Healthy plants from healthy soils

<u>Re</u>silience and <u>st</u>ability of <u>or</u>ganic cropping systems

(RESTOR)

(Application Number 2015-65)

Project management report

For the first project year: February 2016 – January 2017

Thomas F. Döring, Christian Bruns, Dagmar Werren, Imre Vágó, János Kátai, Magdolna Tállai, Christine Watson

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Contact:
Thomas Döring, thomas.doering@agrar.hu-berlin.de
Tel. 0049 (0) 30 2093 46467
Humboldt-Universität zu Berlin, Fachgebiet Acker- und Pflanzenbau
Albrecht-Thaer-Weg 5, 14195 Berlin, Germany

Contents

1 Summary

In this project we study the resilience of soils and crops, i.e. their ability to regain functionality after exposure to stress. The function of soils that we have selected for this study is the ability to suppress soil-borne plant diseases. In the context of resilience this important biological function of soils has previously not been studied. In order to represent a large degree of variation in terms of soil properties, we use soils and crop data from different long-term agricultural field experiments across a large geographic range, namely from North-Eastern Germany, Scotland and Hungary.

The **experimental biotest on soil resilience**, which makes up the largest part of the project, has been completed in the past project year. However, the originally planned number of soil samples could not be included in the biotests due to capacity limits of the testing facility and restrictions with regard to taking soil samples from the long-term field trials. Statistical analysis of the collected data from the soil resilience tests is nearly complete. The preliminary data analyses of the biotests show novel and highly interesting results. We found that different soils strongly differ in their ability to suppress diseases. Our results also show that a stress event combined heat and drought can strongly reduce this disease suppressiveness of soils. Further, the response of suppressiveness to the heat stress depends on the provenience of the soil. The Scottish soils were much more affected than the German and Hungarian soils. Thereby, the data analysed so far suggest that microbial communities responsible for suppressiveness are adapted to prevailing climate, which has potentially severe consequences for the impact of climate change upon plant health. With regard to resilience, we found that after being exposed to stress, (some) soils were able to regain suppressiveness after some time. Finally, our results do *not* seem to support the hypothesis that general indicators of soil fertility are positively linked to either suppressiveness or soil resilience. This is surprising and warrants further investigations into the mechanisms of suppressiveness.

The planned **chemical and microbiological analysis** of the soil samples has been completed, except for a small number of soil samples that will have to be re-analysed for conducting plausibility checks. In-depth statistical data analysis is pending, but initial results confirm extremely large variation in critical soil fertility parameters, which will help to interpret the data collected in the biotests.

For the **crop resilience analysis**, data collection and compilation is partly completed. Data analysis for measuring resilience in annual crops has started on two subsets of crop data. The accompanying analysis of yield stability has been started and is complete for one data subset. A peer-reviewed publication based on this stability analysis is expected to be submitted by about May 2017. Furthermore, novel methodologies for analysing resilience and stability have been developed, resulting in a further paper to be submitted in April 2017. However, due to limited data availability from some of the long-term experiments, the overlap between soil resilience data and crop resilience data is small, thereby restricting the possibilities to test our final hypothesis that resilience data and crop resilience are linked. Therefore, we plan to fill this gap with supplementary experiments in spring and summer of 2017. This will require the project duration to be extended to end of October 2017. We therefore **request a cost-neutral extension of the project** until 30/10/2017.

2 Background and project aims

The promotion and maintenance of health is one of the key principles of organic agriculture. A strong criterion of health that is recognised for all domains of agriculture is resilience, i.e. the ability to recover after disturbance or stress. This project aims to fill three critical gaps in the understanding of resilience in organic (and non-organic) cropping systems. First, the resilience of soils has so far almost exclusively been studied in terms of the decomposition of organic matter, whereas the resilience of other critical soil functions has largely been ignored. Second, key factors that influence the resilience of annual crops to climatic stress events are currently not well understood. Third, it is unclear whether – and how – soil resilience and crop resilience may be linked, e.g. through common underlying drivers. In this project we investigate the resilience of the soil's ability to suppress plant diseases and the resilience of annual crops in response to climatic stress factors, using a set of long-term field experiments from three countries with a large eco-geographic range. We expect that our results may help farmers to design more reliable and resilient cropping systems and that our findings will improve the understanding of what factors are responsible for system health in ecological farming. By exploring potential links between soil health and plant health, we aim to widen the currently insufficient scientific basis underlying the principle of health. The main objectives of this project can be summarized as follows:

(a) to investigate the **resilience of the soil's ability to suppress plant diseases**; in particular, to identify factors that determine the resilience of soils in their ability to support plant health and to gauge the potential pre-adaptation of soil microbial communities to climatic disturbances;

(b) to study the **resilience of annual crops** in response to climatic stress factors; in particular, to investigate the ability of annual crop species to respond to stress experienced within the growing season by compensatory growth of subsequent yield components later on;

(c) to explore the potential **links between soil health and plant health**, by joining up results from the soil resilience and crop resilience experiments.

This will improve the understanding of what factors are responsible for system health in ecological farming. In our project application we identified four key hypotheses:

1. Soil resilience (in its function to suppress plant pathogens and in response to climatic stress) is dependent on specific management factors, especially soil organic matter (SOM) management.
2. Soil resilience is further determined by the geographic location and site conditions that are independent from management; specifically, soils from sites with a high prevalence of climatic stress are pre-adapted to recover, regaining their suppressiveness faster after being stressed.
3. Crop resilience to climatic stress is influenced by agronomic management factors and geographic location; as before, SOM management plays a key role, with high SOM input associated with higher crop resilience.
4. Across sites and different agronomic management treatments, soil resilience is positively correlated with crop resilience in response to climatic stress events.

