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– Preliminary results

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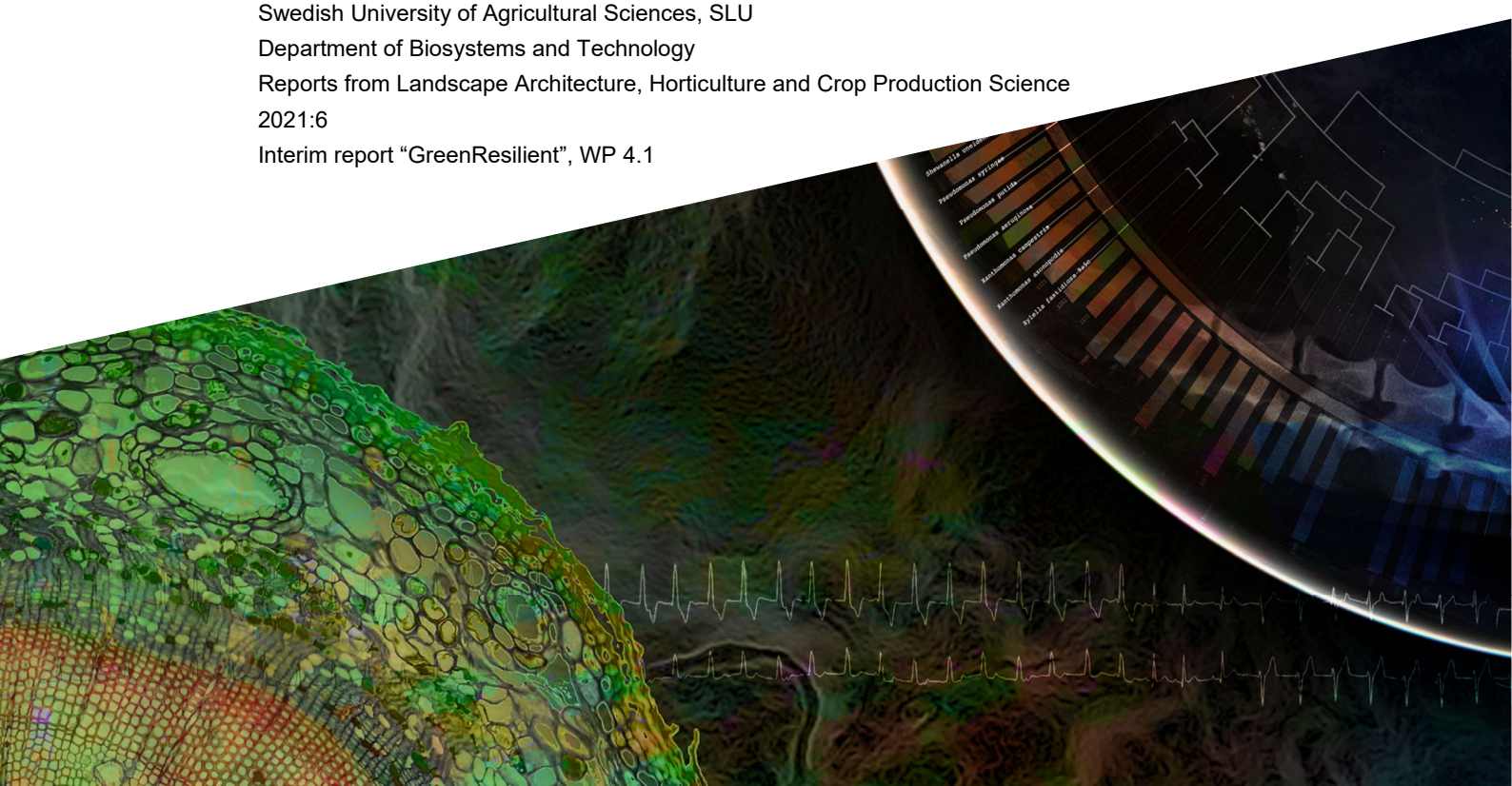

Insights in interaction between soil biodiversity and root disease suppression in organic production systems *preliminary results*

