Healthy plants from healthy soils

Resilience and stability of organic cropping systems

(RESTOR)

Funded by the Ekhaga Foundation, Sweden

Application Number 2015-65

Final report

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

Submitted: 3rd May 2019

Contact:
Thomas Döring, tdoering@uni-bonn.de
Tel. 0049 (0) 228 73 5143
University of Bonn, Agroecology and Organic Farming Group
Auf dem Hügel 6, 53121 Bonn, Germany

Contents

1 Summary

The ability of soils and plants to recover functionality after exposure to external stresses, i.e. their resilience, plays a critical role for the development of sustainable agroecosystems. In addition to resilience, another important desired property of agroecosystems is high temporal stability, in particular stability of yields. This project aimed to investigate the resilience of the soil's ability to suppress plant diseases and the resilience of annual crops in response to stress factors, and to explore the potential links between the soil's resilience and plant resilience. In addition, it aimed to quantify temporal stability of crop yields in response to management and geographic factors. According to the main research hypothesis, a high content of soil organic matter was expected to promote both crop resilience and soil resilience.

The main function of soils 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. To represent a large degree of variation in terms of soil properties, we used soils and crop data from different long-term agricultural field experiments across a large geographic range, namely from North-Eastern Germany, Scotland and Hungary.

Results of this part of the project showed that soils strongly differed in their ability to suppress diseases. A stress event (combined heat and drought) significantly reduced this disease suppressiveness of soils. Further, the response of suppressiveness to combined heat and drought stress depended on the provenience of the soil. The soils from Scotland were much more negatively affected than the German and Hungarian soils. Thereby, our results suggest that microbial communities responsible for suppressiveness may be adapted to prevailing climate, which has potentially severe consequences for the impact of climate change on plant health. In particular, with respect to the function of disease suppressiveness, tolerance to heat stress may be low in Northern soils, thereby highlighting that temperature extremes and heat waves may entail a previously unknown risk to the health of crop plants via reduced disease suppressiveness of soils. With regard to resilience, we found that after being exposed to stress, only one soil out of eight test soils was able to regain suppressiveness significantly after some time (six weeks). Remarkably, we also found that the tolerance to stress was negatively related to resilience.

As expected, the chemical and microbiological analysis of the soil samples revealed extremely large variation in critical soil fertility parameters depending on soil management. However, among the soils subjected to the suppressiveness tests there were no clear and unequivocal relationships between these parameters and the disease suppressiveness of the soils, their tolerance or resilience to the stress event. Thus, our results do *not* seem to support the hypothesis that general indicators of soil fertility (such as organic matter content) are necessarily positively linked to either suppressiveness or tolerance or resilience of this function to abiotic stress. This is surprising and warrants further investigations into the mechanisms of disease suppressiveness. To this end, soil samples have been archived and are available to be subjected to a functional microbiological analysis.

For the crop resilience analysis, a first necessary research step was the development of a conceptual framework for quantifying compensation as the main mechanism contributing to resilience in crops. In this research we showed the close link between compensation and competition and generalized the framework for quantifying compensation for the application to both monocultures and crop mixtures. Data analysis from long-term experiments revealed that resilience decreases within the growing season. We also detected a strong positive effect of liming on crop resilience, and differences between crop species in their resilience.

However, the relationship between soil carbon and crop resilience was not significant in the analysis of a long-term field experiment.

The accompanying analysis of temporal yield stability first developed a new approach of quantifying stability that takes into account scale effects. This approach revealed small but significant decreases of temporal yield stability in cereals and grain legumes over time. Further, significant differences between crop species were shown in terms of yield stability. Contrary to widely-held beliefs, grain legumes were revealed to have a similar yield stability as other spring sown crop species. Also against expectations, no consistent relationship was detected between soil organic carbon and yield stability across various long-term experiments.

Due to limited data availability from some of the long-term experiments, the overlap between soil resilience data and crop resilience data was small, thereby restricting the possibilities to test our hypothesis that soil resilience and crop resilience are linked. To fill this gap, we conducted supplementary experiments in spring and summer of 2017, testing the growth forage legumes for their resilience to drought stress and soil infiltration ability for resilience to compaction. Again, these experiments showed that contrary to expectations, resilience was not necessarily linked to soil organic matter content.

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 were used to identify potential covariates of soil 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). 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 in long-term trials

Data was collected from the German long term trials and analyses on two long-term experiments were performed on crop resilience. 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 methodology for quantifying compensation as a key mechanism underlying resilience was further developed (see manuscript Döring & Elsalahy, attached in the appendix). This method was applied to the IOSDV long-term N fertilization trial to quantify compensation of yield against reduction in ear density.

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 meant that the overlap between soil resilience data and crop resilience data was considerably smaller than anticipated. This restricted the possibilities to test our final, and central, hypothesis that resilience data and crop resilience are linked. Therefore, we conducted supplementary pot trials in 2017 at Humboldt University Berlin. In particular, we performed two related experiments, in which we used the same soils and soil amendments. Here, the aim was ultimately to see if the same treatments (amendment with compost) lead to improved resilience.

3.5.1 Pot experiment on crop resilience

In this experiment, we tested the ability of two species of forage legumes (Black medic = BM, *Medicago lupulina* and Alsike clover = AC, *Trifolium hybridum*) to re-grow after a sustained drought event in monoculture and mixture, and on differently treated soils (with and without compost amendment; with and without soil compaction). In total six treatments were tested (Table 4): Three treatments without compost and without compaction allowing us to compare the two species in monoculture and sown as a mixture; and three further treatments with one of the species (Black medic) to allow a factorial comparison of the effects of compost amendment and soil compaction.

Table 4: Treatments in the pot experiment on crop resilience

Trt	Compost	Compaction	Kg soil / pot	Average density (g/cm³) (below 3 cm depth)	Additional substrate (g) before sowing	Additional substrate after sowing (g)	species
A	without	Without	3.4	1.432	None	108 g	BM
B	without	Without	3.4	1.432	None	108 g	AC
C	without	Without	3.4	1.432	None	108 g	Mix (1:1)
D	with	Without	3.1	1.305	None	100 g	BM
E	without	With	3.4	1.670	486 g	108 g	BM
F	with	With	3.1	1.523	443 g	100 g	BM

About 1.5 t of soil (sandy loam) was obtained at the end of May 2017 from the SW end of the S5 field located at the field experimental station of Humboldt-Universität zu Berlin in Berlin-Dahlem (geographic

coordinates: 52° 27' 58" N 13° 18' 7.5", 49 m asL) from the top soil (upper 10-15 cm). The soil was left to dry in the open, with a roof to protect it from precipitation. The dry soil was sieved through a sieve with 0.5 cm mesh size and the dry sieved soil was kept in big sacks until it was used in the pot experiments. On 19.6.2017 the soil was filled into plastic cylinders of 24 cm length and 12 cm diameter, with a cap at the bottom. In 40 kg batches, a proportion of the soil was thoroughly mixed with compost at 1 parts of compost to 9 parts of soil (i.e. 10% w/w). 3.4 kg of substrate (soil) was used for treatments without compost; 3.1 kg of soil with compost. The inner surface of the cylinders was marked with a waterproof pen to indicate a filling height of 21 cm. When the fixed amount (kg) of soil had been filled into the cylinder, it was gently knocked vertically onto a hard surface to reduce the substrate's volume exactly to the filling height of 21 cm, thus making sure that this initial filling volume was the same in all cylinders (of all treatments).

For the compaction treatment we used a hydraulic hand-operated press. Cylinders were put under the compressor with a circular stamp of 11.8 cm diameter, and the volume of the soil was reduced by pressing the stamp down by 3 cm. The cylinders were then filled up to the original filling height with 3/21*3.4 kg = 486 g substrate for treatment without compost and 3/21*3.1 kg = 443 g amended substrate for treatment with compost (Table 2). The filling height for this additional substrate was again adjusted by gently knocking the cylinder down on a hard surface 1-2 times.

Sowing took place on 26.6.2017. First, the soil was covered with a circular filter paper and 500 ml of tap water was poured carefully onto the filter paper. This was done to avoid silting up of the soil. Filter paper was removed after the water had completely infiltrated. Organically produced seed of black medic (*Medicago lupulina* cv. Ekola) and Alsike clover (*Trifolium hybridum*, cv. Dawn), was carefully placed on top of the wet substrate in a regular pattern (using a sowing stencil to mark the positions of the seeds). Per pot, 24 seeds were sown. In the mix, 12 seeds of Alsike clover and 12 seeds of Black medic were spatially alternated to ensure maximal interaction between the two species. Then, 108 g of dry soil and 100 g of dry compost amended soil were then loosely spread evenly on top of the seeds. This was equivalent to sowing depth of about 6-7 mm. Filter paper was again put on top of the surface and 25 ml of water were then gently poured on 27.6. 2017. By this time the moisture from below the sowing depth had visibly risen to the top through capillary rise. The pots were put in a randomized complete block design with 5 replicates per treatment. There were 60 pots per block: 6 treatments x 2 stress treatments (without and with temporary drought stress) x 5 harvest times. Emerging weed seedlings were removed daily from the pots.

Before the drought, plants received a total of 975 ml of irrigation water per pot. The drought event lasted two weeks. During this time, the non-stressed plants received 1580 ml of water per pot, while the drought-stressed plants received only 150 ml water per pot. After the drought event, the stressed and non-stressed plants were again treated equally, as before the onset of the stress event and received daily watering at libitum, usually at 100 or 200 ml per pot per day. Destructive above-ground sampling of the plants took place on five harvest times (just before onset of the drought stress (18.7.17), at the end of the drought stress event (1.8.17) and three times afterwards, to record recovery from the stress (8.8., 22.8.17 and 31.8.17). Resilience was determined following Orwin & Wardle (2004) for harvest 3 and harvest 4.

3.5.2 Pot experiment on soil resilience

Soil and soil amendments, as well as the pots and the facility for compaction were used as in the pot trial described above. In this experiment though, no plants were grown. Briefly, the soil was filled into the cylinders, and either compacted with the stamp or left uncompacted. In this trial, compaction was used as the stress event (not the drought as before). The function to be tested was infiltration after irrigation, with

shorter time required for complete infiltration indicating better infiltration capacity. The soil was irrigated from above with a drip irrigation device for 60 seconds. It was recorded after how many seconds all water had infiltrated into the soil. In addition, soil moisture was recorded at two depths in each column. As soil moisture results were highly correlated with the infiltration times, we only display results of the latter variable. Three replicates were taken for each treatment. The infiltration measurements were conducted twice, once in mid to late September 2017 and then again about three months later between mid-December and beginning of January, in order to measure recovery after the stress event (compaction).

3.6 Determining crop yield stability

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 and further data sets could be used. Several long-term trials were used for the stability analysis, including (a) the fertilization trial Dikopshof near Wesseling, University of Bonn established 1904 on a Loess soil; (b) the D3 long-term soil management experiment in Berlin-Dahlem, established 1923 on a sandy loam, (c) the IOSDV long-term nitrogen fertilization trial at Berlin-Dahlem, established 1984 on a sandy loam. In addition, the methodology for analysing yield stability was further developed (see paper Döring & Reckling 2018).

4 Results and discussion

4.1 Determining resilience of soil suppressiveness ('biotest')

Detailed results including statistical analysis are presented in the enclosed manuscript (see appendix). Here we summarize the main results. 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.

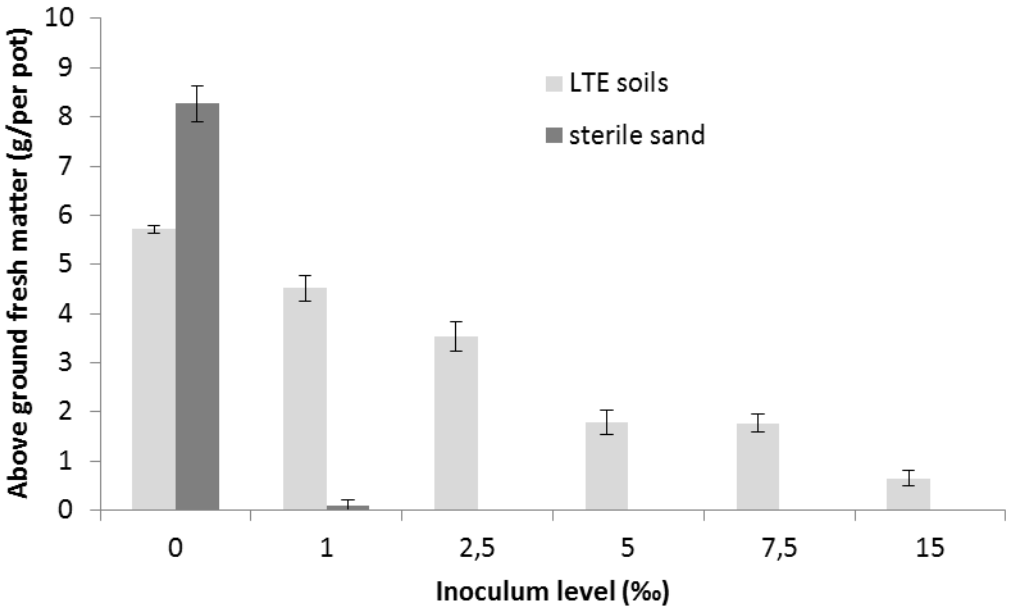


Fig. 1: Above ground fresh matter of peas in the biotest (mean and s.e.), depending on the inoculum level with *Pythium* mixed into the substrate.

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.

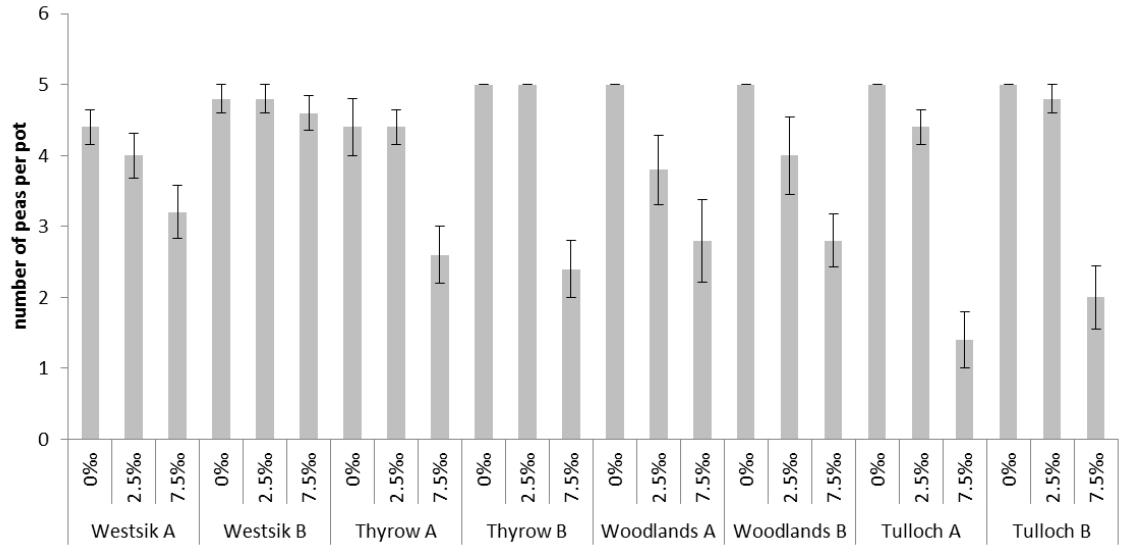


Fig. 2: Main test I: Suppressiveness of the eight tested soils, measured as response of the number of peas per pot (means and s.e.) to the inoculum level (‰).

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.

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.

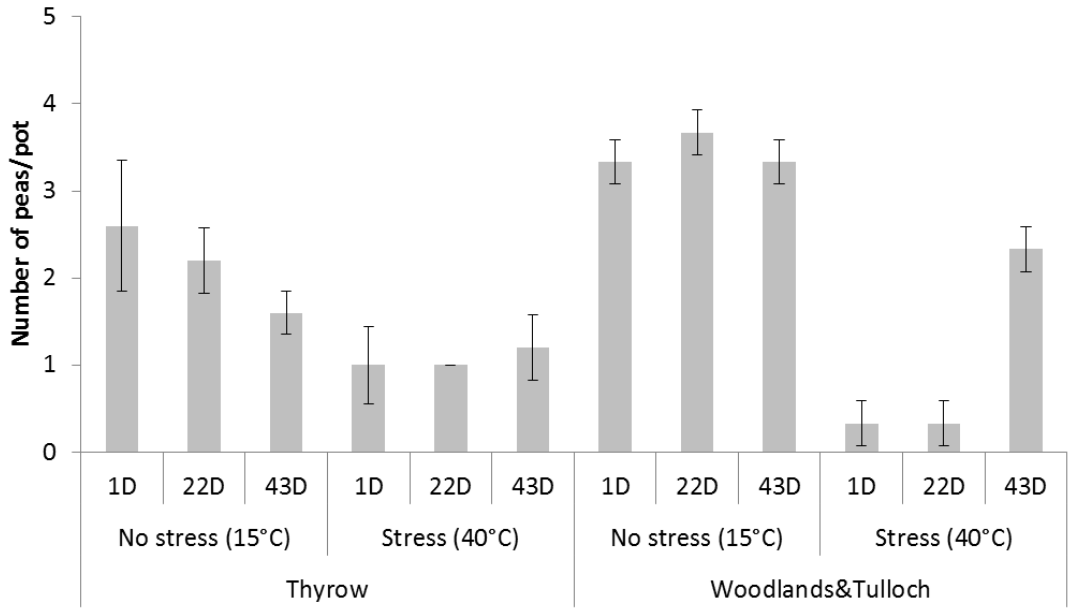


Fig. 3: Selected results from pre-test II: Number of peas per pot (means and s.e.) depending on site (Thyrow vs. Scottish soils pooled from Woodlands and Tulloch), stress level of soil (with and without combined temperature and drought stress), and time (1 days, 22 days and 43 days after stress event); 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.

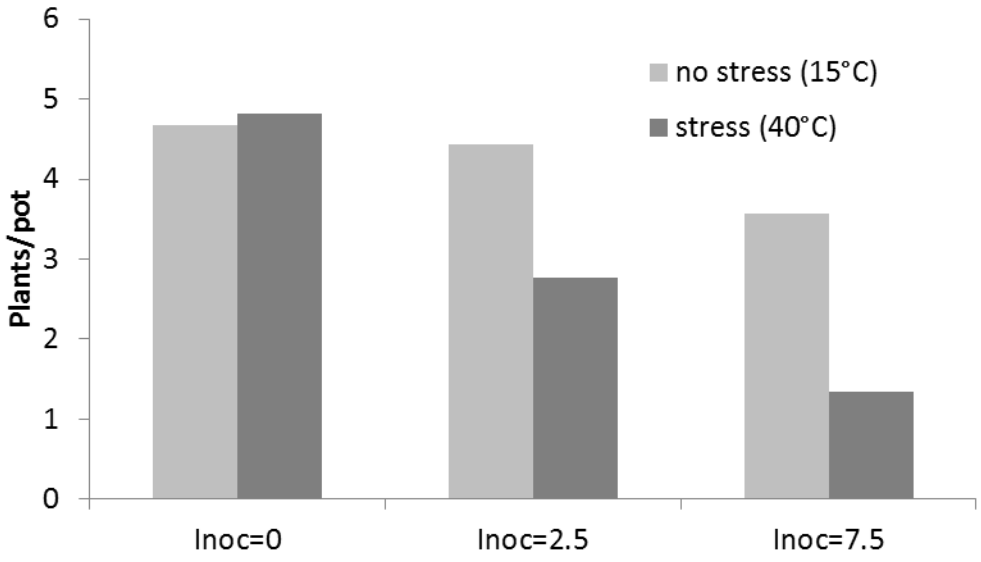


Fig. 4: Results of Main test II: Number of plants per pot depending on inoculum level and stress level.

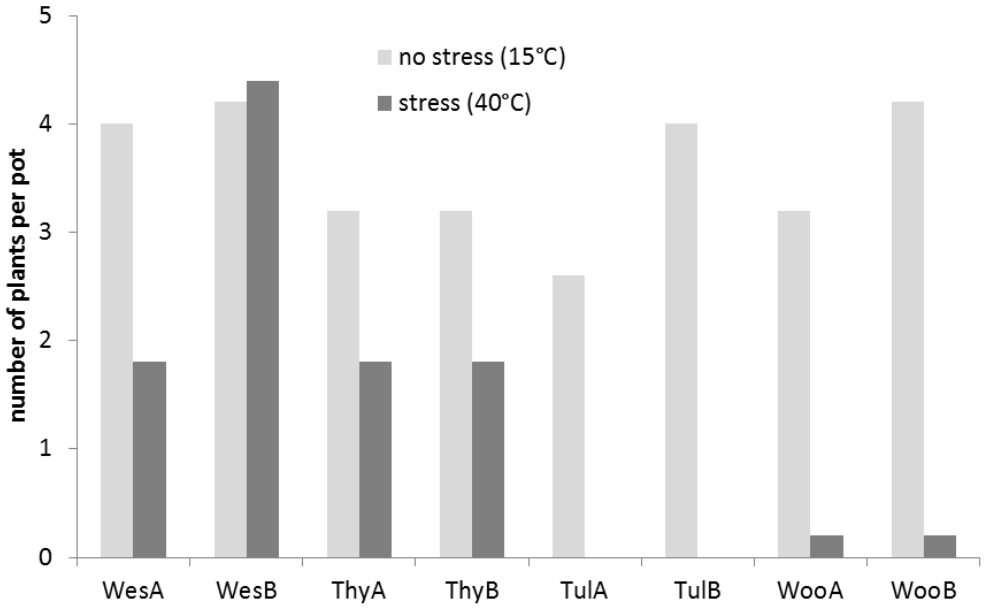


Fig. 5: Effect of combined heat and drought stress applied to soil on number of pea plants after inoculation with a concentration of 7.5 ‰.

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 "day 1 soil" and "day 43 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**, $P<0.05$).

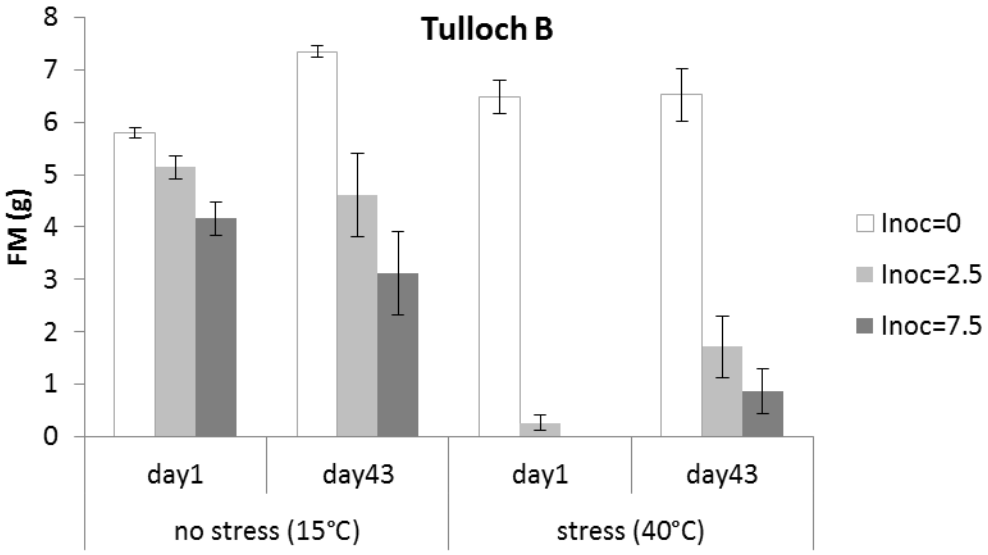


Fig. 6: Fresh matter of pea plants on the Tulloch B test soil, depending on stress level and the number of days elapsed since the stress event.

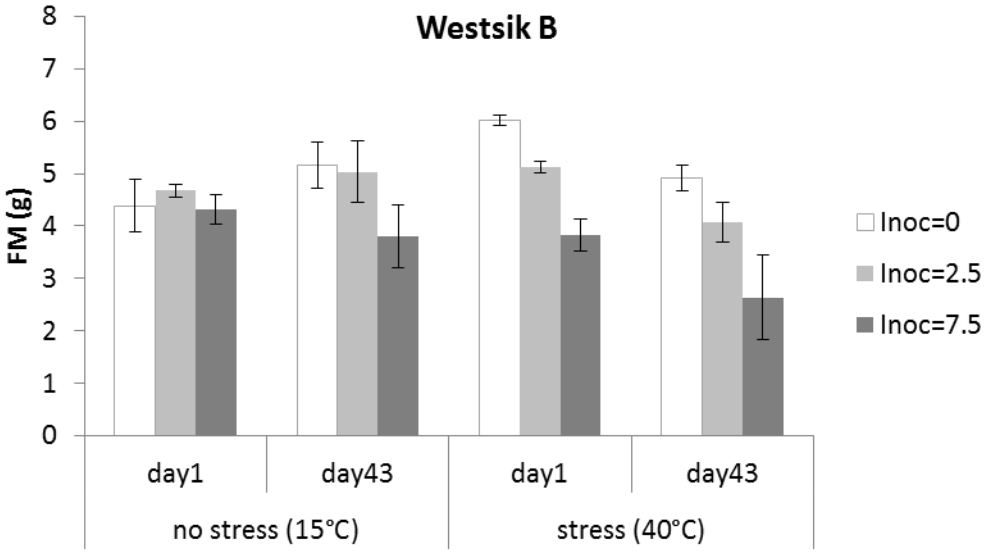


Fig. 7: Fresh matter of pea plants on the Westsik B test soil, depending on stress level and the number of days elapsed since the stress event.

4.2 Chemical and microbiological characterisation of soils

Results of the characterisation of soils are published in Katai et al. 2018. Here we summarize and complement these published results. 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).

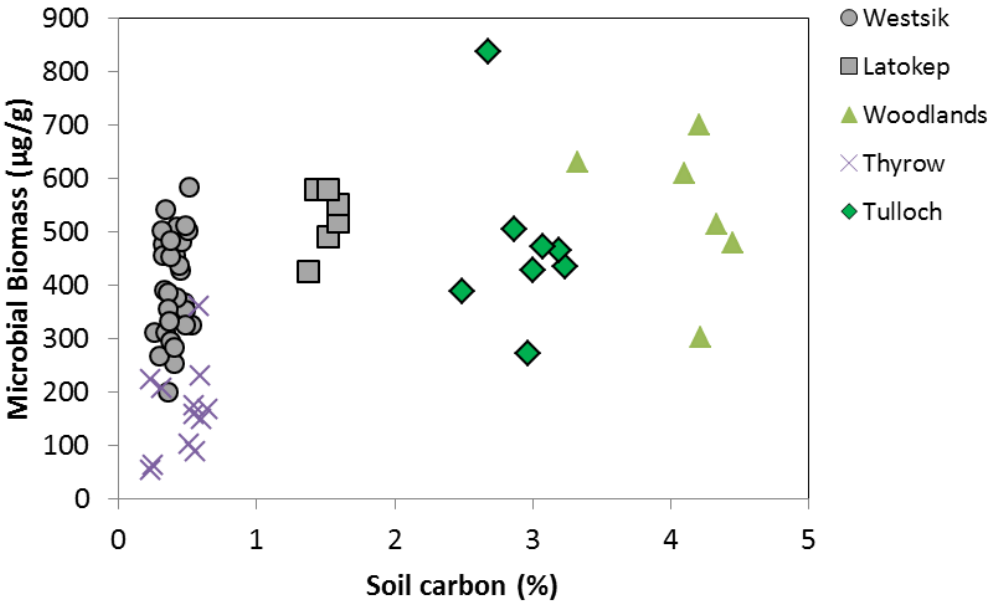


Fig. 8: Soil carbon (%) and microbial biomass (µg/g soil) in the analysed soil samples.

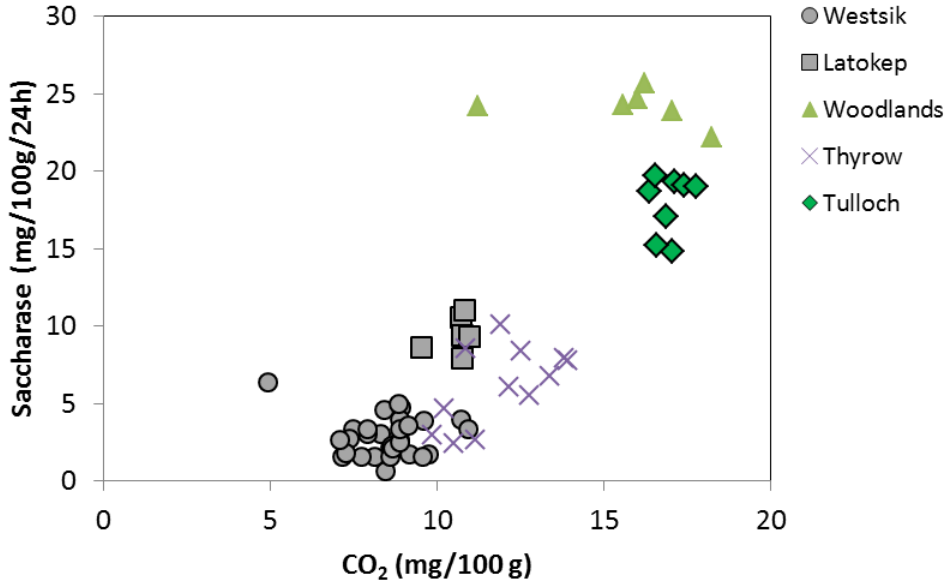


Fig. 9: Soil respiration (measured as CO_2 production) and activity of the enzyme saccharase in the analysed soil samples.

4.3 Determining crop resilience in long-term trials

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**).

In the IOSDV trial, resilience was measured by compensation of grain yield against loss of ear density. Here, we observed no significant correlation between total soil C content and compensation ability across the different treatments (**Fig. 12**).