3 Methods

3.1 Sample selection from long-term field trials

At the project start in February 2016, the project partners met in Berlin to discuss in detail and agree on the methodology for the resilience tests. Further, we selected those long-term field experiments (LTFE) that would be suitable for the resilience tests. The resilience tests required large amounts of soil per trial, which restricted the selectable trials, because the long-term nature and value of the trials forbids exploitative soil sampling that would endanger the integrity of the trial for future studies. The final selection of LTFEs was not only based on plot size (as a proxy for the possibility to take sufficient amounts of soil), but also on data availability, trial design, and trial factors. In addition, it was decided which particular treatments from the selected LTFEs were to be included in the biotests and which ones were to be analysed for chemical and microbiological properties. Four LTFEs from three countries were selected for soil resilience biotests, with two treatments each. Two LTFEs were from Eastern Scotland (called 'Tulloch' and 'Woodlands', both near Aberdeen), one from North-Eastern Germany (called 'Thyrow D41', near Berlin), and one from Eastern Hungary (called 'Westsik', near Debrecen). Thereby the selected trials span a geographic distance of nearly 2,400 km. Additional trials from Hungary (called 'Latokep') and from Germany (called 'Thyrow ABS') were selected to supplement the analysis of chemical and microbiological parameters. Further, the analysis of crop resilience was supplemented by trials from Germany (called 'Dahlem D3' and 'Dahlem E-Feld'). Thus, in total eight LTFEs were selected to be included in this project. Three of these LTFEs are conducted according to organic farming standards (Tulloch, Latokep) or contain organic farming treatments (Thyrow ABS). In the selected trials, the predominant trial factor is crop fertilisation. Table 1 shows some general information about the selected LTFEs.

Table 1: Characterisation of the selected long-term field experiments; X: included; -: not included

Country	Trial Name	Start year	Management	Treatment factors	Soil resilience tests
UK	Tulloch	1991	Organic	Rotation	X
	Woodlands	1922	Conventional	Fertilisation	X
Germany	Thryow D41	1937	Conventional	Fertilisation	X
	Thryow ABS	2005	Organic/Conventional	Management, crop species	-
	Dahlem E-Feld	1954	Conventional	Crop species	-
	Dahlem D3	1923	Conventional	Fertilisation, tillage, rotation	-
Hungary	Latokep	2000	Organic	Management, crop species	-
	Westsik	1929	Conventional	Fertilisation, rotation	X

3.2 Determining resilience of soil suppressiveness ('biotest')

With regard to the biotest, necessary pre-tests (for ensuring robustness and reliability of the methods) and the general experimental design (see below) meant that large amounts of soil were required from each selected treatment of the four chosen LTFEs. A full test of resilience required five factors to be combined: (A) site (with at least 2 and up to 4 levels: Westsik from Hungary, Thyrow from Germany, and Tulloch and Woodlands from Scotland); (B) treatment within site (with at least 2 levels to represent the effect of management); (C) Stressing of the soil (with 2 levels: with and without combined drought and heat stress); (D) Recovery time of the soil (with at least 2 levels: short vs. long); (E) Inoculum concentration (with at least 3 levels). This resulted in a minimum number of 48 variants per replication. From experience with the biotest, a minimum number of 5 replicates was considered to be necessary for generating robust results.

Samples from the top soil were taken in spring 2016 and transported in cooled containers to the central testing facility in Witzenhausen. All soil samples were then stored at 4°C until immediately before the start of the pre-tests resp. main tests. The soil resilience tests are based on the pea-*Pythium* pathosystem and were conducted in controlled climate chambers. Resilience of the suppressiveness of different soils was studied in five steps:

(1) Subjecting the soils to the climatic stress event was performed by using controlled heat (40°C vs. 15°C) and drought (-50% of moisture content) for a period of 4 days, with subsequent return to baseline temperature and moisture (re-wetting).

(2) Soils were inoculated with different concentrations of a well-characterised isolate of *Pythium ultimum*, as well as a *Pythium*-free control.

(3) Peas were then sown in pots with stressed and non-stressed soils, with different times elapsed after the stress event (1 day, 22 days, 43 days).

(4) The proportion of diseased peas was counted and the biomass of above ground parts of the plants was weighed. Disease severity was also scored and measured (as length of lesions) in selected treatments.

(5) With the collected data, resilience can then be determined by comparing the time-dependence of the recovery of soil suppressiveness against the pathogen. Specifically, after the stress event, we expected that the suppressiveness of the soil decreases initially, and recovers more quickly in some soils.

Before these main tests for quantifying resilience could be performed, pre-tests had to be run (a) to select a suitable pea cultivar; (b) to determine water holding capacity of the different soils; (c) to determine the optimal levels of inoculum so that suppressiveness would in principle be detectable in all candidate soils; and (d) to optimize the time for assessing recovery of the suppressiveness. The biotests were organised in four experiments (**Table 2**). Pretests were conducted with soils which had been taken from the field margins of the long-term trials. This was done to reduce the impact of soil sampling on the integrity of the LTFEs. Although treatments were not identical between the different LTFEs, it was possible to group them according to their general fertility level (**Table 3**).

Table 2: Pre-tests and main tests for determining soil resilience

Test	Soil from	Aim
Pre-test I	Field margin near LTFE, at each site	Determining optimal level of inoculum, 6 levels
Main test I	Plots of LTFEs; partly pooled*	Quantifying effect of treatment and site on suppressiveness
Pre-test II	Field margin near LTFE, two sites**	Determining optimal time for measuring recovery
Main test II	Plots of LTFEs; partly pooled*	Testing resilience (recovery) of suppressiveness

*in the two LTFEs that have proper field replicates, these had to be pooled prior to using the soil in the biotests because (a) the available amount of soil was limited; and (b) equal inoculation of samples could be performed more precisely; **(1) Thyrow and (2) pooled sample from Woodland and Tulloch

