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Grain Yield Stability of Cereal-Legume Intercrops Is Greater Than Sole Crops in More Productive Conditions

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Abstract: The intercropping of two or more crop species on the same piece of land at a given time has been hypothesized to enhance crop yield stability. To address this hypothesis, we assessed the grain yield stability of various barley-pea and wheat-faba bean mixtures grown in seven experimental field trials (locations) across Europe during two years with contrasting weather (2017 and 2018). Three different yield stability measures were used, all based on the expected yield variability of the mixture components grown as sole crops, and the corresponding observed yield variability of the same components grown in 50:50 mixtures in a replacement design. Stability indices were calculated as ratios between the expected and observed variabilities, with values > 1 indicating greater stability of the intercrops. Mean grain yields tended to be higher in intercrops than sole crops. However, in contrast to our hypothesis, the observed (intercrop) yield stability was similar or lower than the expected (sole crop) stability in most locations except one. Furthermore, yield stability significantly increased with increasing mean yields when assessed across differentially productive locations. The results are relevant for the designing of intercropping systems as a means to increase yield stability and the resilience of cropping systems.

Keywords: barley (*Hordeum vulgare*); crop diversification; faba bean (*Vicia faba*); pea (*Pisum sativum*); wheat (*Triticum aestivum*); yield stability; intercropping

1. Introduction

The mixed cropping, or intercropping, of two or more species on the same piece of land can enhance functional biodiversity and resource utilization efficiency in agricultural landscapes, which in turn could enhance yield stability [1,2]. Yield stability refers to how stable the yield of an agricultural system is over time, e.g., from year to year (temporal yield stability) and space, e.g., across different environments (spatial yield stability). The yield stabilizing effect of intercrops could be caused by different component species, or the specific cultivars used, being adapted to different environmental conditions, making them differentially responsive or sensitive to management actions, soil conditions, weather extremes, pests or diseases. For example, if one intercrop component suffers more than the other from a disease or extreme weather, others could partly compensate for the corresponding yield losses by utilizing more of the available resources. However, theoretical and empirical evidence for a general stabilizing effect of more diverse plant stands is inconclusive. For example, it has been shown that the stabilizing effect of more diverse plant stands is strongly influenced by the functional similarity (i.e., synchrony) of the component species and the environmental conditions [3–6]. Furthermore, it has been shown in global analyses that positive plant-plant interactions increase with the intensity of environmental stress [7,8]; suggesting, in an agricultural context, that a stabilizing effect of crop species mixtures could be expected to be more apparent in low productivity than high productivity environments. Another relevant theory derived from natural plant communities is the "insurance hypothesis", predicting that more diverse plant stands will be more resistant or resilient to perturbations such as extreme climate events, because a more diverse stand is more likely to hold species or genotypes that are better capable of withstanding a perturbation [9]. Whilst most of the theories making predictions on diversity-stability relationships are based on more diverse (natural) vegetation than is generally present in agricultural fields, these fields can provide interesting cases to test whether, and under which conditions, diversity-stability benefits can be achieved in agricultural crops.

All plant adaptations to different environmental conditions and stresses are accomplished through suites of traits, and often involve trade-offs between these traits [10]. These suites of traits can include traits enhancing tolerance or resistance properties while not significantly influencing growth rate and seed output or grain yield, but also traits resulting in decreased growth rate and/or reduced seed output or grain yield as adaptations per se to more stressful conditions. Trade-offs between various stress response traits are commonly observed and many of them probably cannot be offset by plant breeding, which results in many different alternative outcomes of stress responses [10,11]. From an intercropping perspective, this implies first that the individual components in a mixture are likely to have different suites of stress responses, resulting in different temporal and spatial yield variability (or stability) patterns; and second that the most pronounced yield-stabilizing effects from a crop stand basis could be expected for mixtures in which the components have asynchronous stress response patterns [12] reflected by smaller yield reductions of one component when others are suffering greater yield reductions, and vice versa.

Yield-stabilizing effects in crops and cropping systems have been investigated in field experiments, but results have been inconclusive [13,14]. Part of the problem is that yield stability in cropping systems has been assessed with different statistical methods, and it is often advisable to use more than one stability (or variability) measure in order to come to conclusive results [15,16]. Evidence for increased stability in mixtures is occasionally claimed based on the comparison of standard coefficients of variance (CV) for mean yields assessed in mixed vs. sole cultures [17]. However, the uncritical use of CV as a stability measure is problematic especially when comparing sole crops and intercrops, first because the CV is influenced by both the mean and the variability [18]; and second because the CV observed in an intercrop can be expected to be lower than the corresponding sole crop simply due to a statistical averaging effect [19]. For example, the admixing of two contrasting components can ameliorate the extreme values observed for the intercrop components when they are grown separately as sole crops even when no biological effect is invoked [20]. Suitable alternative variability measures not influenced by the mean value include the variance [21] and the adjusted CV (aCV) [22]. Thus, it has been shown that crop yield data often follow a power-law relationship between the sample variance and the sample mean, a relationship known as Taylor's Power Law (TPL) [18]; and this implies that the CV tends to decrease with increasing mean. The potential dependence of the standard CV from the mean can be removed by adjusting the slope (b) of the TPL regression to a value of 2, as was demonstrated previously [22]. Other statistical approaches to analyze yield stability include the regression of $\log_e$-transformed yield data obtained from different treatments (e.g., the regression of sole cropping vs. intercropping yields) and the subsequent testing of the regression coefficients (slopes, *b*) against a predicted value (e.g., $b = 1$) [23].