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Abstract

Soil health and biodiversity are fundamental features for both organic production systems and of an agroecological approach. Soil health and functional biodiversity are effects of the dynamics imposed by environmental factors and crop management, and are closely related to the dynamics in soil organic matter. Within the framework of CORE Organic project ”GreenResilient”, crop rotations for “business as usual” (BAU) and innovative (INN) organic greenhouse cropping systems were designed according to local preconditions in the five countries hosting the experimental sites: Belgium (BE), Switzerland (CH), Denmark (DK), France (FR) and Italy (IT). The choice and order of crops within the various BAU and INN systems differed between countries. Soil microbial activity and biodiversity were analysed using fluorescein diacetate activity (FDA) and metagenomic analysis (Illumina MiSeq) of fungi (ITS) and bacteria (16S) at three key events, namely start, midterm and end of the two-year crop rotation. Samples were collected from three blocks (in some cases four) at a density of three replicates (resulting in 9 or 12 replicate samples per treatment). Specific disease suppressiveness was evaluated with respect to *Fusarium oxysporum* f.sp. *lycopersici* (FOL). There was no general trend with respect to microbial activity for the different cropping systems. Interestingly, the microbial activity initially rose in many of the systems (midterm) but decreased to a lower level mostly similar or insignificantly higher to the starting point. A general significant decrease in microbial activity was found at all cropping systems in IT from the start to the end of the experiment. Likewise, soil bacterial and fungal alpha diversity varied between the different sampling incidents with respect to both species richness and evenness (Chao1 index, Shannon diversity index). Interestingly, a strong shift towards richer fungal community was found for the CH-BAU systems as compared to the CH-INN systems over time (based on % change from initial sample). CH-INN systems displayed a richer bacterial community than CH-BAU systems. Similar observations were found sporadically in other systems, displaying changes in beta-diversity between systems over time. Shifts in relative abundance was found for some phyla over time within systems, but no general trend applying to all BAU or INN was registered. A presence of several types of fungal pathogens were observed in all countries, independent of production system. Microbial activity did not conclusively explain variations in microbial diversity for fungi or bacteria. No differences were found in plant performance when assessing specific suppressiveness towards FOL. As plant performance in control samples was much better than those detected in fresh soil samples, with or without amendment of FOL, the general build-up of pathogenic organisms during the crop rotations might mask direct effects.

Keywords: crop rotation, disease suppressiveness, Fusarium oxysporum *f.sp.* lycopersici *(FOL), greenhouse, microbial activity, microbial diversity, organic farming, soil bacterial and fungal communities*

Preface

This interim report displays preliminary workpackage results obtained from the ERA-NET Co-fund project Core Organic 2016EU "Organic and biodynamic vegetable production in low-energy GREENhouses – sustainable, RESILIENT and innovative food production systems" (GreenResilient). The project was approved in 2017, started in 2018 and ended in October 2021. The ambition of the project was to demonstrate the potential and feasibility of an agroecological approach to greenhouse production, considering the climatic and pedological conditions prevailing in the different participating countries. The main expected result of GREENRESILIENT was the definition of innovative and resilient cropping systems for Mediterranean, Central and Northern European protected organic production. Both production potential and sustainability aspects were in focus. This was addressed through a multi- and interdisciplinary approach involving expertise within agronomy/horticulture, agroecology, soil chemistry, entomology, plant pathology, weed science, ecology and environmental sciences from eight European countries each with relatively large areas of protected organic production.
Crop rotation experiments were set up in organic greenhouses in five countries, namely Belgium (BE), Switzerland (CH), Denmark (DK), France (FR) and Italy (IT) where cropping systems managed according to the common organic practice of the country were compared to an innovative approach reinforcing resilience. The systems were analysed with respect to soil fertility aspects and aspects considering soil health and functional biodiversity.

This report displays the preliminary findings concerning soil microbial activity, soil microbial diversity and specific disease suppressiveness. The work was conducted within one task of workpackage 4 "Soil health and functional biodiversity". Leader for WP4 was Beatrix Alsanius. The task on "Soil borne diseases and soil biodiversity assessment" (WP4.1) was lead by Anna Karin Rosberg. It involved participants from the Council for Agricultural Research and Economics (CREA) as well as the private company La Colombaia, Italy, the Research group on organic farming (GRAB), France, Agroscope, Switzerland, the Vegetable Research Centre Kruishoutem as well as the Flanders Institute for Agriculture, Fisheries and Food, Belgium, Århus university, Denmark and SLU, Sweden.

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Abbreviations

AGROEC	Cropping system based on an agroecological approach
ASC	Agricultural service crop
BAU	Cropping system managed according to common standards for organic farming in the respective country “Business as usual”
BIODYN	Cropping system managed according to common standards for biodynamic organic farming in Italy
BE	Experimental site in Belgium
CH	Experimental site in Switzerland
DK	Experimental site in Denmark
FDA	Fluorescein diacetate
FOL	Fusarium oxysporum f.sp. lycopersici
FR	Experimental site in France
INN	Cropping system using an innovative approach
IT	Experimental site in Italy
PLFA	Phospho-lipid fatty acid

1. Background

Soil health and biodiversity are fundamental features for both organic production systems and of an agroecological approach. Soil health and functional biodiversity are a function of the dynamics imposed by environmental factors and crop management (Elliot, 1997) and closely related to the dynamics in soil organic matter. Well-studied in various open field ecosystems, these two phenomena have been much less investigated within the framework of organic greenhouse ecosystems. As cropping system parameters (crop choices, crop rotation intensities) as well as environmental factors (temperature, precipitation/irrigation, humidity) used in organic greenhouse horticulture vary substantially from the ones prevailing under open field conditions, dynamics in soil biodiversity and health cannot be translated from open field conditions. Figure 1 displays interactivities and feedback loops in the soil to be considered within the framework of soil biodiversity and health.