Table 3: Treatments selected for soil resilience tests

	Site							
Criterion	**Westsik**		**Thyrow**		**Tulloch**		**Woodlands**	
Treatment	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
Name	[X]	[I]	[a3:NPK+FYM]	[a8:PK]	[Grass]	[Stockless]	[NPK]	[PK]
Cereal yield (rel.)	100	48.2[a]	100	17.7[b]	100	ca. 90[c]	100	ca.90[d]
C_{org} (%)	0.41	0.30	0.55	0.25	~2.94[e]		4.21	4.34

[a]: winter rye; [b]: spring barley; [c]: spring oats (Watson et al 2011, Organic Agriculture); [d]: spring oats (Walker et al. 2010); [e]: re-analysis pending

3.3 Chemical and microbiological characterisation of soils

A total of 66 soil samples from the LTFEs were subjected to standard chemical and microbiological characterisation by the soil laboratory at Debrecen University. The measurement programme included soil moisture, organic carbon (C_{org}), microbial biomass carbon (C_{mic}), soil respiration, pH, and enzyme activity (saccharase, dehydrogenase, phosphatase). In subsequent data analyses these data will serve to identify covariates that have a potential impact on soil resilience resp. crop resilience.

Soil pH was measured in distilled water (H_2O) and 1 M KCl, with a ratio of soil to water of 1/2.5 w/w. Soil organic carbon was determined according to Szekely et al. (1960)[1]. The rate of soil respiration (CO_2-production) was measured after 10 days incubation with NaOH trapping (Öhlinger, 1996). Microbial biomass carbon (MBC) was assessed by the fumigation-extraction method according to Vance et al. (1987). Phosphatase activity was determined by the Krámer-Erdeiné method (cit. Szegi, 1979). Saccharase activity was measured according to Frankenberger and Johanson (1983) and dehydrogenase activity by the method described in Mershi (1996).

3.4 Determining crop resilience

Compiling data on yield and yield components of crops in the selected long-term trials is partly completed. Data from the German trials is finalised and analyses on two long-term experiments have been performed on crop resilience. These analyses will be completed over the coming months. Resilience was measured in the long term trial D3 in Berlin Dahlem as the ability of winter wheat to compensate the reduction in a particular yield component by subsequent yield components. Similar analyses were run for the so-called E-Feld in Berlin Dahlem with various crop species. Among the datasets of this project, the E-Feld and the D3 are the two long-term trials with the largest data sets on yield components. The D3 combines five trial factors (tillage depth, liming, farm yard application, phosphorus application, rotation). The E-Feld has no agronomic treatments but provides continuous measurements of yield and yield components for seven crop species.

In addition, the resilience analysis was supplemented by an analysis of temporal yield stability. This analysis was integrated with a parallel research project, so that a larger data set could be used. A peer-reviewed publication based on this stability analysis is expected to be submitted by about May 2017. In addition, the methodologies for analysing resilience and stability have been further developed, which resulted in a paper to be submitted in April 2017.

3.5 Linking soil resilience and crop resilience

The limited data availability from some of the long-term experiments combined with the restrictions on taking large amounts of soils from these values field experiments means that the overlap between soil resilience data and crop resilience data is smaller than anticipated. This restricts the possibilities to test our final hypothesis that resilience data and crop resilience are linked. Therefore, we plan to fill this gap with supplementary experiments in spring and summer of 2017 at Humboldt University Berlin. This will require the project duration to be extended until the end of October 2017, but will not entail any additional costs.

[1]. References are provided upon request.

4 Preliminary results

4.1 Determining resilience of soil suppressiveness ('biotest')

Pretest I showed that the experimental system was robust, with a clear dose-response relationship between the inoculum level of the pathogen and the biomass of the pea plants (Fig. 1). In comparison to sterile sand, the tested soils showed clear evidence for disease suppressiveness. While no peas survived in sterile sand when the inoculum level was 2.5‰ or higher, disease was suppressed by the tested soils even at the highest inoculum level of 15‰. Based on these pre-tests, it was decided to select three inoculation levels (0‰, 2.5‰ and 7.5‰) out of the six tested levels for further tests. At 0‰, the pea biomass in the soils was found to be lower than in sterile sand; this is thought to be a combined effect of nutrients in the sand and biotic factors in the soil.

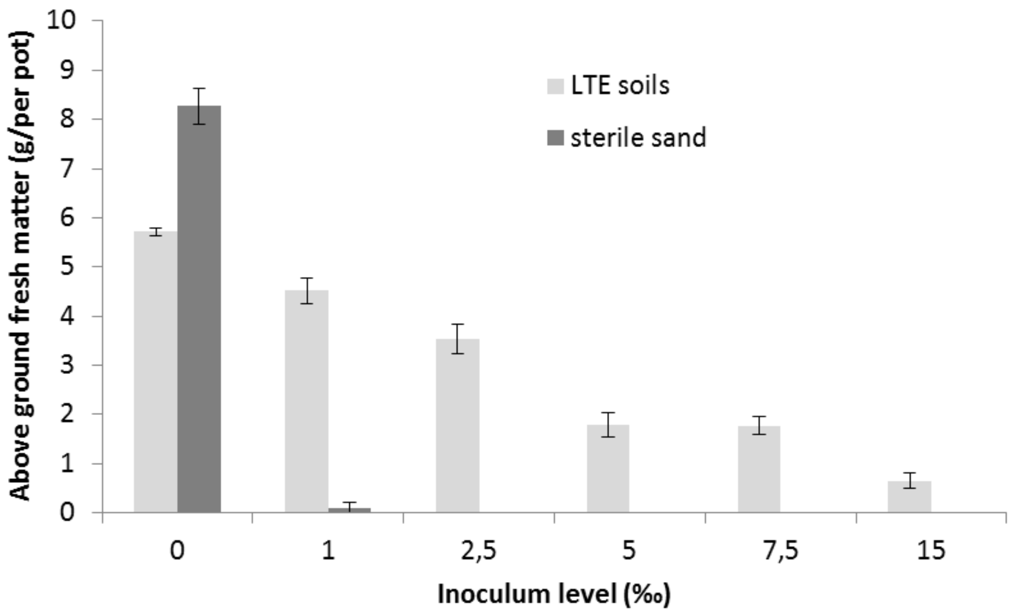

Fig. 1: Above ground fresh matter of peas in the biotest (mean and s.e.), depending on the inoculum level with *Pythium* mixed into the substrate.