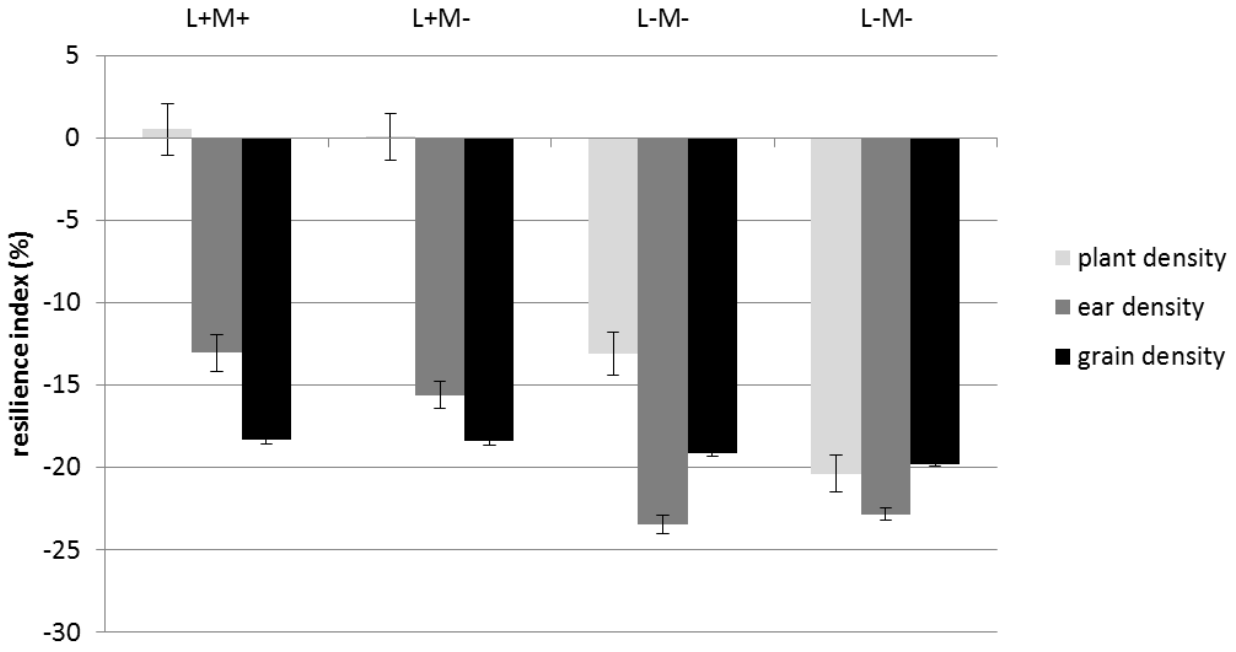


Fig. 10. Effect of fertilisation on resilience in winter wheat. Analysis based on 15 years between 1970 and 2003 from the long term experiment D3, Berlin Dahlem. Treatments are encoded as L+ (With Lime), L- (without Lime), M + (with farm yard manure), and M- (without farm yard manure). Error bars represent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

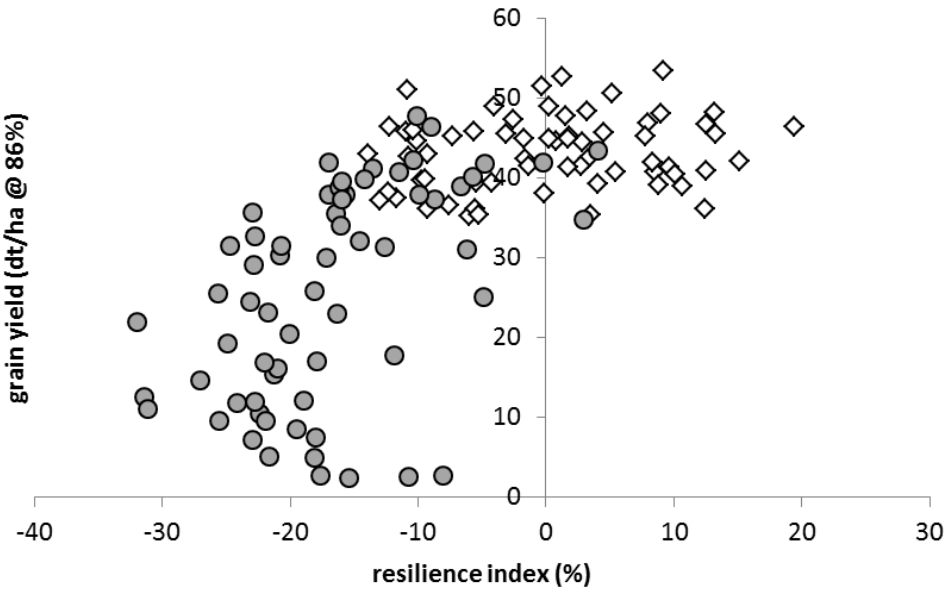


Fig. 11. Mean grain yield of winter wheat (at 86% moisture) plotted against resilience with regard to reduced plant density; analysis based on 15 years between 1970 and 2003 from the long term experiment D3, Berlin-Dahlem. The resilience index measures by how much yield changes when plant density is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

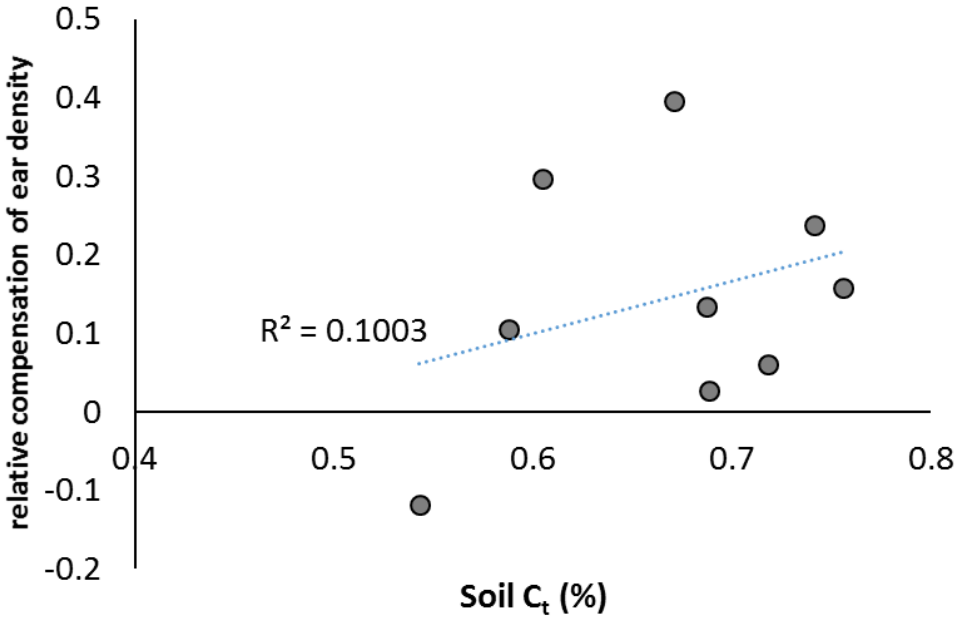


Fig. 12: Relationship between soil total carbon (%) and relative compensation of grain yield against loss of ear density. Positive values of compensation mean that reduced ear density leads to less than proportional losses of grain yield. A value of 0 indicates that reduced ear density is translated completely proportionally into grain yield reduction. The relationship between the two variables (soil C_t and compensation) is positive but statistically not significant. In this trial, the soil is free of carbonates so that C_t can be considered to equal organic C content in the soil.

4.4 Soil resilience and crop resilience in pot experiments

4.4.1 Pot experiment on crop resilience

In almost all cases, crop resilience in response to the drought event was negative, i.e. recovery almost never led the drought-stressed plants to reach the level of the unstressed plants over time (Fig. 13). Further data analysis, comparing the two legume species, revealed that black medic (BM) exhibited higher resilience to drought than Alsike clover (AC); this is likely because under stress BM produced higher biomass than AC, while the situation was reversed under non-stress conditions. Especially at harvest 3, partial land equivalent ratios (PLER) showed that BM is able to cope better after drought stress conditions. Further observations made during the harvests indicated that BM formed multiple 'tillers', whereas AC formed only few. With regard to the mixing effect - contrary to expectations – the Land Equivalent Ratio (LER) did not increase under drought stress. Contrary to expectations, mixing the two legume species did not lead to an increase in resilience, in comparison to the resilience of the two sole crops (comparison between treatment C and the average of A and B, Fig. 12). Further, and again contrary to expectations, the addition of compost did not increase plant resilience to drought (compare treatment D against A, and F against E). The reason of the low resilience in compost treatments is probably that the non-stressed version of compost reaches much higher biomass than that of non-compost. However, compost did lead to higher biomass; one reason is probably better water holding capacity of the soil (as seen e.g. in lower dry matter content of foliage in compost treatment). Compaction had no clear effect on resilience of the plants; however, it did lead to higher biomass, the reason is probably higher soil mass and, concurrently, higher amount of water (and nutrients). Analysis of the soil moisture before onset of the drought event showed the positive effects of compost on soil moisture retention, but also increased water content in the compacted soil due to higher amount of soil in the pots (Table 5).

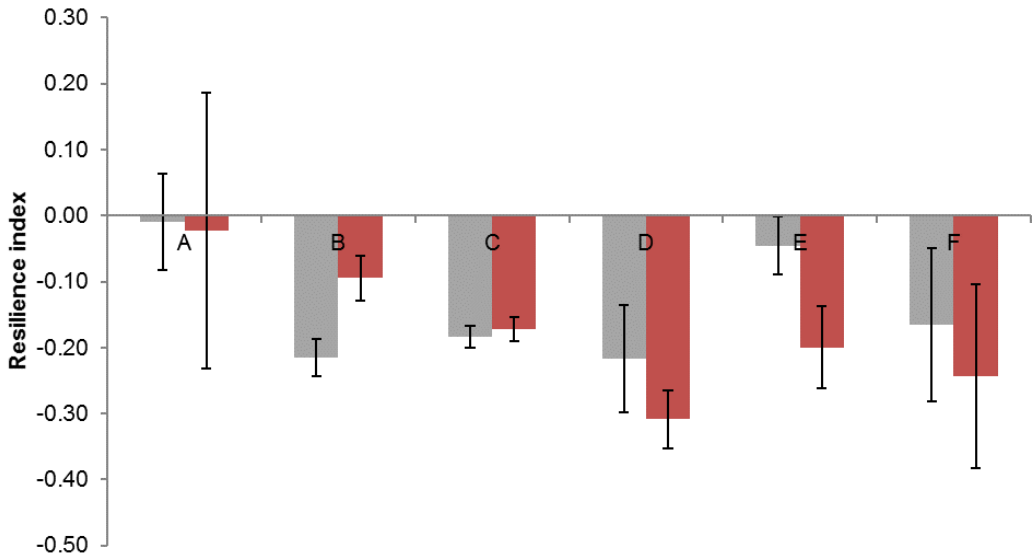


Fig. 13: Resilience index (after Orwin & Wardle 2004), for harvest 3 (grey bars) and harvest 4 (red bars) of forage legume above-ground biomass, following a two-week drought event. Higher values indicate stronger recovery from drought in relation to unstressed plants. Treatments: A-C: without compost and without compaction; A: Black medic, B: Alsike clover; C: Mixture; D-F: Black medic, D: with compost, without compaction; E: without compost, with compaction; F: with compost, with compaction. Bars show means and error bars denote standard errors, n=5.

Table 5: Soil moisture before onset of the drought event

Factor	trt1	trt2	Diff. (abs, ml)	Abs. change of moisture (%)	Possible explanation
Compaction	A	E	16	-0.1	more soil holds more water, but apart from that, no effect
Compost	A	D	85	3.1	organic matter holds water like a sponge
Species	A	B	92	2.6	Alsike clover has slower growth rate than Black medic, therefore lower transpiration
Mixture	C	AB	-16	-0.5	Black medic is slightly dominant (>50%) in mixture, thus more transpiration
Compost when compacted	E	F	64	2.1	organic matter holds water like a sponge
Compaction when compost added	D	F	-5	-1.0	Better growth thus higher transpiration

In general, the experiment showed that caution is needed with regard to *apparent resilience*: This situation can arise when the growth of plants slacks in the unstressed control (because of limited resources) while the previously stressed treatment has not exhausted resources and numerically this situation results in high resilience, but this probably needs to be seen as an artefact.

4.4.2 Pot experiment on soil resilience

In the soil resilience experiment, amending the soil with compost led to faster, i.e. better infiltration, but compost did not consistently result in better recovery of infiltration, after disturbing the soil via compaction (Fig. 14). In soils that were kept dry during the recovery period, compost amendment improved recovery, but this was not the case in soils that were occasionally irrigated with small amounts of water during the recovery period.

The resilience index after Orwin & Wardle (2004) could not directly be applied to the infiltration times, because in the case of infiltration, larger values indicate lower functionality. Application of the resilience index to inverse infiltration times did not lead to conclusive results, most likely because of mathematical artefacts.

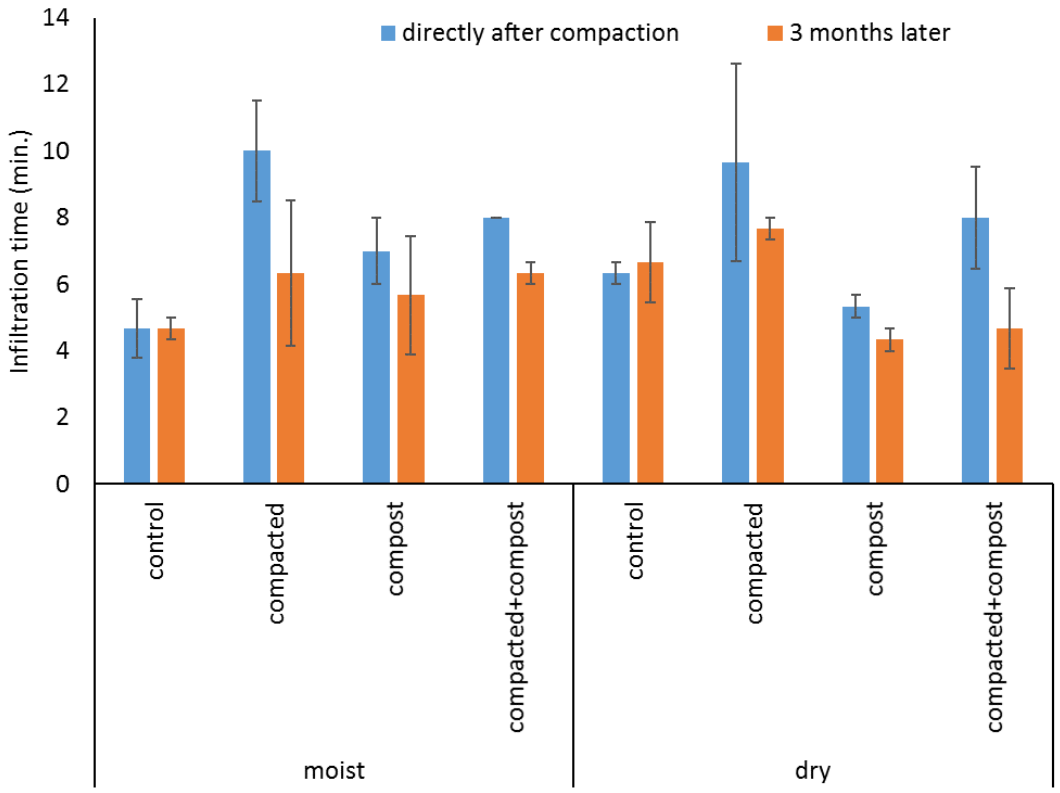


Fig. 14: Infiltration time in minutes in differently treated soils, directly after compaction and three months later. Half of the cylinders were kept dry over the three months, whereas the other received occasional irrigation treatments.

4.4.3 Linking the two pot experiments on resilience

After considerable experimental effort that went into in the pot trials, data analysis within each of the trials showed that even substantial amendments of soils with organic matter – in the form of compost – does not consistently lead to improved resilience in crops or soils. Frustrating our attempt to link up soil resilience and crop resilience, the experiments showed that the dynamic and complex nature of the growth processes often makes the straight-forward quantification of resilience difficult. The most illustrative example is the possibility of apparent resilience explained above.

4.5 Crop yield stability

In the analysis of wheat yield stability over time in the D3 long-term trial, results 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.

In the IOSDV trial, we found a significant positive correlation between total soil carbon content (C_t, %) and the mean yield level across the different fertilization treatments (Table 6). However, there was not significant correlation between C_t and yield variability indices (POLAR and aCV).

Table 6: Regression analysis of total soil carbon content (C_t, %) again yield stability indices

Dependent variable	Estimate	Standard error	P-value	Significance
Mean yield	118.6	43.7	0.03	*
Yield variability (POLAR)	-0.36	0.33	0.30	ns
Yield variability (aCV, %)*	-8.9	9.22	0.36	ns

*scale-adjusted coefficient of variation see Döring & Reckling 2018

Similarly, there was no effect of additional organic manure application on yield variability in the Dikopshof long-term fertilization trial (Table 7).

Table 7: Effect of additional farm yard manure on yield stability in the Dikopshof long-term fertilization trial for the years 2000-2008. Negative values indicate lower yield variability in the organically fertilized variant.

	Absolute difference (%) in temporal yield variability between treatments with and without additional farm yard manure application				
Basic fertilization level	**potato**	**Sugar beet**	**wheat**	**rye**	**mean**
NPKCa	-3.4	-2.7	1.9	3.2	-0.2
NPKX	-4.6	5.7	3.1	0.7	1.2
NPXCa	-5.7	4.7	-10.5	1.9	-2.4
NXKCa	0.6	7.5	5.3	-2.1	2.8
XPKCa	3.8	0.3	-1.5	4.9	1.9
XXXX	3.9	6.9	2.0	-1.0	2.9
mean	**-0.9**	**3.7**	**0.1**	**1.3**	**1.0**

5 Conclusions

The project has allowed us to make substantial progress in the quantification and characterisation of resilience and stability in agricultural contexts. A new yield stability index was developed (Döring & Reckling 2018), and several insights into the factors determining stability could be gained: Over the last few decades, yield stability has slightly but significantly decreased. Crop species differ significantly in their yield stability (Reckling et al. 2018); but against expectations, soil organic matter content had no significant or consistent effect on yield stability.

With regard to resilience, a new approach was developed for quantifying compensation as a main contributor to resilience. Experimental work showed that suppressiveness of soils against plant diseases is negatively affected by combined drought and heat stress but only one in eight tested soils showed signs of recovery from this stress in its function to suppress crop disease. Recovery and tolerance to the stress were inversely related. Further, neither the tests on suppressiveness nor other pot experiments conducted in our study, nor the analysis of data from a long-term trial could find significant positive effects of soil organic matter on crop resilience or soil resilience.

We conclude that although multiple benefits are known for soil organic matter, resilience and stability are possibly *not* among these benefits. This means that approaches other than soil organic matter management need to be identified for making agro-ecosystems more stable and more resilient. Further, this project was unable to empirically establish a link between resilience in plants and resilience in soils. However, we consider this research still very much as work in progress, since the aspects of resilience investigated in this study cover only a fraction of the multiple possibilities of quantifying resilience in agricultural contexts.

6 References

Döring TF, Reckling M. 2018. Detecting global trends of cereal yield stability by adjusting the coefficient of variation. European Journal of Agronomy 99: 30-36.

Frankenberger, W.T., Johanson, J.B. 1983. Method of measuring invertase activity in soils. Plant and Soil. 74. 301-311.

Kátai J, Döring T, Tállai M, Balla-Kovács A, Henzsel I, Makádi M, Sándor Z, Vágó I. 2018. Influence of alternative plant nutrition methods on soil microbial characteristics in long-term experiments. Agrochemistry and Soil Science 67, 79-90.

Mershi, VW 1996. Dehydrogenase activity with the substrate INT. In: Schinner F, Öhlinger R, Kandeler E, Margesin R (eds.) Methods in Soil Biology. Springer-Verlag Berlin – Heidelberg. 243-245.

Öhlinger R, Kandeler E, Margesin R. (eds.) 1996. Methods in Soil Biology. Springer-Verlag Berlin Heidelberg. pp. 20-26., 32-36

Orwin KH, Wardle DA. 2004. New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances. Soil Biology & Biochemistry 36: 1907–1912.

Reckling M, Döring TF, Bergkvist G, Stoddard FL, Watson CA, Seddig S, Chmielewski F-M, Bachinger J. 2018. Grain legume yields are as stable as other spring crops in long-term experiments across northern Europe. Agronomy for Sustainable Development 38: 63.

Székely Á, Schlick B, Szabó T. 1960. On the photometric and colorimetric determination of organic carbon. (In Hungarian). Agrokémia és Talajtan, 9, 111-120.

Szegi J. 1979. Soil Microbiological Methods. (In Hungarian) Mezőgazda Kiadó, Budapest. pp. 250-256.

7 Appendix

7.1 Project-related output

The main experiment of the project, focussing on resilience of soil suppressiveness, led to two papers, one of which is published already (Katai et al., see list below, 8.1.2), while the other is close to submission to Applied Soil Ecology. Both papers are also enclosed in this report. Further, as stated in the project description, data from long-term experiments was not only used for resilience analysis but also for yield stability analysis. This led to a series of publications, also thanks to the collaboration with researchers outside the project (Reckling et al. 2018, Döring & Reckling 2018, Reckling & Döring 2019, all listed below). In addition, research work in the course of this project revealed that for quantifying resilience in agro-ecosystems, a critical precursor is the concept of compensation. Therefore, in collaboration with another research project (PhD thesis Heba Elsalahy at Humboldt University Berlin) we developed a framework for quantifying compensation in plant monocultures and mixtures. Finally, the main results of the project were presented at a 2-day German organic farming conference in Saxony in March 2019 (about 50 participants).

7.1.1 Publications in preparation

Döring TF, Rosslenbroich D, Giese C, Athmann M, Watson C, Vágó I, Kátai J, Tállai M, Bruns C. Disease suppressive soils vary in recovery from stress. Manuscript in preparation for submission to Applied Soil Ecology. (***attached #1***)

Döring TF, Elsalahy H. Quantifying compensation in plant mixtures. Manuscript in preparation for submission to Journal of Applied Ecology. (***attached #2***)

Ahrends HE, Gaiser T, Siebert S, Rezaei EE, Seidel SJ, Hüging H, Ewert F, Döring T, Eugster W. Yield stability of four major European food crops along a gradient of long-term nutrient supply. Submitted to Scientific Reports.

7.1.2 List of publications generated through the project

Kátai J, Döring T, Tállai M, Balla-Kovács A, Henzsel I, Makádi M, Sándor Z, Vágó I. 2018. Influence of alternative plant nutrition methods on soil microbial characteristics in long-term experiments. Agrochemistry and Soil Science 67, 79-90. DOI: 10.1556/0088.2018.67.1.6 (***attached #3***)

Reckling M, Döring TF, Bergkvist G, Stoddard FL, Watson CA, Seddig S, Chmielewski F-M, Bachinger J. 2018. Grain legume yields are as stable as other spring crops in long-term experiments across northern Europe. Agronomy for Sustainable Development 38: 63 (DOI: 10.1007/s13593-018-0541-3)

Döring TF, Reckling M. 2018. Detecting global trends of cereal yield stability by adjusting the coefficient of variation. European Journal of Agronomy 99: 30-36. (***attached #4***)

Döring TF, Wolfe MS. 2019. Plant Breeding and Genetics in Organic Agriculture. In: Atkinson D, Watson CA (Eds.): The Science Beneath Organic Production. Wiley. DOI: 10.1002/9781119568988.ch13.

Reckling M, Döring TF, Bergkvist G, Chmielewski F-M, Stoddard FL, Watson CA, Seddig S, Bachinger J. 2018. Grain legume yield instability has increased over 60 years in long-term field experiments as measured by a scale-adjusted coefficient of variation. Aspects of Applied Biology 138: 15-20. (***attached #5***)

Reckling M., Döring TF. 2019. International long-term experiments for the analysis of grain legume yield stability. LTFE-Meeting 03. 2019, University of Gießen, Germany.

7.1.3 Other output: Conference talks and teaching

Döring, T. 2019. Die Rolle von Humus für die Resilienz und Stabilität von Agrarökosystemen. Presentation at Hofgut Eichigt, Saxony, 22. March 2019.

Döring, 2018. Resilienz. Two lectures in Bachelor module “Agroecology” at University of Bonn, Summer Term 2018.

Disease suppressive soils vary in recovery from stress

Thomas F. Döring[1,2]*, Dagmar Rosslenbroich[3], Christian Giese[1], Miriam Athmann[1], Christine Watson[4,5], Imre Vágó[6], János Kátai[6], Magdolna Tállai[6], Christian Bruns[3]

*Corresponding author; e-mail: tdoering@uni-bonn.de; Tel. 0049 (0) 228 73 5143

[1]Agroecology and Organic Farming Group, Faculty of Agriculture, University of Bonn, Auf dem Hügel 6, 53121 Bonn, Germany

[2]Department of Agronomy and Crop Science, Faculty of Life Sciences, Humboldt University of Berlin, Albrecht-Thaer-Weg 5, 14195 Berlin, Germany

[3]Faculty of Organic Agriculture Sciences, University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany

[4]Crop and Soil Systems, Scotland's Rural College, Aberdeen AB21 9YA, UK

[5]Department of Crop Production Ecology, Swedish University of Agricultural Sciences, 750 07 Uppsala, Sweden

[6]Institute of Agricultural Chemistry and Soil Science, University of Debrecen, H-4032 Debrecen, Böszörményi út 138, Hungary

Abstract

Soil-borne plant diseases are a major source of crop losses. Biologically active soils have the ability to suppress pathogenic infections of plants, but little is known how this essential soil function might be affected by abiotic stresses. Using a model system with pea and its fungal pathogen *Pythium ultimum* we studied how the suppressiveness of different soils from a wide geographic range responds to combined heat and drought stress. We found that different soils strongly differ in their ability to suppress diseases and that a stress event of combined heat (40°C) and drought (-50% moisture) can strongly reduce this disease suppressiveness. Further, the response of suppressiveness to the stress depended on the provenience of the soil. Soils from a cool-climate site in Scotland were much more negatively affected than soils from warmer sites in Germany and Hungary. After being exposed to stress, some soils were able to regain suppressiveness after several weeks while others were not, thereby showing different degrees of recovery from the stress. Stress tolerance was negatively related to recovery. Our results 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.

Key words: legumes; *Pythium ultimum*; resilience; soil-borne diseases; stress; suppressiveness

Introduction

Soil-borne plant pathogens decrease the yield potential of all economically relevant crops across the world [1-3], sometimes causing total yield loss [4, 5]. In addition, soil-borne pathogens negatively impact on cropping systems in indirect ways, e.g. by impeding the adoption of legume cropping, thereby reducing the potential of biological nitrogen fixation as an alternative of nitrogen fertilizers [6, 7].

However, soils are able to suppress diseases [8, 9] by affecting the establishment, development and persistence of pathogens [10, 11]. While abiotic soil characteristics including soil pH, moisture and clay content may play a role in suppressiveness [12], the main reason of suppressiveness is attributed to antagonistic mechanisms such as predation, parasitism, competition and antibiosis [13]. A well-studied example is the antagonism between *Pseudomonas* bacteria and the soil-borne pathogen *Gaeumannomyces graminis* var. *tritici* causing the take-all disease in cereals [14]. The suppressiveness of soils against plant diseases can be supported in various ways, e.g. through organic amendments such as compost [15, 16].

While rising temperatures in the course of climate change are expected to increasingly disrupt crop production [17, 18] little is known about how soil suppressiveness may react to the projected changes [19, 20]. So far, research has focussed on how plants or pathogens respond to (projected) climate change, e.g. soil-borne *Phytophthora cinnamoni* [21]. A study modelling the response of *P. cinnamoni* to climate change assumed no reaction of suppressiveness to climate change [22], but this assumption is currently not rooted in empirical evidence. In fact, van der Voort et al. [23] demonstrated a change in the composition of the soil microbial community, especially bacteria, after heat treatment of the soil at 50°C, and a concurrent loss of suppressiveness against the pathogen *Rhizoctonia solani* [24]. Currently, however, it is unclear whether stress-induced loss of suppressiveness is also relevant under lower, i.e. more realistic soil temperatures, and in which way the tolerance of suppressiveness to stress varies amongst different soils.

In addition, soil microbial communities may also recover after the cessation of a stress event [25]. So far, the recovery and resilience of soils, i.e. their ability to regain functionality after stress has mainly been studied in terms of decomposition [25], while other soil functions have been neglected in resilience studies. In view of the importance of soil-borne plant diseases for crop yields, and the key function of soils to reduce these diseases through various mechanisms of suppressiveness, it is essential to investigate the recovery of disease suppressiveness after stress.

This study therefore investigates the tolerance and recovery of the soil's ability to suppress plant diseases in response to abiotic stress. We compare soils from long-term experiments with different management and geographic origin for their ability to suppress the pathogen *Pythium ultimum* infecting pea (*Pisum sativum*) seedlings. In a second step, we analyse the tolerance of soils against combined heat and drought and their ability to regain suppressiveness afterwards. In particular, we hypothesise that (i) combined heat and drought stress negatively affects suppressiveness; (ii) tolerance of suppressiveness to this abiotic stress varies among different soils; and (iii) the ability to regain suppressiveness also varies among different soils. Specifically, after the stress event, we expected that the suppressiveness of the soil decreases initially, and recovers more quickly in some soils.

Material and Methods

Overview

Our main aim was to determine tolerance and resilience of disease suppressiveness in response to combined heat and drought stress in a range of contrasting soils. The soil suppressiveness bioassays are based on the pea-*Pythium* pathosystem [26] and were conducted under controlled conditions in climate chambers. The bioassays were organised in four experiments. Experiment 1 aimed to determine the optimal levels of inoculum so that suppressiveness would in principle be detectable in all candidate soils. The aim of experiment 2 was to test soils of contrasting provenience and different management for differences in their suppressiveness in the absence of stress; inoculum levels for this experiment were chosen based on results from experiment 1. In experiment 3, we intended to determine the optimal time for measuring recovery from the abiotic stress event. Finally, experiment 4 used the selected time for testing the recovery of suppressiveness to compare the tolerance and resilience of different soils.

Sample selection from long-term field trials

We used soils from active long-term field experiments (LTFE) so that soil properties would be in equilibrium with regard to agricultural management [27]. The bioassays 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 selection of LTFEs was mainly 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. Four LTFEs from three countries were chosen for the bioassays, with two treatments each. Two LTFEs were from Eastern

Scotland (here abbreviated with 'Tulloch' and 'Woodlands'), one from North-Eastern Germany ('Thyrow'), and one from Eastern Hungary ('Westsik') (**Table 1**).

Table 1: Characterisation of sites; all sampled soils are from long-term field experiments

Site name	Woodlands	Tulloch	Thyrow	Westsik
Country	UK		Germany	Hungary
Latitude	57°11'00'N	57°10'35''N	52° 16' N	47°58'36'' N
Longitude	2°12'0.5''W	2°15'32''W	13° 12' E	21°42'20'' E
Altitude (m asl)	125	125	40	103
Annual temperature (°C)*	7.9		8.9	10.0
Mean July temperature (°C)*	14.1		18.3	20.5
Annual precipitation (mm)*	816		495	618
Trial name	Old rotation	Organic rotation trial	Static nutrient depletion trial	Westsik rotation experiment
Start year	1922	1991	1937	1929
Soil type	Dystric Cambisol	Leptic Podzol	Albic Luvisol	Arenosol
Soil texture	Sandy loam	Sandy loam	Silty sand	Sand
Reference	[28]	[29]	[30]	[31]

*multi-annual average, 1971-2000

The Tulloch LTFE is the youngest trial with 25 years of continuous trialling at the sampling time for the present study and is conducted according to organic farming standards. The other trials are run according to locally typical conventional practice, or modified as by the respective treatments. We selected two treatments from each of the trials to represent contrasting fertility levels within site, mainly according to previously obtained results on soil organic matter levels and crop yields.

Experiment 1 was conducted with uncropped bare soil directly adjacent to the plots of each of the four study sites, i.e. not from the core trial area, in order to reduce the impact of soil sampling on the integrity of the LTFEs. For the same reason, experiment 3 was performed only with soil from the margin of the Thyrow site and a pooled sample from the neighbouring trials of Woodland and Tulloch which are approx. 3.5 km apart. In experiments 2 and 4, samples from the two LTFEs that have field replicates (Thyrow and Tulloch) were pooled across replicates prior to using the soil in the replicated bioassays, because the available amount of soil was limited and by pooling the field repetitions equal inoculation of samples could be performed more precisely.

Although treatments were not identical between the different LTFEs, it was possible to group them according to their relative yield level (**Table 2**). Thus, within each site, level A represents the higher and level B the lower yield of a cereal test crop. However, the primary interest of the current study was not the comparison of different treatments on each site; instead, different treatments were selected to increase the variability of soil properties among the test soils.

Table 2: Treatments selected for soil bioassays in experiments 2 and 4; sites are sorted by decreasing C_{org}-level, and within sites by descending relative yield level.