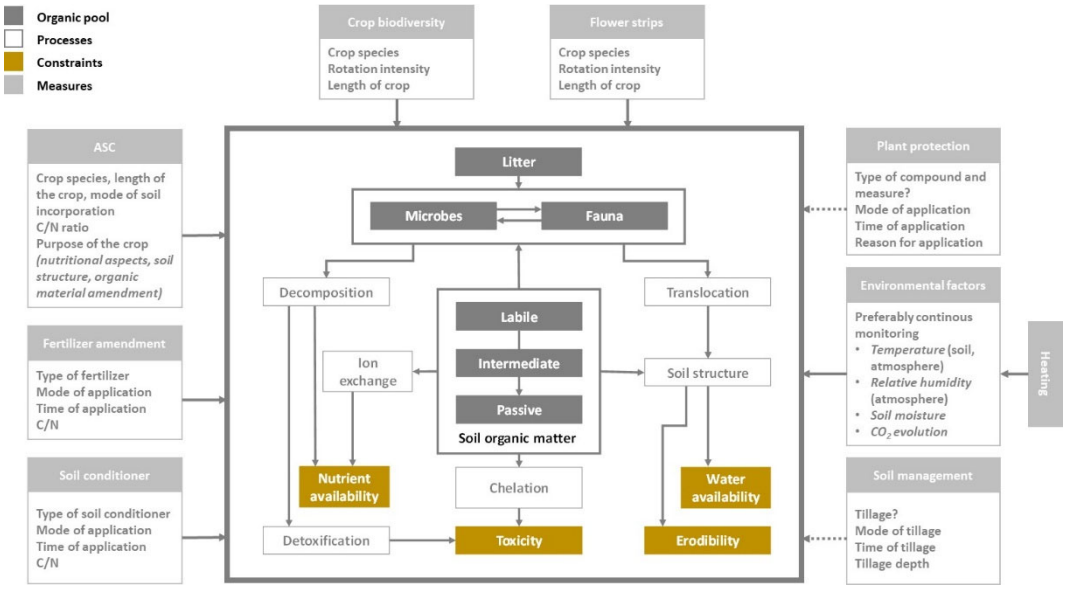

Figure 1 Organic pools, processes, constraints and measures as well as their interactivities in the different soil ecosystems studied in GreenResilient (ASC: agricultural service crop) (based on Pankhurst et al., 1997, modified; illustration: B. Alsanius).

We base our study on the following definitions given for soil health as "…the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air

and water environments, and maintain plant, animal, and human health" (Pankhurst et al., 1997). And the definition for biodiversity as "…the variability among living organisms from all sources, including, 'inter alia', terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part: this includes diversity within species, between species and of ecosystems…" (United Nations (UN), 1992). As indicated by the UN definition, biodiversity as phenomenon is very complex. The introduction of recently developed technology, such as next generation sequencing and related statistical analysis methods, in combination with traditional methods, allow for a differentiation between morphological biodiversity, taxonomic biodiversity (species biodiversity), ecological biodiversity (variations within the ecosystem), and functional biodiversity (measure of the number of functionally disparate species within a population based on feeding preferences or mechanisms, motility and predation).

Soil functions and soil-based ecosystem services are interrelated. This project task report considers soil microbial parameters. These are illustrated in Figure 2.

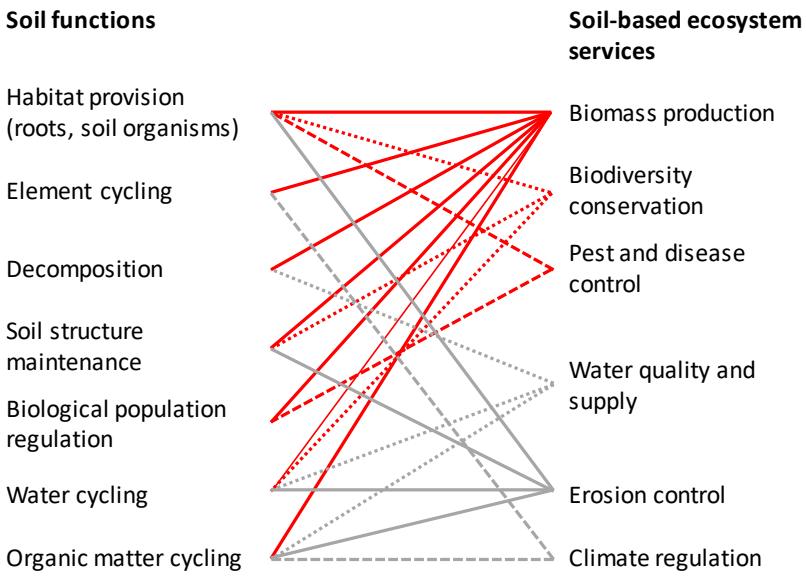

Figure 2 Interactivities between soil functions and soil-based ecosystem services involving soil microbiota (modified from (Bünemann et al., 2018)). Red lines indicate the target parameters and grey lines denote other interrelated soil functions/processes and soil-based ecosystem services.

The present report does not consider an evaluation of ecosystem function beyond soil microbial activity, diversity and health as illustrated in Figure 3. Such task needs to be performed in collaboration with all other tasks within this work package (4) and work package 3.

Figure 3 State-Impact-Response framework of soils (modified from (Brussaard et al., 2007) and (Bünemann et al., 2018).