As in the pre-test, the different soils showed clear evidence of suppressiveness in main test I. Peas survived to different degrees, depending on the level of inoculum (Fig. 2), but also differences among soils were evident. For example, at the highest inoculation levels, pea survival was greatest in the Westsik soils, and smallest in the organically managed Tulloch soils. This was unexpected, since much lower levels of soil organic carbon were measured in the Westsik soils than in the Scottish soils (see section 4.2. below). Also within sites, suppressiveness was not linked to general parameters of soil fertility. For example, the unfertilized control soil Westsik B showed higher levels of suppressiveness than the fertilised soil Westsik A from the same long-term experiment.

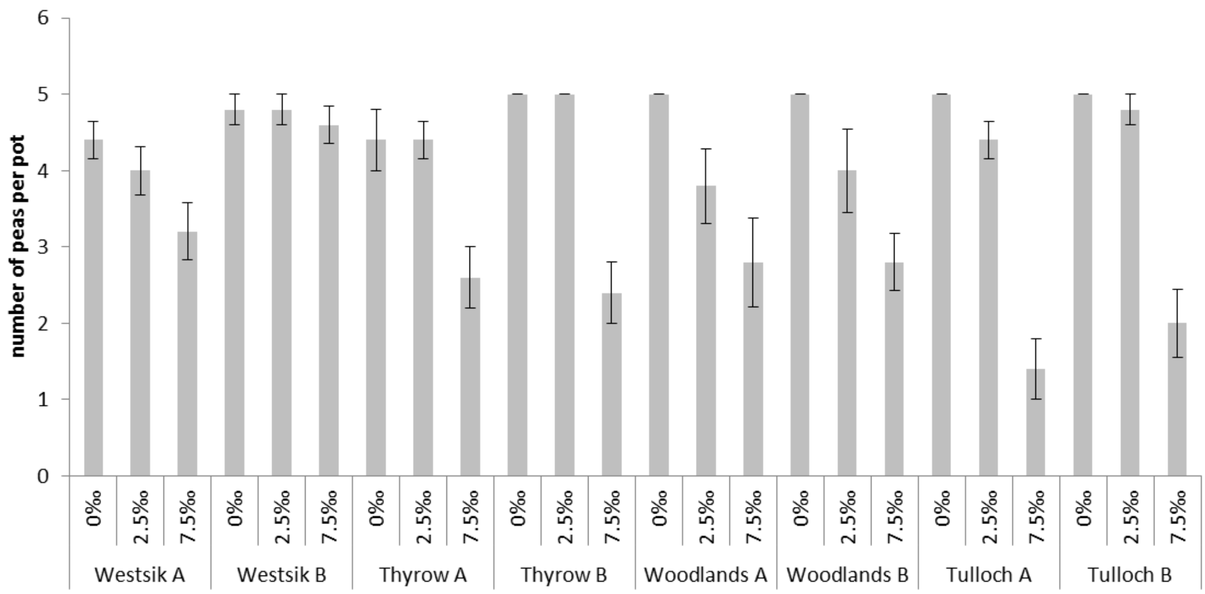

Fig. 2: Main test I: Suppressiveness of the eight tested soils, measured as response of the number of peas per pot (means and s.e.) to the inoculum level (‰).

Pretest II aimed at determining the optimal timing for measuring recovery from stress (**Fig. 3**). Stressing the soils with heat (40°C) and drought reduced the ability of the soil to suppressive the disease; immediately after the stress event, the soil from Thyrow was less strongly affected than the soil mixed from the two Scottish sites. However, the ability to suppress disease was later regained by the soil from Scotland, thereby showing clear signs of resilience.

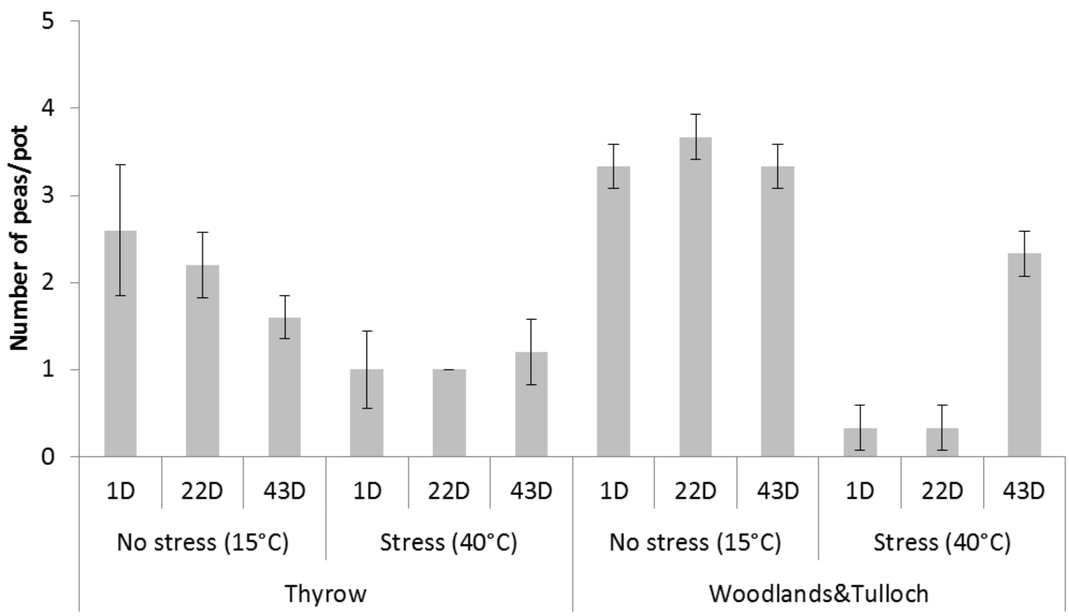

Fig. 3: Selected results from pre-test II: Number of peas per pot (means and s.e.) depending on site (Thyrow vs. Scottish soils pooled from Woodlands and Tulloch), stress level of soil (with and without combined temperature and drought stress), and time (1 days, 22 days and 43 days after stress event); only results from the high level of inoculum (7.5 ‰) are displayed. At 0 ‰ all peas survived in all treatments (i.e. they reached the maximum number of 5). At 2.5 ‰ results were at intermediate levels.