Criterion	Woodlands		Tulloch		Thyrow		Westsik	
Treatment	A	B	A	B	A	B	A	B
Original treatment name	3	5	T4	T3	a3	a8	X	I
Treatment explanation[e]	NPK	PK	Mixed	Stockless	NPK+FYM	PK	FYM	Control
Cereal yield (%)	100	92.8[a]	100	87.4[b]	100	17.7[c]	100	48.2[d]
pH (in H_2O)	6.0	6.9	6.0	6.1	6.8	7.0	6.0	5.4
C_{org} (%)	4.10	4.34	2.94	2.94	0.55	0.25	0.41	0.26

[a]: spring oats [32]; [b]: spring oats [29]; [c]: spring barley [d]: winter rye; [e]: full treatment description see **Table S1** in Supporting Information

Soil sampling, transport and sample preparation

Samples from the top soil (< 15 cm depth) were taken with hand trowels in spring 2016 (mid-March to beginning of April) from all four sites. The fresh soil was immediately put in plastic bags of approx. 20 l for transport in cooled containers from the sampling sites to the central testing facility at Kassel University, Germany. All soil samples were then stored at 5 to 7°C until immediately before the start of the experiments. Before the tests, the soils were sieved (mesh size 10 mm). Experiment 1 started in May 2016 and was finished at the end of June 2016; experiment 4 was finished at the end of November 2016.

Maintenance of pathogen and inoculation procedure

Soils were inoculated with different concentrations of a well-characterised isolate of *Pythium ultimum*, as well as a *Pythium*-free control. In parallel, all tests were also run with sand autoclaved at 121°C for 20 min, as an additional substrate. For maintenance, the pathogen was grown on a 1.5% maize agar medium (see Electronic Supplementary Material).

Design of experiments

Before the experiments for quantifying suppressiveness and resilience were performed, pre-tests were run to select a suitable pea cultivar and to determine water holding capacity of the different soils. Peas (*cv.* Alvesta, non-treated conventionally produced seed from KWS, Germany) were sown in 500 ml pots (5 seeds per pot, sowing depth 2 cm.).

All four experiments were run in a randomized complete block design with five replicates. In experiment 1, sterile sand and soil taken directly adjacent each of the four LTFEs at Woodland,

Tulloch, Thyrow and Westsik, i.e. five different substrates, were inoculated with 0, 1, 2.5, 5, 7.5, and 15‰ of *Pythium*, resulting in a total of 150 pots. In experiment 2, eight test soils (2 treatments from each site, **Table 2**) were inoculated with 0, 2.5, and 7.5‰ of *Pythium* without prior stressing of the soil. In addition, in order to ensure robustness of the testing system, sterile sand was also included as a further substrate in this experiment.

In experiment 3, soils from Thyrow and a mixed substrate from the two Scottish sites were each subjected to stress conditions (see below) or remained unstressed; these soils were then left for different amounts of time (1, 22 and 43 days) under non-stressed conditions before being inoculated with 0, 2.5, and 7.5‰ of *Pythium*. In addition, sterile sand was included as a further substrate to monitor robustness of the testing procedure. Finally, in experiment 4 we subjected all eight test soils from the four LTFEs to stress or non-stress conditions (see below) and selected two recovery times (1 and 43 days after the stress event). All soils were then inoculated with 0, 2.5, and 7.5‰ of *Pythium*. Again, sterile sand was included as an additional test substrate.

Soil stressing

Subjecting the soils to the abiotic stress event was performed by using controlled heat (40°C) vs. 15°C in non-stressed soils, and drought (-50% of moisture content, w/w) vs. no loss of moisture in non-stressed soils. Stress conditions were maintained for a period of 4 days (96 h), with subsequent return to baseline temperature (15°C) and moisture. For the period of the stress event, stressed and non-stressed soils were kept in open aluminium trays (dimensions 30 x 23 x 6 cm, 2 l soil per tray, 4 cm filling depth). Non-stressed soils were occasionally sprayed with water during the 4-day period to avoid drying out of the surface. Full re-wetting of the soils after stress was done carefully with a Gloria hand-held sprayer in 50 l containers to return back to pre-stress water content. Over the four days, the non-stressed soil lost only small amounts of water (0% to 2.5% w/w), which were also compensated by rewetting. The choice of stress temperature followed other studies on the resilience of soils [33-35].

Testing and assessments

Pots were kept in three growth chambers with a daily regime of 16h at 20°C and 10.000 Lux, and 8h at 16°C in the dark; irrigation was performed as required. Per individual treatment combination, five replicates (pots) were randomized within the growth chambers. Plant growth was monitored daily. 21 days after sowing, 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, following established protocols [36].

Calculations and statistical analysis

Mortality of pea plants was considered as the main parameter of interest. Mortality data is displayed as % of sown peas and was analysed using a binomial error structure based on the number of peas, and in case of over-dispersion with quasi-binomial models. In some rare cases when mortality was 100% in all five replicates, i.e. when standard errors were 0, the analyses were supplemented with non-parametric Wilcoxon rank sum tests.

The relative change y of plant dry matter in response to the inoculation was calculated as $y = (x_i - x_0)/x_0$, where x_i is the dry matter in the inoculated treatment and x_0 is the dry matter in the non-inoculated treatment. Tolerance of suppressiveness against stress was defined as the absolute pea plant fresh matter difference between the stress treatment and the respective non-stress treatment. Recovery of suppressiveness was defined as the absolute pea plant fresh matter difference between the 43 days and 1 day after stress treatment. Resilience of suppressiveness was determined by comparing the time-dependence of the recovery of soil suppressiveness against the pathogen.

Analyses of variance were performed on fresh matter, dry matter and the relative change of dry matter. Homoscedasticity was examined with the Fligner-Killeen test, and normality of model residuals with the Shapiro-Wilk test. In experiment 1, because of deviations from normality and homoscedasticity, we used the non-parametric two-sample Wilcoxon rank sum test for each inoculum level.

In multifactorial trials, model reduction followed AIC as model selection criterion [37]; non-significant terms were removed from the model. Multiple comparison tests were performed with Tukey's Honest Significant Difference test. All statistical calculations were performed with the programme R, version 3.4.2 (R Development Team 2017).

Results

When testing the soils from the margins of all four field trial sites (experiment 1), we found a clear dose-response relationship between inoculum level of the pathogen and pea plant mortality, demonstrating that the experimental system was robust (**Fig. S1**). In comparison to sterile sand, the tested soils showed clear evidence for disease suppressiveness. While at 0‰, pea mortality in both sterile sand and the soils was zero, no peas survived in sterile sand when the inoculum level was 2.5‰ or higher, but disease was significantly suppressed by the tested soils even at the highest inoculum level of 15‰. Suppressiveness of the soils was also evident when fresh matter of peas was measured

(Fig. S2). Based on these data, we selected three inoculation levels (0‰, 2.5‰ and 7.5‰) for further experiments.

Evidence of the soils' suppressiveness was confirmed in experiment 2. Peas survived to different degrees, depending on inoculum level. In comparison to the non-inoculated control, the loss of plant dry matter was significantly greater in soils than in sterile sand (data not shown). In addition, differences among soils were evident (Fig. 1). Pea survival was greatest and loss of dry matter smallest in the soils from Westsik. Within sites, suppressiveness was not linked to general parameters of soil fertility in this experiment (Table 2).

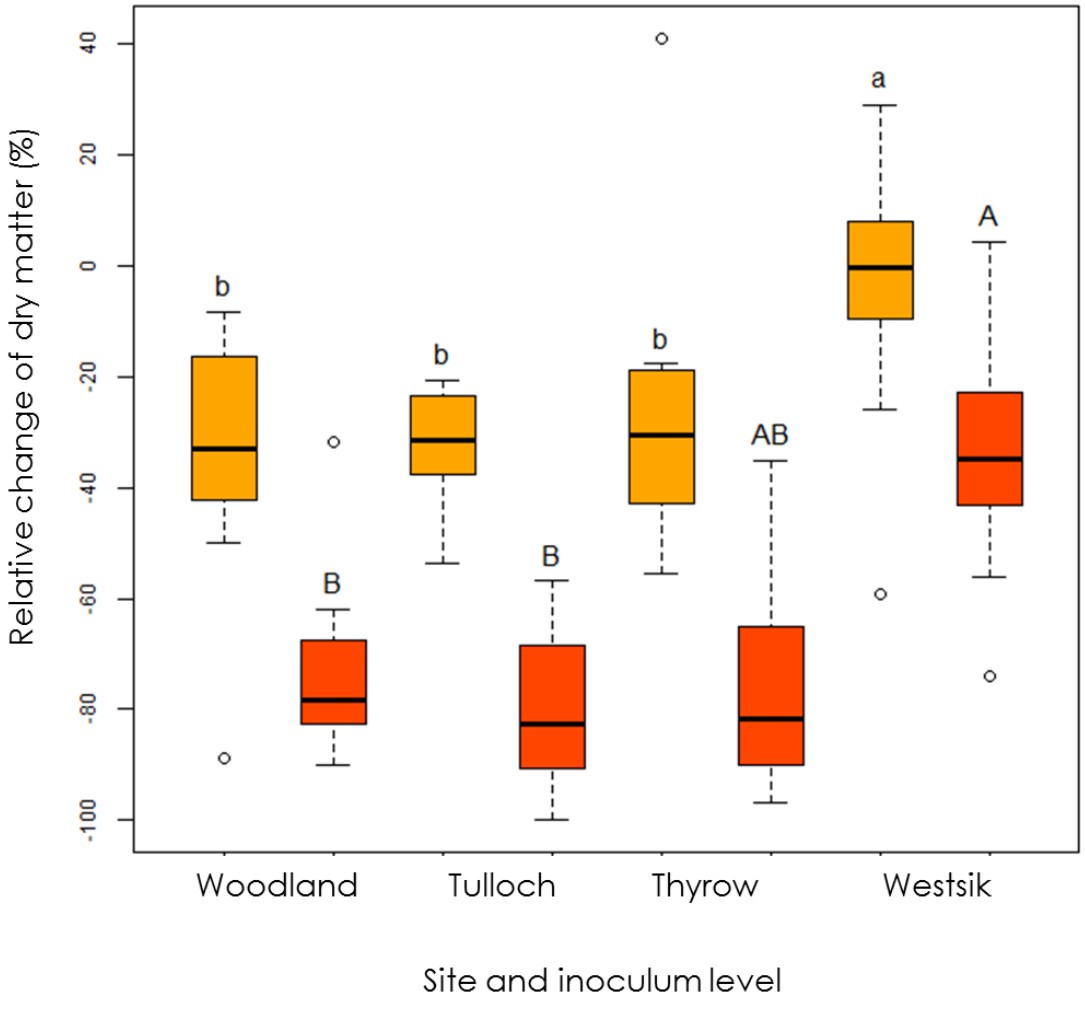


Fig. 1: Relative change of dry matter as percentage of non-infected treatment, depending on sampling site and on inoculum level (2.5‰, orange boxes, and 7.5‰, red boxes, experiment 2). A value of -100% equals complete loss of dry matter, so suppressiveness of the soils corresponds to deviation from -100%. Bars topped with

different letters are significantly different within each inoculum level at P<0.05 following Tukey's HSD test. Treatments within sites had no significant effect on relative change in dry matter in this experiment.

Stressing soils with combined heat and drought in experiment 3 significantly reduced the soils' ability to suppress disease (**Fig. 2**). Across both tested substrates, the stress induced a reduction of the number of surviving plants by 73.9% (P<0.001). However, test soils also differed in the degree to which they were affected by the stress. Immediately after the stress event, the soil from Thyrow was less strongly affected than the soil mixed from the two Scottish sites: pea fresh matter was reduced by 76.5% in comparison to non-stressed soils in the Thyrow soil, and by 91.9% in the Scottish soil. However, suppressiveness was later regained by the test soil from Scotland, which thereby demonstrated evidence of resilience. At 43 days after the stress event, suppressiveness was significantly higher than immediately after the stress, or at 22 days (P<0.05). Based on these results we selected the time of 43 days after the stress event for experiment 4 for quantifying recovery of suppressiveness.

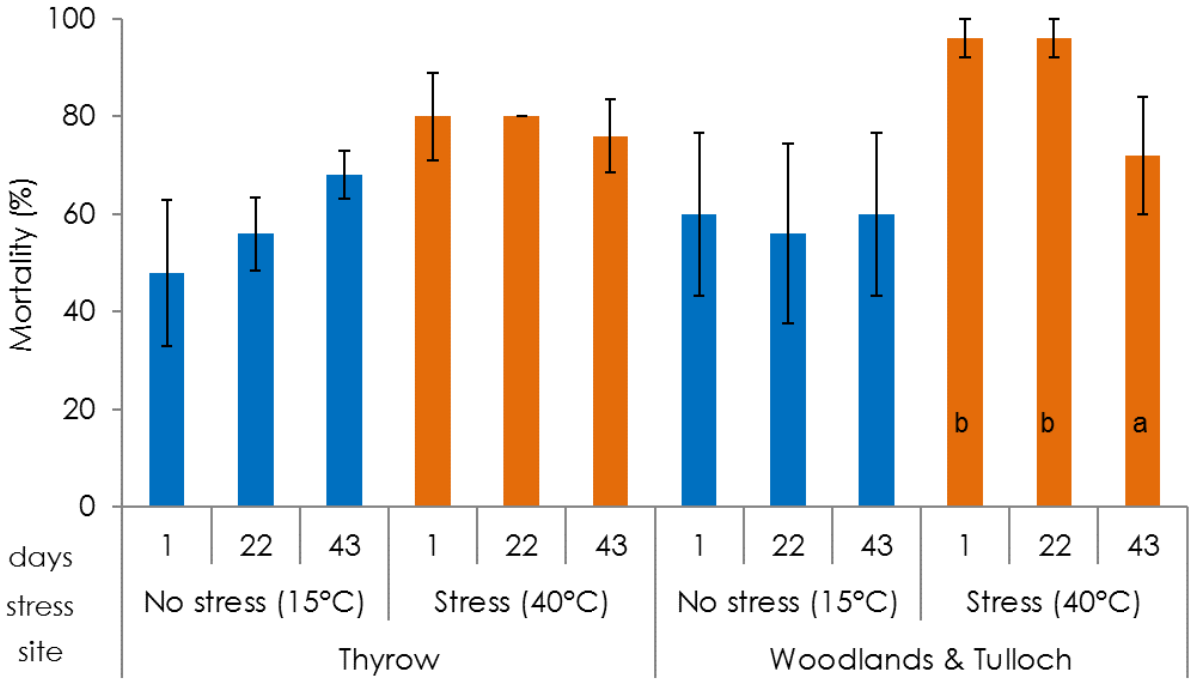


Fig. 2: Mortality (%, means and s.e.) depending on site (Thyrow vs. Scottish soils pooled from Woodlands and Tulloch), stress level of soil (with and without combined temperature and drought stress), and time (1 days, 22 days and 43 days after stress event); only results from the high level of inoculum (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. Within each site and stress treatment, bars marked with different letters are significantly different at P<0.05 (Tukey HSD test).

Experiment 4 confirmed the impact of the combined heat and drought stress on the suppressiveness of soils. When inoculated with *Pythium*, stressed soils exhibited a lower survival of plants (**Fig. 3**), a higher disease score and a lower fresh matter (**Fig. S3**) than non-stressed soils, but this effect was strongly and significantly dependent on site (**Fig. 4**, P<0.001, **Table S2**). In particular, the Scottish soils (sites Woodland and Tulloch) were much more negatively affected by stress application than the German and Hungarian soils. Fertility treatments had no consistent effect, as they did not affect the number of peas in this trial except for Westsik, where the heat stress decreased the number of peas only in the high fertility soil.

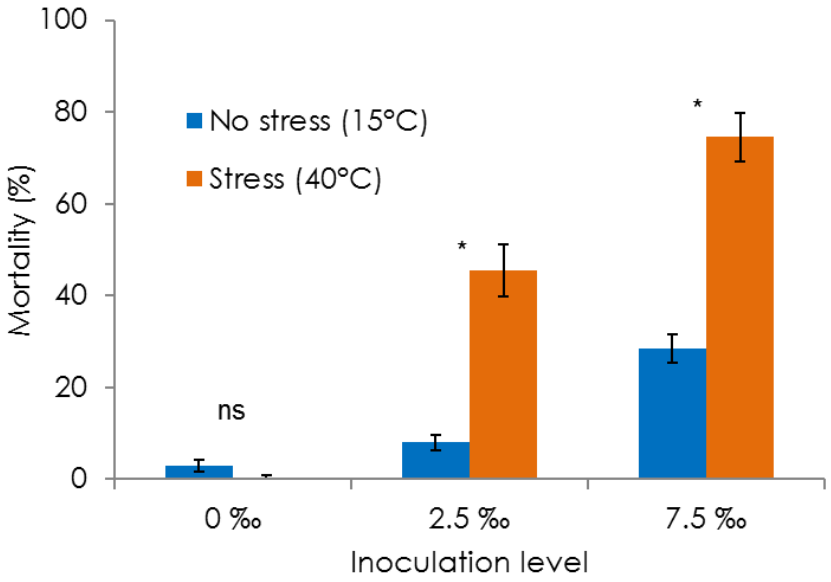
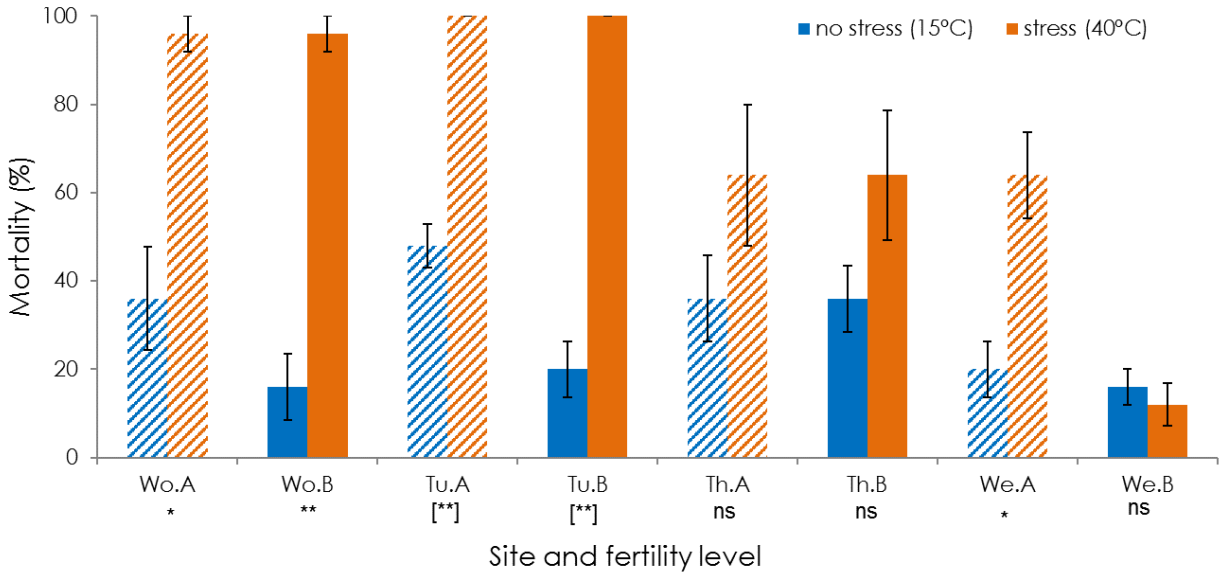


Fig. 3: Mortality of pea seedlings (% of sown), means and standard errors across eight test soils from the four sites, for three different *Pythium* inoculum levels in stressed (red bars) vs. non-stressed soils (blue bars) (experiment 4); sowing was done one day after stress or control event. Significance level of difference between stress treatments at each inoculum level, according to general linear model with quasibinomial error distribution: ns: not significant; *: P<0.05; for full statistical analysis see Supporting Information. Mortality of peas in sterile (autoclaved) sand was 0%, 92% and 100% at 0‰, 2.5‰ and 7.5‰ inoculation, respectively (n=5).



252

253 **Fig. 4**: Mortality of pea plants (means and standard errors, n=5) in eight different soils which were inoculated
254 with 7.5‰ *Pythium* (experiment 4). Soils were inoculated one day after keeping soils at 15°C (blue bars) or
255 after combined heat (40°C) and drought stress was applied to the soils (red bars); Wo: Woodlands, Tu: Tulloch,
256 Th: Thyrow, We: Westsik; A: High fertility, hashed; B: low fertility, filled; *: P<0.05; **: P<0.01; ns: not
257 significant according to general linear model with quasibinomial error distribution. Pea mortality in non-
258 inoculated treatments was 3.0±1.3% and 0.5±0.5% in non-stressed soils and stressed soils, respectively.

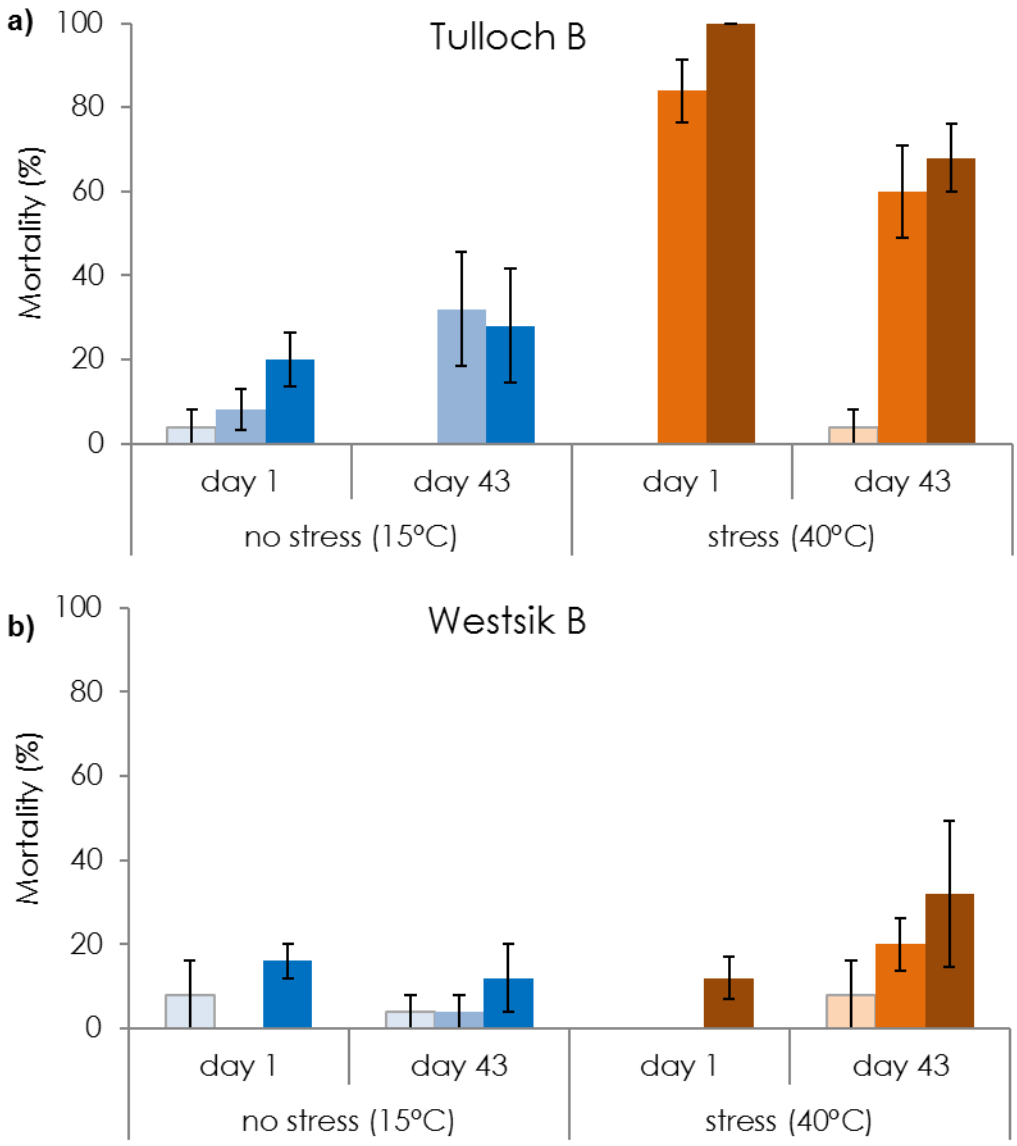


Fig. 5: Mortality of pea plants (means and standard errors, n=5) on the Tulloch B (a) test soil and Westsik B (b) soil, depending on stress level and the number of days elapsed since the stress event (experiment 4); *Pythium* levels: without inoculation (light bars), 2.5‰ (intermediate bars) and 7.5‰ (dark bars).

With regard to recovery, the picture was more complex. Significant recovery of the ability to suppress diseases was only found in one out of eight soils (Tulloch B, **Fig. 5a,** difference between "day 1 soil" and "day 43 soil" significant, $P<0.01$). 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 suppressiveness was significantly weaker 43 days after the stress event than 1 day after it (**Fig. 5b**, $P<0.05$).

Further, there was a significantly negative correlation between recovery from stress (measured as plant fresh matter difference between 43 days and 1 day after the stress event) and tolerance to stress (measured as plant fresh matter difference between the 40°C and the 15°C treatment) (**Fig. 6**).

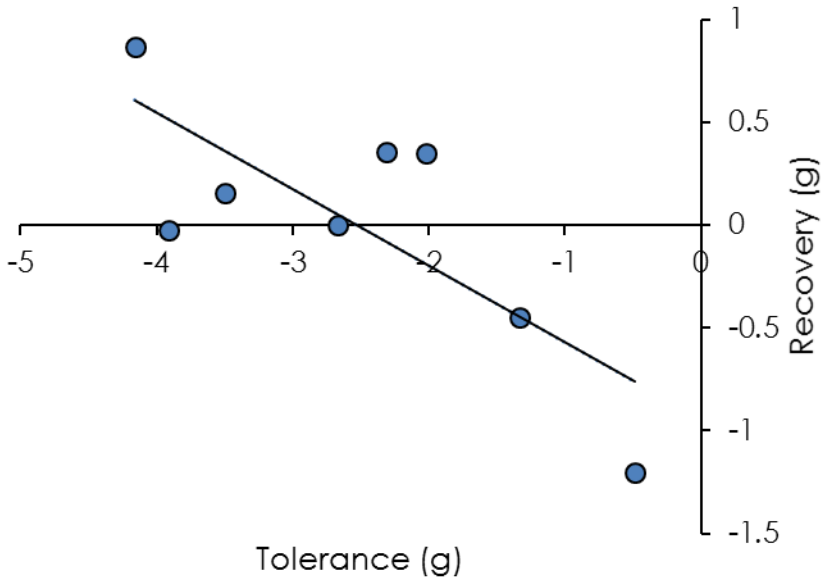


Fig. 6: Relationship between tolerance to stress and recovery from stress (experiment 4) with tolerance being defined as absolute pea plant fresh matter difference between 40°C treatment and 15°C treatment and recovery defined as absolute pea plant fresh matter difference between 43 days and 1 day after stress treatment; each point represents 5 replicates; adjusted R^2=0.529, $P<0.05$, df=6.

Discussion

Our experiments provide evidence of suppressiveness in different soils against the model pathogen *Pythium ultimum* infecting peas (**Figs. 1, S1**). Soil-borne diseases such as *Pythium ultimum* are a strong yield limiting factor in grain legumes such as peas [38-40], thereby negatively affecting protein supply in food and feed, but also nitrogen supply in crop rotations [41]. Because soil borne diseases increase the necessary number of break years between successive legume crops [42], suppressiveness of soils has important indirect impacts in that it helps to maintain higher concentrations of legumes in crop rotations. Apart from legumes, however, suppressiveness of soils is also important in multiple other crops [13]. Confirming earlier research on suppressiveness of soils from long-term experiments [43], we found that suppressiveness against *Pythium ultimum* was affected by soil type (**Fig. 1**).

Further, we demonstrate that the disease suppressiveness of soils is negatively affected by combined heat and drought stress (**Fig. 2-5**), bearing particular relevance under conditions of a warming climate

with an increasing prevalence of droughts and more extreme weather events. Our results are in line with a similar study which found reduced suppressiveness against the pathogen *Rhizoctonia solani* infecting sugar beet seedlings following shorter (1h) and much stronger (50°C and 80°C) heat stress events [23, 24]. As the results presented here demonstrate, loss of suppressiveness can already be effected by lower temperatures, i.e. at 40°C which lies within a more realistic bracket for soil surface temperatures [44] than previously tested.

In our study it is not possible to disentangle heat effects from drought effects, but these two stress factors often co-vary anyway, because high temperatures increase evaporation and are associated with low precipitation. Previous research has shown that climate change affects plant diseases through multiple mechanisms [20], including accelerated evolution of pathogens, shorter periods of incubation, earlier incidence of first infections within the season, expansion of the geographic range of pathogen occurrence, and higher susceptibility of plants to diseases under abiotic stresses. Our results highlight a further mechanism how plant diseases can be affected by climate changes. As we show, a heat and drought pulse can lead to reduced ability of the soil to suppress diseases, which increases the severity of climate change impacts on plant health, and adds further complexity to the pathosystem.

In our study, different soils were affected differently by the stress (**Fig. 1, 4**), which may be explained by variability in the composition of soil microbial communities [23]. The Scottish soils were more negatively affected than the German and Hungarian soils. A plausible explanation for this observation is a differential adaptation of the microbial community to climatic situation (**Table 1**). High summer temperatures experienced by soil microbial communities at the Hungarian site may have pre-adapted them to the experimental heat (and drought) conditions. This means that soils from higher latitudes, where temperature increases are predicted to be stronger in the future will be particularly vulnerable to decreased suppressiveness against plant diseases. On the long run, soils may also adapt to changed climatic conditions.

Our experiments provide evidence of recovery of the suppressiveness function at least in one soil, the Tulloch B soil (**Fig. 5**). Previous research on resilience and recovery in soils has concentrated on only a few functions, with the main focus being on decomposition [25]. To our knowledge, resilience or recovery of suppressiveness has so far not been investigated. Yet how quickly suppressiveness is regained in soils is highly relevant for crop production because of the relatively short time period in which plants are infected by soil-borne pathogens. Resilient soils may regain suppressiveness after a stress event before the susceptible period begins, thereby reducing the risk of disease infection, whereas less resilient soils could recover eventually from the stress, but recovery may be too late for

plants to benefit from regained suppressiveness. A conceptual model for the reassembly of the soil microbial community after heat stress was presented recently [23]. According to this idea, reassembly depends on tolerance against the stress and growth rate of individual species. Insofar as a pre-requisite of high resilience is high growth rate for quick recovery, our results support and expand this concept. The Westsik soil showed greatest stress tolerance (**Fig. 4**) but low recovery (**Fig. 5**), whereas the opposite was the case for the Tulloch soil; in fact, tolerance was negatively correlated with recovery (**Fig. 6**), indicating a trade-off between tolerance against combined heat and drought stress and the ability to recover from such stress.

Finally, both suppressiveness and the response of this function to stress were not positively affected by soil organic matter (SOM) content or other parameters typically associated with high soil fertility (**Fig. 4**). This was true within sites and amongst sites, i.e. the higher soil fertility treatments within individual sites (e.g. with addition of farm yard manure) were not associated with higher suppressiveness or stress tolerance. For example, pea survival was greatest in the Westsik soils (**Fig. 1**), which showed lowest levels of soil organic carbon among the test sites (**Table 2**). Similarly, in a survey of 26 soils across Scotland, organic carbon content was not correlated to resilience after heat [45]. These observations are unexpected, since addition of organic matter such as compost to soil is known to increase disease suppression, though different composts can affect diseases differently [46]. The fact that in our study, soil suppressiveness and response of this function to abiotic stress was not associated with high SOM levels means that there may be a trade-off between different functions of biological parameters of soil fertility. In particular, previous research on resilience and response to (abiotic) stresses has focused on decomposition; our study suggests that this function may not necessarily be aligned with suppressiveness.