Soil health is one factor describing the output of a well-functioning ecosystem. However, soil health is a very complex phenomenon and various bioindicators have been used for description, among these

i. soil microbial parameters (soil microbial activity, soil microbial biomass, activity of various enzymes related to degradation of organic matter and/or nutrient cycling)
ii. nematode community structure (including bacteri-, fungi-, herbi-, omnivores as well as predators)
iii. soil arthropod structure
iv. plant root pathogens

This report is limited to the first group of indicators. Soil disease suppressiveness is another output indicating a soil ecosystem health. In suppressive soils, plant root pathogens will either

i. not establish, establishes but causes little or no disease, or
ii. establishes and causes disease at first but then the disease declines with successive cropping of a susceptible host despite the presence of the pathogen (Schlatter et al., 2017).

Soil suppressiveness is microbially mediated. Disease suppressiveness may be general or specific. In the present case, specific suppressiveness was estimated with respect to *Fusarium oxysporum* f.sp. *lycopersici* (FOL). However, soil health is strongly interlinked with the abiotic and biotic soil factors and thus need to be considered for soil health assessment. As indicated in figure 1, soil structure and texture, soil organic matter and nutrient content are important features. These soil health interactivities need to be analysed in collaboration with WP3, where collected data from WP4 will be analysed using multivariate statistical approaches to identify decisive factors discriminating soil health-promoting and counteracting strategies.

2. Material and methods

2.1. Sampling

Soil samples were taken for analysis of microbial activity and biomass as well as assessment of biodiversity at the start and end of the rotation at the five experimental sites in Belgium (BE), Switzerland (CH), Denmark (DK), France (FR), Italy (IT). An intermediate sample was also taken which was outside the original plan and which was funded by supplementary funds (Royal Swedish Academy of Agriculture and Forestry; Project ID: GFS2018-0059). All samplings comprised at least one BAU and one INN system. Samplings within the various rotations at the different sites are displayed in Table 1.

2.2. Microbial activity, biomass and diversity analysis

The first soil sampling was performed before sowing of the first crop, but after the soil had been irrigated. At all sampling events, three replicate soil samples were collected within each treatment block, avoiding the outermost edges of the blocks. For each replicate sample, eight soil cores were sampled and mixed together thoroughly in a clean bucket. From the mixed soil, three individual 50-ml centrifuge tubes were filled to the brim, marked with the sample ID and placed in a freezer (-20°C) until transport of the frozen material to Sweden (see Figure 4).

Analysis with respect to microbial activity based on fluorescein diacetate analysis (FDA), microbial biomass based on phospholipid fatty acid analysis (PLFA), and microbial diversity using metagenomics were carried out following the methods described by Green et al. (2006), Frostegård et al. (1993) and Alsanius et al. (2017), respectively.

Table 1 Sampling events within the various crop rotations at the five experimental sites in Belgium (BE), Switzerland (CH), Denmark (DK), France (FR) and Italy (IT)

Experi-mental site	Start		Mid-term			End		
	Event	Treat-ment	Event	Treat-ment	Preceding crop	Event	Treat-ment	Preceding crop
BE*	Apr 2018	BAU INN	May 2019	BAU INN	Radish Radish	Oct 2020	BAU INN	Tomato Tomato
CH	Apr 2018	BAU INN	Sept 2019	BAU1 BAU2 INN1 INN2	Tomato Tomato Melon Melon	Sept 2020	BAU1 BAU2 INN1 INN2	Tomato Tomato Tomato Tomato
DK	Apr 2018	BAU INN	Oct 2019	BAU INN	Tomato Tomato + ASC	Oct 2020	BAU INN	Tomato Tomato + ASC
FR	Apr 2018	BAU INN	Oct 2019	BAU INN	Eggplant Eggplant + pepper	Aug 2020	BAU INN	Cucumber Tomato + cucumber
IT	Apr 2018	BAU INN1 INN2	Nov 2019***	BAU INN1 INN2	Early lettuce after sola-rization Early lettuce after ASC Early lettuce after ASC	Oct 2020	BAU INN1 INN2	Squash Squash Squash

**At the BE site four samplings were conducted; the additional sampling was done in Sept 2018 in BAU after winter purslane and INN after winter purslane/Swiss chard*