At 43 days after the stress event, the suppressiveness was much higher than immediately after the stress, or at 22 days. Based on these results we selected the time of 43 days after the stress event for main trial II.

In main trial II, the impact of the combined heat and drought stress on the suppressiveness of the soil was found again. Stressed soils had a lower survival of plants than non-stressed soils (**Fig. 4**), but this effect was strongly and significantly dependent on site (**Fig. 5**, P<0.001). In particular, the Scottish soils were much more negatively affected by the heat application than the German and Hungarian soils.

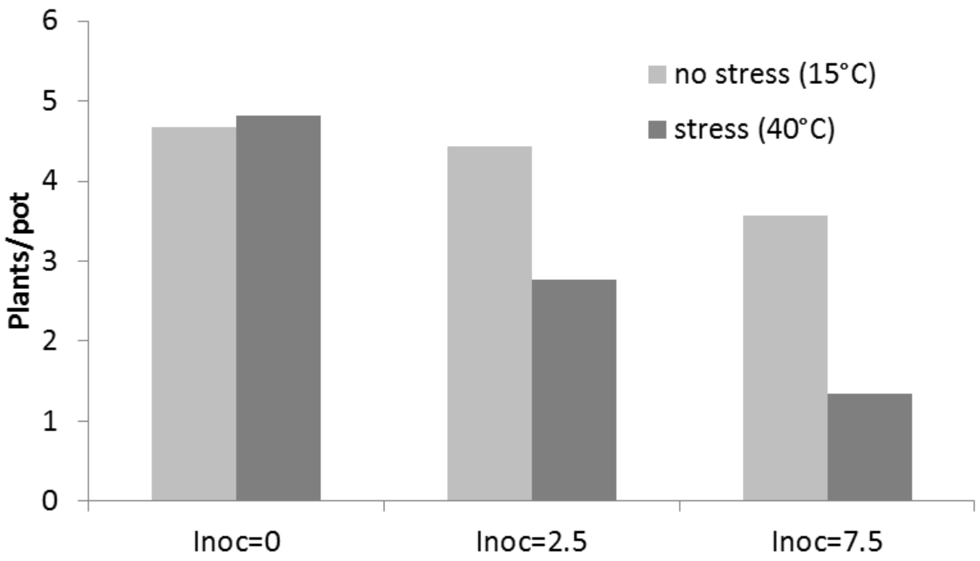

Fig. 4: Results of Main test II: Number of plants per pot depending on inoculum level and stress level.

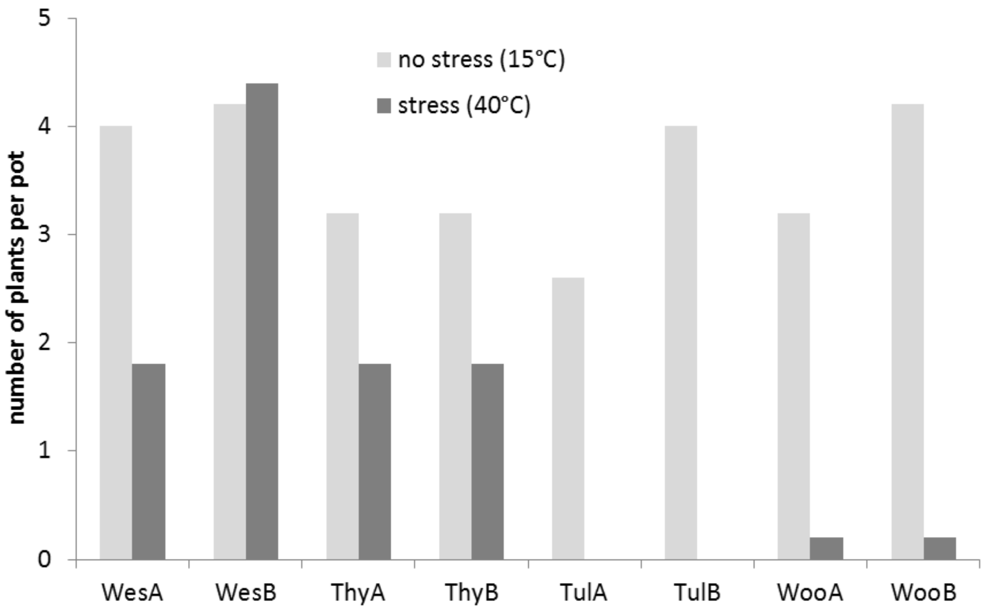

Fig. 5: Effect of combined heat and drought stress applied to soil on number of pea plants after inoculation with a concentration of 7.5 ‰.

With regard to resilience, the picture was more complex. Significant recovery of the ability to suppress diseases was only found in one out of the eight test soils (Tulloch B, Fig. 6, difference between "day1 soil" and "day43 soil" significant at P<0.01). In six other soils, there was no difference in suppressiveness between soils that had been stressed 43 days before and soils tested immediately after the stress event. In the Westsik B soil, the ability to suppress the disease was significantly weaker 43 days after the stress event than 1 day after it (Fig. 7, P<0.05).