Recently Griffiths & Philippot [25] suggested that resistance and resilience of the soil microbial community are governed by soil physico-chemical structure through its effect on microbial community composition and physiology. However, the effect of soil texture and structure on suppressiveness, resistance and resilience is not considered in most studies. Gregory et al. [47] argue that soil biological and physical resilience is closely linked, as biological processes in soil depend on the physical pore structure that defines microbial habitats. In their study, grassland soils with high organic matter were more resistant and resilient to heat, copper and compaction than arable soils. An arable soil with 65% clay was found to be highly resilient also, pointing towards soil texture as another parameter affecting soil resilience. Kuan et al. [45] found that soil resilience to heat stress could be distinguished by soil class, which is related to soil texture. Heijnen and van der Veen [48] showed that addition of clay minerals increased survival of rhizobia inoculated into a sandy loam and attributed this effect to the

creation of protective microhabitats, inaccessible to protozoa predating on rhizobia. Plant growth promoting rhizobacteria (PGPR) associated with suppressiveness, like e.g. *Pythium oligandrum*, which has been identified as one antagonist of *Pythium ultimum* [49], could be better protected from heat in small pores. In turn, the soil fauna and microbial community may contribute to stabilize soil structure, e.g. via earthworm burrowing or fungal hyphae. How exactly soil structure is linked to microbial community composition, physiology and function, certainly needs further research. Thus, to shed light on the mechanisms underlying the detected differences in tolerance to and recovery from abiotic stress, future studies should include soil structure and its interactions with soil microbes.

Concluding, this study demonstrates that the ability of the soil to suppress diseases is affected by stress events, such as combined heat and drought stress which lead to short-term negative effects on plants by reducing disease suppressiveness of soils. This response strongly varies among different soils, with soils from sites with a high prevalence of climatic stress being affected more strongly but tending to show greater degree of recovery. While some soils are pre-adapted to stress, others may compensate their lack of adaptation by higher growth rates and regaining their suppressiveness faster after being stressed. The underlying mechanisms need further research.

Acknowledgements

We thank the Ekhaga Foundation, Stockholm for funding the project RESTOR (Application Number 2015-65).

Authors' Contributions

CB, CW, DR, IV, JK and TFD designed the experiments; DR conducted the bioassay experiments; MT conducted the soil chemical and microbiological analysis, TFD analyzed the data; and CB, CG, CW, MA and TFD wrote the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

References

[1] Le, C., Hoang, T., Thai, T., Tran, T., Phan, T. & Raaijmakers, J. 2018 Isolation, characterization and comparative analysis of plant-associated bacteria for suppression of soil-borne diseases of field-grown groundnut in Vietnam. *Biological Control* **121**, 256-262.

[2] Marzano, S.-Y.-L., Villamil, M.B., Wander, M.M., Ugarte, C.M., Wen, L. & Eastburn, D.M. 2015 Organic transition effects on soilborne diseases of soybean and populations of Pseudomonadaceae. *Agron. J.* **107**, 1087-1097.

[3] van Toor, R., Chng, S., Warren, R., Butler, R., Cromey, M., Craigie, R. & McCloy, B. 2016 Wheat cultivar susceptibility to Gaeumannomyces graminis var. tritici and influence on *Pseudomonas fluorescence* numbers in the rhizosphere. *Australasian Plant Pathology* **45**, 595-607.

[4] Okubara, P.A., Dickman, M.B. & Blechl, A.E. 2014 Molecular and genetic aspects of controlling the soilborne necrotrophic pathogens *Rhizoctonia* and *Pythium*. *Plant Science* **228**, 61-70.

[5] Schroeder, K., Okubara, P., Tambong, J., Lévesque, C. & Paulitz, T. 2006 Identification and quantification of pathogenic Pythium spp. from soils in eastern Washington using real-time polymerase chain reaction. *Phytopath.* **96**, 637-647.

[6] Löbmann, M.T., Vetukuri, R.R., de Zinger, L., Alsanius, B.W., Grenville-Briggs, L.J. & Walter, A.J. 2016 The occurrence of pathogen suppressive soils in Sweden in relation to soil biota, soil properties, and farming practices. *Appl. Soil Ecol.* **107**, 57-65.

[7] Watson, C.A., Reckling, M., Preissel, S., Bachinger, J., Bergkvist, G., Kuhlman, T., Lindström, K., Nemecek, T., Topp, C.F. & Vanhatalo, A. 2017 Grain legume production and use in European agricultural systems. In *Adv. Agron.* **144**, 235-303.

[8] Campos, S.B., Lisboa, B.B., Camargo, F.A., Bayer, C., Sczyrba, A., Dirksen, P., Albersmeier, A., Kalinowski, J., Beneduzi, A. & Costa, P.B. 2016 Soil suppressiveness and its relations with the microbial community in a Brazilian subtropical agroecosystem under different management systems. *Soil Biol. Biochem.* **96**, 191-197.

[9] Mazzola, M. 2002 Mechanisms of natural soil suppressiveness to soilborne diseases. *Antonie van Leeuwenhoek* **81**, 557-564.

[10] Kwak, Y.-S. & Weller, D.M. 2013 Take-all of wheat and natural disease suppression: a review. *Plant Path. J.* **29**, 125.

[11] Mazzola, M. 2007 Manipulation of rhizosphere bacterial communities to induce suppressive soils. *J. Nematol.* **39**, 213.

[12] Jambhulkar, P.P., Sharma, M., Lakshman, D. & Sharma, P. 2015 Natural mechanisms of soil suppressiveness against diseases caused by Fusarium, Rhizoctonia, Pythium, and Phytophthora. In *Organic Amendments and Soil Suppressiveness in Plant Disease Management* pp. 95-123, Springer.

[13] Gómez Expósito, R., de Bruijn, I., Postma, J. & Raaijmakers, J.M. 2017 Current insights into the role of rhizosphere bacteria in disease suppressive soils. *Front. Microbiol.* **8**, 2529.

[14] Weller, D.M., Raaijmakers, J.M., Gardener, B.B.M. & Thomashow, L.S. 2002 Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Ann. Rev. Phytopath.* **40**, 309-348.

[15] Bonanomi, G., Antignani, V., Capodilupo, M. & Scala, F. 2010 Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. *Soil Biol. Biochem.* **42**, 136-144.

[16] Pérez-Piqueres, A., Edel-Hermann, V., Alabouvette, C. & Steinberg, C. 2006 Response of soil microbial communities to compost amendments. *Soil Biol. Biochem.* **38**, 460-470.

[17] Dawson, T.P., Perryman, A.H. & Osborne, T.M. 2016 Modelling impacts of climate change on global food security. *Climatic Change* **134**, 429-440.

[18] Wiebe, K., Lotze-Campen, H., Sands, R., Tabeau, A., van der Mensbrugghe, D., Biewald, A., Bodirsky, B., Islam, S., Kavallari, A. & Mason-D'Croz, D. 2015 Climate change impacts on agriculture in 2050 under a range of plausible socioeconomic and emissions scenarios. *Environ. Res. Lett.* **10**, 085010.

[19] Chakraborty, S., Pangga, I.B. & Roper, M.M. 2012 Climate change and multitrophic interactions in soil: the primacy of plants and functional domains. *Glob. Change Biol.* **18**, 2111-2125.

[20] Pautasso, M., Döring, T.F., Garbelotto, M., Pellis, L. & Jeger, M.J. 2012 Impacts of climate change on plant diseases – opinions and trends. *Eur. J. Plant Pathol.* **133**, 295-313. (DOI 10.1007/s10658-012-9936-1).

[21] Burgess, T.I., Scott, J.K., Mcdougall, K.L., Stukely, M.J., Crane, C., Dunstan, W.A., Brigg, F., Andjic, V., White, D. & Rudman, T. 2017 Current and projected global distribution of *Phytophthora cinnamomi*, one of the world's worst plant pathogens. *Glob. Change Biol.* **23**, 1661-1674.

[22] Thompson, S.E., Levin, S. & Rodriguez-Iturbe, I. 2014 Rainfall and temperatures changes have confounding impacts on *Phytophthora cinnamomi* occurrence risk in the southwestern USA under climate change scenarios. *Glob. Change Biol.* **20**, 1299-1312.

[23] van der Voort, M., Kempenaar, M., van Driel, M., Raaijmakers, J.M. & Mendes, R. 2016 Impact of soil heat on reassembly of bacterial communities in the rhizosphere microbiome and plant disease suppression. *Ecol. Lett.* **19**, 375-382.

[24] Mendes, R., Kruijt, M., De Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J.H., Piceno, Y.M., DeSantis, T.Z., Andersen, G.L. & Bakker, P.A. 2011 Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science*, 1203980.

[25] Griffiths, B.S. & Philippot, L. 2013 Insights into the resistance and resilience of the soil microbial community. *FEMS Microbio. Rev.* **37**, 112-129.

[26] Hagn, A., Engel, M., Kleikamp, B., Munch, J.C., Schloter, M. & Bruns, C. 2008 Microbial community shifts in *Pythium ultimum*-inoculated suppressive substrates. *Biol. Fert. Soils* **44**, 481-490. (doi:DOI: 10.1007/s00374-007-0230-x).

[27] Geisseler, D. & Scow, K.M. 2014 Long-term effects of mineral fertilizers on soil microorganisms – A review. *Soil Biol. Biochem.* **75**, 54-63. (doi:https://doi.org/10.1016/j.soilbio.2014.03.023).

[28] Walker, R.L., Edwards, A.C. & Watson, C.A. 2014 Nutrient input and climatic effects on the dry biomass productivity of a crop rotation over 60 years. In *European Society of Agronomy XIIIth Congress* (Debrecen, Debrecen University.

[29] Watson, C., Baddeley, J., Edwards, A., Rees, R., Walker, R. & Topp, C. 2011 Influence of ley duration on the yield and quality of the subsequent cereal crop (spring oats) in an organically managed long-term crop rotation experiment. *Organic Agriculture* **1**, 147.

[30] Ellmer, F. & Baumecker, M. 2005 Static nutrient depletion experiment Thyrow. Results after 65 experimental years. *Arch. Agron. Soil Sci.* **51**, 151–161.

[31] Lazányi, J. 2000 Sustainable rye production in the Westsik crop rotation experiment. *Acta Agronomica Hungarica* **48**, 271–277.

[32] Walker, R., Edwards, A., Watson, C.A., Campbell, C., Hillier, S. & Morrice, L. 2010 Nutrent cycling and soil fertility information gained from various long-term field experiments established on a granitic derived soil located in NE Scotland.

[33] Kuan, H.L., Hallett, P.D., Griffiths, B.S., Gregory, A.S., Watts, C.W. & Whitmore, A.P. 2007 The biological and physical stability and resilience of a selection of Scottish soils to stresses. *European Journal of Soil Science* **58**, 811-821.

[34] Griffiths, B., Bonkowski, M., Roy, J. & Ritz, K. 2001 Functional stability, substrate utilisation and biological indicators of soils following environmental impacts. *Appl. Soil Ecol.* **16**, 49-61.

[35] Wertz, S., Degrange, V., Prosser, J.I., Poly, F., Commeaux, C., Guillaumaud, N. & Le Roux, X. 2007 Decline of soil microbial diversity does not influence the resistance and resilience of key soil microbial functional groups following a model disturbance. *Envir. Microbiol.* **9**, 2211-2219.

[36] Pflughöft, O. 2008 Pilzkrankheiten in Körnerfuttererbsen (*Pisum sativum* L.) – Diagnose, Epidemiologie, Ertragsrelevanz und Bekämpfung. PhD-Thesis, University of Göttingen, Germany.

[37] Burnham, K.P. & Anderson, D.R. 2002 *Model Selection and Multimodel Inference - a Practical Information-Theoretic Approach*. New York, Springer.

[38] Emden, H.F., Ball, S.L. & Rao, M.R. 1988 Pest, disease and weed problems in pea, lentil, faba bean and chickpea. In *World crops: Cool season food legumes* (ed. R.J. Summerfield), pp. 519-534, Springer Netherlands.

[39] Saeed, M.F., Baćanović, J., Bruns, C., Schmidt, H. & Finckh, M.R. 2017 Seed health of organic peas and faba beans and its effects on the health of the harvested grains. *J.Plant Dis. Prot.* **124**, 331-337.

[40] Seethapathy, P., Kurusamy, R. & Kuppusamy, P. 2017 Soil borne diseases of major pulses and their biological management. *Innovative Farming* **2**, 1-11.

[41] Reckling, M., Bergkvist, G., Watson, C.A., Stoddard, F.L., Zander, P.M., Walker, R.L., Pristeri, A., Toncea, I. & Bachinger, J. 2016 Trade-offs between economic and environmental impacts of introducing legumes into cropping systems. *Front. Plant Sci.* **7**, 669.

[42] Döring, T.F. 2015 Grain Legume Cropping Systems in Temperate Climates. In *Grain Legumes - Handbook of Plant Breeding* (ed. A. De Ron), pp. 401-434. New York, Heidelberg, Springer.

[43] Tamm, L., Thürig, B., Bruns, C., Fuchs, J.G., Köpke, U., Laustela, M., Leifert, C., Mahlberg, N., Nietlispach, B. & Schmidt, C. 2010 Soil type, management history, and soil amendments influence the development of soil-borne *(Rhizoctonia solani*, *Pythium ultimum*) and air-borne (*Phytophthora infestans*, *Hyaloperonospora parasitica*) diseases. *Eur. J. Plant Pathol.* **127**, 465-481.

[44] Ramier, D., Boulain, N., Cappelaere, B., Timouk, F., Rabanit, M., Lloyd, C.R., Boubkraoui, S., Métayer, F., Descroix, L. & Wawrzyniak, V. 2009 Towards an understanding of coupled physical and biological processes in the cultivated Sahel–1. Energy and water. *J. Hydrol.* **375**, 204-216.

[45] Kuan, H., Hallett, P., Griffiths, B., Gregory, A., Watts, C. & Whitmore, A. 2007 The biological and physical stability and resilience of a selection of Scottish soils to stresses. *Eur. J. Soil Sci.* **58**, 811-821.

[46] Termorshuizen, A.J., van Rijn, E., vander Gaag, D.J., Alabouvette, C., Chen, Y., Lagerlöf, J., Malandrakis, A.A., Paplomatas, E.J., Rämert, B., Ryckeboer, J., et al. 2006 Suppressiveness of 18 composts against 7 pathosystems: Variability in pathogen response. *Soil Biol. Biochem.* **38**, 2461-2477.

[47] Gregory, A.S., Watts, C.W., Griffiths, B.S., Hallett, P.D., Kuan, H.L. & Whitmore, A.P. 2009 The effect of long-term soil management on the physical and biological resilience of a range of arable and grassland soils in England. *Geoderma* **153**, 172-185.

[48] Heijnen, C. & Van Veen, J. 1991 A determination of protective microhabitats for bacteria introduced into soil. *FEMS Microbiol. Ecol.* **8**, 73-80.

[49] Martin, F. & Hancock, J. 1986 Association of chemical and biological factors in soils suppressive to *Pythium ultimum*. *Phytopath.* **76**, 1221-1231.

Quantifying compensation in plant mixtures

Thomas Döring[1,2] & Heba Elsalahy[2,3]*

[1] University of Bonn, Agroecology and Organic Farming Group, Auf dem Hügel 6, 53121 Bonn, Germany

[2] Humboldt-University of Berlin, Department of Agronomy and Crop Science, Albrecht-Thaer-Weg 5, 14195 Berlin, Germany

[3] Botany and Microbiology Department, Faculty of Science, Assiut University, Assiut 71516, Egypt

*corresponding author

Abstract

Compensation is a key mechanism with relevance for biological and ecological systems. From genes to individuals to communities, compensatory processes ensure functionality, stability and resilience of these systems. However, while there is a rich literature on compensation in natural and semi-natural communities, quantification of compensation in the context of relatively simple agro-ecosystems, such as binary plant mixtures, has remained entirely underdeveloped. Here, we elaborate a method of quantifying compensation which is consistent across systems of only one component (monocultures) and two component-systems (mixtures). Using plant communities and their response to loss of plant density as an example, we show how this concept can be applied to experimentally grown monocultures and mixtures of grain legumes and cereals. Further, we generalize this approach – through the 'CROC' model of compensation – by characterizing four variables, the Contributor (e.g. plant density), the Reductor (e.g. a mortality factor reducing plant density), the Outcome (e.g. grain mass per unit area), and the Compensator (e.g. the grain mass per remaining plant).

Keywords: competition, density, intercropping, compensation

1 Introduction

Compensation is a process by which an entity (an individual or system or a group of individuals) is able to regain or restore the normal state of functionality in response to losing a part of its structure or a number of individuals, after disturbance or stress. With this concept in mind, the quantification of compensation is an important tool to assess different options of restoring functionality, whether at the individual, the population or the community level.

Compensatory processes ensure functionality in systems scaling from genetics to ecology through all levels of biological organisation, i.e. genes (El-Brolosy & Stainier, 2017), cells (Gonzalvez et al., 2013), organs, (Horiguchi & Tsukaya, 2011 Hisanaga et al., 2015), individuals (de Jong & Lin, 2017), populations (Bayu *et al.*, 2005; Marlow *et al.*, 2016), and communities (Bai *et al.*, 2004). At the individual level, e.g. in plants, when the whole shoot is occasionally removed as a result of insect damage (Forbes & Rosenheim, 2011), grazing (Liu *et al.*, 2011), specialist herbivory (de Jong & Lin, 2017), or other causes, the plant is adapted to the defoliation by compensation mechanisms.

At the population level, e.g. when a seed-borne pathogen kills a proportion of the sown plants, the remaining plants compensate the reduced density, i.e. the failure of their neighbours, by increased growth. At the community level, compensation occurs in both artificial and natural community when some species are lost from the community by removal or by a change in the environmental conditions (Marlow et al., 2016). The remaining species may then compensate for the lost species to sustain the productivity of the community (Adler & Bradford, 2002). Also the loss or decline of some species as a result of climatic change can be compensated by

the growth of other species (Gonzalez & Loreau, 2009). Consequently, productivity depends on the remaining species and their abundance after environmental perturbations (Adler & Bradford, 2002).

As a concept, compensation plays a particularly important role in ecological research on the effects of variations in biological diversity (Isbell *et al.*, 2009). The use of increased diversity in managed ecosystems, e.g. arable cropping systems (Hauggaard-Nielsen *et al.*, 2008; Lithourgidis *et al.*, 2011) or grassland (Scherer-Lorenzen *et al.*, 2003) is often promoted because of the multiple benefits of diversified systems in comparison to monocultures. These advantages include increased grain yield (Yu *et al.*, 2016; Reiss & Drinkwater, 2018), reduced incidence of pests (Tooker & Frank, 2012), diseases (Mitchell, 2002; Mundt, 2002) and weeds (Bedoussac *et al.*, 2015), as well as increased resource use efficiency (Scherer-Lorenzen *et al.*, 2003). Prominent examples of systems with increased diversity include intercropping grain legumes and cereals (Hauggaard-Nielsen *et al.*, 2008; Bedoussac *et al.*, 2015), cultivar mixtures (Reiss & Drinkwater, 2018), composite cross populations (Döring *et al.*, 2011), or agroforestry systems (Smith *et al.*, 2012).

Understanding which mechanisms are responsible for diversity effects is important for designing and optimizing diversified cropping systems for maximizing the services they provide. One of the requirements for improving such understanding is a conceptually convincing and practicable way of quantifying compensation. The quantification of compensation is therefore of great importance for disentangling various mechanisms underlying diversity effects and to clarify the relationship between compensation on the one hand and other desirable system properties, such as stability, tolerance to stress or resilience.

So far, however, the various approaches developed in ecological and biological research for the quantification of compensation effects have mainly been applied to individual plants (de Jong & Lin, 2017; McNickle & Evans, 2018), or natural or semi-natural ecosystems (Adler & Bradford, 2002; Bai *et al.*, 2004; Ranta *et al.*, 2008), while they have failed to have noticeable impact in research on managed agro-ecosystems such as arable intercropping systems. At present it is even unclear which of the several metrics of compensation are applicable to the specific situations of diversified cropping systems, where the 'diversified system' often means that just two species are cropped together instead of one, and where dynamics, in contrast to natural ecosystems are re-set each new season.

Several approaches for quantification of compensation in communities build on the notion that asynchrony of population dynamics between different species indicates compensation, and therefore use negative correlation between partners as a compensation metric (Bai *et al.*, 2004). A different but conceptually similar approach is the "ratio of the relationship between the variance of the aggregate variable [typically the sum of the component populations] and the variances of the individual species populations" (Gonzalez & Loreau, 2009).

For the scenario of multi-species experiments on the relationship between diversity and productivity, Adler & Bradford (2002) suggested a concept of quantifying compensation with the perspective of removing species from a species pool. In their study, they defined an index of compensation, C, as the difference between the observed and expected biomass divided by the expected loss of biomass following extinction.

In our paper we propose a variation and generalization of the approach suggested by Adler & Bradford (2002) and apply this to the typical scenario of an intercropping study, where the combination of two species (a grain legume and a cereal) is compared with the two respective sole crops (Helenius & Jokinen, 1994). Further, we attempt a generalization of the compensation concept, calling the resulting framework the '*CROC*' model of compensation.

2 Concept of quantifying compensation

2.1 Quantifying compensation in monocultures at individual plant densities

We first consider the case in which there is no compensation. In monocultures, no compensation would mean that biomass per unit area (i.e. yield) is exactly proportional to plant density. For example, losing half the plants from the sown density (e.g. through pathogen infection or reduced seed viability) would result in half the yield in comparison to full density, since no compensation means that the remaining plants do not show compensatory growth. Yield y in this hypothetical no-compensation scenario can be expressed by the linear equation

$$y = y_{max}\, D_{abs}/D_{max} \qquad \text{(eqn. 1)},$$

where D_{abs} is the actual absolute plant density measured as the number of plants per unit area, D_{max} is the maximum plant density and y_{max} is the biomass per unit area at maximal density. For this and further calculations, it is useful to define relative plant density as $D = D_{abs} / D_{max}$ with $D \in [0;1]$. Note that the absolute value of the maximum plant density D_{max} is dependent on the empirical context, i.e. the density range chosen by the experimenter.

Density dependence of plant biomass in single or mixed stand plant communities is often expressed by the fitting the non-linear equation

$$w = \frac{k}{1+aD} \qquad \text{(eqn. 2)},$$

to the data, e.g. following Shirtliffe & Johnston, (2002), where D is relative plant density, w is the biomass per individual plant and k and a are parameters to be estimated from the data. This equation represents the increasing competition with increasing plant density, resulting in a biomass function degressively increasing with increasing density: If $f(D)$ is the function that relates plant yield y, measured as biomass per unit area, to relative plant density D, the competition function in the monoculture can be described by

$$y = f(D) = wD = \frac{kD}{1+aD} \qquad \text{(eqn. 3)},$$

If plants compete with each other, at least at high densities, a will be > 0; further, because biomass cannot be negative, k will also be >0. At the highest and lowest relative density, the values are, $f(1) = \frac{k}{1+a}$ and $f(0) = 0$, respectively.

We now represent the actual observed yield in the monocultures by fitting eqn. 3 to the data of each monoculture. When plant density is decreasing, this function can be interpreted as a result of competitive release.

Following eqns. 1 and 3, the no-compensation function n that simulates a proportional decrease of yield with each unit of D decreasing from 1 can now be re-written as

$$n(D) = \frac{kD}{1+a} \qquad \text{(eqn. 4)}.$$

Thus, $n(D)$ is a linear function with an intercept of zero and a slope of $f(1) = k/(1+a)$.

The compensation in the monoculture at a particular plant density can then be calculated as the difference between the actual yield in the monoculture and the hypothetical non-compensation function, i.e. the compensation function $c(D)$ is defined as the difference between actual yield $f(D)$ and the yield expected for the case of no compensation, i.e.

$$c(D) = f(D) - n(D) \qquad \text{(eqn. 5)}.$$

The expression in eqn. 5 represents the yield amount that goes above the yield that is exactly proportional to plant density. Further, we can summarize the compensation across all different plant densities by calculating the area between the two lines (see section 2.3).

In order to find the density D_{c_max} at which compensation is maximal, we calculate the derivative of $c(D)$.

$$c'(D) = \left(\frac{kD}{1+aD}\right)' - \left(\frac{kD}{1+a}\right)' = k\left(\frac{1}{(1+aD)^2} - \frac{1}{1+a}\right) \quad \text{(eqn. 6)}.$$

The maximum compensation is found by setting $c'(D) = 0$. This leads to the solution at

$$D_{c\_max} = \frac{-1+\sqrt{1+a}}{a} \quad \text{(eqn. 7)}.$$

In this model, the density at which compensation is maximal is dependent on only one parameter, a. Across all values of density at which compensation peaks, it is then also possible to determine the maximum overall density at which compensation peaks.

If $z(a) = \frac{-1+\sqrt{1+a}}{a}$ then de l'Hospital's rule can be used to find

$$\lim_{a\to 0} z(a) = \frac{1}{2} \quad \text{(eqn. 8)}.$$

In other words, when compensation is overall very small, i.e. when a approaches 0, the density at which this small compensation peaks approaches $D_{max\_c} = 0.5$. From this value, D_{max_c} decreases with increasing value of a, approaching zero as a approaches infinity. This means that with this model as defined by eqns. 3, 4 and 5, compensation will always peak below half the maximum plant density.

2.2 Quantifying compensation in mixtures at individual plant densities

For a binary mixture, we introduce subscripts for the partners A and B. Here, we envisage a situation of one species having a *fixed* density, i.e. no disturbance experienced, whereas the other one loses a *varying* proportion of the sown density due to a given stress or disturbance factor. The partner with the variable density (against which the compensation takes place) is set as A, so that the partner with the fixed density is B. For sake of brevity we call A the variable partner and B the fixed partner (Figure 1).

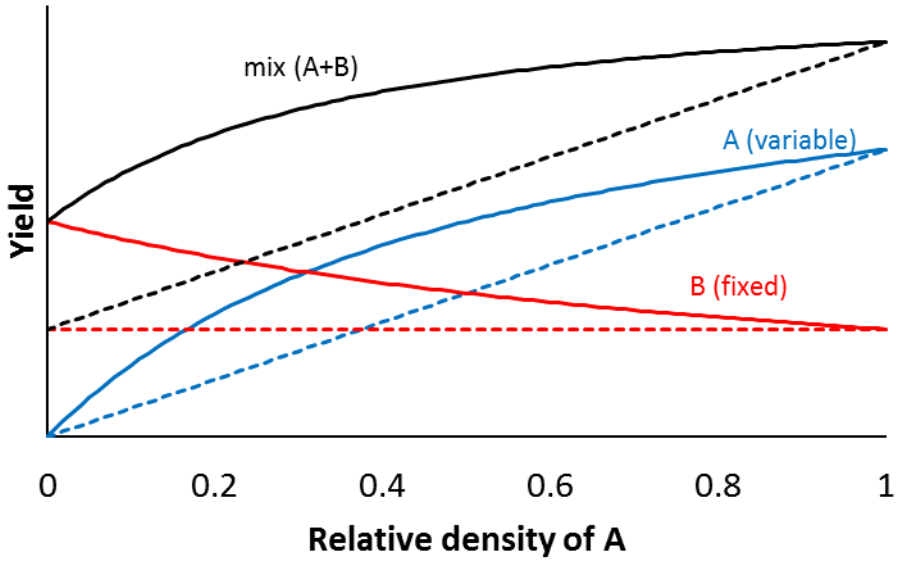


Fig. 1: Yield response to variation in density of one of the partners in a mixture; dashed lines: no compensation; solid lines: observed yield; blue: A, the partner with variable density; red: B, the partner with the fixed density, unaffected by the stress; black: mix of A and B.

As in the monocultures (eqns. 2 and 3), we follow the same non-linear functions to model biomass per plant and biomass per unit area, respectively, and define the compensation function as the difference between observed biomass and a theoretical no-compensation situation.

If the density of A is reduced in the mixture (Fig. 1, going from right to left on the x-axis), while the density of B is kept fixed we can specify theoretical expectations for the case of no compensation. For A itself, this is again just a proportional decrease of yield with decreasing A density in analogy to eqn. 1, as it follows the same process as in the monoculture. For the species with fixed density, no compensation means that whatever the density of A, yield of B remains constant. At zero density of A, the only "remaining" plants are from partner B, i.e. this corresponds to a monoculture of B. Conceptually, however, this monoculture is also a mixture where one partner, namely A, has completely failed.

The difference between the solid red line and the dashed red line shows that at low densities of A, partner B compensates through relaxed competition. In addition, the level of yield of B represented by the horizontal dashed red line can be seen as compensation through presence of B. Even if there is no compensatory growth through relaxed competition, losing all plants of partner A would still yield this level just because B is still present.

In analogy to eqn. 5, the compensation function of the variable partner can then be written as

$$c_A(D_A) = \frac{k_A D_A}{1+a_A D_A} - \frac{k_A D_A}{1+a_A} \qquad \text{(eqn. 9a)}.$$

For the fixed partner in the mixture, the compensation function is

$$c_B(D_A) = \frac{k_B D_B}{1+a_B D_A} - \frac{k_B D_B}{1+a_B} \qquad \text{(eqn. 9b)}.$$

Note that D_B is fixed whereas D_A is variable, i.e. when modelling the biomass of the species with the fixed density (eqn. 9b), the model uses the varying density of the other species as input variable. Therefore, c_B is a function of D_A.

With decreasing density of A (higher rates of failure), the summed compensatory effects tend to become larger (Fig. 1). We can use the Fig. 1 to deconstruct the compensation effects in mixtures. These are (i) the presence of B, (ii) the compensatory growth of B and (iii) the compensatory growth of A itself. The observed biomass in the mixture (with varying densities of one species, and a fixed density of the other species) is again simply the sum of the mixture's components (eqn. 10). This is the compensation function c_M of the mixture, i.e. the sum of the two partners:

$$c_M(D_A) = c_A(D_A) + c_B(D_A) \quad \text{(eqn. 10)}.$$

The derivative of the compensation function in the mixture can be calculated but leads to a complex fourth order polynom; it can be solved for zero, i.e. for the density at which compensation is maximal, but the expression is unwieldy and it may be easier to solve this numerically.

2.3 Compensation across the entire range of densities

In order to quantify overall compensation across all values of density, i.e. to summarize compensation for all relative densities between 0 and 1, we calculate the integral of the compensation function. In the competition function of the monoculture (compare eqn. 3), this leads to

$$\int f(D)dD = \int \frac{kD}{1+aD} dD = \frac{k}{a^2}(aD + 1 - \ln(aD+1)) \quad \text{(eqn. 11)},$$

with

$$\int_0^1 \frac{kD}{1+aD} dD = \frac{k}{a}\left(1 - \frac{\ln(1+a)}{a}\right) \quad \text{(eqn. 12)}.$$

Integrating the no-compensation function of the monoculture (eqn. 4) leads to

$$\int n(D)dD = \int \frac{kD}{1+a} dD = \frac{1}{2} D^2 \frac{k}{1+a} \quad \text{(eqn. 13)},$$

with

$$\int_0^1 \frac{kD}{1+a} dD = \frac{k}{2(1+a)} \quad \text{(eqn. 14)}.$$

Therefore, the compensation area C, i.e. the compensation across all relative densities (from 0 to 1) can be determined as

$$C = \frac{k}{a}\left(1 - \frac{\ln(1+a)}{a}\right) - \frac{k}{2(1+a)} \quad \text{(eqn. 15)}.$$

Total biomass Y_{max} at the theoretically maximal compensation across all densities is

$$Y_{max} = \frac{k}{1+a} \quad \text{(eqn. 16)}.$$

Relative compensation can then be defined as

$$r = \frac{C}{Y_{max}} = \frac{\left[\frac{k}{a}\left(1 - \frac{\ln(1+a)}{a}\right) - \frac{k}{2(1+a)}\right]}{\frac{k}{1+a}} = \frac{1 - \frac{\ln(1+a)}{a} - \frac{a}{2(1+a)}}{\frac{a}{1+a}} \quad \text{(eqn. 17)}.$$

The maximal relative compensation is

$$\lim_{a\to\infty} r(a) = \frac{1}{2} \quad \text{(eqn. 18)}.$$

An approximating function $r^*(a)$ can be found for $r(a)$ that is easier to calculate:

$$r^*(a) = \frac{ua}{1+ta} \quad \text{(eqn. 19)}.$$

For r = 0 up to r = 0.4, this function is an ideal approximation of $r(a)$ with u = 0.1375 and t = 0.3049.