*** INN1 = biodynamic; INN2 = agroecosystem; hereafter called BIODYN and AGROEC*

**** The samples were retaken due to problems with delivery of the frozen material*

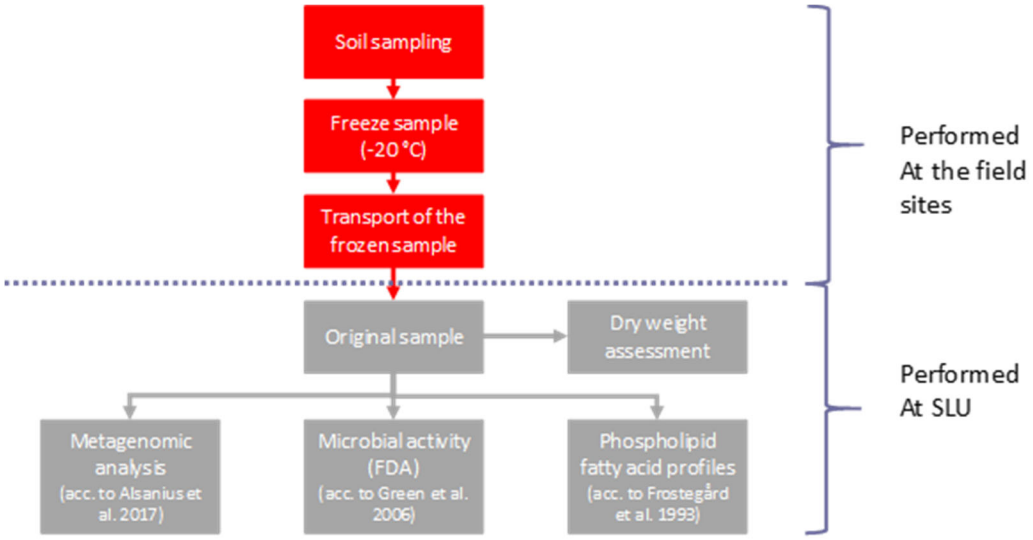

Figure 4 Flow chart of soil samplings and analyses

2.3. Soil suppressiveness bioassay

Sampling with respect to specific soil suppressiveness was only performed at the end of the rotation and was planned to comprise all sites. However, no soil samples for this analysis were received from the site in DK. Samples from the site in BE had been pooled over all systems and could therefore not be used to estimate soil suppressiveness.

Fresh soil samples (5 kg) were taken at the end of the rotation from all sites and treatments where soil samples have previously been taken (see exception above). The samples were kept at ambient temperature, transported with overnight shipment to Alnarp where they were kept at 4-8 °C and dark. All sites reported observed disease problems in the tomato crop. For the bioassay, tomato was used as a model plant and FOL as model pathogen. To investigate the suppressive effect of the soils, the soil from each site and treatment was divided into 12 trays. Half of the trays were subjected to a heat treatment in order to sterilize the soil, and half were kept in room temperature. Tomato seeds (cv. 'Moneymaker') were surface sterilized with NaOCl and washed five times with sterile distilled water to ensure pathogen free starting material. Seeds were sown in both the heat sterilized and the fresh soils (Figure 5). After 7 days of growth, the first row of seeds in half of the trays were inoculated with a suspension of *Fusarium* microconidia with a concentration of 10^6 spores per ml. Plants were grown in a greenhouse at 24°C and 70 % humidity, with a daylength of 16 husing high pressure sodium lamps, for three weeks before plant fresh and dry weight (105°C for 72 h) were assessed. One-way ANOVA was used to calculate significant differences of fresh and dry weight of the plants.

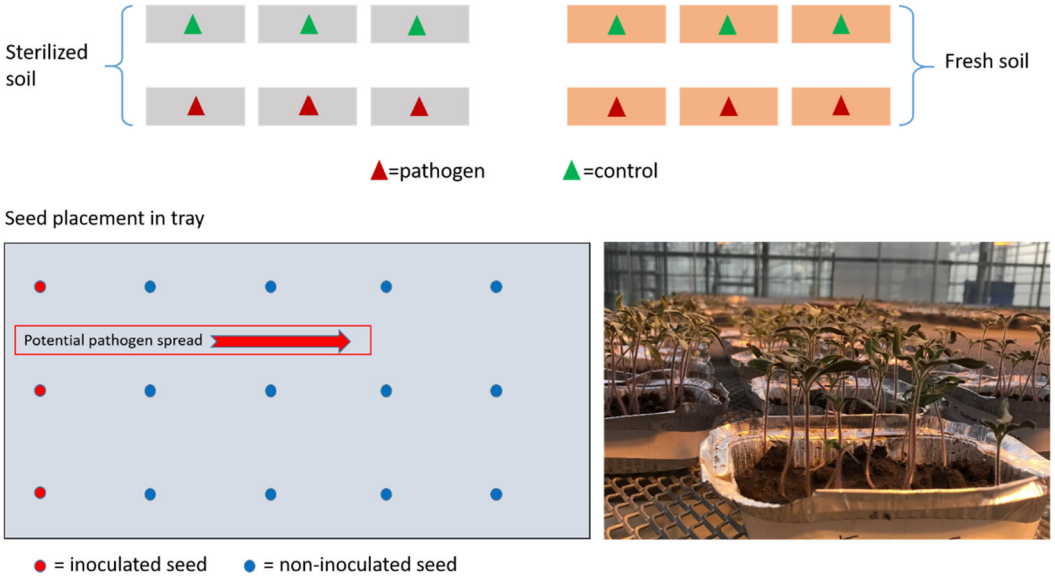

Figure 5 Suppressiveness bioassay overlay. Soil from each treatment and experimental site was divided into 12 aluminum trays. Six trays were thermally sterilized, while the remaining six trays were kept in room temperature. Fifteen seeds were sown in in each tray, and after one week of growth the first row of seeds in three of the sterilized trays and in three fresh soil trays were inoculated with a microspore suspension of Fusarium oxysporum *f.sp.* lycopersici*. (Illustration: AK Rosberg).*

3. Results and Discussion

As the crop rotations differed between the different BAU and INN treatments and between the sites, the results are displayed for each country separately. The results for the bacterial and fungal microbial diversity indices and the microbial activity are displayed in Figure 6.