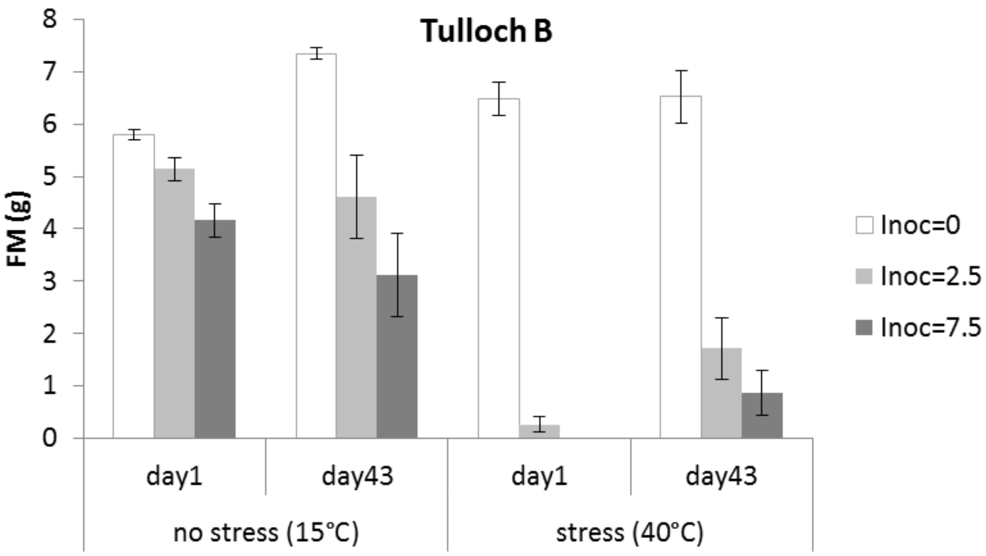

Fig. 6: Fresh matter of pea plants on the Tulloch B test soil, depending on stress level and the number of days elapsed since the stress event.

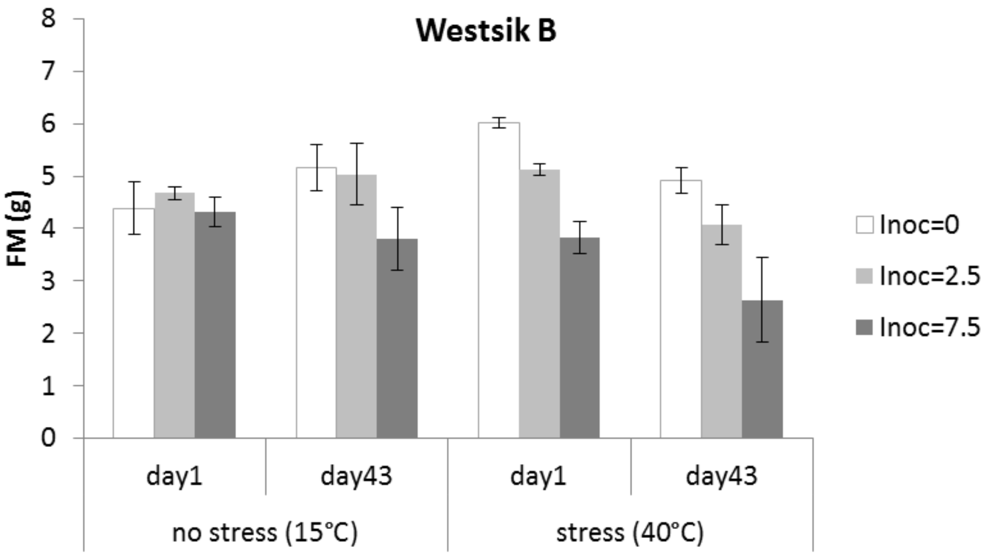

Fig. 7: Fresh matter of pea plants on the Westsik B test soil, depending on stress level and the number of days elapsed since the stress event.

4.2 Chemical and microbiological characterisation of soils

In the soil samples from the selected long-term trials there was a large range of values for both soil carbon and microbial biomass (Fig. 8). Amongst the samples there was a positive correlation between soil respiration and saccharase levels (Fig. 9). Further data analysis will be performed when the pending re-analysis of some of the soil samples is complete.

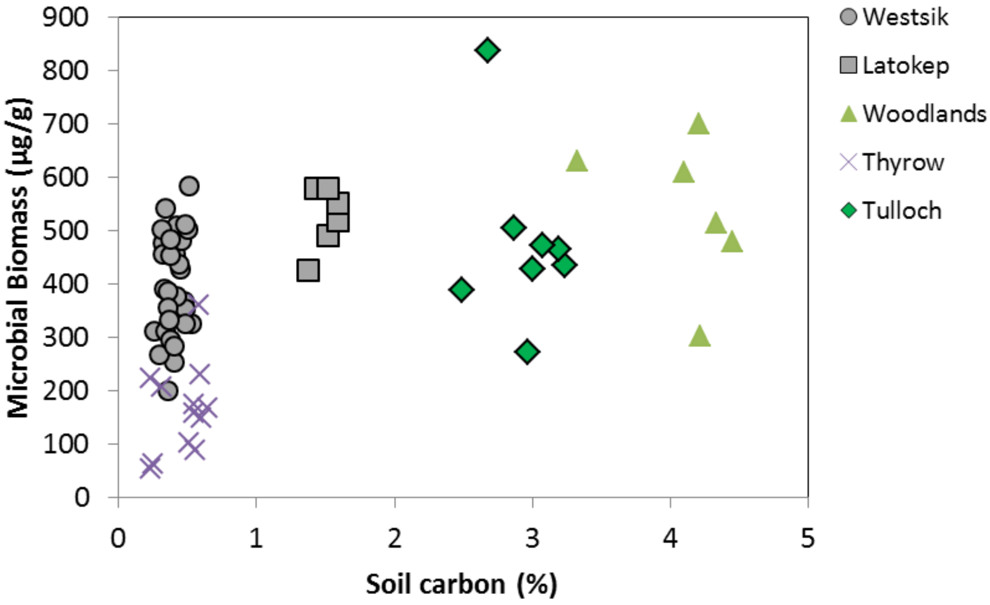

Fig. 8: Soil carbon (%) and microbial biomass (µg/g soil) in the analysed soil samples.