In the mixtures, as in the monocultures, the effect of compensation across all plant densities can be summarized by the area between the solid lines (observed data) and dashed lines (theoretical expectation if there is no compensation). For the variable partner, these areas correspond to eqns. 12 and 14, respectively. For the fixed partner, the no-compensation function is constant (Fig. 1), with the no-compensation area across all densities equaling

$$\int_0^1 \frac{k_B D_B}{1+a_B} dD_A = \frac{k_B D_B}{1+a_B} \quad \text{(eqn. 20)},$$

while the area under the competition function is

$$\int_0^1 \frac{k_B D_B}{1+a_B D_A} dD_A = \frac{k_B D_B \ln(1+a_B)}{a_B} \quad \text{(eqn. 21)},$$

so that the compensation area C_M of the mixture equals

$$C_M = \frac{k_A}{a_A}\left(1 - \frac{\ln(1+a_A)}{a_A}\right) - \frac{k_A}{2(1+a_A)} + \frac{k_B D_B \ln(1+a_B)}{a_B} - \frac{k_B D_B}{1+a_B} \quad \text{(eqn. 22)},$$

Because the densities are relative and go from 0 to 1, the areas are numerically equal to the average biomasses of the respective functions.

3 Application: Compensation in a mixture of oats and beans

We apply this model to published grain yield data from a field experiment with faba beans (*Vicia faba*) and oats (*Avena sativa*), which were sown in monocultures and mixtures at a range of different densities (Helenius & Jokinen, 1994). We use different subsets of the data to generate scenarios where one partner has a fixed and the other a variable density. The dataset allows us to choose both oats or beans as the fixed partner in alternative scenarios. The non-linear function represented by eqn. 3 was fitted to the data using the *nls* procedure of the programme R (v. 3.4.2). The difference between the resulting function and the no-compensation function (eqn. 1) represents compensation for loss of density (Fig. 2).

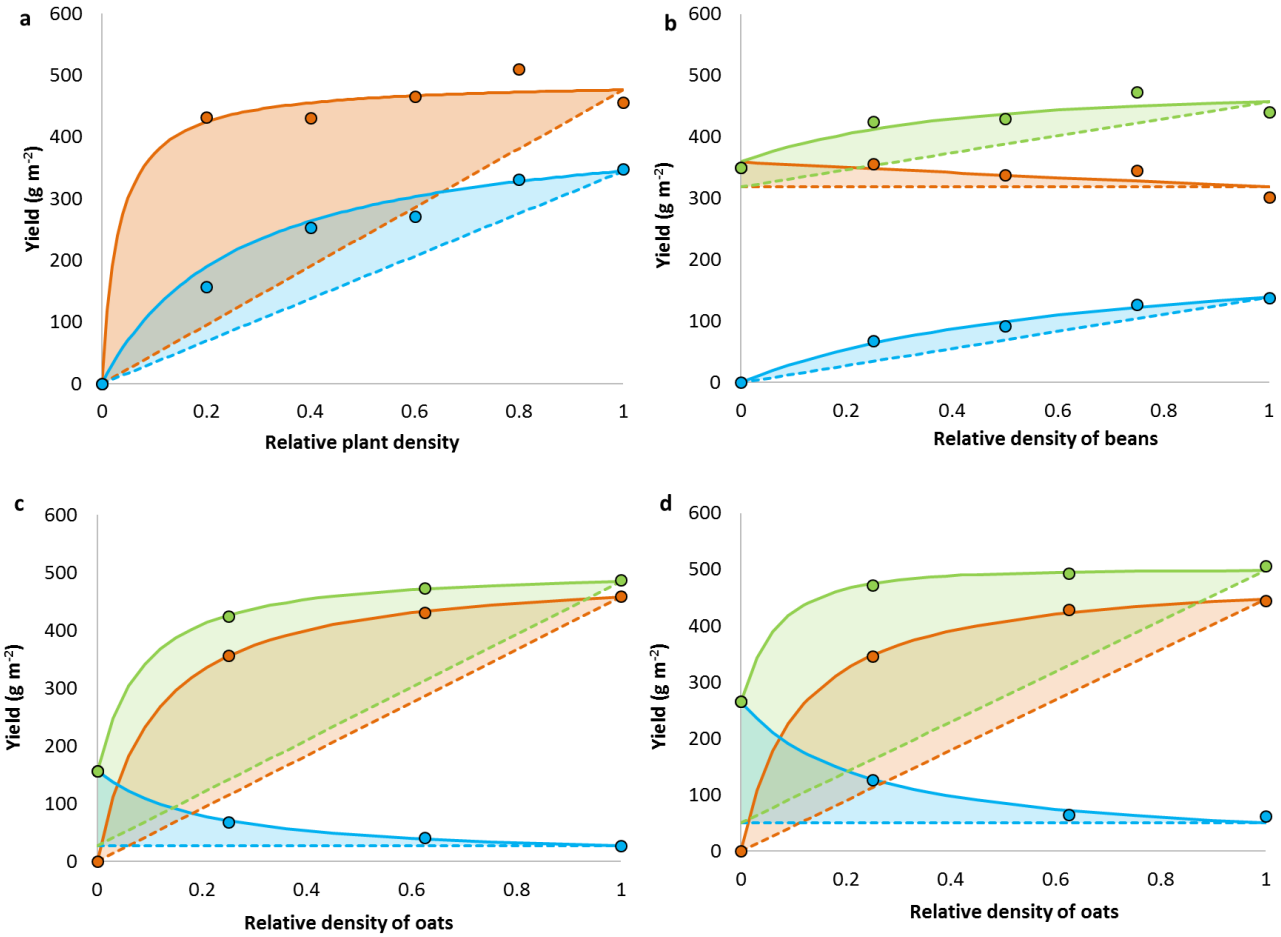


Fig. 2: Grain yields (in g dry matter m^{-2}) of oats (red), bean (blue) and their sum (green) in monocultures (a) and mixtures (b-d), depending on the relative plant density of the variable partner, with oats density fixed at 100 seeds m^{-2} (b), beans density fixed at 20 seeds m^{-2} (c), and bean density fixed at 60 seeds m^{-2} (d). Panels show individual data points from the original study (coloured points), non-linear functions according to eqn. 3 fitted to the data (solid lines), the no-compensation functions (broken lines, eqn. 1) and compensation represented by the difference between the two functions (shaded areas, corresponding to eqns. 12 and 14); data from (Helenius & Jokinen, 1994).

The analysis shows that compensation in the monocultures is stronger in oats than in bean (Fig. 2a). If compensation is expressed relative to total biomass across all densities (i.e. by dividing shaded area by the total area under the curve), the oat and bean monocultures compensate 46% and 33% of grain yield in the monocultures, respectively. Compensation in the mixture is small (9%) when the density of the beans is variable (Fig. 2b), i.e. competitive release following loss of bean density does not lead to substantial compensation. In this case, beans and oats contribute about equally to the compensation in the mixtures. If the bean density is fixed at 20 or 60 seeds per m^2, compensation (by both partners together) makes up a substantial proportion of the total grain yield in the mixture (Fig. 2c and d), amounting to 42% in both cases.

Results across all combinations of densities show that compensation for loss of bean density is much smaller than compensation for loss of oats density (Fig. 3). Further, the degree of compensation for loss of bean density strongly depends on the density of oats, i.e. the fixed partner in this case, with compensation strongly decreasing from the bean monoculture (oats fixed at 0) to mixtures with higher

densities of the cereal. Remarkably, in the case of fixed bean density, the mixtures (with bean density fixed at 20, 40 or 60 seeds per m²), do not show a higher degree of compensation than the oats monoculture (i.e. where bean density is fixed at zero seeds). Adding beans to an oats crop suffering from various degrees of seed mortality therefore does not increase compensation for the loss of oats density. In the converse case, i.e. if oats are added to a bean crop that is afflicted by seed mortality factor, relative compensation for loss of bean density even decreases.

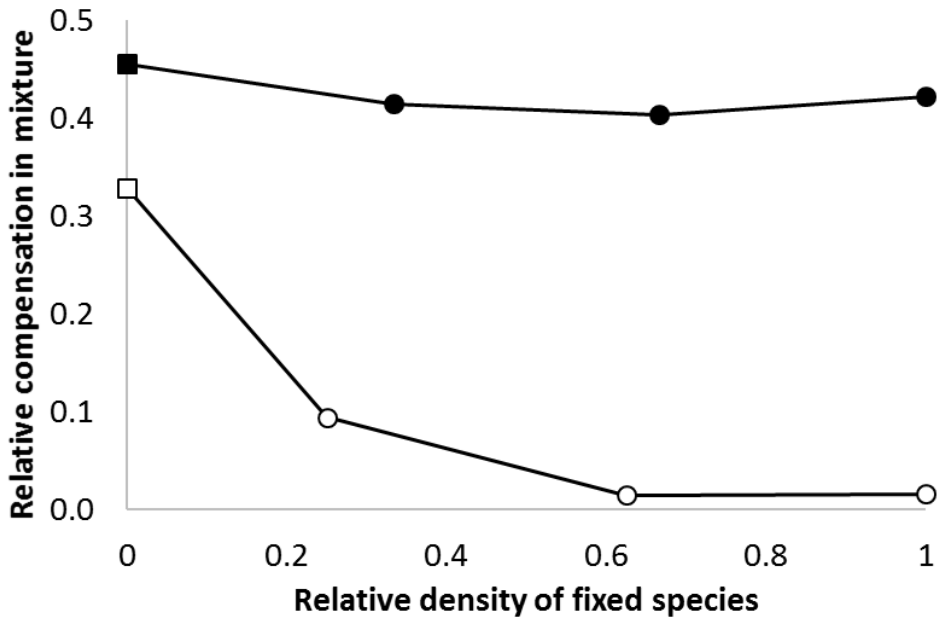


Fig. 3. Compensated grain yield in mixtures (circles) and monocultures (square symbols) of beans and oats, relative to total grain yield, in case of fixed bean density (filled symbols, oats with variable density) and fixed oats density (open symbols, beans with variable density), so that bean monoculture is represented by open square, and oats monoculture by filled square; for better comparison of the species, densities are expressed as relative to the maximal tested density; data from (Helenius & Jokinen, 1994). Note that monoculture can be conceived as a mixture where the other partner is fixed at density of 0.

4 Generalization

4.1 The 'CROC' model of compensation

So far we have concentrated on the reduction of plant density, i.e. number of plants per unit area, and the effect of this reduction on biomass yield as the variable constituting the outcome of interest. In our example this loss of plant density is compensated for by an increased growth of the remaining plants, a mechanism based on competitive release.

For generalizing the concept of compensation, we introduce four terms: (1) The '*contributor*' is the variable positively contributing to the final outcome. In the applications above, the contributor is plant density, but the contributor could also be green leaf area, the biomass obtained in the previous harvest or other variables with a causal and positive link to the final outcome Further examples are listed in Table 1. (2) The '*reductor*' is the stress or disturbance factor reducing the contributor. In the examples discussed above, this was an arbitrary mortality factor leading to seed or seedling mortality, e.g. seed predators or pathogens. (3) The '*outcome*' is the target variable, in our examples above this is biomass per unit area; other examples include, among a multitude of potential variables, the biomass of an individual plant, or the number of successfully pollinated flowers. Finally, (4) the '*compensator*' it is the factor by which the contributor needs to be multiplied to obtain the outcome variable. The compensator is the variable directly linked to the mechanism of compensation; in our

example the compensator is the biomass per plant. The concatenation of the four initials of these variables leads to the name of this concept as the '*CROC'* model of compensation.

As we have shown above, this concept can be applied to sole crops (monocultures), and to mixtures. More generally, we can apply this to systems containing just one component or systems with more components. With systems composed of more than one component, it makes sense to separate these components into a party that is affected by the reductor, and another, unaffected party. Note that parties can include more than one components, e.g. more than one species. In the examples on plant density elaborated above, the affected and unaffected party correspond to the variable and the fixed partner, respectively. This scenario may be further generalized by allowing both parties to be variable in their response to the reductor, i.e. by being affected to different degrees. Table 1 lists further examples to which this concept may be applied.

4.2 Using different functions for density dependence

Another way of generalizing the compensation metric is provided by replacing the non-linear relationship between contributor (e.g., density) and outcome (e.g., yield). In the example above, this relationship is encapsulated in eqn. 2, but this could be replaced by a different function, e.g. the power function $w = \alpha D^{\gamma}$, where D is again relative plant density, w is the biomass per individual plant and the parameters α and γ are estimated from the data (Watkinson, 1980). Accordingly, the yield function $y = f(D)$ is $wD = \alpha D^{\beta}$, with $\beta = \gamma+1$. We only consider the case of $0<\beta<1$, in which case the yield function is degressively increasing with increasing D; with $\beta=1$, compensation is nil, and compensation becomes negative with $\beta>1$. The no-compensation function $n(D)$ equals $f(1)D = \alpha D$. The compensation at a particular plant density is then $c(D) = f(D) - n(D) = \alpha D\,(1 + D^{\beta-1})$. This function is maximal at

$$D_{c\_max} = \beta^{\frac{1}{1-\beta}} \qquad \text{(eqn. 23)}.$$

As $\beta<1$ approaches 1, D_{c_max} approaches a value of $e^{-1} \approx 0.3679$. Comparing this with the value obtained from eqn. 8, the scenario building on the power function yields a smaller value for the maximal density at which compensation peaks. In both cases, compensation peaks at relatively small densities, i.e. when competition is not intense.

Calculating compensation across all densities is done as above (eqns. 11-14) by integrating the respective functions, in this case, $\int f(D)dD = \frac{\alpha}{1+\beta}D^{\beta+1}$ with $\int_0^1 f(D)dD = \frac{\alpha}{1+\beta}$, and $\int n(D)dD = \frac{\alpha}{2}D^2$, with $\int_0^1 n(D)\,dD = \frac{\alpha}{2}$. Therefore, the compensation (in the monoculture) across all densities is

$$C = \frac{\alpha}{2}\frac{(1-\beta)}{(1+\beta)} \qquad \text{(eqn. 24)}.$$

Because in this case total biomass at the theoretically maximal compensation across all densities is $Y_{max} = \alpha$, the actual compensation relative the theoretically maximal compensation to is $r = \frac{(1-\beta)}{2(1+\beta)}$, which becomes maximal with a value of 0.5 as $\beta>0$ approaches 0.

In the mixture, calculations analogous to the procedure developed in eqn. 10 to 15 and 20 to 22, lead to compensation C in the mixtures equaling

$$C_M = \frac{\alpha_A}{2}\frac{(1-\beta_A)}{(1+\beta_A)} + D_B\alpha_B\left(\frac{1}{\beta_B} - 1\right) \qquad \text{(eqn. 25)}.$$

5 Discussion

5.1 The relationship between competition and compensation

In the context of plant mixtures, compensation is the effect that the presence of one species or cultivar compensates for the failure of another component in the mixture. If one component fails (or experiences reduced growth), the other one 'takes its place'. Compensation is also evident in monocultures: if one plant individual fails, another one may compensate the failure by increased growth, which is possible through reduced competition among the remaining individuals. Thus, plant individuals respond to reduced plant density (i.e. relaxed competition) by compensatory growth. Compensation in response to a given disturbance requires plants individuals (of the same or different species) to vary in their response to that disturbance factor. E.g. a seed-borne pathogen kills a proportion of the sown plants; the remaining plants compensate the reduced density, i.e. the failure of their neighbours, by increased growth. Because compensation is basically response to loss, it is expected to become particularly evident as plant density *decreases*.

Our approach to quantify compensation in plant stands builds on the close relationship between competition and compensation, i.e. competitive release as the mechanism underlying compensation (Creissen *et al.*, 2013). In Figs. 1 and 2, this can be thought of as moving from low to high density when competition is in the focus, whereas the opposite direction, from high to low density is the perspective for compensation.

Eqns. 8 and 23 above demonstrate that compensation is peaks between zero density and half the maximum density. This means that as density increases beyond this (low) value, competition increases and compensation decreases. Below this point of maximal compensation however, both competition and compensation decrease as density decreases. This is because at very low densities, although competition amongst plants is small, individual plants are reaching a limit in translating the higher amount of resources available per plant into increased individual plant yield.

5.2 Comparison with other metrics of compensation

Possibly following the close conceptual relationship between competition and compensation, previous attempts to quantify compensation in mixed plant stands have sometimes used negative correlation between different species as a metric for compensation (Bai *et al.*, 2004) or similar approaches (Akatov *et al.*, 2016). However, this approach has several drawbacks. (1) First, correlation is symmetrical and is therefore unable to help deciding which of the two species (or, more generally, parties) contributes to observed compensatory effects. (2) Further, as long linear models are used, the use of negative correlation is limited because the non-linearity of the interactions between species and in response to the reductor, is not captured. (3) Correlational approaches to the quantification of compensation also have the disadvantage that the metric is unitless, whereas the framework developed here is able to report compensation effects in units of biomass or yield, or any other unit measuring the outcome. (4) Another reasons why regression or correlation approaches are not satisfactory is that they are not applicable to monocultures, whereas the model proposed here of quantifying compensation is consistent in both mixed stands and monocultures.

Similar arguments are valid in the case of the variance ratio, which divides the variance of the aggregate variable, e.g. the mixture, by the sum of the variances of the components, e.g. the monocultures (Gonzalez & Loreau, 2009). Again, this approach returns a unitless metric, is symmetric, and cannot be used in monocultures. In addition, scale effects according to Taylor's power law may have to be taken into account with the variance approach (Döring *et al.*, 2015b).

As a metric of compensation for diversity-productivity experiments, Adler & Bradford (2002) used "the difference between the observed and expected biomass divided by the expected loss of biomass following extinction". Our model is similar in that it compares observed against expected outcomes. Translated to our model, the notion of extinction would mean that the contributor (e.g. plant density) is set to a value of zero. The model suggested by Adler & Bradford (2002) is therefore unable to model the compensatory effects from the remaining individuals of the party affected by the stress, because it is completely extinct. In the applied context

of intercropping, however the scenario of complete loss, i.e. corresponding to extinction, is relatively rare (though not unheard of).

The procedure developed here, generalized in the *CROC* model, has the advantage of relating compensation to a specific contributor, such as density, and can therefore help to elucidate mechanisms of compensation. However, the flipside of this advantage is that the model is relatively demanding in terms of data input. For example, in case of plant density being the contributor to compensation in a two-species mixture (intercropping) scenario, the compensation calculations require a set of various density combinations to be tested. So far, published studies on intercropping have rarely produced the experimental set-ups required for such calculations.

5.3 Compensation and its relationship with other effects of interaction in plant mixtures

Mixtures of different plant species or cultivars are known for a multitude of benefits when compared to their respective monocultures, including increased yield (Nyfeler *et al.*, 2009; Yu *et al.*, 2016; Reiss & Drinkwater, 2018), reduced incidence of weeds (Suter *et al.*, 2017), pests (Döring, 2014), and diseases (Wolfe, 1985; Finckh *et al.*, 2000; Zhu *et al.*, 2000), as well as increased resource use efficiency through complementarity (Scherer-Lorenzen *et al.*, 2003). Here, we discuss the relationship between compensation on the one hand and two of the various mechanisms underlying the observed effects in mixtures on the other, namely selection and complementarity.

To explain advantages in mixtures over monocultures, an important mechanism is selection, especially in the context of plant disease reduction (Finckh & Mundt, 1992). Selection means that the partner with the higher performance, e.g. due to stronger disease resistance, increases its proportion in the mixture beyond the initially sown proportion. More specifically, it has been defined as 'the difference between the disease reduction calculated based on planted frequencies and the reduction based on harvested frequencies' (Finckh & Mundt, 1992). To have a positive effect on grain in the mixture, selection requires compensatory processes by individual plants: because the absolute density of the resistant partner does not change, the reduced performance of the susceptible partner can only result in grain yield above the average of the monocultures if individual plants of the resistant partner exhibit increased per-plant yield. So if there is no compensation, increased production via selection is not possible.

Complementation occurs when different individuals use complementary niches; their resource use in the mixture is greater than the average resource use of the monocultures. Competition among plant individuals is decreased through reduced niche overlap. Thus, complementation becomes particularly evident at *increasing* plant densities because then competition effects are strongest. This is therefore in contrast to compensation which we have shown is more important at lower densities. Complementation could be measured as the difference between biomass in mixture at full density and biomass in monoculture at full density. This leads to two values for complementation for each species, i.e. it is not symmetrical. An alternative is to compare mixture performance simply against the average of the monocultures.

5.4 Compensation, stability and resilience

In addition to the benefits mentioned above, mixtures are associated with reduced production risk, which is often referred to as the insurance hypothesis (Yachi & Loreau, 1999). A large number of studies has demonstrated that increased diversity in plant communities may lead to higher stability of the aggregate yield (Tilman *et al.*, 2006; Isbell *et al.*, 2009; Döring *et al.*, 2015a; Raseduzzaman & Jensen, 2017), though there are also counter-examples and studies with mixed results (Clay & Allard, 1969; Fukai & Trenbath, 1993; Tracy & Sanderson, 2004). Compensation can be seen as a main mechanism for reduced risk (Creissen *et al.*, 2013) and increased stability. The application of our model to a set of experimental two-component mixtures and monocultures of arable crops highlights that compensation does not only happen in mixtures but also in monocultures; in fact, compensation can be even higher in monocultures than in mixtures. The CROC model may provide a framework for understanding the complex relationship between diversity and stability by experimentally targeting specific contributors and reductors and their compensatory relationships to yield or other outcome variables.

Our model allows to deconstruct compensation in mixtures into three parts: First, compensation through competitive release by the affected party; second, compensation through competitive release by the unaffected; and third, compensation through 'presence' of the unaffected party. Notably, this third part does not require any interaction between the partners, therefore this scenario could, for example, be represented by two neighbouring fields. If one but not the other partner (the affected party) fails, the other will simply still be there. Conceptually, it could be argued that this third part of compensation is not 'true' compensation, but our model allows disentangling the different components and is therefore independent of the decision whether or not this passive 'still being there' should be thought of as part of compensation.

Compensation is not only closely related to stability but is also similar to the concept of resilience, i.e. the ability of a system to rebound after a stress event (Döring *et al.*, 2015c). In the terms of the *CROC* model, following a stress event (corresponding to the reductor), a resilient system regains functionality (the outcome) via the compensator. The main difference between the concepts of resilience and compensation is the focus of resilience on the temporal dynamics of the system. In addition, in the *CROC* model compensation can only be calculated if there are several values for the reductor, e.g. if there is a stress gradient, while resilience can also be determined by comparing a stressed and unstressed variant (Griffiths & Philippot, 2013).

As for the relationship between diversity and stability, there is also strong interest in the contribution of diversity to resilience (Reusch *et al.*, 2005). It has been suggested that response diversity, i.e. the diversity in response to the stress is required for resilience (Elmqvist *et al.*, 2003; Folke *et al.*, 2004). Interestingly, however, response diversity (in terms of response of the contributor to the reductor) is not necessary for compensation. Even if both parties are affected by the reductor they can both compensate through competitive release as in the monocultures.

6 Conclusions

The *CROC* model and the equations laid out above provide a framework for in-depth research on compensation and its relationship to resilience and stability. In particular, the analysis and quantification of compensation in mixtures can help to further develop the large potential of diversification for improving multifunctional performance of agro-ecosystems, especially in response to biotic and abiotic stresses. Ultimately this may help to identify actions necessary to develop resilient and stable production systems, both in agriculture and forestry.

Acknowledgements

We are grateful for support from the Jameel Scholarship at Humboldt University Berlin to HE and for support from the Ekhaga foundation, Sweden to TFD.

References

Adler PB, Bradford JB. 2002. Compensation: an alternative method for analyzing diversity-productivity experiments. *Oikos* **96**(3): 411-420.

Akatov V, Akatova T, Eskina T, Zagurnaya YS, Sazonets N, Chefranov S. 2016. The effect of density compensation in species-poor plant communities of mainland areas (on the example of the Western Caucasus). *Biology Bulletin Reviews* **6**(5): 386-399.

Bai Y, Han X, Wu J, Chen Z, Li L. 2004. Ecosystem stability and compensatory effects in the Inner Mongolia grassland. *Nature* **431**(7005): 181-184.

Bayu W, Rethman N, Hammes P. 2005. Growth and yield compensation in sorghum (Sorghum bicolor L. Moench) as a function of planting density and nitrogen fertilizer in semi-arid areas of northeastern Ethiopia. *South African Journal of Plant and Soil* **22**(2): 76-83.

Bedoussac L, Journet E-P, Hauggaard-Nielsen H, Naudin C, Corre-Hellou G, Jensen ES, Prieur L, Justes E. 2015. Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming. A review. *Agronomy for Sustainable Development* **35**(3): 911-935.

Clay RE, Allard RW. 1969. A comparison of performance of homogeneous and heterogeneous barley populations. *Crop Science* **9**(4): 407-&.

Creissen HE, Jorgensen TH, Brown JK. 2013. Stabilization of yield in plant genotype mixtures through compensation rather than complementation. *Annals of Botany* **112**(7): 1439-1447.

de Jong TJ, Lin T. 2017. How to quantify plant tolerance to loss of biomass? *Ecology and evolution* **7**(9): 3080-3086.

Döring TF. 2014. How aphids find their host plants, and how they don't. *Annals of Applied Biology* **165**(1): 3-26.

Döring TF, Annicchiarico P, Clarke S, Haigh Z, Jones HE, Pearce H, Snape J, Zhan J, Wolfe MS. 2015a. Comparative analysis of performance and stability among composite cross populations, variety mixtures and pure lines of winter wheat in organic and conventional cropping systems. *Field Crops Research* **183**: 235-245.

Döring TF, Knapp S, Cohen JE. 2015b. Taylor's power law and the stability of crop yields. *Field Crops Research* **183**: 294-302.

Döring TF, Kovacs G, Wolfe MS, Murphy K. 2011. Evolutionary plant breeding in cereals– into a new era. *Sustainability* **3**: 1944-1971.

Döring TF, Vieweger A, Pautasso M, Vaarst M, Finckh MR, Wolfe MS. 2015c. Resilience as a universal criterion of health. *Journal of the Science of Food and Agriculture* **95**: 455–465.

Elmqvist T, Folke C, Nyström M, Peterson G, Bengtsson J, Walker B, Norberg J. 2003. Response diversity, ecosystem change, and resilience. *Frontiers in Ecology and Environment* **1**(9): 488–494.

Finckh MR, Gacek ES, Goyeau H, Lannou C, Merz U, Mundt CC, Munk L, Nadziak J, Newton AC, de Vallavieille-Pope C, et al. 2000. Cereal variety and species mixtures in practice, with emphasis on disease resistance. *Agronomie* **20**: 813-837.

Finckh MR, Mundt CC. 1992. Plant competition and disease in genetically diverse wheat populations. *Oecologia* **91**: 82-92.

Folke C, Carpenter S, Walker B, Scheffer M, Elmqvist T, Gunderson L, Holling CS. 2004. Regime shifts, resilience, and biodiversity in ecosystem management. *Annual Review of Ecology, Evolution, and Systematics* **35**: 557-581.

Forbes AA, Rosenheim JA. 2011. Plant responses to insect herbivore damage are modulated by phosphorus nutrition. *Entomologia Experimentalis et Applicata* **139**(3): 242-249.

Fukai S, Trenbath B. 1993. Processes determining intercrop productivity and yields of component crops. *Field Crops Research* **34**(3-4): 247-271.

Gonzalez A, Loreau M. 2009. The causes and consequences of compensatory dynamics in ecological communities. *Annual Review of Ecology, Evolution, and Systematics* **40**: 393–414.

Griffiths BS, Philippot L. 2013. Insights into the resistance and resilience of the soil microbial community. *FEMS Microbiology Reviews* **37**: 112-129.

Hauggaard-Nielsen H, Jørnsgaard B, Kinane J, Jensen ES. 2008. Grain legume–cereal intercropping: the practical application of diversity, competition and facilitation in arable and organic cropping systems. *Renewable Agriculture and Food Systems* **23**(01): 3-12.

Helenius J, Jokinen K. 1994. Yield advantage and competition in intercropped oats (*Avena sativa* L.) and faba bean (*Vicia faba* L.)" Application of the hyperbolic yield-density model. *Field Crops Research* **37**: 85-94.

Isbell FI, Polley HW, Wilsey BJ. 2009. Biodiversity, productivity and the temporal stability of productivity: patterns and processes. *Ecology Letters* **12**(5): 443-451.

Lithourgidis AS, Dordas CA, Damalas CA, Vlachostergios DN. 2011. Annual intercrops: An alternative pathway for sustainable agriculture. *Australian Journal of Crop Science* **5**(4): 396-410.

Liu Y, Pan Q, Liu H, Bai Y, Simmons M, Dittert K, Han X. 2011. Plant responses following grazing removal at different stocking rates in an Inner Mongolia grassland ecosystem. *Plant and Soil* **340**(1-2): 199-213.

Marlow N, Thomson P, Rose K, Kok N. 2016. Compensatory responses by a fox population to artificial density reduction in a rangeland area in Western Australia. *Conserv. Sci. West Aust* **10**.

McNickle GG, Evans WD. 2018. Toleration games: compensatory growth by plants in response to enemy attack is an evolutionarily stable strategy. *AoB Plants* **10**(4): ply035.

Mitchell C, E., David Tilman, James V. Groth. 2002. Effects of grassland plant species diversity, abundance, and composition on foliar fungal disease. *Ecology* **83**(6): 1713-1726.

Mundt CC. 2002. Use of multiline cultivars and cultivar mixtures for disease management. *Annual Review of Phytopathology* **40**: 381-410.

Nyfeler D, Huguenin-Elie O, Suter M, Frossard E, Connolly J, Lüscher A. 2009. Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology* **46**(3): 683-691.

Ranta E, Kaitala V, Fowler MS, Laakso J, Ruokolainen L, O'Hara R. 2008. Detecting compensatory dynamics in competitive communities under environmental forcing. *Oikos* **117**(12): 1907-1911.

Raseduzzaman M, Jensen ES. 2017. Does intercropping enhance yield stability in arable crop production? A meta-analysis. *European Journal of Agronomy* **91**: 25-33.

Reiss ER, Drinkwater LE. 2018. Cultivar mixtures: a meta-analysis of the effect of intraspecific diversity on crop yield. *Ecological Applications* **28**(1): 62-77.

Reusch TBH, Ehlers A, Hämmerli A, Worm B. 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences* **102**: 2826–2831.

Scherer-Lorenzen M, Palmborg C, Prinz A, Schulze ED. 2003. The role of plant diversity and composition for nitrate leaching in grasslands. *Ecology* **84**: 1539-1552.

Smith J, Pearce BD, Wolfe MS. 2012. A European perspective for developing modern multifunctional agroforestry systems for sustainable intensification. *Renewable Agriculture and Food Systems* **27**: 323-332.

Suter M, Hofer D, Lüscher A. 2017. Weed suppression enhanced by increasing functional trait dispersion and resource capture in forage ley mixtures. *Agriculture, Ecosystems & Environment* **240**: 329-339.

Tilman D, Reich PB, Knops JMH. 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature* **441**: 629-632.

Tooker JF, Frank SD. 2012. Genotypically diverse cultivar mixtures for insect pest management and increased crop yields. *Journal of Applied Ecology* **49**(5): 974-985.

Tracy BF, Sanderson MA. 2004. Productivity and stability relationships in mowed pasture communities of varying species composition. *Crop Science* **44**(6): 2180-2186.