3.1. Microbial activity

Fluorescein diacetate (FDA) is hydrolyzed by several different enzymes such as proteases, lipases and esterases, which are products of microbial metabolism. The product of the enzymatic reaction is fluorescein which is released into the soil and can be measured with a spectrophotometer. Since the soil samples at the different sites were taken before any actual treatments had started, the results shown below display the baseline, which will serve for forthcoming comparisons at a later point (mid- and end-sampling).

Microbial activity was high in all sites already at the beginning of the experiment. Microbial activity was highest at the FR site (range: 359-377 mg/kg soil) and lowest at the BE (221-242 mg/kg soil) and DK site (234-238 mg/kg soil) in the start of the experiment. Significant differences between the treatments at start were only observed at the Swiss site (range: 245-349 mg/kg soil). Mean values fluctuated between the sampling events, which may be an influence from the previous crop or management practice. Therefore, it is difficult to draw conclusions on the development of microbial activity over the course of the crop rotation. For the BE site, a general trend towards increased microbial activity was found in the BAU, but not in the INN system. At the site in CH, microbial activity fell from the start to the midterm sampling in all treatments apart from the INN1 which displayed a strong increase. Significantly higher microbial activity was also found in the final sample in the CH-INN2 treatment. Microbial activities at the DK experimental site displayed similar dynamics with a drop from the start to the mid-term sampling but increase towards the end. At the French site, there was a trend towards decreasing microbial activities in the BAU system over time, whereas the one in the INN system remained at the same level from the start to the end. Decreasing microbial activities were also noted for the three IT treatments. There was a considerable decrease in activity in both the BAU and BIODYN system from the start to the

intermediate sampling and activities remained at the lower level also in the final sample. A decrease was also found for the AGROEC system. Microbial activity results at the IT site differed significantly between start and end sample.

3.2. Microbial diversity

Along the course of the experiment at the Belgian site, species richness decreased in both treatments. Species richness slightly recovered towards the end of the experiment in both treatments (BAU>INN) but did not regain its initial level. On the contrary, no differences were found with respect to diversity and evenness (Shannon H index) over the four sampling events and BAU and INN rotations, respectively. The contrary was observed for fungal alpha diversity, where species richness remained stable, but diversity and evenness decreased at the end of the rotation (sampling event 4).

No differences could be detected for beta diversity, i.e., diversity between systems, of the bacterial communities. Shifts could be found for the BAU system at the fourth sampling event with respect to fungal beta diversity. This shift might be explained by the considerably higher abundance of two fungi, namely *Verticillium biguttatum* and *Mortierella minutissima*. Both are interesting from a plant health point of view. *V. biguttatum* is ascribed antagonistic effects towards *Rhizoctonia solani* (Morris et al., 1995), a fungal pathogen common on tomato while *M. minutissima* has chitinolytic activities (Ozimek and Hanaka, 2021), an important function with respect to fungal interactions. With respect to the fungal community structure, it is worthwhile to mention that *Fusarium oxysporum* was present in all systems and at all sampling events. However, the analysis did not account to subspecies level and the displayed group may comprise both non-pathogenic and pathogenic *Fusarium oxysporum*. Also, *Monographella cucumerina*, a pathogen on pumpkin, melon (Infantino et al., 2021) and rocket (Gilardi et al., 2018), increased in relative abundance after cultivation of melon. Network analysis at the end of the rotation indicated a microbial network with stronger coherence for the INN system as compared to the BAU system.

Figure 6 Soil microbial activity, as well as fungal and bacterial diversity indices (Shannon diversity index bacteria and fungi: Shannon B, Shannon F; Chao1 for bacteria and fungi: Chao1B, Chao1F) the five experimental sites in Belgium (BE), Switzerland (CH), Denmark (DK), France (FR) and Italy (IT). At each site samples were taken at the beginning, midterm and at the end of the two-year rotation in the business as usual (BAU) and innovative (INN) system from three blocks with three replicates. Two intermediate samples were taken at the site in Belgium. At the Belgian, Danish and French sites samples were collected in one BAU and one INN system. At the Swiss and Italian site samples were taken in two BAU (CH) and two INN (CH, IT) systems. The IT-INN1 was managed according to biodynamic principles, the IT-INN2 followed an agroecological approach. Results are expressed as % of the start value (=100%)

At the Swiss (CH) site, bacterial alpha biodiversity was changed significantly in the BAU systems when considering both richness as well as diversity and evenness. The same trend was found in both BAU systems. Species richness decreased significantly in both BAU systems, ending up at a Chao1 index almost 50% of the

initial one. Species richness decreased also in the two INN systems at the CH site, from the first to the second sampling event, but recovered and reached a level similar to the start sampling event. Also, the Shannon index was significantly reduced in the two BAU systems from the start to the end of the rotation. No large deviations were found for diversity and evenness for the two INN systems at the CH site. In contrast, an increase in fungal richness was detected for the two BAU systems and the INN1 system during the course of the experiment. Fungal diversity and evenness fluctuated in all systems, and significant reductions in Shannon index were found for BAU2 and the two INN systems when comparing start and final soil samples.