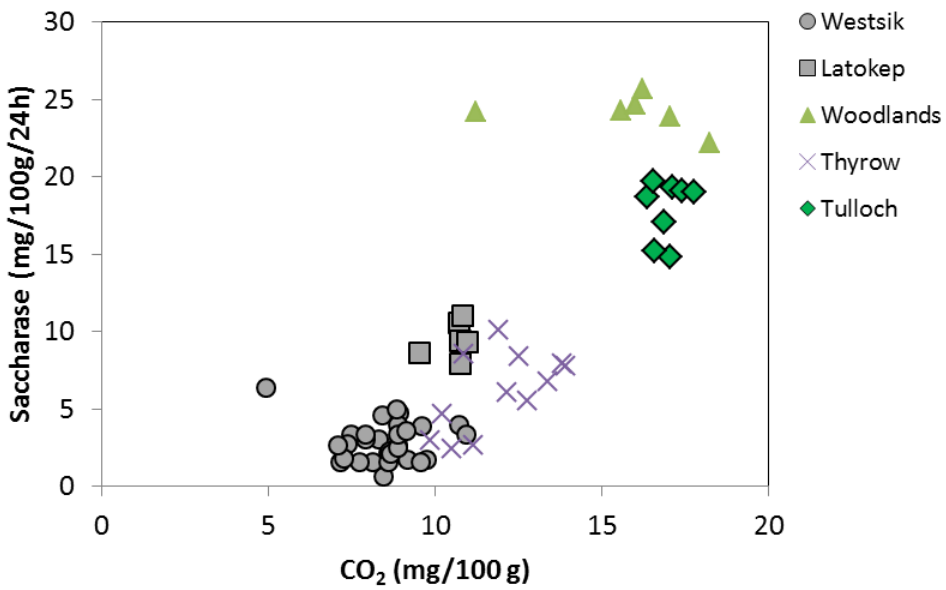

Fig. 9: Soil respiration (measured as CO_2 production) and activity of the enzyme saccharase in the analysed soil samples.

4.3 Determining crop resilience and stability

Resilience was measured in the long term trial D3 in Berlin-Dahlem as the ability of winter wheat to compensate the reduction of a particular yield component by increase in subsequent yield components. For example, reduction in plant density (P) may be compensated later in the season by increased ear density (E), increased number of grains per ear (N) and increased thousand grain weight (G). Reduced E can only be compensated by increased N or G. Finally, resilience against reduced grain density (D = E N) may only happen through increased G. As an index of resilience we used the relative amount by how much yield changes when a particular yield component is reduced by 20 %. In this case, total compensation corresponds to 0%, overcompensation to positive values and the case of no compensation is represented by a value of -20%.

Crop resilience varied for the different yield components and over time (within the season). Resilience against reduction in plant density strongly differed among liming and manure treatments. With the application of lime, reduction in plant density was completely compensated by later yield components, i.e. by yield per single plant (**Fig. 10**). Resilience against reduced plant density was lowest in the treatment that had no lime and no manure. Treatment differences were much smaller for resilience against reduced ear density, but liming still showed positive effect on resilience for this yield component. No treatment differences were observed for resilience against reduced grain density. Here, reductions can only be compensated by increase in thousand grain weight, which is known to have a relatively low plasticity. In contrast to liming and manure application, the other trial factors (P application, rotation and tillage depth) had no consistent effects on the resilience of wheat. Further, there was a positive relationship between resiience and grain yield within the non-limed treatments, but not within the limed treatments (**Fig. 11**).