Watkinson AR. 1980. Density-dependence in single-species populations of plants. *Journal of Theoretical Biology* **83**: 345-357.

Wolfe MS. 1985. The current status and prospects of multiline cultivars and variety mixtures for disease resistance. *Annual Review of Phytopathology* **23**: 251-273.

Yachi S, Loreau M. 1999. Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *Proceedings of the National Academy of Sciences of the United States of America* **96**(4): 1463-1468.

Yu Y, Stomph T-J, Makowski D, Zhang L, van der Werf W. 2016. A meta-analysis of relative crop yields in cereal/legume mixtures suggests options for management. *Field Crops Research* **198**: 269-279.

Zhu YY, Chen HR, Fan JH, Wang YY, Li Y, Chen JB, Fan JX, Yang SS, Hu LP, Leung H, et al. 2000. Genetic diversity and disease control in rice. *Nature* **406**(6797): 718-722.

Tables

Table 1: Examples of compensation according to the 'CROC' model, defining the contributor, the reductor, the outcome and the compensator.

Nr.	Number of components	Example	Contributor (x-axis)	Reductor	Outcome (y-axis)	Compensator
1	1 ('monocultures')	Cereal monoculture	Plant density	Mortality of young plants due to mechanical weeding	Grain mass per unit area	Grain yield per plant
2			Ear density	Reduced tillering due to limited nutrient supply	Grain mass per unit area	Grain weight per ear
3			Grain density	Drought-induced loss of grain density	Grain mass per unit area	Thousand kernel weight
4	2 ('binary mixtures')	Mixture of different forage legume species	Plant density	Reduced germination ability in partner A	Biomass per unit area	Biomass per plant
5		Mixture of a grain legume and a cereal	Plant density	Pathogen-induced seed mortality in partner A	Grain mass per unit area	Grain mass per plant
6		Species mixture of grass and clover	Plant density	Reduced germination ability in partner A	Ground cover	Cover per plant
8		Mixture of different wheat cultivars	Green leaf area	Leaf pathogen (e.g. yellow rust) in partner A	Grain mass or leaf mass per unit area	Leaf area of partner B
9		Community of pollinators	Abundance of pollinators	Insecticide	Number of pollinated plants	Successful visits per pollinator
10		Grasshopper community in grassland	Abundance in year 1	Mortality due to cutting of grassland	Abundance in year 2	Fecundity of remaining grasshoppers
11		Multiple species mix from two functional groups in grassland	Species richness	Different number of species from one functional group (i.e. partner A) fail	Biomass per unit area	Biomass of remaining species

536

AGROCHEMISTRY AND SOIL SCIENCE
67 (2018) 1, **79-90**
DOI: 10.1556/0088.2018.67.1.6

Influence of alternative plant nutrition methods on soil microbial characteristics in long-term experiments

[1]János KÁTAI – [2]Thomas DÖRING – [1]Magdolna TÁLLAI – [1]Andrea BALLA-KOVÁCS – [3]István HENZSEL – [3]Marianna MAKÁDI – [1]Zsolt SÁNDOR – [1]Imre VÁGÓ

[1] Institute of Agricultural Chemistry and Soil Science, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Hungary;
[2] Albrecht D. Thaer-Institut für Agrar- und Gartenbauwissenschaften, Lebenswissenschaftliche Fakultät, Humboldt-Universität zu Berlin, Germany;
[3] Research Institute of Nyíregyháza, Institutes for Agricultural Research and Education Farm, University of Debrecen, Hungary

Abstract

The size of the arable land is constantly decreasing all over the world due to severe anthropogenic disorders. Plant production therefore has to be adapted to changing environmental conditions along with the proper selection of crop varieties and the application of sustainable environmental technologies which also consider economic aspects. The investigations were carried out in the Westsik long-term fertilization experiment near Nyíregyháza, East Hungary, which was set up in 1929 (89 years ago). Alternative forms of nutrient supplies (A) (green manure, straw with and without fermentation, organic fertilizer with and without inorganic fertilizer supplements) were used in different crop rotations. The test plant was potato (*Solanum tuberosum* L.) and the soil type sand with a low humus content (Arenosols). A further long-term experiment is located on calcareous chernozem soil (Chernozems) in Debrecen (set up in 1983, 35 years ago). In one part of this experiment, organic farming (OF) has been carried out with a pea, winter wheat and maize crop rotation for over 15 years with no inorganic fertilization. In another block in this experiment, changes in soil properties as a result of the medium and high doses of fertilizers applied in intensive farming (I) were evaluated with a maize (*Zea mays L.*) monoculture as the test plant.

The results obtained with alternative nutrient supplies (green manure, fermented and unfermented straw, farmyard manure, fertilization) proved that the soil organic carbon content increased to varying degrees in humus-poor, acidic sand soil. The organic matter content of the soils increased in response to the treatments, contributing to a significant enhancement in soil microbial parameters (MBC, saccharase, dehydrogenase and phosphatase enzyme activities).

The carbon dioxide production and saccharase enzyme activity in organic plots (OF) were significantly lower than in intensively farmed (I) soils. At the same time, in the case of organic farming (OF) the microbial biomass carbon, phosphatase and dehydrogenase activity were significantly higher in OF plots than in I plots.

Postal address: JÁNOS KÁTAI, University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Agricultural Chemistry and Soil Science, Böszörményi út 138., H-4032 Debrecen, Hungary.
E-mail: katai@agr.unideb.hu

Compared to the control soil, MBC was 7-8 times higher in organic plots and 1.3-3.8 times higher in intensive plots.

Organic farming on chernozem soil generally resulted in higher microbial activity (MBC, phosphatase, saccharase and dehydrogenase enzyme activity) than in either intensively farmed chernozem or in the case of alternative farming (A) on sandy soil.

Introduction

The aim of crop production all over the world is to maintain or increase crop yields in order to produce larger quantities of better quality food and feed raw materials. Crop production has to adapt to changing environmental conditions through the selection of varieties and the application of environmentally sound, economical technologies (LOCH and NOSTICZIUS 2004).

Among the ecological factors, the physical, chemical and biological properties of the soils are of paramount importance, so the complex knowledge of these properties is essential (KÁTAI, 1992). Without this knowledge it is impossible to achieve rational soil use, sustainable soil fertility, and profitable farming (BIRÓ, 2005).

However, the direction and intensity of the transformation of organic matter depends not only on the soil humus content and the organisms living in the soil, but also on the natural fertility of the soil, the effects of environmental factors and the technology applied. The intensity and speed of humification and mineralization determine the amounts of both organic matter and plant-available minerals in the soil (FÜLEKY and RAJKAINÉ, 1999).

Various organic substances and fertilizers (plant residues, manure, biogas fermentation residues, green manure, compost and slurry) generally have favourable effects on soil biodynamics (MÜLLER, 1991; KÁTAI et al., 1999; KÁTAI 2000; SZILI-KOVÁCS et al., 2009), providing direct nutrient sources for saprotrophic microbes. Other positive effects are caused by humification, better soil structure and improved air and water management.

The effects of fertilizer application on the chemical and microbiological properties of soils have been investigated in many long-term experiments (JANUSAUSKAITE et al., 2013; GEISSELER and SCOW, 2014; KÁTAI et al., 1999, 2014; SZILI-KOVÁCS et al., 2009; POWLSON and JOHNSTON, 1994; SIMON and CZAKO, 2014; ZHAO et al., 2013),which demonstrated that a balanced nutrient supply has a positive effect on the nutrient cycles, energy flow, biodynamics and activity of soil organisms, and thus on the growth and development of plants.

The requirements of organic, integrated and intensive farming vary considerably. Organic farming is based on organic fertilization and biological plant protection, without the use of artificial fertilizer or dangerous plant protection agents (SCHRAMA et al., 2018). The aim of integrated management is to optimize soil fertility and plant nutrition to achieve the desired crop yield (SÁRDI, 2011). The purpose of intensive farming is to achieve the greatest yield. Different plant

nutrition methods have many direct and/or indirect effects on soil conditions, on nutrient reserves and nutrient content, and on microbiological processes.

The purpose of this study was to compare different forms of nutrient supplies (green manure, livestock manure, inorganic fertilizers and various combinations of these) in organic and conventional farming systems. It was hypothesised that i) organic farming increases soil microbiological biomass and activity compared to conventional farming; ii) there is a close connection between soil nutrient status and microbial biomass and activity, and that this correlation is determined by the farming system; iii) the soil type has a defining role in the intensity of microbial processes. To prove this hypothesis, investigations were made on chemical soil properties and on microbial biomass and activity.

Materials and methods

Experimental area

Experiment I.

One part of the investigations was carried out in a long-term fertilization experiment, set up in 1929 by Vilmos Westsik near Nyíregyháza, in the eastern part of Hungary. In this experiment various forms of nutrient supply (green manure, fermented or non-fermented straw, animal manure) were used with or without inorganic fertilizers in different crop rotations on sand with low humus content (WRB: Arenosols) and acidic pH. The test plant was potato. Six of the 15 treatments were selected for the present experiment:

I. AC: control, fallow, without cultivation or fertilization
II. AGM-N: with main-crop lupine as green manure, combined with N fertilizer
IV. AS-NPK: with fermented straw and NPK fertilizer
VII. AS: with straw but no inorganic fertilizers
XI. AF-N: with farmyard manure and N fertilizer
XIV. AGM-PK: with second-crop lupine as green manure, ploughed in soil, in autumn, and PK fertilizer

Experiment II.

In another long-term fertilization experiment, set up in 1983 on a highly fertile calcareous chernozem soil (WRB: Chernozems) in the Hajdúság loess area west of Debrecen in East Hungary, organic farming has been carried out in one block for more than 15 years, with no inorganic fertilizer and a constant maize-wheat-pea crop rotation. The other block involved intensive farm management with medium and high doses of fertilizer. The test crop here was a maize monoculture.

Treatments:

1. Control (C) (maize, without fertilization)

Organic farming (OF)

2. OF, P (pea)
3. OF, M (maize)

Intensive farm management (using solid NH_4NO_3, $NH_4H_2PO_4$ and KCl fertilizers)

4. Medium dose of fertilizers (IMF1): 120, 90, 90 kg ha^{-1} N, P_2O_5, K_2O, resp.
5. Large dose of fertilizers (IMF2): 240, 180, 180 kg ha^{-1} N, P_2O_5, K_2O, resp.

Soil samples were taken from 5 points/treatments at a depth of 2-20 cm on 10 May 2016.

Chemical and biological soil properties investigated

Laboratory analyses on moisture content (105°C), pH (BUZÁS, 1988), organic carbon content (OC) (SZÉKELY et al., 1960), soil respiration (CO_2 production) (ÖHLINGER, 1996), microbial biomass carbon (MBC) (VANCE et al, 1987), dehydrogenase activity (MERSHI, 1996), phosphatase activity (SZEGI, 1979) and saccharase activity (FRANKENBERGER and JOHANSON, 1983) were carried out in four replications.

Table 1

Effect of alternative management (A) on some physical and chemical parameters of humiferous sandy soil (Arenosol) (Nyíregyháza, May 2016); test plant: potato

Treatment code	Moisture content %	pH in distilled water	pH in 1.0 M KCl solution	Humus content %	Organic C content g kg^{-1}
I. Control AC	5.49	5.40	3.88	0.51a	3.0a
II. AGM-N	5.97	4.76	3.51	0.54a	3.1a
IV. AS-NPK	5.66	4.37	3.51	0.62ab	3.6ab
VII. AS	6.58	6.24	4.93	0.64ab	3.7ab
XI. AF-N	5.64	5.37	4.44	0.83c	4.8c
XIV. AGM-PK	7.58	5.18	3.74	0.83c	4.8c
$LSD_{5\%}$	-	-	-	0.09	0.5

For treatment details, see Materials and methods

Statistical analyses

One-way ANOVA was used for determining treatment effects and the standard deviation (S.D.) of the means was calculated using the SPSS 13.0 program at the 5.0 % significance level. After ANOVA, Duncan's multiple range test was used to compare the means. Values indicated with different letters were significantly different from each other at the 5.0 % significance level. Pearson correlation analysis was used to reveal correlations between microbiological and chemical soil properties.

Results and discussion

Westsik research experiment (Experiment I)

The soils in the Westsik experiment in Nyíregyháza had very low moisture content at the sampling date (*Table 1*). Based on the pH values, the soils could be classified as acidic, with the exception of the AS-F treatment, which was only slightly acidic. The soils also had low or very low organic carbon content, which rose slightly compared to the control in response to green manure (AGM-N), straw

(AS) and straw + nitrogen fertilizer (AS-N), and increased significantly in the AF-N and AGM-PK treatments.

The carbon dioxide production of the soils increased slightly in all the treatments (*Table 2*), but only showed a significant increase in the AF-N treatment. Microbial biomass carbon (MBC) significantly increased in all the treatments, with particularly high values in the green manure treatments (AGM-N, AGM-PK). The humiferous sandy soil had very low saccharase enzyme activity in the control plot, but the values were more than doubled by the straw (AS-NPK) and green manure plus PK fertilizer (AGM-PK) treatments. The highest phosphatase activity was found in the green manure – mineral fertilizer (AGM-PK) combination, followed by the farmyard manure plus mineral N fertilizer (AF-N) treatment. All treatments with the exception of unfermented straw (AS-NPK) resulted in significant increases (1.1 to 1.8 fold). Similarly high activity was measured for dehydrogenase in the AGM-PK and AF-N treatments, but except for the AGM-N treatment, a significant increase was observed in all the treatments compared with the control.

Table 2
Effect of alternative management on some soil microbial characteristics of humiferous sand (Arenosols) (Nyíregyháza, May 2016)

Treatment	CO_2 mg/100g soil/10 days	MBC µg g^{-1}	Saccharase glycose mg $100g^{-1}$	Phosphatase mg $100g^{-1}$ $2h^{-1}$	Dehydrogenase INTF µg $100g^{-1}$
I. Control AC	8.65a	134.21a	2.12a	11.23a	35.13a
II. AGM-N	8.89a	251.14b	2.52a	15.37b	37.73a
IV. AS-NPK	8.90a	192.89c	4.76b	12.21a	39.80b
VII. AS	8.87a	226.56d	3.97c	15.93b	40.15b
XI. AF-N	9.59b	163.17e	3.86c	17.13b	47.43c
XIV. AGM-PK	8.85a	256.62b	4.97b	20.22c	48.40c
$LSD_{5\%}$	0.62	23.94	0.62	1.29	3.65

For treatment details, see Materials and methods
INTF = reduced iodonitrotetrazolium formazan

Debrecen-Látókép experimental site (Experiment II.)

The soils in the Debrecen-Látókép experiment had higher than average moisture content at the time of sampling (*Table 3*). The soil $pH_{(H_2O)}$ ranged from 5.93 to 6.27, and could be classified as slightly acidic. Compared to the untreated control, the soils of both organic (OF) and intensive farming (I) were more acidic. Compared with the control, the fertilizer treatments (IMF1, IMF2) used in intensive farming (I) caused a significant increase in the organic carbon content. On the organic plots (OF) the soil organic carbon content was lower than in the intensive plots, but did not differ significantly from the control.

Table 3

Effect of organic farming (OF) and intensive management (I) on some physical and chemical parameters of calcareous chernozem soil (Chernozems) (Debrecen-Látókép, May 2016)

Treatment	Crop	Moisture content %	pH in distilled water	pH in 1.0 M KCl solution	Humus content %	Organic C content g kg^{-1}
a. Control MC	Maize	19.30	6.27	5.09	2.76a	16.0a
Organic farming						
b. OF Pea	Pea	20.56	5.99	4.88	2.62a	15.2a
c. OF Maize	Maize	22.43	5.93	5.12	2.75a	15.9a
Intensive farming						
d. Medium dose IMF1	Maize	19.19	6.04	4.85	3.03a	17.4b
e. Large dose IMF2	Maize	19.15	5.95	4.71	3.17ab	18.2b
$LSD_{5\%}$		-	-	-	0.31	0.82

For treatment details, see Materials and methods

Table 4

Effect of organic farming (OF) and intensive management (I) on some microbial parameters of calcareous chernozem soil (Chernozems) (Debrecen-Látókép, May 2016)

Treatment	CO_2 mg 100g $soil^{-1}$ 10 $days^{-1}$	MBC µg g^{-1}	Saccharase glycose mg $100g^{-1}$	Phosphatase mg $100g^{-1}$ $2h^{-1}$	Dehydrogenase INTF µg $100g^{-1}$
a. Control MC	19.53a	36.95a	14.43a	12.75a	25.90a
Organic farming					
b. OF Pea	10.71b	245.50b	10.55b	20.47b	50.58b
c. OF Maize	10.76b	275.55b	9.39b	21.44b	53.10b
Intensive farming					
d. Medium dose IMF1	19.88a	51.37a	13.13a	13.67a	33.20c
e. Larger dose IMF2	19.98a	143.33c	13.16a	15.52a	33.75c
$LSD_{5\%}$	1.07	34.30	1.65	3.85	3.85

INTF = reduced iodonitrotetrazolium formazan

The carbon dioxide production and saccharase enzyme activity of the soil were significantly lower in the case of organic farming (OF) than for intensive farming (I) (*Table 4*). At the same time, the microbial biomass carbon, phosphatase and dehydrogenase activity were significantly higher on organic (OF) than on intensive plots (I). The highest phosphatase and dehydrogenase activity was measured in the organically farmed soils. Compared to the control soil, *MBC* was 7-8 times higher in the case of organic farm management (OF) and 1.3-3.8 times higher for intensive farming (I). Inorganic fertilizers (IMF1, IMF2) significantly increased the MBC values in the soil.

In intensive farming (I), the carbon dioxide production, saccharase and phosphatase enzyme activity were similar to the control values. Mineral fertilization (IMF1, IMF2) resulted in higher values for both enzymes compared to the control, but this increase was only slight for phosphatase. The dehydrogenase enzyme activity showed a significant increase in these treatments.

Table 5

Pearson's correlations between soil properties Management system A (Nyíregyháza): n=24; Management systems OF and I (Debrecen-Látókép): n=20

Measured parameters	Manage-ment system	Mois-ture content	pH (H2O)	Orga-nic car-bon	CO_2 produc-tion	MBC	S-ase activity	P-ase activity
a. Moisture content	A OF, I							
b. $pH_{(H2O)}$	A OF, I	-- 						
c. Organic carbon	A OF, I	- -	- -					
d. CO_2 production	A OF, I	- -	- -	- 0.775				
e. MBC	A OF, I	0.666 0.698	- 0.699	- -	- -0.885			
f. Saccharase activity	A OF, I	- -0.751	- 0.572	0.603 -	- 0.830	 -0.822		
g. Phosphatase activity	A OF, I	0.684 0.591	- -0.58	0.728	- -0.824	0.615 0.843	 -0.757	
h. Dehydrogenase activity	A OF, I	- 0.635	- 0.713	0.871 -	- -0.874	- 0.885	0.620 -0.830	0.757 0.832

All he given correlations are significant at the 0.01 level (2-tailed).
Management systems: organic (OF), intensive (I) and alternative (A) farming
MBC=microbial biomass carbon, S-ase= saccharase, P-ase=phosphatase

Correlations between physico-chemical and biological parameters

Pearson's correlations were calculated to reveal correlations between the chemical and biological properties of the soil (*Table 5*), and the correlations found between microbial biomass carbon and phosphatase enzyme activity, and between phosphatase and saccharase enzyme activity are depicted in *Figures 1* and *2*, respectively, from which it can be seen that these pairs of parameters were in close connection with each other.

Studies in the Westsik long-term experiment (KÁTAI et al., 1999) showed that the use of white lupine straw or green manure supplemented with NPK enhanced the nitrate, AL-soluble phosphorus and potassium contents of the soil. The addition of straw, green manure or livestock manure had a stimulating effect on the activity of the saccharase and phosphatase enzymes to varying degrees.

Among the microbiological properties of the soil, the value of MBC and the activitiy of the saccharase, urease and dehydrogenase enzymes were significantly increased by alternative nutrient supplies (green manure, farmyard manure and straw) in the acidic, humus-poor sandy soil tested in the Westsik experiment. These results were consistent with previous results (KÁTAI et al., 2002).

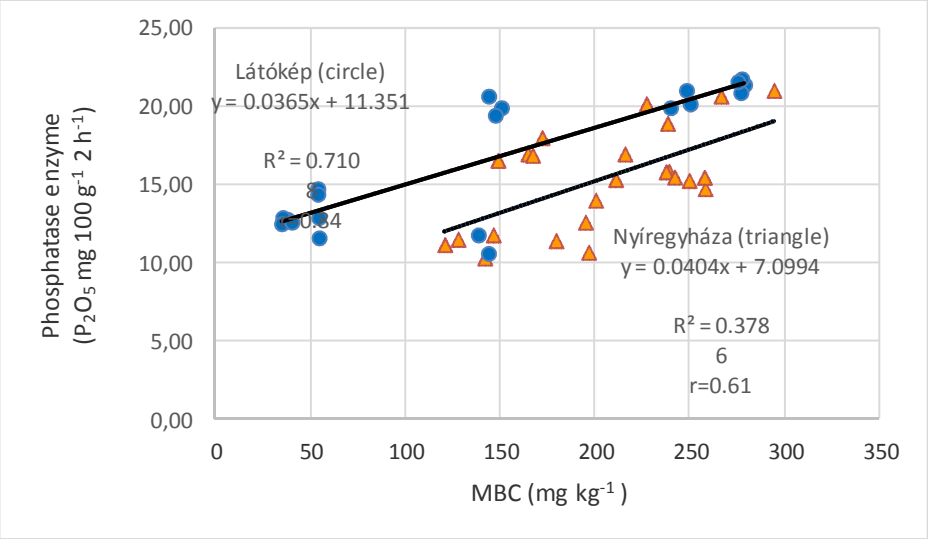


Figure 1
Correlation between MBC and phosphatase enzyme activity (Pearson's correlations, Nyíregyháza n=24; Debrecen – Látókép n=20)

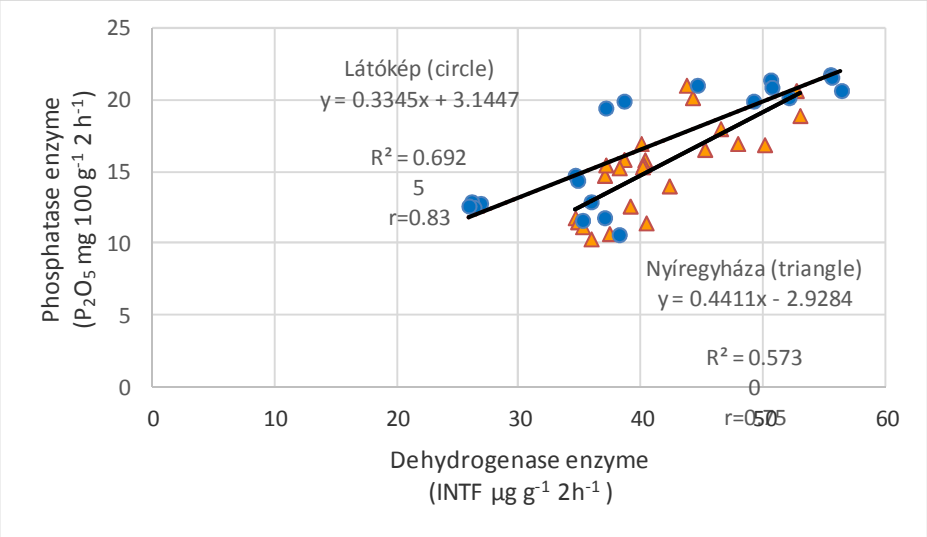


Figure 2
Correlation between the dehydrogenase and phosphatase enzyme activities (Pearson's correlations, Nyíregyháza n=24; Debrecen – Látókép n=20)

PIOTROWSKA-DLUGOSZ and WILCZEWSKI (2015) found a similar phenomenon: green harvestings (catch crop, pea, *Pisum sativum* L.) increased MBC and the activity of soil enzymes, including saccharase, so these authors recommended greening for the stimulation of microbial activity. A similar conclusion was drawn by BOLTON et al. (1985) and LOSAKOV et al. (1986).

Comparison between organic and intensive farming conditions

When assessing the effects of organic and intensive farming on soil microbiological processes, the values of MBC, phosphatase and dehydrogenase activity were found to be higher in organic farming than in intensive farming, while higher carbon dioxide production and saccharase activity were measured in intensive farming.

Organic manure plus inorganic fertilizer increased the microbial biomass carbon by 74-99% (LIU et al., 2013). STEVLIKOVA et al. (2002) also demonstrated that MBC values were higher in organic than in intensive farming.

LUKÁCSNÉ and ZSUPOSNÉ (2008) reported higher values of phosphatase and dehydrogenase activity, MBC and CO_2 production in organic farming than in traditional farming systems. As in the present work, the saccharase enzyme activity proved to be higher in the traditional farming system.

BALEZENTIENE and KLIMAS (2009) found that nitrogen application stimulated the activity of saccharase, but the lowest values were measured with inorganic fertilization, suggesting that saccharase (and urease) activity is a bioactive property, usually positively correlated with soil nutrient content. In their view, the activity of

the two enzymes is a bio-indicator of soil fertility, responding well to the type of manure used and the system of nutrition management.

In summary, it can be concluded that straw, green manure and farmyard manure had a potent effect on the soil microbiological characteristics studied. Organic farming also contributed significantly to the increased activation of soil microbiological processes.

Conclusions

As a result of alternative nutrient supplies (A), the soil organic carbon content increased in all the treatments compared to the control. This increase was significant in the AF-M (inorganic fertilizer plus farmyard manure) and AGM-F (green manure plus inorganic fertilizer) treatments. The higher organic matter contents resulting from the treatments contributed to the significant enhancement of soil microbial parameters (MBC, saccharase, dehydrogenase and phosphatase enzyme activities).

The organic (OF) and intensive (I) cultivation treatments in the long-term experiment on calcareous chernozem soil (Debrecen-Látókép) had shorter life periods, only existing since 2000 and 1983, respectively.

Comparing the organic and intensive farming systems, it can be concluded that the carbon dioxide production and saccharase activity of organic plots (OF) were significantly lower than on intensive plots (I). In contrast, the microbial biomass carbon and the phosphatase and dehydrogenase activity were significantly higher in organic farm management systems (OF) than in the intensive farming system (I). These results are in harmony with data from the literature and also highlighted the need for sustainable production technologies.

Acknowledgements

This research was financially subsidised by “EKHAGA Stiftelsen”, Stockholm, Sweden. Project title: “Healthy plants from healthy soils: Resilience and stability of organic cropping systems”. Project number: 2015-65.

References

BALEZENTIENE, L., KLIMAS, E. 2009. Effect of organic and mineral fertilizers and land management on soil enzyme activities. Agronomy Research **7**. (Special issue I) 191-197.p.

BIRÓ, B. 2005. The soil as a living substance. In: The importance and value of soils in the 21st century. (eds. STEFANOVITS P. & MICHÉLI E.). (In Hungarian) MTA, Budapest. p. 141-169.

BOLTON, H., ELLIOTT, L. F, PAPENDICK, R. I., BEZDICEK, D. F. 1985. Soil microbial biomass and selected soil enzyme activities: effect of fertilization and cropping practices. Soil Biology and Biochemistry. **17**. (3) 297-302. p.

BUZÁS, I. (ed.) 1988. Methods of Soil and Agricultural Chemistry Analysis 2. Physico-chemical and Chemical Methods of Soil Analysis. (In Hungarian) Mezőgazdasági Kiadó, Budapest. pp. 90-93.

FÜLEKY, G., RAJKAINÉ VÉGH, K. 1999. Nutrient supplying ability of the soil. (ed. G. FÜLEKY). In: soil-nutrient-management. (In Hungarian) Mezőgazdasági Kiadó, Budapest. p. 112-120.

FRANKERBERGER, W.T., JOHANSON, J.B. 1983. Method of measuring invertase activity in soils. Plant and Soil. **74**. 301-311.

GEISSELER, D., SCOW, K.M. 2014. Long-term effects of mineral fertilizers on soil microorganisms. A review. Soil Biology and Biochemistry. **75**. 54-63.

JANUSAUSKAITE, D., ARLAUSKIENE, A., MAIKTENIENE, S. 2013. Soil mineral nitrogen and microbial parameters as influenced by catch crops and straw management. Zemdirbyste Agriculture, **100** (1) 9-18.

KÁTAI, J. 1992. Correlation among the physical, chemical characteristics and microbiological activities of some soil types. In: Functioning and Dynamics of Perturbed Ecosystems. eds. BELLAN, D., BONIN, G., EMIG, C. Lavoisier Publishing, Paris. p. 137-158.

KÁTAI, J., LAZÁNYI, J., VERES, E. 1999. Investigation of soil microbiological parameters in Westsik long-term field experiment. In: Tiszántúli Mezőgazdasági Tudományos Napok. (In Hungarian). Debrecen, 1999. okt. 28-29. p. 175-184.

KÁTAI, J. 2000. Comparative investigations on soil microbiological parameters in a long-term fertilizer experiment. For the 80th anniversary of the birth of Prof. Ernő Bocz. (In Hungarian). Debrecen. p. 51-63.

KÁTAI, J., ZSUPOSNÉ OLÁH, Á., SÁNDOR, Z., TÁLLAI, M. 2014. Comparison of soil parameters of the carbon and nitrogen cycles in a long-term fertilization field experiment. Agrokémia és Talajtan. **63**. 129-138.

LIU, E., YAN, C., MEI, X., ZHANG, Y. FAN, T. 2013. Long–term effect of manure and fertilizer on soil organic carbon pools in dryland farming in Northwest China. PlosOne. https://doi.org/10.1371/journal.pone.0056536

LOCH, J., NOSTICZIUS, Á. 2004. Agro-chemistry and Plant Protection Chemistry. Mezőgazda Kiadó, Budapest. 19-22, 114.

LOSAKOV, V. G., EMCEV, V.T., NICÉ, L. K., IVANOVA, SZ. F., ROGOVA, T. A. 1986. Biologicseszkaja aktivnoszt pocsvy v szpecinivnogo sziderata i szolomy v kacsesztve udobrenij. Izv. TSZHA. Moszkva 4. pp. 10-17.

LUKÁCSNÉ VERES E. – ZSUPOSNÉ OLÁH Á. 2008. Mészlepedékes csernozjom talaj fontosabb paramétereinek alakulása hagyományos és ökológiai gazdálkodási rendszerekben. Talajvédelem különszáma (ed.: SIMON L.) 455-464. p.

MÜLLER, G. 1991. Soil microbiological aspects of agroecology and intensive agricultural production. (In Hungarian) Agrokémia és Talajtan. **40**. 263-272.

MERSHI, V. W. 1996. Dehydrogenase activity with the substrate INT. In: SCHINNER, F., ÖHLINGER, R., KANDELER, E., MARGESIN, R. (eds.) Methods in Soil Biology. Springer-Verlag Berlin – Heidelberg. 243-245.

ÖHLINGER, R., KANDELER, E., MARGESIN, R. (eds.) 1996. Methods in Soil Biology. Springer-Verlag Berlin Heidelberg. pp. 20-26., 32-36

PIOTROWSKA-DLUGOSZ, A., WILCZEWSKI, E. 2015. Influences of catch crop and its corporation time on soil carbon and carbon-related enzymes. Pedosphere. **25**. (4) 569-579. p.

POWLSON, D. S., JOHNSTON, A. E. 1994. Long-term Field Experiments: their Importance in Understanding Sustainable Land Use. Soil Resilience and Sustainable Land Use. CAB International. 367-394.p.

SÁRDI, K. 2011. Soil-nutrient management. Debreceni Egyetem, Nyugat-Magyarországi Egyetem, Pannon Egyetem (on-line teaching material). (In Hungarian).