With respect to fungal beta diversity, the two INN systems grouped separate in the midterm and final samples. Bacterial beta diversity clustered for all systems and samplings, except for BAU1 and INN1 at the last sampling event as well as BAU2 at the second sampling event.

Bacterial relative abundances, assessed on genus level >2%, showed considerable shifts in both BAU systems with respect to unclassified Gemmamonadaceae, which increased towards the end of the experiment. A similar shift, but less pronounced was found for this genus in INN1. This might be due to the changes observed for subgroup 6 (unclassified) which went into the opposite direction. In both INN systems the relative abundance of nitrifying bacteria increased towards the end of the rotation. This effect was most pronounced for INN2.

Fluctuations occurred also for fungal relative abundances. For both INN systems, the class Eurotiomycetes (unclassified) became more abundant. Also, the occurrence of *Cladosporium sphaerospermum*, *Chryosporium lobatum* and *Chrysosporium* sp. increased towards the end of the experiment. *C. sphaerospermum* is known as a decomposer of organic matter and observed on decaying branches and leaves in the Mediterranean region (Osono et al., 2004, Pereira et al., 2002). The increased presence of *C. lobatum* can only be speculated about. It has been described in the context of parasitism of mosquito larvae (Mohanty and Prakash, 2008). It would be interesting to follow up on the fungus capacity to antagonize other soil insects. The pathogen, *Micrographella cucumerina* was more abundant in soil samples from INN2 at the end of the rotation. Likewise, *Verticillium biguttatum* increased.

At the Danish (DK) site, there was a trend towards increased bacterial species richness in both systems, however these are trends and not significantly different for all sampling events. No change occurred with respect to bacterial diversity and evenness. Whereas fungal richness did not show any differences over the course of the rotations in the systems, a significant decrease in fungal diversity and evenness was found in both BAU and INN system. The Shannon index decreased quicker in the BAU system as compared to the INN system. Shifts were observed both for the

bacterial and beta diversity. From high similarity of the start samples, diversity increased during the course of the experiment. This was more pronounced for fungal than for bacterial analyses. At the end of the rotations, both bacterial and fungal samples of the INN system deviated significantly from the start samples. At the end of the experiment, the relative abundances on genus level were substantially higher for *Bacillus* sp. in the INN system as compared across systems and sampling events. An increased abundance of subgroup 6 (unclassified) was observed for both systems at the end of the rotation, as compared to previous sampling events. With respect to relative abundance of soil fungi on genus level, *Fusarium oxysporum* was present in both systems at the start but vanished below 2% of relative abundance during the course of the experiment. Notably, high abundances of yeasts were monitored in samples from both systems. Yeasts are important for degradation of less recalcitrant organic matter components.

At the French (FR) site, alpha diversity of soil bacteria and fungi oscillated but remained on a stable level. There was a trend towards increase species richness in the FR BAU system, and a slight trend towards decreased species richness in the FR INN system. With respect to beta diversity of soil bacteria, a shift from the start to the end was found for both systems and final samples clustered outside the ones of the start samples. Whereas no distinct change in beta diversity could be found for the FR BAU system, the soil fungal samples taken from the INN system clustered individually at the three samplings.

Relative abundance of bacteria shifted most dramatically for subgroup 6 (unclassified), which decreased, and Gemmatimonadaceae (unclassified) and *Gemmatimonas* which increased in both systems. The latter one is interesting from a mineralization point of view (Li et al., 2017). Gemmatimonadaceae is still a quite unstudied family, but one representative, *Gemmatimonas aurantica* has been shown to be a phosphate accumulating organism (Zhang et al., 2003). The present analysis was performed on genus level, hence discussion regarding presence of a certain species of bacteria, or its function, are speculative.. Furthermore, the relative abundance of nitrifying bacteria decreased in both systems over time, whereas Chitinophagaceae (unclassified) and the genus *Olivibacter* increased. The latter one is known as a colonizer of ligneous material and referred to as organic matter decomposer (Haidar et al., 2021). A representative of the *Olivibacter* genus has been ascribed to be able to degrade diphenol (*Olivibacter sitiensis*) (Ntougias et al., 2014).

Fungal relative abundance fluctuated between the systems and samplings. However, there are two observations that appear relevant to comment on, namely the increase in relative abundance of the genus *Glomus* and *Microascus*. The genus Glomus hosts mycorrhizal fungi. The genus *Microascus* hosts some degraders of organic matter (Sandoval-Denis et al., 2016).

