- Reduced tilled favoured taxa belonging to Gigasporaceae, Diversisporaceae and Claroideoglomeraceae.

- Winter Wheat with WOSR as preceding crops incremented the percentage of taxa belonging to Glomeraceae
- Not visible changes at family level for both faba bean and sugar bean when used as preceding crops.

The soil AMF community was dominated by Glomeraceae followed by Diversisporaceae and Gigasporaceae families. Acaulosporaceae and Claroideoglomeraceae as well as members belonging to Archaeosporaceae, Ambisporaceae and Paraglomeraceae represented extremely rare taxa.

These proportions of AMF groups extend the knowledge learned from our previous study and other reports in high-input managed agro-ecosystems as well as other natural ecosystems.
At the higher resolution, Glomus was the dominant genus in the AMF assemblage. AMF of this genus survive and propagate more easily because of the ability to colonize via pieces of mycelium or mycorrhizal root fragments. Such characteristics can partially explain the dominance of Glomus over other members in the agro-ecosystem with repetitive severe physical disturbance, such as ploughing between crop cycles. Besides Glomus, a large number of environmental and unclassified Glomeraceae were also found in arable soils. This phenomenon indicates that a phylogenetically diverse not yet identified Glomeraceae community may inhabit the high-input managed agro-ecosystem.
A more detailed investigation of the shifts in AMF taxa (genus/species level) compositions mirroring different treatments can guide us toward an effective balance between high input management systems and reduced ones. The analyses are in progress.

In parallel, the 2012 spring samples were also investigated to characterize the overall fungal communities present in soil using pyrosequencing on the nuclear ribosomal ITS regions. The set of primers ITS3m (Zhong et al. 2010) and ITS4 (White et al. 1990) with barcodes was used to amplify the ITS2 of the internal transcribed spacer (ITS) rDNA region.
Some results of these analyses were reported in the poster "**Lumini E., Ghignone S., Bianciotto V.** (2013) Different molecular approaches reveal how arbuscular mycorrhizal fungal communities thrive in a winter-wheat rotation management system" presented at Proceedings of the Second International conference on Microbial Diversity 2013- Microbial Interactions in Complex Ecosystems, MD2013, Torino, October 23-25, 2013. 399-400. ISBN: 978-88-908636-5-3).

# 10. Annex V – Nematode community

Aad Termorshuizen (BLGG AgroXpertus and BLGG Research)

**Material and methods**

Free-living nematodes were extracted from 100 ml field-moist soil, using the Oostenbrink elutriator
(Oostenbrink, 1960). After total numbers were counted microscopically, nematodes were fixed in hot formaldehyde 4%, and 150 randomly selected nematodes were identified. Nematode genera and species were assigned to trophic groups (Yeates et al., 1993), and to colonizer-persister groups (cp-groups) (Bongers, 1990; Bongers et al., 1995). In addition, the Nematode Channel Ratio (NCR) was calculated as B/(B+F) where B = # bacterivorous and F = # fungivorous nematodes (Yeates, 2003). The Maturity Index was calculated as the weighted mean of the individual cp-values (Bongers, 1990; Korthals et al., 1996).

**Results and Discussion**

The number of nematodes encountered was, averaged over all samples, 1743 /100 ml fresh soil. The coefficient of variation (= standard deviation / average × 100%) varied for these year counts between 23 and 39%. The first sampling resulted in significantly higher counts (2858) than the other samplings (1487, 1738, and 1625 respectively; $P<0.001$, paired t-tests; all /100 ml fresh soil). Such variation is not remarkable, given that nematode communities strongly depend on moisture content and thus weather conditions. 9.7% of the counts consisted of unidentifiable dauer larvae.

The majority of nematodes encountered were bacterivores (48%) or plant parasites (45%) (Table 1). Among the plant parasites, many were not identifiable to the species level (e.g. 17.3% of the plant pathogens were counted as *Pratylenchus* and 25.3% *Tylenchidae*). The most commonly counted plant pathogen was *Pratylenchus neglectus* (8.6%); in lower densities other primary plant pathogens were encountered as well, including the quarantaine species *Meloidogyne chitwoodi* (1.3%), *Pratylenchus penetrans* (0.2%). A minor pathogens, *Tylenchorhynchus dubius*, was present at 0.7% and probably the7.8% counted as *Tylenchorhynchus* probably also belonged to *T. dubius.* The 4.4% counted as juveniles Heterodidae very likely were sugar beet nematodes (*Heterodera betae* or *H. schachtii*), as their numbers were in the sugar beet containing field by far the highest. Direct comparisons of counts of plant parasitic nematodes can be somewhat arduous since significant numbers may be present in root remnants.

Table 1. Breakdown of nematode data into functional groups.

| functional group | share (%) |
|---|---|
| bacterivores[1] | 48.0 |
| fungivores | 4.3 |
| carnivores | 1.8 |
| algaevores | 0.1 |
| omnivores | 1.4 |
| plant parasites | 44.5 |

[1] Dauerlarvae were assigned to the bacterivorous nematodes.