SCHRAMA, M., DE HAAN, J.J., KROONEN, M, VERSTEGEN, H., VAN DER PUTTEN, W. H. 2018. Crop yield gap and stability in organic and conventional farming systems. Agriculture, Ecosystems and Environment. **256**. 123-130.

SIMON, T., CZAKO, A. 2014. Influence of long-term application of organic and inorganic fertilizers on soil properties. Plant Soil and Environment. **60**. 314-319.

STEVLÍVKOVÁ, T., JAVOREKOVÁ, S., VJATRÁKOVÁ, J. 2002. Soil biological activity within integrated and ecological management of soil. Agrártudományi Közlemények 1. Acta Agraria Debreceniensis. Debrecen. 47-52. p.

SZÉKELY, Á., SCHLICK, B., SZABÓ, T. 1960. On the photometric and colorimetric determination of organic carbon. (In Hungarian). Agrokémia és Talajtan, **9**. 111-120.

SZEGI, J. 1979. Soil Microbiological Methods. (In Hungarian) Mezőgazda Kiadó, Budapest. pp. 250-256.

SZILI-KOVÁCS, T., ZSUPOSNÉ OLÁH, Á., KÁTAI, J., VILLÁNYI, I., TAKÁCS, T. 2009. Correlation between biological and chemical properties in soils from long-term experiments. Agrokémia és Talajtan (In Hungarian). **58**. 309-325.

VANCE, E.D., BROOKES, P.C., JENKINSON, D.S. 1987. An extraction method for measuring soil microbial biomass-C. Soil Biol. Biochem. **19**. 703-707.

ZHAO, B., CHEN, J., ZHANG, J., XIN, X., HAO, X. 2013. How different long-term fertilization strategies influence crop yield and soil properties in a maize field in the North China Plain. Journal of Plant Nutrition and Soil Science. **176**. 99-109.

European Journal of Agronomy 99 (2018) 30–36

Contents lists available at ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja

Detecting global trends of cereal yield stability by adjusting the coefficient of variation

Thomas F. Döring[a,b], Moritz Reckling[c,d,*]

[a] Department of Agronomy and Crop Science, Albrecht-Thaer-Institute of Agricultural and Horticultural Sciences, Humboldt University of Berlin, Berlin, Germany
[b] Agroecology and Organic Farming Group, Institute of Crop Science and Resource Conservation, University of Bonn, Bonn, Germany
[c] Leibniz Centre for Agricultural Landscape Research, Müncheberg, Germany
[d] Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

ARTICLE INFO

Keywords:
Cropping systems
Crop yields
Rye
Stability index
Taylor's power law
Wheat

ABSTRACT

High stability of crop yields is a key objective in crop production and breeding, especially under the conditions of a changing climate. Reliable indices are therefore needed for quantifying yield stability. Recently it was shown that some frequently used indices of yield stability, such as the coefficient of variation (CV) may be wrongly interpreted if there is a systematic dependence of the variance σ^2 from the mean yield μ following Taylor's power law. Here we propose a method to adjust the standard CV to account for the systematic dependence of σ^2 from μ. This adjusted CV can be used as a stability index that is expressed in units that are equivalent to the standard CV, as a percentage of the mean, and can therefore be used in agronomic studies that aim to provide guidance for farmers and advisors. Applying this adjusted CV (called aCV) to FAO cereal yield data, we show that the temporal yield stability of both wheat and rye has weakly but significantly decreased over the last five decades and this trend was not picked up with the standard CV in wheat, and was more marked with the aCV than with the standard CV in rye. For the intensifying research on yield stability in agronomy, the suggested method is a novel alternative to estimate yield stability more conclusively, allowing straight-forward interpretation and providing the basis for developing cropping systems with higher yield stability in the future.

1. Introduction

High crop yield stability is an important goal shared by farmers, breeders and consumers. In the face of global change and increasing environmental variability, working towards this goal is becoming ever more imperative (Peltonen-Sainio et al., 2010; Reidsma et al., 2010). Substantial efforts are therefore dedicated to reducing variability in crop performance, e.g. through plant breeding (Mühleisen et al., 2014; Chamekh et al., 2015) and agronomic management (e.g. Smith et al., 2007). Adaptation strategies are also related to the socio-economic conditions and farm management (Reidsma et al., 2010). One of the critical issues in this endeavour is the use of appropriate measures of yield stability. Over the past few decades, scores of yield stability indices have been proposed (Eberhart and Russell, 1966; Becker, 1981; Becker and and Léon, 1988; Huehn, 1990; Eghball and Power, 1995; Piepho, 1998; Dehghani et al., 2008). An extensive literature deals with the comparison of various stability indices (e.g. Becker and and Léon, 1988; Crossa, 1988; Ferreira et al., 2006). Often, however, the interpretation of the results from stability analyses is not easy. This is partly because different stability indices may lead to contrasting conclusions as they reflect different concepts of stability (Dehghani et al., 2008). In addition, the relatively high complexity of the calculations involved in quantifying stability makes it difficult to separate 'true' effects from mathematical artefacts. In agronomic and ecological research, a popular index of yield stability is the coefficient of variation (CV) (Francis and Kannenberg, 1978; Küchenmeister et al., 2012; Ray et al., 2015; Di Matteo et al., 2016). The CV is defined as the standard deviation σ divided by the mean μ, and is expressed as percentage of the mean: $CV = \sigma/\mu \cdot 100\%$.

Recently it has been shown that under certain conditions, the unguarded interpretation of the CV of crop yield data may be misleading (Döring et al., 2015). In particular, it was demonstrated that crop yield data, especially when it spans over a large range, may often follow a power-law relationship between the sample variance σ^2 and the sample mean μ, and in this case the CV tends to typically decrease with increasing mean, according to the data published so far. The power-law relationship, $\sigma^2 = A\mu^b$, is known as Taylor's Power Law (TPL). Logarithmic transformation of TPL results in a linear relationship, expressed as the equation $\log(\sigma^2) = a + b \log(\mu)$ with $a = \log(A)$. The relationship was first extensively described by the British ecologist and

* Corresponding author at: Leibniz Centre for Agricultural Landscape Research, Eberswalder Str. 84, 15374, Müncheberg, Germany.
E-mail address: moritz.reckling@zalf.de (M. Reckling).

https://doi.org/10.1016/j.eja.2018.06.007
Received 22 November 2017; Received in revised form 8 June 2018; Accepted 11 June 2018
Available online 15 June 2018

entomologist Roy Taylor (Taylor, 1961) and has been detected in hundreds of data sets from population ecology (Cohen et al., 2012, 2013) and multiple other contexts and sciences (Duch and Arenas, 2006; Eisler et al., 2008). Because TPL has also been found in crop yield data (Döring et al., 2015), caution is needed when interpreting the CV. In particular, if TPL holds, then $CV = \mu^{b/2-1} g^{a/2}$, where a and b are the regression parameters (intercept and slope) of the TPL log-log regression and g is the basis of the logarithm. Thus, the CV may change in a non-linear manner with increasing mean, unless $b = 2$. For most crop yield data sets analysed for the presence of TPL-like relationships between mean and variance, the slope b has been found to be < 2 (Döring et al, 2015). In these cases, the CV systematically decreases with increasing μ in a nonlinear way.

Stability parameters need to show independence from the mean, though. Otherwise it would not be possible to differentiate true effects of stability from changes in the mean; information on stability per se would be confounded by information contained in the mean. Specifically, when using the CV, large means will often (if $b < 2$) be automatically associated with low CVs, just because of a mathematical artefact, rather than biologically or agronomically meaningful mechanisms. To solve this problem, it is necessary to account for systematic dependence of the variance from the mean. One method is the stability index POLAR, which calculates the residuals from the linear regression of $\log(\sigma^2)$ against $\log(\mu)$ (Döring et al., 2015). The new method presented here is an adjusted coefficient of variation (aCV) that removes systematic dependence of the standard CV from the mean. In contrast to the units of POLAR stability that are the logarithm of squared yield units, the aCV is expressed in percentages of the mean and thereby facilitates application in agronomic studies that aim to provide guidance to farmers and advisors. In particular, the aCV enables users to interpret results on yield stability more easily and thereby to make decisions on adjusting management.

The objective of this article is to demonstrate a novel method to quantify yield stability by adjusting the standard CV such that dependence from the mean yield is removed. We apply the novel stability index that we call 'scale-adjusted coefficient of variation' using a publicly available data set of cereal yields, obtained from the Food and Agriculture Organisation (FAO) statistics website, to examine global trends of cereal yield stability.

2. The adjusted coefficient of variation

Here we show how the scale-adjusted coefficient of variation (aCV) is calculated in four steps:

2.1. First step

Means ($\hat{\mu}$) and variances ($\hat{\sigma}^2$) are calculated for a subset of data. This creates a list of means $\hat{\mu}_i$ paired with variances $\hat{\sigma}^2_i$, that is, each pair (with index i) consists of a mean and a variance. Following TPL, a linear regression is calculated for $\log_{10}$ of the variance over the $\log_{10}$ of the mean. With $v_i = \log(\hat{\sigma}^2_i)$ and $m_i = \log(\hat{\mu}_i)$, the linear regression is $v = a + bm$.

2.2. Second step

The residuals u_i from this regression line, the i.e. the Power Law Residuals (POLAR), are calculated as

$$u_i = v_i - (a + bm_i) \quad (1)$$

2.3. Third step

We adjust the logarithm of the variance which is subsequently used for calculating the coefficient of variation. The adjusted logarithm of the variance $\tilde{v}_i$ is

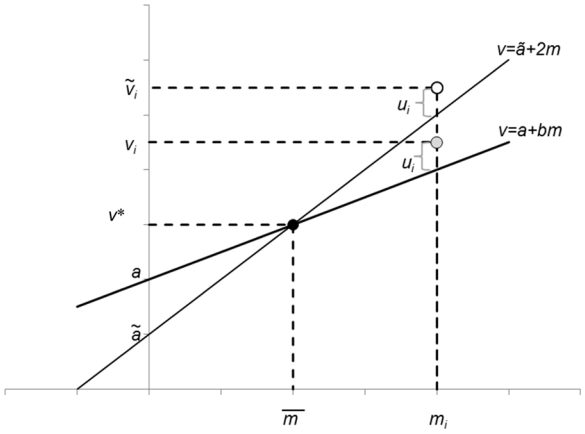


Fig. 1. Example illustrating the procedure for correcting the $v = \log(\hat{\sigma}^2)$ by setting the TPL slope to $b = 2$. The original TPL regression follows the equation $v = a + bm$, where $m = \log(\hat{\mu})$, in this case with $b < 2$ (bold line). Individual points $(m;v)$ (e.g. the grey point) deviate from the regression by u_i. To set $b = 2$, the original regression line is rotated around the average value $\overline{m}$ over all m, resulting in the thin line represented by the equation $v = \tilde{a} + 2m$. Because $v^* = a + b\,\overline{m} = \tilde{a} + 2\,\overline{m}$, the adjusted intercept $\tilde{a}$ can be calculated as $\tilde{a} = a + (b - 2)\,\overline{m}$ (see Eq. (5)). The adjusted value for $\log(\hat{\sigma}^2)$ (represented by the white point) can then be calculated by inserting m into the new $v = \tilde{a} + 2m$ and adding u_i. This results in $\tilde{v}_i = 2m_i + \tilde{a} + u_i$.

$$\tilde{v}_i = \tilde{a} + 2m_i + u_i \quad (2)$$

$$\text{With} \quad \tilde{a} = a + (b-2)\overline{m} \quad (3)$$

where $\overline{m} = \frac{1}{n}\sum m_i$.As illustrated and explained in Fig. 1, this procedure adjusts v by setting the TPL slope to $b = 2$, and rotating it around $\overline{m}$.

2.4. Fourth step

The final step is using the adjusted logarithm of the variance for calculating the adjusted coefficient of variation $\tilde{c}_i = aCV_i$.

$$\tilde{c}_i = \frac{\sqrt{g^{\tilde{v}_i}}}{\hat{\mu}_i} \cdot 100\% \quad (4)$$

where g is the basis of the logarithm (which was 10 in our case). Combining and simplifying the Eqs. (1)–(4) leads to

$$\tilde{c}_i = \frac{1}{\hat{\mu}_i}\left[10^{(2-b)m_i + (b-2)\overline{m} + v_i}\right]^{0.5} \cdot 100\% \quad (5)$$

These calculations are exemplified in Table 1 for a small subset of the wheat yield data.

If TPL holds, then for a particular point i, the *anticipated* coefficient of variation $\hat{c}_i$ is

$$\hat{c}_i = \hat{\mu}_i^{\frac{b}{2}-1} g^{\frac{a}{2}} \cdot 100\% \quad (6)$$

Here, the TPL slope a and the intercept b are used to calculate the coefficient of variation that would be expected for $\hat{\mu}_i$ if there was no deviation from the TPL regression line (i.e. if u = 0). When $b < 2$, the anticipated CV decreases non-linearly with increasing mean. For adjusting the coefficient of variation we have removed the dependence of the CV from the mean by setting the slope b to 2 in Eqs. (2) and (3), so that $\hat{\mu}_i^{\frac{b}{2}-1} = \hat{\mu}_i^{0} = 1$.

In short, the adjusted coefficient of variation sets the TPL slope to 2, so that the dependence from the mean disappears.

Table 1
Example for calculating the adjusted coefficient of variation based on wheat yields (units in t ha^{-1}) from FAOstat for selected countries. For this example, the TPL regression is calculated over the 15 data points only; the resulting regression parameters are slope $b = 1.0513$; intercept $a = -1.6271$. The average over all $\log(\hat{\mu}_i)$ is $\overline{m} = 0.3327$. The adjusted CV is calculated according to Eq. (5).$\widetilde{c}_i = \frac{1}{\hat{\mu}_i}[10^{(2-b)m_i+(b-2)\overline{m}+v_i}]^{0.5} \cdot 100\%$.

Country	Start year of decade	Mean = $\hat{\mu}_i$	Variance = $\hat{\sigma}^2_i$	$\text{Log}_{10}(\mu_i) = m_i$	$\text{Log}_{10}(\hat{\sigma}^2_i) = v_i$	POLAR = u_i	Standard CV[%] = c_i	Adjusted CV [%] = $\widetilde{c}_i$
Algeria	1964	0.5891	0.0064	−0.2298	−2.1933	−0.32	13.59	7.35
	1974	0.6169	0.0141	−0.2098	−1.8517	0.00	19.23	10.63
	1984	0.7944	0.0218	−0.1000	−1.6620	0.07	18.58	11.58
	1994	1.0310	0.0318	0.0133	−1.4979	0.12	17.29	12.20
	2004	1.5032	0.0314	0.1770	−1.5034	−0.06	11.78	9.94
Germany	1964	3.8610	0.0766	0.5867	−1.1160	−0.11	7.17	9.46
	1974	4.6969	0.0620	0.6718	−1.2078	−0.29	5.30	7.68
	1984	6.1148	0.0822	0.7864	−1.0851	−0.28	4.69	7.70
	1994	7.1526	0.1605	0.8545	−0.7945	−0.07	5.60	9.90
	2004	7.5348	0.1986	0.8771	−0.7019	0.00	5.92	10.72
Spain	1964	1.2229	0.0157	0.0874	−1.8055	−0.27	10.23	7.83
	1974	1.6321	0.0593	0.2127	−1.2268	0.18	14.92	13.09
	1984	2.4293	0.0565	0.3855	−1.2483	−0.03	9.78	10.36
	1994	2.5011	0.1997	0.3981	−0.6996	0.51	17.87	19.19
	2004	3.0161	0.2715	0.4794	−0.5662	0.56	17.28	20.28

3. Application of the adjusted coefficient of variation

3.1. Data sets and data filtering

Data sets were analysed to represent crop yield variation at a national level (national yield statistics from the Food and Agriculture Organization database, FAOStat) (FAOSTAT, 2014). The FAO dataset was downloaded in November 2014 from http://faostat3.fao.org/download/. For wheat and rye, data were extracted for area (recorded in ha) and yield (recorded in hg ha^{-1}). In the database, there is no distinction made between spring and winter sown wheat. The downloaded data were then filtered prior to further calculations. Only countries with a complete set of the last fifty years (1964–2013) were retained in the dataset. Also, countries were excluded where the average area of the selected crop in any decade was below 10,000 ha. To exclude data that indicated low reliability, data was removed from the dataset for which yields were rounded to 1000 hg ha^{-1} in two or more of 50 years. In addition, countries were removed from the dataset, where yields were equal in consecutive years in two or more cases over the 50-year period. For wheat and rye these combined filters resulted in final datasets of crop yields from 77 and 19 countries, respectively.

3.2. Calculations and statistical analysis

All analyses were performed with the programme R, version 3.0.0. Yield data were first grouped into five consecutive decades (1964–1973, 1974–1983 etc.). The data were then detrended (Ray et al., 2015) for each decade and each country, i.e. linear trends within each decade were removed so as to avoid penalising intended yield increases over time due to agronomic and technical progress, which would have led to higher variances. Detrending followed the equation

$$y_{d,x} = y_{o,x} - \hat{y}_{x,D} + \overline{y}_D \quad (7)$$

where $y_{d,x}$ is the detrended yield for year x, $y_{o,x}$ is the original yield for year x, $\hat{y}_{x,D}$ is the yield estimated for year x assuming a linear trend of y over the years within decade D, and $\bar{y}_D$ is the average yield for decade D.

Means ($\hat{\mu}$) and variances ($\hat{\sigma}^2$) were calculated per country within each decade D and for each country. For comparison with the adjusted coefficient of variation, the *standard* coefficient of variation c_i is calculated as:

$$c_i = \frac{\hat{\sigma}_i}{\hat{\mu}_i} \cdot 100\% \quad (8)$$

Finally, the aCV was tested for significant changes over time by a linear mixed effects model with the *lme* function in R, using country as a random factor and decade as a fixed factor.

3.3. Results of the application

For both wheat and rye, there was a highly significant linear relationship between log(mean) and log(variance), i.e. in both cases TPL

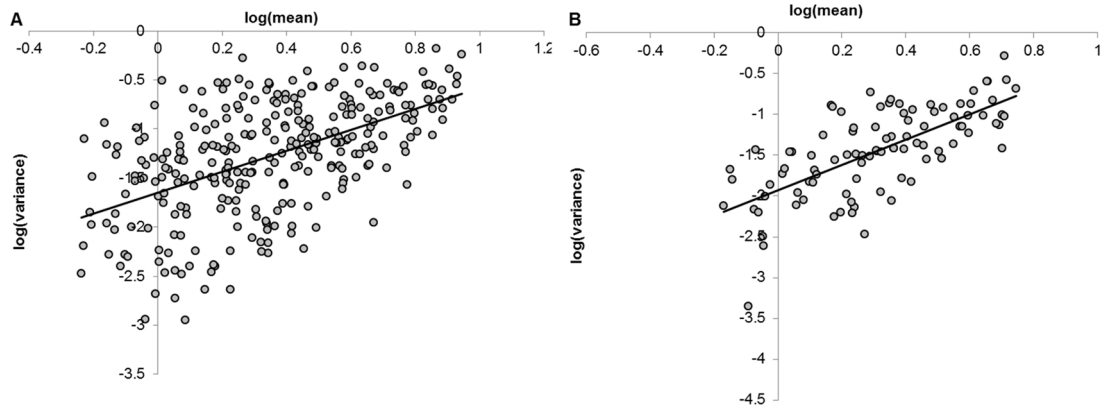


Fig. 2. Linear regression of $\log_{10}$ of mean against $\log_{10}$ of variance (original data in t ha^{-1}) for wheat (**A**) and rye (**B**), following Taylor's Power Law. The regression follows $y = a + bx$ with $a = -1.651 \pm 0.045$ (s.e.) and $b = 1.066 \pm 0.100$ (df = 288, Adjusted $R^2 = 0.279$, $P < 0.001$) for wheat, and $a = -1.928 \pm 0.066$ and $b = 1.546 \pm 0.167$ (df = 93, Adjusted $R^2 = 0.474$, $P < 0.001$) for rye.

Table 2
Regression statistics (estimate and standard error (SE) for slope and intercept of linear regression, as well as P-value and Adjusted R^2) for standard and adjusted coefficient of variation, based on yield data of wheat (df = 288) and rye (df = 93) from FAOstat.

Relationship		Wheat Estimate	SE	P-value	Adj. R^2	Rye Estimate	SE	P-value	Adj. R^2
Standard CV ~ mean	intercept	18.35	0.84	< 0.001	0.187	12.97	0.99	< 0.001	0.088
	slope	−2.08	0.25	< 0.001		−1.15	0.36	< 0.01	
Adjusted CV ~ mean	intercept	12.67	0.67	< 0.001	0.003	10.65	0.94	< 0.001	< 0
	slope	−0.28	0.20	0.165		−0.23	0.34	0.506	
Adjusted CV ~ standard CV	intercept	3.85	0.31	< 0.001	0.779	1.24	0.30	< 0.001	0.919
	slope	0.64	0.02	< 0.001		0.87	0.03	< 0.001	

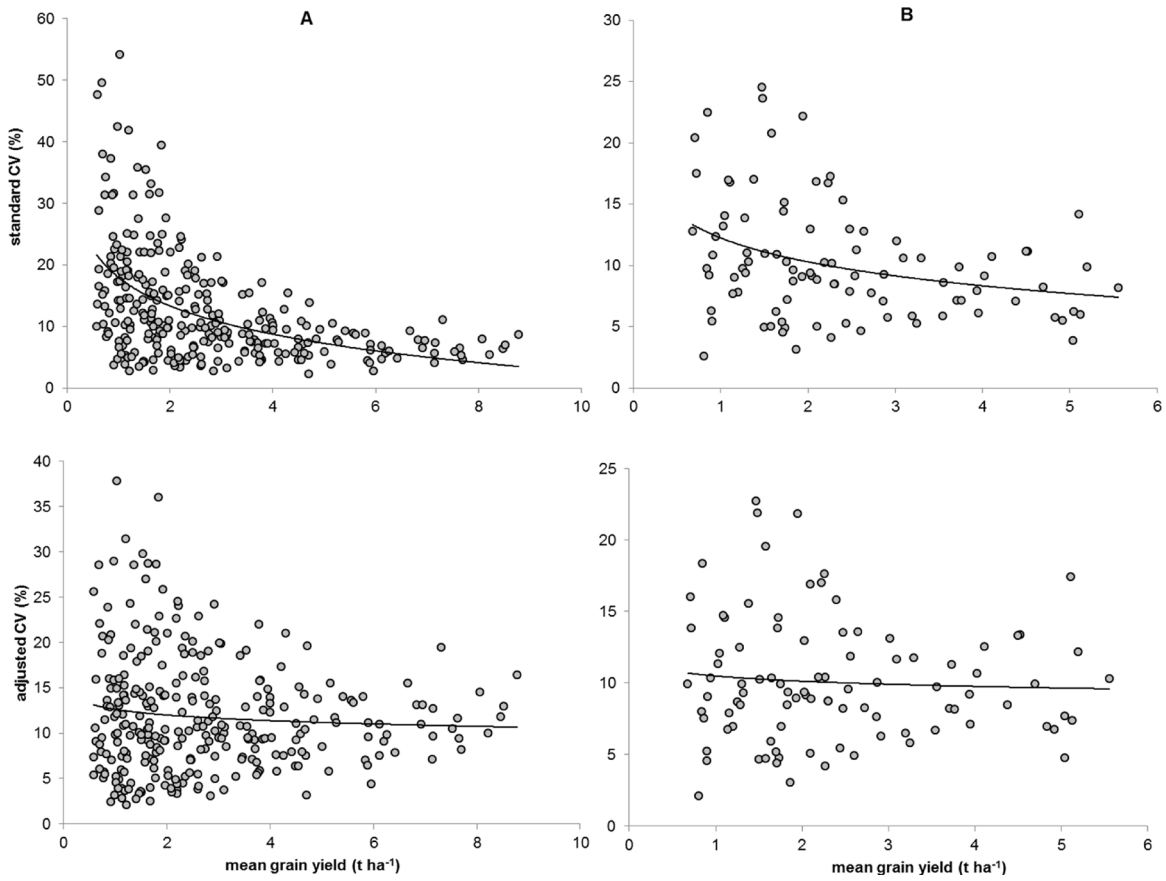


Fig. 3. Standard coefficient of variation (CV) and adjusted CV plotted against mean grain yield for yield data of wheat (**A**) and rye (**B**) from 1964 to 2013 with data from FAOSTAT (2014) for 77 countries (wheat) and 19 countries (rye).

was valid, with regression slopes *b* being > 1, but < 2 (Fig. 2). Accordingly, the standard CV significantly decreased with increasing mean for both wheat and rye (Table 2). Larger yields i.e. > 4 t ha^{-1} tended to have a lower standard CV of 2–15% and lower yields tended to have higher and a larger range of CV values from 3% to 54% in both wheat and rye (Fig. 3). Adjusting the CV for the dependence of the variance on the mean, resulted in no decrease of the adjusted CV with increasing mean (Table 2 and Fig. 3).

We also tested the relationship between the adjusted CV and the standard CV (Table 2). For both wheat and rye, the regression slope was > 0 but < 1, meaning that there was positive correlation between the two indices, but that large standard CV were adjusted down, and small ones were adjusted up, as expected.

Over the five decades and at the global level, the adjusted CV increased significantly in both wheat and rye (Fig. 4 and Table 3), i.e. yield instability increased over time. However, the size of this increase was small. On average, the adjusted CV increased by an absolute amount of 0.7 ± 0.1% per decade in wheat and by 1.0 ± 0.3% per decade in rye. In contrast, when using the standard CV, no significant change over time was observed for wheat, and for rye, the change over time (0.8 ± 0.3% per decade) was smaller than when the adjusted CV was used. This global trend of increasing yield instability was also observed for several individual but not for all countries (Fig. 5). Yield instability increased in Spain, Germany and Argentina for both wheat and rye, decreased in Brazil for wheat and increased in rye, and there was no trend observed in the United States and Turkey.

Since the standard CV significantly depended on the mean yield, we also tested whether mean yield increased over time across all countries included in the dataset. This was the case in both crops, with a stronger increase in wheat (0.410 ± 0.024 t ha^{-1} per decade) than in rye (0.294 ± 0.026 t ha^{-1} per decade) (Table 3).

4. Discussion

The standard coefficient of variation systematically decreases with increasing mean yield, when yield data follow Taylor's Power Law

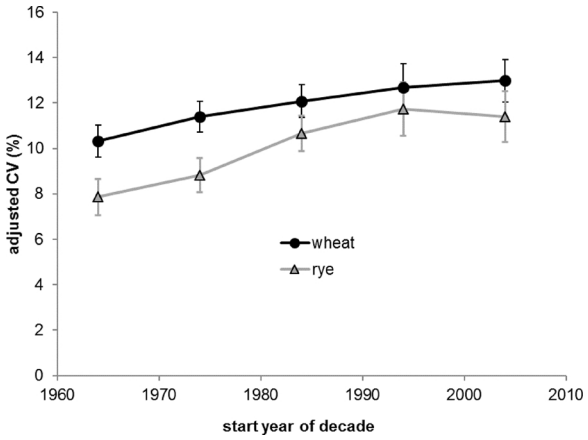


Fig. 4. Adjusted coefficient of variation (%) (mean and S.E.) for yield data of wheat (black circles) and rye (grey triangles) from 1964 to 2013 for five consecutive decades, data from FAOSTAT (2014) for 77 countries (wheat) and 19 countries (rye).

Table 3
Statistics (estimate and standard error (SE) for slope, as well as P-Value) in linear mixed effects models, testing effects of start year of decade (as fixed effect) on standard CV, adjusted CV and mean yield.

Relationhsip (Fixed)	Wheat Estimate	SE	P-value	Rye Estimate	SE	P-value
Standard CV ~ start year	−0.0089	0.0226	0.6935	0.0776	0.0267	< 0.01
Adjusted CV ~ start year	0.0660	0.0178	< 0.001	0.1000	0.0256	< 0.001
mean ~ start year	0.0410	0.0024	< 0.001	0.0294	0.0026	< 0.001

(TPL) with a regression slope of $b < 2$. As shown here, wheat and rye yields from national yield surveys do show these properties (Fig. 2), confirming previous accounts of yield data following TPL (Döring et al., 2015). Consequently, the use of the standard coefficient of variation (CV) for analysing yield stability is problematic when mean yield levels are different, because it is not possible to decide whether any differences in CV are genuine or just due to underlying differences in the mean which would make its use less appropriate in agronomy. In cases where the difference between yield levels is negligible, the standard CV is probably valid and adjustments are not needed.

In a study on the effect of different cropping systems on wheat, maize and soybean yields it was shown that yield variability in wheat and soybean, measured with the standard CV, was significantly higher in an organic than in a conventional cropping system (Smith et al., 2007). While the mean wheat yield was substantially and significantly lower in the organic than in the conventional system, mean yields of soybean were statistically equivalent in the two systems. Given the potential dependence of the standard CV from the mean, this finding points to the possibility that in the study by Smith et al. (2007) the differences in soybean yield stability between the systems may have been agronomically more meaningful than for wheat yield stability, since the differences in wheat CV may have been an artefact caused by the differences in means. Similarly, synergies between plant productivity and yield stability proposed in ecological research (e.g. Tilman, 1996) may at least partly be artefacts due to TPL.

Conversely, it can currently not be excluded that the properties of the yield data found here, in particular the TPL slope < 2, are not caused by genuine factors that simultaneously drive yield variability down and mean yield up. Indeed, both plant breeding and crop management aim for high mean yield *and* high yield stability. However, because TPL (with $b < 2$) is so widespread, and occurs in datasets of various structures (Döring et al., 2015), the standard CV needs to be interpreted with caution whenever the mean yields span a large range as is the case in global yield datasets. On the other hand, when means in a yield dataset do not cover large ranges, accurately estimating the parameters for the TPL regression line may become difficult. In any case, it should be checked whether there are indications of systematic dependence between means and variances in a given dataset.

We have further demonstrated how the potential dependence of the standard CV from the mean can be removed, i.e. by adjusting the slope b to a value of 2. In comparison to the previously suggested POLAR stability index (Döring et al., 2015), the adjusted CV has the advantage that it is expressed in straight-forward units, i.e. as percentage of the mean. In this study, we have applied the adjusted CV to wheat and rye data and were able to quantify the decrease in temporal yield stability to be ≤ 1%-point per decade, which seems more meaningful and easier to interpret than absolute changes in the POLAR stability index.

The finding of long-term increase of yield variability of wheat and rye (Fig. 4) confirms earlier results that yields have become more variable over time in wheat, rice and maize in the Southern Hemisphere (though not at the global scale) (Iizumi et al., 2014), and for main crops in large parts of Europe (Peltonen-Sainio et al., 2010; Olesen et al., 2011), but not for the northernmost European conditions of Finland, where no change in yield variability was recorded (Peltonen-Sainio et al., 2008). Again, however, caution is due when interpreting results where yield variability was assessed with the standard CV, and where yields were compared across regions and different crop species because

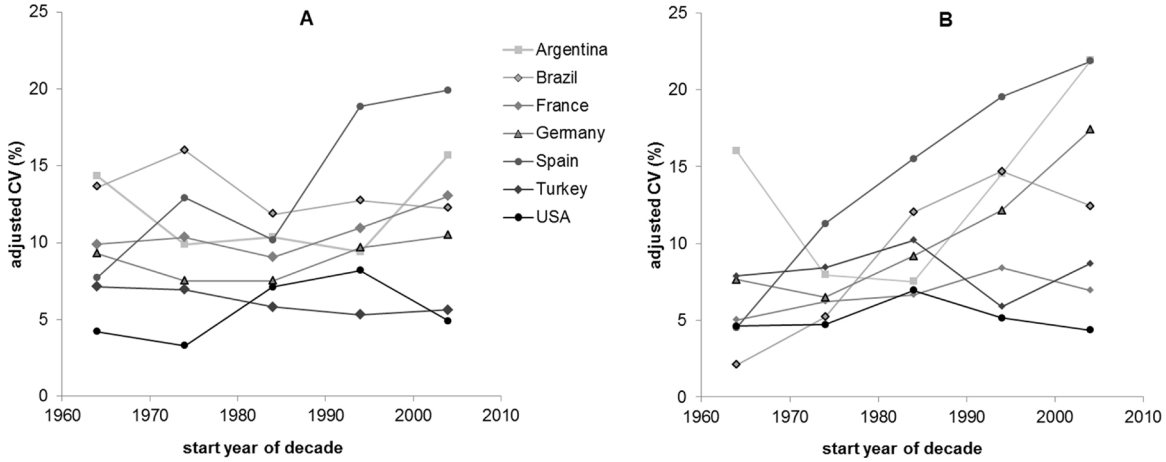


Fig. 5. Adjusted coefficient of variation (%) for yield data of wheat (**A**) and rye (**B**) from 1964 to 2013 for five consecutive decades, data from FAOSTAT (2014) for seven selected countries.

high means appear to correspond with low CV values. Reckling et al. (2018) also found an increase in yield variability for grain legumes, cereals and broad-leaved crops over 60 years in two long-term field experiments from Sweden and Germany, applying the adjusted CV presented in this paper.