At the Italian (IT) site, bacterial and fungal alpha diversity was affected by the treatments. Bacterial species richness decreased in all three systems during the run of the experiment. The drop from start to the end was most pronounced for the BAU system, but differences were also significant for the other two systems. Likewise, bacterial diversity and evenness decreased during the run of the experiment in all three sites. As for richness, the change became evident already at the midterm sampling for BAU but was distinct for all three systems at the end of the rotations. Fungal alpha biodiversity was fluctuating when considering species richness. The BAU and AGROEC system displayed contrasting trends in the midterm sampling but ended at richness levels similar to the start sample. For the BIODYN system, also fungal richness dropped over the course of the experiment. Fungal diversity and evenness remained stable in the AGROECO system but decreased in both BAU and BIODYN during the course of the rotations. Beta diversity analysis of soil bacteria did not reveal any distinct pattern. However, deviations in beta diversity were found for the BAU system which displayed increased grouping outside the main cluster during the course of the experiment.

Considerable changes were found in microbial community structure of the run of the experiment at the IT site. Interestingly, the relative abundance of Bacillus fell in all three systems. This observation was most pronounced in BAU. Likewise, the abundance of *Sphingomonas* increased. *Fusarium oxysporum* was present in most samplings and also *Monographella cucumeria* was recurrently abundant.

The survey of biodiversity and community structure is very comprehensive. There are some signs indicating shifts during this two-year-period of observation. However, it is important to keep in mind, that consistent shifts to become distinct usually require longer periods of observations. From the present set-up within the project, no comparison can be done between countries. The different systems cannot be compared other than on a system base. From the present data it cannot be concluded on single treatments within the crop rotation or individual fertilizers or additives. When concluding on the results, it is of utter importance to be aware of the latest crop grown before the sampling was done. Land use are considered as microbial community drivers and will have an impact on the microbial community structure (Lori et al., 2017). These may partly explain differences between sampling events within a system.

It would be valuable to look into the results from a functional perspective. In contrast to nematology, functional databases for microbial soil microorganisms have not reached this stage (terragenome, (Vogel et al., 2009)). It would be interesting to revisit the data and add this dimension to the material further on.

3.3. Suppressiveness against *Fusarium oxysporum* f.sp. *lycopersici* (FOL)

Concerning the results from the suppressiveness bioassay the assay could be carried out for samples coming from the IT, FR and CH experimental sites. The results from the Italian site showed that there were no significant differences in either fresh weight or dry weight measurements between any of the site treatments. In addition, there were no significant differences between Fusarium-inoculated, healthy, or sterilized soils either. For the French samples, 3 different site treatments were evaluated for suppressiveness: INN, BAU and an intermediary treatment called "Inter". Both fresh- and dry weights of the INN treatment were significantly higher compared to Inter. BAU did not significantly differ from either of the other two treatments. This was the case for the inoculated and healthy soils that had been sterilized before the assay while there were no significant differences between the fresh soil samples irrespective of treatment. A trend in both the French and Swiss soil samples were that the plants grown in the sterilized soils had a higher fresh and dry weight. This could be caused by a release of nitrogen in the soil during the sterilization process leading to an increased growth potential of the plants. However, the reduced growth in the fresh soil samples also points to a presence of pathogens in the soils already before they were inoculated with FOL. Due to the production practice of growing tomato every year, or every other year, it is highly plausible that there has been a build-up of root pathogens for a long period of time. Data from metagenomic sequencing showed the presence of a range of pathogenic organisms in all experimental sites at rather high abundances. While the soils from the different sites did not show a clear suppressive effect against FOL, it does not necessarily mean that the soils have no suppressive effect at all. In this trial only one pathogen was tested, due to its importance in the production of a high-value crop, but suppression could be present for other disease-causing organisms. The lack of significant differences in the fresh and dry weights of tomato plants from inoculated and non-inoculated soils could also be due to the type of suppressiveness where a pathogen establishes but does not cause a growth reduction. While the plant weights were not significantly different, the weights of the *Fusarium*-inoculated plants were generally lower than those of the non-inoculated ones. A longer growing period might have been beneficial for observing the long-term effect of the pathogen.

4. Conclusion

The present results need to be cautiously interpreted. As the environmental preconditions at the five experimental sites in BE, CH, DK, FR, IT deviate, results from different countries cannot be compared with each other. Furthermore, as the crop sequence in the crop rotations vary also within the countries with respect to BAU and INN system, the general trends on system level are in focus. The fact that crops are driving soil microbial communities, and that consistent changes in soil microbiota takes longer than two years, is imperative for interpretation of the results.

- No consistent interactions between microbial activity and microbial alpha diversities for bacteria or fungi could be found. Shifts in beta diversity were found for some systems during the two-year-long experimental period.
- Metagenomic data indicated the presence of the target pathogen (Fusarium oxysporum f.sp. lycopersici) used for the suppressiveness test in all soils. In general, plant performance from inoculated and non-inoculated control (heat treated) plots was higher than the ones using untreated soil. No specific disease suppressiveness towards *Fusarium oxysporum* f.sp. *lycopersici* could be stated in BAU or INN systems.
- Metagenomic analysis show a potential build-up of pathogens in soils.
- Data from microbial analyses needs to be related to data on soil nutrient content, as well as the presence of nematodes and weeds for a clearer understanding of cause and effect in the different production systems.

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