The fungivorous nematodes occurred in higher densities in the labour than in the tcs treatment (significant in samples 1 and 4; $P<0.05$, t-tests).

Table 2. Numbers (/100 g of fresh soil) of omnivorous nematodes in the labour vs. the tcs samples.

| sampling | labour | tcs | sign. |
|---|---|---|---|
| 1 | 40.7 | 14.7 | * |
| 2 | 49.0 | 25.9 | |
| 3 | 18.9 | 11.5 | |
| 4 | 30.9 | 9.4 | * |

. = P<0.10, *=P<0.05, **=P<0.01, ***=P<0.001.

Table 3. Maturity Index(2-5) in the labour vs. the tcs samples.

| sampling | labour | tcs | sign. |
|---|---|---|---|
| 1 | 2.4 | 2.2 | * |
| 2 | 2.5 | 2.4 | |
| 3 | 2.2 | 2.1 | |
| 4 | 2.4 | 2.2 | ** |

. = P<0.10, *=P<0.05, **=P<0.01, ***=P<0.001.

With respect to the responses of specific nematode species or groups, the effects of pre-crop outnumbered those of soil tillage treatment. The most striking examples are presented here. With the bacterivorous genus *Eucephalobus*, numbers in wosr as pre-crop were, in general, higher than those in faba which in turn were higher than in sugar beet as pre-crop (Table 4). Although found in small amounts, the omnivorous *Dorylaimoidea* were significantly higher in wosr than in sugar beet in two years and a tendency to be higher in one year (Table 5).

Table 4. Amount of *Eucephalobus* relative to the total amount of nematodes excl. Dauerlarvae in the labour treatments of three pre-crops. Different letters show, per row, significant differences at P=0.05 (t-tests).

| sampling | sugar beet | faba | wosr |
|---|---|---|---|
| 1 | 2.5a | 7.0b | 5.3a |
| 2 | 4.2a | 6.2b | 11.7c |
| 3 | 4.3a | 2.5a | 12.9c |
| 4 | 3.6a | 5.4a | 14.2b |

Table 5. As Table 4, but *Dorylaimodea* (omnivorous).

| sampling | sugar beet | wosr | sign. |
|---|---|---|---|
| 1 | 1.5 | 0.8 | |
| 2 | 1.8 | 3.6 | . |
| 3 | 0.0 | 1.1 | * |
| 4 | 0.6 | 3.4 | ** |

. = P<0.10, *=P<0.05, **=P<0.01, ***=P<0.001.

Among the plant parasites, the genus *Pratylenchus* was pre-crop-dependent, with amounts in wosr as pre-crop generally higher than in sugar beet (Table 6).

Table 6. As Table 4, but *Pratylenchus* (plant parasitic).

| sampling | sugar beet | wosr | sign. |
|---|---|---|---|
| 1 | 8.0 | 14.7 | * |
| 2 | 2.9 | 8.6 | |
| 3 | 4.5 | 14.7 | . |
| 4 | 6.5 | 13.5 | ** |

. = P<0.10, *=P<0.05, **=P<0.01, ***=P<0.001.

On the contrary, systems effects (labour vs. tcs) were much less clear or absent. However, some interesting observations included the number of omnivorous nematodes tending to be higher in the labour treatments (Table 7). This largely also resulted in comparable differences between labour and tcs in the maturity index (2-5). So, if higher amounts of omnivorous nematodes or a higher maturity index is regarded as a positive soil quality attribute (#REF), then the results indicate that labour certainly does not have a *lower* soil quality.

Table 7. As Table 4, but total number of omnivorous nematodes.

| sampling | labour | tcs | sign. |
|---|---|---|---|
| 1 | 40.7 | 14.7 | * |
| 2 | 49.0 | 25.9 | |
| 3 | 18.9 | 11.5 | |
| 4 | 30.9 | 9.4 | * |

. = P<0.10, *=P<0.05, **=P<0.01, ***=P<0.001.

Overall, the results indicate that pre-crop species has a stronger selection pressure on the nematode community than system treatment (i.e., labour vs. tcs).

**References**

Bongers, T. 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83: 14-19.

Bongers, T., De Goede, R.G.M., Korthals, G.W., Yeates, G.W. 1995. Proposed changes of c-p classification for nematodes. Russ. J. Nematol. 3: 61-62.

Korthals, G.W., de Goede, R.G.M., Kammenga, J.E., Bongers, T., 1996. The maturity index as an instrument for risk assessment of soil pollution. In N.M. van Straalen, N.M., D.A. Krivolutsky, (eds.), Bio-indicator Systems for Soil Pollution. Kluwer, Dordrecht, pp. 85-93.

Oostenbrink, M. 1960. Estimating nematode populations bysomeselected methods. In: J. Sasser, W.R. Jenkins (eds.), Nematology. Chapel Hill, University of North Carolina Press, pp. 85-102.

Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W., Georgieva, S.S. 1993. Feeding habits in soil nematode families and genera – an outline for soil ecologists. J. Nematol. 25: 315-331.

Yeates, G.W., 2003. Nematodes as soil indicators: function and biodiversity aspects. Biol. Fertil. Soils 37: 199-210.