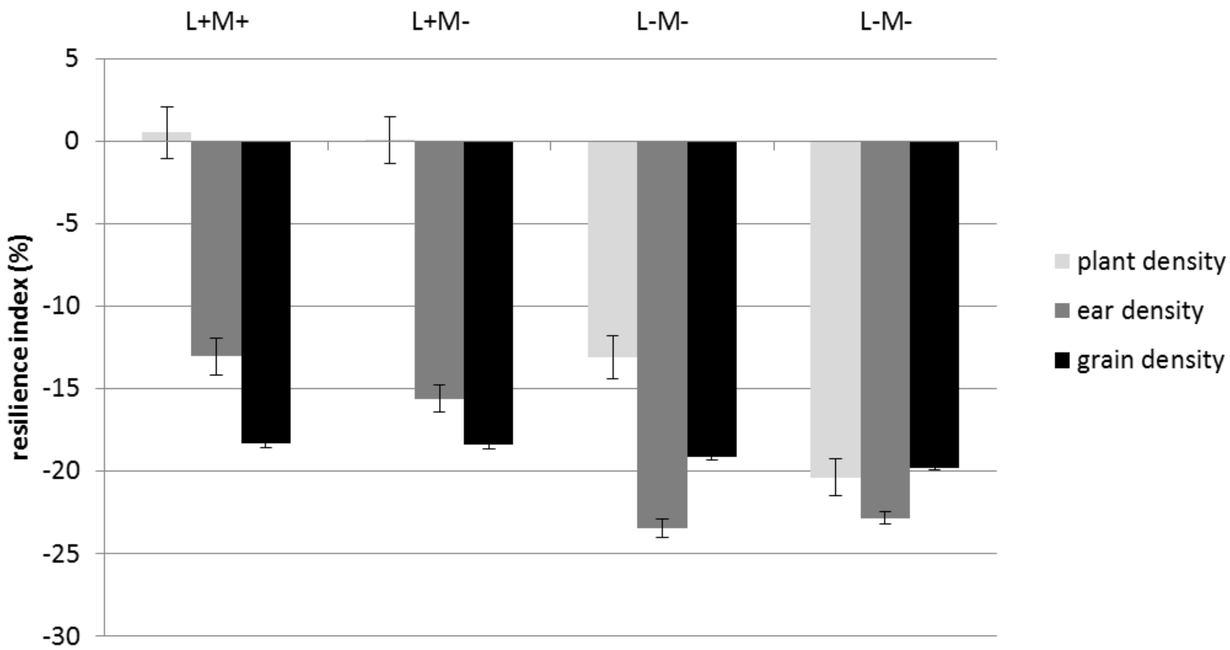

Fig. 10. Effect of fertilisation on resilience in winter wheat. Analysis based on 15 years between 1970 and 2003 from the long term experiment D3, Berlin Dahlem. Treatments are encoded as L+ (With Lime), L- (without Lime), M + (with farm yard manure), and M- (without farm yard manure). Error bars represent the standard error of the mean.

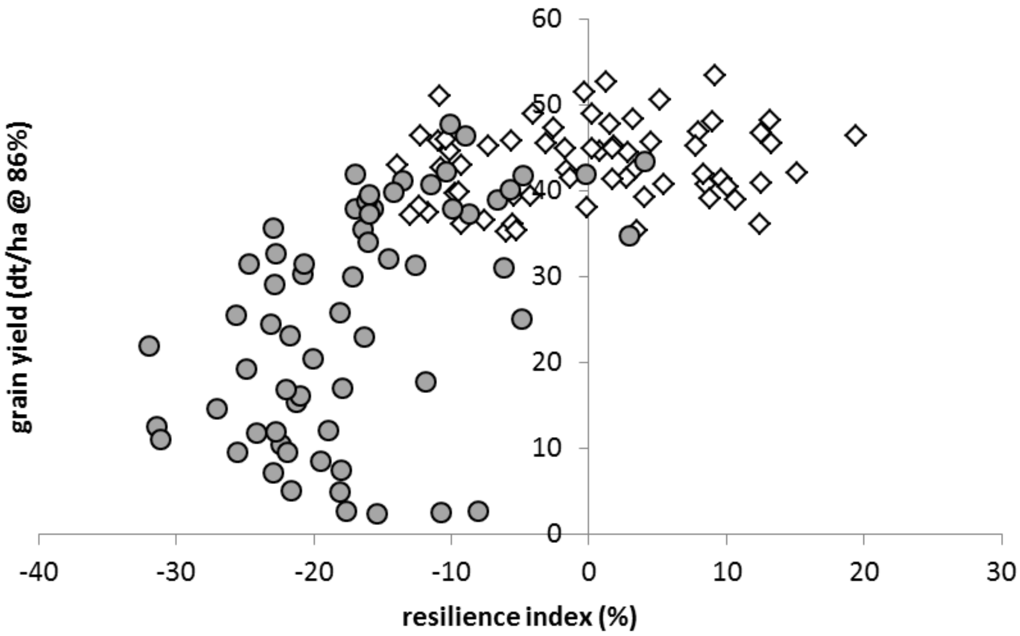

Fig. 11. Mean grain yield of winter wheat (at 86% moisture) plotted against resilience with regard to reduced plant density; analysis based on 15 years between 1970 and 2003 from the long term experiment D3, Berlin-Dahlem. The resilience index measures by how much yield changes when plant density is reduced by 20 %. With lime (open diamonds) and without lime (grey circles).

With regard to the stability of wheat yields over time, analyses showed that liming was the only trial factor with a consistent effect. In the period between 1968 and 1989, the treatment with lime had a corrected coefficient of variation of 30.4%, while the value was 35.3% in the unlimed treatment. In the second period between 1991 and 2013, the difference between the treatments was even higher, with 29.1% in the limed and 42.1% in the unlimed treatment.

4.4 Linking soil resilience and crop resilience

For this step there are currently no results yet.

5 Time plan

The project was originally planned to run from February 2016 to May 2017 (Table 4), but **we request a no-cost extension until end of October 2017**. The project started on time and preparatory analyses (tasks 1.1-1.3) have been completed (see results, section 4.2), with the exception of a few (<10) samples that will have to be re-analysed for chemical and microbiological properties (task 1.3). This is planned to be completed by the end of May 2017.

The biotest for quantification of soil resilience (task 2.1) has been completed. The data analysis is mostly completed (see results, section 4.1.), but additional statistical analyses are planned to be conducted on further collected data (e.g. disease scores) until the end of May 2017. Data compilation for the crop resilience (task 3.1) is ongoing is expected to be completed by end of April 2017. The delay in this task was caused by contractual issues which affected access to data, but this has now been sorted. The analysis of data from long term trials (task 3.2) is currently ongoing (see results, section 4.3) and will be expanded to other datasets (further crops from the D3 trials, as well as other long-term trials). Finally, the analysis will be supplemented by non-linear approaches.

Joint data analysis (task 4.1) and synthesis meeting by the consortium (task 5.1) are scheduled for June and July 2017 and dissemination to relevant stakeholder (task 5.2 and 5.3) for August to October 2017.

Table 4: Original time plan

	Year/Month												**2017**				
Nr.	**Task**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**
1.1	Sample selection by project partners																
1.2	Sampling and sample transport																
1.3	Chemical and microbiological characterisation of samples																
2.1	Biotest for soil resilience (Pythium-pea system): experiments																
2.2	Biotest for soil resilience (Pythium-pea system): data analysis																
3.1	Crop resilience: data compilation																
3.2	Crop resilience: data analysis																
4.1	Joint data analysis (linking crop and soil resilience)																
5.1	Project meeting to discuss experimental results																
5.2	Dissemination and knowledge exchange events (on-farm)																
5.3	Writing final project report and scientific papers																