The long-term increase of mean yields has also been reported earlier on a global level (Iizumi et al., 2014) and may mainly be attributed to genetic improvements (Laidig et al., 2014). In Europe yield stagnation has however been observed in the last two decades associated with recent climatic changes (Olesen et al., 2011) and the lack of break crops in cereal dominated cropping systems (Brisson et al., 2010), though multiple other reasons may also have played a contributing role to the observed stagnation.

Another of our findings is the lower yield stability in wheat than in rye which has also been observed by Reckling et al. (2015) in a low-input cropping system on sandy soils and by Cernay et al. (2015) for a national data set from Eastern Europe but not in Western Europe. At the supra-national level, this may partly be explained by wheat being grown as a spring and winter crop and rye being grown predominantly as a winter crop. Spring crops tend to have lower yield stability due to a shorter growing period and more dependence on the water availability during spring. Another reason could be the larger data set for wheat (77 countries) with a larger distribution of yield levels across sites compared to rye (19 countries). In addition, rye is known to be less sensitive to drought than wheat, which could also explain difference in yield stability. However, most important are the different yield levels between the countries, which seem to be larger for wheat.

For the interpretation of all of our results, however, caution is due, since the FAO data set combines low spatial resolution with potential problems in the comparability of data collection methods over time and across countries. The data set was chosen because it is publicly available and, at the global level, it is currently the best available long-term data series of crop yields. More importantly, the focus of our study is the description of a new method for calculating yield stability. So for making application easy and calculations transparent, public availability of the dataset was considered essential. The approach shown here can be further developed by analysing the contribution of other factors to yield stability, such as geographic region, country size or the development level of country. Further analysis of the underlying reasons for decreasing yield stability as observed in our study will benefit from using data with higher spatial resolution, climate and weather data, as well as information on cropping systems e.g. using long-term field experiments.

The new indicator offers the characteristics needed to be integrated into existing cropping system frameworks to evaluate impacts of alternative production systems (Reckling et al., 2016a, b) and to design more resilient cropping systems.

5. Conclusion

Research on climate change adaptation including the assessment of yield stability is increasing in plant sciences. The suggested method of scale-adjustment of the coefficient of variation is a novel alternative to estimate yield stability more conclusively than the frequently applied unadjusted coefficient of variation and it allows straight-forward interpretation by farmers, advisors and researchers. Because all stability indices have their specific advantages and disadvantages, the comprehensive evaluation of yield stability will always have to rely on a set of multiple stability indices. With the adjusted coefficient of variation we provide a new addition to this set that can be used to develop cropping systems with higher yield stability in the future.

Author contributions

TD and MR designed the study and wrote the manuscript. TD performed the statistical analysis. TD and MR prepared the figures and interpreted the results.

Acknowledgements

We thank the Ekhaga Foundation Sweden (project RESTOR 2015-65) and the Federal Ministry of Education and Research, Germany for funding through the FACCE-ERA-NET + project Climate-CAFE (Grant PTJ-031A544). We thank Johannes Forkman for helpful comments on earlier versions of this manuscript.

References

Becker, H.C., 1981. Correlations among some statistical measures of phenotypic stability. Euphytica 30, 835–840.
Becker, H.C., Léon, J., 1988. Stability analysis in plant breeding. Plant Breed. 101, 1–23.
Brisson, N., Gate, P., Gouache, D., Charmet, G., Oury, F.X., Huard, F., 2010. Why are wheat yields stagnating in Europe? A comprehensive data analysis for France. Field Crops Res. 119, 201–212.
Cernay, C., Ben-Ari, T., Pelzer, E., Meynard, J.-M., Makowski, D., 2015. Estimating variability in grain legume yields across Europe and the Americas. Sci. Rep. 5, 11171.
Chamekh, Z., Karmous, C., Ayadi, S., Sahli, A., Hammami, Z., Fraj, M.B., Benaissa, N., Trifa, Y., Slim-Amara, H., 2015. Stability analysis of yield component traits in 25 durum wheat (Triticum durum Desf.) genotypes under contrasting irrigation water salinity. Agric. Water Manag. 152, 1–6.
Cohen, J.E., Xu, M., Schuster, W.S., 2012. Allometric scaling of population variance with mean body size is predicted from Taylor's law and density-mass allometry. Proc. Natl. Acad. Sci. 201212883.
Cohen, J.E., Xu, M., Schuster, W.S., 2013. Stochastic multiplicative population growth predicts and interprets Taylor's power law of fluctuation scaling. Proc. R. Soc. B: Biol. Sci. 280, 20122955.
Crossa, J., 1988. A comparison of results obtained with two methods for assessing yield stability. Theor. Appl. Genet. 75, 460–467.
Dehghani, H., Sabaghpour, S.H., Sabaghnia, N., 2008. Genotype × environment interaction for grain yield of some lentil genotypes and relationship among univariate stability statistics. Span. J. Agric. Res. 6, 385–394.
Di Matteo, J.A., Ferreyra, J.M., Cerrudo, A.A., Echarte, L., Andrade, F.H., 2016. Yield potential and yield stability of Argentine maize hybrids over 45 years of breeding. Field Crops Res. 197, 107–116.
Döring, T.F., Knapp, S., Cohen, J.E., 2015. Taylor's power law and the stability of crop yields. Field Crops Res. 183, 294–302.
Duch, J., Arenas, A., 2006. Scaling of fluctuations in traffic on complex networks. Phys. Rev. Lett. 96, 218702.
Eberhart, S.A., Russell, W.A., 1966. Stability parameters for comparing varieties. Crop Sci. 6, 37–40.
Eghball, B., Power, J.F., 1995. Fractal description of temporal yield variability of 10 crops in the United States. Agron. J. 87, 152–156.
Eisler, Z., Bartos, I., Kertész, J., 2008. Fluctuation scaling in complex systems: Taylor's law and beyond. Adv. Phys. 57, 89–142.
FAOSTAT, 2014. "Crop Production Data", (Ed.) Food and Agriculture Organization of the United Nations. http://faostat3.fao.org/).
Ferreira, D.F., Demétrio, C.G.B., Manly, B.F.J., de Almeida Machado, A., Vencovsky, R., 2006. Statistical models in agriculture: biometrical methods for evaluating phenotypic stability in plant breeding. Cerne 12, 373–388.
Francis, T., Kannenberg, L., 1978. Yield stability studies in short-season maize. I. A descriptive method for grouping genotypes. Can. J. Plant Sci. 58, 1029–1034.
Huehn, M., 1990. Nonparametric measures of phenotypic stability. Part 1: theory. Euphytica 47, 89–194.
Iizumi, T., Yokozawa, M., Sakurai, G., Travasso, M.I., Romanenkov, V., Oettli, P., Newby, T., Ishigooka, Y., Furuya, J., 2014. Historical changes in global yields: major cereal and legume crops from 1982 to 2006. Glob. Ecol. Biogeogr. 23, 346–357.
Küchenmeister, F., Küchenmeister, K., Wrage, N., Kayser, M., Isselstein, J., 2012. Yield and yield stability in mixtures of productive grassland species: does species number or functional group composition matter? Grassl. Sci. 58, 94–100.
Laidig, F., Piepho, H.-P., Drobek, T., Meyer, U., 2014. Genetic and non-genetic long-term trends of 12 different crops in German official variety performance trials and on-farm yield trends. TAG. Theor. Appl. Genet. Theoretische Und Angewandte Genetik 127, 2599–2617.
Mühleisen, J., Piepho, H.-P., Maurer, H.P., Longin, C.F.H., Reif, J.C., 2014. Yield stability of hybrids versus lines in wheat, barley, and triticale. Theor. Appl. Genet. 127, 309–316.
Olesen, J.E., Trnka, M., Kersebaum, K.C., Skjelvåg, A.O., Seguin, B., Peltonen-Sainio, P., Rossi, F., Kozyra, J., Micale, F., 2011. Impacts and adaptation of European crop production systems to climate change. Eur. J. Agron. 34, 96–112.
Peltonen-Sainio, P., Jauhiainen, L., Hakala, K., 2008. Are there indications of climate change induced increases in variability of major field crops in the northernmost European conditions? Agric. Food Sci. 218 206–222%V.
Peltonen-Sainio, P., Jauhiainen, L., Trnka, M., Olesen, J.E., Calanca, P., Eckersten, H., Eitzinger, J., Gobin, A., Kersebaum, K.C., Kozyra, J., Kumar, S., Marta, A.D., Micale, F., Schaap, B., Seguin, B., Skjelvåg, A.O., Orlandini, S., 2010. Coincidence of variation in yield and climate in Europe. Agric. Ecosyst. Environ. 139, 483–489.
Piepho, H.P., 1998. Methods for comparing the yield stability of cropping systems - a review. J. Agron Crop. Sci. 180, 193–213.

Ray, D.K., Gerber, J.S., MacDonald, G.K., West, P.C., 2015. Climate variation explains a third of global crop yield variability. Nat. Commun. 6.
Reckling, M., Döring, T., Stein-Bachinger, K., Bloch, R., Bachinger, J., 2015. Yield stability of grain legumes in an organically managed monitoring experiment. Asp. Appl. Biol. 128, 57–62.
Reckling, M., Bergkvist, G., Watson, C.A., Stoddard, F.L., Zander, P.M., Walker, R., Pristeri, A., Toncea, I., Bachinger, J., 2016a. Trade-offs between economic and environmental impacts of introducing legumes into cropping systems. Front. Plant Sci. 7, 669.
Reckling, M., Hecker, J.-M., Bergkvist, G., Watson, C.A., Zander, P., Schläfke, N., 2016b. A cropping system assessment framework—evaluating effects of introducing legumes into crop rotations. Eur J. Agron. 76.
Reckling, M., Döring, T.F., Bergkvist, G., Chmielewski, F.-M., Stoddard, F.L., Watson, C.A., Seddig, S., Bachinger, J., 2018. Grain legume yield instability has increased over 60 years in long-term field experiments as measured by a scale-adjusted coefficient of variation. Asp.Appl. Biol. 138, 15–20.
Reidsma, P., Ewert, F., Lansink, A.O., Leemans, R., 2010. Adaptation to climate change and climate variability in European agriculture: the importance of farm level responses. Eur. J. Agron. 32, 91–102.
Smith, R.G., Menalled, F.D., Robertson, G.P., 2007. Temporal yield variability under conventional and alternative management systems. Agron. J. 99, 1629–1634.
Taylor, L., 1961. Aggregation, variance and the mean. Nature 189, 732–735.
Tilman, D., 1996. Biodiversity: population versus ecosystem stability. Ecology 77, 350–363.

Aspects of Applied Biology ***138****, 2018*
Advances in Legume Science and Practice

Grain legume yield instability has increased over 60 years in long-term field experiments as measured by a scale-adjusted coefficient of variation

By MORITZ RECKLING[1,2], THOMAS F DÖRING[3,4], GÖRAN BERGKVIST[2], FRANK-M CHMIELEWSKI[3], FREDERICK L STODDARD[5], CHRISTINE A WATSON[2,6], SYLVIA SEDDIG[7] and JOHANN BACHINGER[1]

[1]*Leibniz Centre for Agricultural Landscape Research, Germany*
[2]*Dept of Crop Production Ecology, Swedish University of Agricultural Sciences, Sweden*
[3]*Dept of Agronomy and Crop Science, Humboldt-University of Berlin, Germany*
[4]*Dept of Agroecology and Organic Farming, University of Bonn, Germany*
[5]*Dept of Agricultural Sciences, Viikki Plant Science Centre, University of Helsinki, Finland*
[6]*Crop and Soil Systems, Scotland's Rural College, Aberdeen, UK*
[7]*Institute for Resistance Research and Stress Tolerance, Julius Kuehn Institute, Germany*
Corresponding Author Email: moritz.reckling@zalf.de

Summary

Grain legumes produce high quality protein for food and feed, and provide ecosystem services contributing to sustainable cropping systems. Yet yield instability is perceived to be high, resulting in low adoption by farmers in Europe, where grain legumes were cultivated on only 1.5% of the arable land in 2014. The objective of this study was to assess whether grain legume yield instability has changed over time between 1953 and 2015. We used data from two long-term field experiments in Europe and accounted for yield differences between crops by applying Taylor's Power Law (TPL) in a scale-adjusted coefficient of variation (aCV). The results showed that grain legume yield instability was higher than that of autumn-sown cereals, but similar to those of broad-leaved crops and spring-sown cereals. Temporal yield instability of all crops significantly increased over time by more than half from 1960 to 2015 in Borgeby (Sweden) and from 1953 to 2008 in Berlin-Dahlem (Germany). This happened despite progress in plant breeding and technical development in agronomy, and climate change may be an important driver. Our findings contribute to improving the perception of grain legumes in general and point towards necessary improvements to support adaption to climate change.

Key words: Cereals, climate change, inter-annual yield variation, pulses, scaling, Taylor's Power Law, yield variability

Introduction

There is a global market demand for plant-based protein (Wu *et al*., 2014), and in Europe a large proportion of the protein used for feed and human food is imported. On the other hand, the negative environmental impacts caused by current crop production are partly due to the low diversity and

high input intensity of current cropping systems (Tilman *et al*., 2002). Legumes can diversify cropping systems and provide ecosystem services, thus contributing to more sustainable cropping systems (Watson *et al*., 2017), with lower trade-offs between economic and environmental impacts (Reckling *et al*., 2016*a*) and potentially supporting climate change mitigation (Jensen *et al*., 2012).

Grain legume yields are generally perceived to be unstable, as highlighted in farmer surveys (Zimmer *et al*., 2016) and expressed by international experts (EIP-AGRI, 2014). There is not enough empirical evidence available to support or reject these assumptions. Studies using statistical data have found greater yield instability for grain legumes than for cereals (Peltonen-Sainio & Niemi, 2012; Cernay *et al*., 2015). These quantitative studies have a scale-dependent bias, by using (i) aggregated yield data from national statistics and (ii) stability indicators that do not account for yield differences among crop species. Aggregated yield data shows a different stability than that observed in the field, and many stability indicators are related to the size of the mean yield, e.g. the standard coefficient of variation (CV). Hence, grain legumes that are grown on small proportions of land and tend to have lower yields are likely to have lower yield stability indicators for both of these reasons.

In order to overcome the scale-dependent bias of the studies mentioned above, Reckling *et al*. (2018) used field-level data from five long-term field experiments conducted in the United Kingdom, Sweden and Germany and a scale-adjusted coefficient of variation (aCV) that is based on Taylor's Power Law (TPL), the empirical relationship of the variance and the mean (Taylor, 1961). The study showed that the standard CV was negatively correlated with the dry matter yield and that grain legumes with lower grain yields had the largest range of CV values compared to cereals and broad-leaved crops. When the CV was adjusted (aCV) by application of the TPL, it was independent of the yield, and showed much less instability for grain legumes compared to the standard CV. Across sites, yield instability of grain legumes was higher than that of autumn-sown cereals, but lower than that of other spring-sown broad-leaved crops, and only slightly greater than spring-sown cereals. The analysis did not, however, evaluate the change of instability over time.

The objective of this study is to assess whether temporal yield instability of grain legumes changed over time. The hypothesis was that yield instability decreased between 1953 and 2015 due to progress in plant breeding and agronomy.

Material and Methods

In order to test our hypothesis, we used field-level data from two long-term field experiments conducted in Sweden and Germany (Table 1). The data set is based on 1512 site-year combinations that were divided into sub-sets of 8 years (rotation length). We used a scale-adjusted coefficient of variation (aCV) that is based on Taylor's Power Law (TPL). We applied TPL to adjust the standard CV to account for differences in yield between grain legumes and other crop types using the linear relationship between log(mean) and log(variance) from Reckling *et al*. (2018).

The scale-adjusted CV (aCV) is calculated as:

$$aCV_i = \frac{\sqrt{g^{\tilde{v}_i}}}{\mu_i} \cdot 100\%$$

where g is the basis of the logarithm, $\tilde{v}_i$ is the adjusted logarithm of the variance, calculated by setting the slope of log(variance) against log(mean) to 2, which removes systematic dependence of CV from the mean, μ_i are the residuals from the TPL regression line following Döring *et al*. (2015).

The aCV was tested for significant changes over time by a linear model with the *lm* function in R software version 3.3.1 (R Core Team, 2016). Yield instability between crops at each site was tested in R using an ANOVA and the Tukey's HSD test for pairwise comparisons of multiple means.

Table 1. *Characteristics of long-term field experiments*

Long-term field experiment	Agrometeorological field experiment at the Humboldt-University	R4-0002 at the Swedish University of Agricultural Sciences
Site	Berlin-Dahlem, Germany	Borgeby, Sweden
%clay	3	11
%sand	73	63
Annual precipitation (mm)*	555	672
Annual mean air temperature (ºC)*	9.6	8.3
Years	1953–2008	1960–2015
References	Chmielewski & Köhn (1999a)	St-Martin *et al*. (2017)

*Over the years under investigation.

Results

There were large differences in temporal yield instabilities among crops in both experiments. The lowest yield instability was found for autumn-sown cereals, with 19% and 15% for winter wheat and winter rye at Borgeby and Berlin-Dahlem, respectively (Table 2). Temporal yield instabilities of the grain legumes field pea, yellow lupin and faba bean were similar to other crops except winter rye at Berlin-Dahlem, and winter wheat, sugar beet and spring wheat at Borgeby (Table 2).

Table 2. *Adjusted coefficient of variation (aCV) per crop and site*

Site	Crop type	Crop	n	aCV (%)*	
Borgeby	Grain legume	Field pea	14	36	c
	Broad-leaved crop	Sugar beet	21	23	ab
		Winter oilseed rape	21	27	abc
	Cereals	Spring barley	28	29	bc
		Spring wheat	21	22	ab
		Winter wheat	35	19	a
Berlin-Dahlem	Grain legume	Yellow lupin	7	30	bc
		Faba bean	7	36	c
	Broad-leaved crop	Potato	7	35	c
		Sugar beet	7	30	bc
	Cereals	Spring barley	7	20	ab
		Spring oat	7	25	abc
		Winter rye	7	15	a

*Letters indicate significant difference per site at $P<0.05$. n = the number of 8-year subsets per crop and site used to calculate the aCV.

Temporal yield instability of all crops increased significantly over the investigated time period ($P<0.001$) by 14% (absolute) at Borgeby and by 15% at Berlin-Dahlem. There was no difference between the different crop types, grain legumes, cereals and broad-leaved crops increased significantly over time ($P<0.001$) at both sites (Fig. 1). The only individual crops where instability did not change over time were winter wheat at Borgeby and yellow lupin at Berlin-Dahlem.

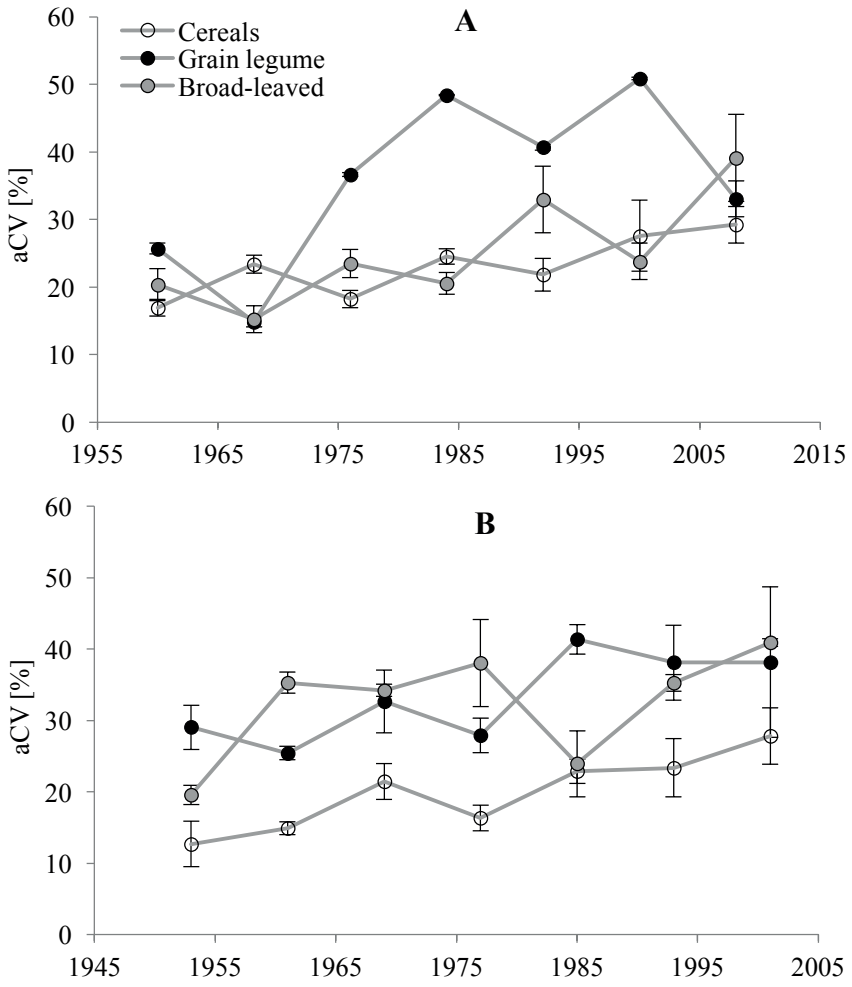


Fig. 1. Adjusted coefficient of variation (aCV %) (mean and SE) for yield data of cereals, grain legumes and broad-leaved crops (A) in the Swedish long-term experiment at Borgeby from 1960 to 2008 and (B) in the German long-term experiment at Berlin-Dahlem from 1953 to 2001. SE are not visible for grain legumes in Fig. A due to few observations.

Discussion

Earlier studies have shown that TPL, a widely verified quantitative pattern in ecology and other sciences (Eisler *et al*., 2008), can be used effectively to compare yield instability between different crop species grown in long-term experiments (Reckling *et al*., 2015; Reckling *et al*., 2018) and to compare differences in production systems (Döring *et al*., 2015). Using the log-linear relationship between yield and variance in the aCV instead of the linear relationship assumed in the standard CV changed the ranking of yield instabilities of crop types (Reckling *et al*., 2018). According to Reckling *et al*. (2018), the yield instability of grain legumes was comparable to that of other spring-sown crops, and only winter-sown cereals were significantly more stable. These novel findings challenge the current perception that yield instability is a major reason for grain legumes not being cultivated on larger areas in Europe (EIP-AGRI, 2014; Zimmer *et al*., 2016).

This present study showed that yield instability in two long-term experiments has increased over time for the grain legumes pea and faba bean (not yellow lupin), cereals (except winter wheat) and other broad-leaved crops. If this is a general trend, the increase in yield instability could have influenced the current perception of grain legume yield stability among farmers, although we have shown that instability also increased for other crops.

Increased yield instability may be due to changes in climate (Peltonen-Sainio *et al*., 2010). Ray *et al*. (2015) found in a global analysis of crop yield statistics for maize, rice, wheat and soybean that climate variability accounted for 32–39% of the observed yield variability. At Berlin-Dahlem, climate explained nearly 60% of the yield variability of spring barley and oat (Chmielewski & Köhn, 1999*b*).

Plant breeding that provides new cultivars with higher yield potential and increased tolerance to some biotic and abiotic stresses, as well as technical developments in agronomy, have contributed to a yield increase (Iizumi *et al*., 2014). In comparison to cereals, however, grain legumes have been neglected by plant breeding, as evidenced by the much lower number of available cultivars. Whether plant breeding has contributed to, or counter-acted the observed increase in yield instability remains speculative. In future research we aim to examine variation in weather variables that may account for the increase in instability and the effect of changes in cultivars in the long-term experiments. Cool-season grain legumes are susceptible to high temperature, water deficit and waterlogging (Stoddard *et al*., 2006), and breeding for broad adaptation is considered desirable in the context of increasing probability of these stresses along with increased atmospheric CO_2 concentration (Vadez *et al*., 2011).

Factors affecting temporal yield stability can be managed by breeding and agronomy, but each species has different tolerances to abiotic stresses and is affected by different diseases. Although crop diversification has been suggested as a tool to reduce yield instability (Gaudin *et al*., 2015), St-Martin *et al*. (2017) found no significant differences in yield stability between less and more diverse cropping systems in data on winter and spring wheat from the Borgeby experiment. All the crop rotations used in the Borgeby and Berlin-Dahlem experiments were quite diverse compared to common farming practice.

We conclude that, in contrast to our expectation of a decrease, yield instability of most major crops including grain legumes has increased through time in two long-term experiments. Greater attention to yield stabilization is needed in plant breeding and agronomy. Hence, and to support adaption to a changing climate, a systems approach is needed to design more resilient cropping systems (Reckling *et al*., 2016*b*).

Acknowledgements

We thank the Swedish University of Agricultural Sciences and the Humboldt-University of Berlin for providing the data from their long-term field experiments. The work was financed by the FACCE-ERA-NET+ project Climate-CAFE (Grant PTJ-031A544).

References

Cernay C, Ben-Ari T, Pelzer E, Meynard J-M, Makowski D. 2015. Estimating variability in grain legume yields across Europe and the Americas. *Scientific Reports* **5**:11171.

Chmielewski F-M, Köhn W. 1999*a*. The long-term agrometeorological field experiment at Berlin-Dahlem, Germany. *Agricultural and Forest Meteorology* **96**:39–48.

Chmielewski F-M, Köhn W. 1999*b*. Impact of weather on yield components of spring cereals over 30 years. *Agricultural and Forest Meteorology* **96**:49–58.

Döring T F, Knapp S, Cohen J E. 2015. Taylor's power law and the stability of crop yields. *Field Crops Research* **183**:294–302.

EIP-AGRI. 2014. EIP-AGRI Focus Group Protein Crops: final report. Access http://ec.europa.eu/eip/agriculture/en/content/protein-crops [19.12.2017].

Eisler Z, Bartos I, Kertész J. 2008. Fluctuation scaling in complex systems: Taylor's law and beyond. *Advances in Physics* **57**:89–142.

Gaudin A C M, Tolhurst T N, Ker A P, Janovicek K, Tortora C, Martin R C, Deen W. 2015. Increasing Crop Diversity Mitigates Weather Variations and Improves Yield Stability. *PLOS ONE* **10**:e0113261.
Iizumi T, Yokozawa M, Sakurai G, Travasso M I, Romanenkov V, Oettli P, Newby T, Yasushi Ishigooka Y, Furuya J. 2014. Historical changes in global yields: major cereal and legume crops from 1982 to 2006. *Global Ecology and Biogeography* **23**:346–357.
Jensen E S, Peoples M B, Boddey R M, Gresshoff P M, Hauggaard-Nielsen H, Alves B J R, Morrison M J. 2012. Legumes for mitigation of climate change and the provision of feedstock for biofuels and biorefineries. A review. *Agronomy for Sustainable Development* **32**:329–364.
Peltonen-Sainio P, Jauhiainen L, Trnka M, Olesen J E, Calanca P, Eckersten H, Eitzinger J, Gobin A, Kersebaum K C, Kozyra J, Kumar S, Marta D A, Micale F, Schaap B F, Seguin B, Skjelvag A O, Orlandini S. 2010. Coincidence of variation in yield and climate in Europe. *Agriculture, Ecosystems & Environment* **139**:483–489.
Peltonen-Sainio P, Niemi J K. 2012. Protein crop production at the northern margin of farming: to boost or not to boost. *Agricultural and Food Science* **21**:370–383.
R Core Team. 2016. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
Ray D K, Gerber J S, MacDonald G K, West P C. 2015. Climate variation explains a third of global crop yield variability. *Nature Communications* **6**:5989.
Reckling M, Bergkvist G, Watson C A, Stoddard F L, Zander P M, Walker R L, Pristeri A, Toncea I, Bachinger J. 2016*a*. Trade-offs between economic and environmental impacts of introducing legumes into cropping systems. *Frontiers in Plant Science* **7**:669
Reckling M, Döring T, Stein-Bachinger K, Bloch R, Bachinger J. 2015. Yield stability of grain legumes in an organically managed monitoring experiment. *Aspects of Applied Biology* **128**, *Valuing long-term sites and experiments for agriculture and ecology*, pp. 57–62.
Reckling M, Döring T F, Stoddard F L, Bergkvist G, Watson C A, Seddig S, Chmielewski F-M, Bachinger J. 2018. Stability of grain legume yields is a matter of scaling. *Landscape 2018*, 12–16 March 2018, Berlin.
Reckling M, Hecker J-M, Bergkvist G, Watson C A, Zander P, Schlaefke N, Stoddard F L, Eory V, Topp C F E, Maire J, Bachinger J. 2016*b*. A cropping system assessment framework - evaluating effects of introducing legumes into crop rotations. *European Journal of Agronomy* **76**:186–197.
St-Martin A, Vico G, Bergkvist G, Bommarco R. 2017. Diverse cropping systems enhanced yield but did not improve yield stability in a 52-year long experiment. *Agriculture, Ecosystems & Environment* **247**:337–342.
Stoddard F L, Balko C, Erskine W, Khan H R, Link W, Sarker A. 2006. Screening techniques and sources of resistance to abiotic stresses in cool-season food legumes. *Euphytica* **147**:167–186.
Taylor L. 1961. Aggregation, variance and the mean. *Nature* **189**:732–735.
Tilman D, Cassman K G, Matson P A, Naylor R, Polasky S. 2002. Agricultural sustainability and intensive production practices. *Nature* **418**:671–677.
Vadez V, Berger J D, Warkentin T, Asseng S, Ratnakumar P, Chandra Rao K P, Gaur P M, Munier-Jolain N, Larmure A, Voisin A-S, Sharma H C, Pande S, Sharma M, Krishnamurthy L, Abdou Zaman M. 2011. Adaptation of grain legumes to climate change: a review. *Agronomy for Sustainable Development* **32**:31–44.
Watson C, Reckling M, Preissel S, Bachinger J, Bergkvist G, Lindstrom K, Nemecek T, Topp C, Vanhatalo A, Zander Z, Murphy-Brokern D, Stoddard F L. 2017. Grain legume production and use in European agricultural systems. *Advances in Agronomy* **144**:235–303.
Wu G, Fanzo J, Miller D, Pingali P, Post M, Steiner J L, Thalacker-Mercer A E. 2014. Production and supply of high-quality food protein for human consumption: sustainability, challenges, and innovations. *Annals of the New York Academy of Sciences*, pp. 1–19.
Zimmer S, Liebe U, Didier J-P, Hess J. 2016. Luxembourgish farmers' lack of information about grain legume cultivation. *Agronomy for Sustainable Development* **36**:2.