The aims of this study are to (i) define a set of three different yield stability indicators for the comparison of temporal and spatial yield stability in sole crops and intercrops grown in contrasting environments; and (ii) apply these indicators to a data set including a large quantity of individual cereal-legume cultivar combinations, here called 'plant teams' grown across Europe (Spain, Italy, Austria, Germany, Denmark, United Kingdom, Sweden). The same cereal-legume plant teams were grown for two years and included intercrops and the corresponding component sole crops of barley (*Hordeum vulgare*) with pea (*Pisum sativum*), and wheat (*Triticum aestivum*) with faba bean (*Vicia faba*). In a crop stand-based approach, we explored the hypotheses that grain yields are more stable in intercrops compared to growing the components separately as sole crops; and that the yield-stabilizing effect of intercrops is more pronounced in less productive conditions. In a single crop-based approach, we explored the hypothesis that plant teams consisting of components with asynchronous yield response patterns across two years with contrasting weather are more stable than those with synchronous yield response patterns.

2. Materials and Methods

2.1. Experimental Sites, Cultivar and Mixture Information, Assessments of Grain Yields

Data from seven experimental field trials (locations) grown across Europe in two consecutive years (2017, 2018) were used for the analyses (Table 1). Mean temperatures during the cultivation periods varied from 13 °C to 19 °C, and the weather conditions during the second year (2018) were warmer and drier in some locations, but cooler and wetter in other locations compared to the first year (2017) (Table 1). Sowing densities and crop management partly varied between the field trials, as is specified in Table 1. For example, "high" and "low" fertilizer levels implied different amounts of nutrient supply in the different locations. Weed management was generally kept at a minimum in all field trials, and was restricted to mechanical weeding or the application of a pre-emergence herbicide prior to sowing in some trials and years. For the analyses in this study, only the data from those cultivars and mixtures which were grown in both years in 50:50 mixtures and under the same fertilization treatments were included in the analysis. The agricultural growing season in the Spanish location normally spans from end of November to June the following year, but in the 2016 to 2017 season, crops could be sown only in early March, and the resulting very short growing season generated very low yields (<0.8 Mg ha^{-1}) in 2017. For this reason, the Spanish data were excluded from the analyses relating yield stability to mean yields. Various spring-sown cultivars of barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.) were grown as sole crops and intercrops (50:50 barley-pea and wheat-faba bean mixtures) in the seven locations, ranging from one to 16 different cultivar combinations (i.e., plant teams) per location (Supplementary Table S1). In some of the locations, the barley-pea sole crops and intercrops were not grown in the exactly same sites as the wheat-faba bean sole crops and intercrops, which however did not impact on our analyses because the two crop combinations were here analyzed separately. Only eight of the 44 plant teams in total (and their corresponding component sole crops) were grown in more than one location. Grain dry matter yields (Mg ha^{-1}) were assessed in plots ranging in size between 9 and 12 m^2 using combine harvesters in most cases except the German location, in which harvests of 1 m^2 plots were carried out manually (Table 1).

Table 1. Overview of the experimental cereal-legume field trials grown across Europe (from South to North) in 2017 and 2018 and used in the analyses. Four replicate plots in randomized block designs were applied in each trial. Sowing densities are given separately for barley (B) & pea (P) and wheat (W) & faba bean (F) sole crops and intercrops. Mean temperatures and precipitation sums refer to the individual cultivation periods of the seven trials. n/a—not applied.

Location **(Plot Size for Grain Yield Assessments, m)**	**Latitude, Longitude**	**Fertilizer * Treatments (kg N ha^{-1})**	**Sowing Densities ** (Seeds m^{-2})**	**Year**	**Cultivation Period**	**Mean Temp. (°C)**	**Precip. Sum (mm)**	**Days with Precip. > 1 mm**
Spain (Córdoba)	37°47′15″ N	BP 134, 268	B350, P80	2017	1 Mar–12 Jun	17.1	279	26
(5 × 2 or 10 × 1)	5°3′13″ E	WF 134, 268	W440, F50	2018	27 Nov §–8 Jun	12.6	466	47
Italy (Ancona)	43°32′42″ N	BP 50, 100	B400, P100	2017	17 Feb–21 Jul	16.4	227	22
(9 × 1.2)	13°21′34″ E	WF 80, 160	W ¥ 400, F50	2018	1 Feb–3 Jul	13.8	480	45
Austria (Gleisdorf)	47°6′49″ N	BP n/a	BP n/a,	2017	11 Apr–9 Aug	16.3	356	54
(8 × 1.3)	15°42′0″ E	WF 0	W440, F40	2018	12 Apr–8 Aug	18.6	535	64
Germany (Münster)	51°58′32″ N	BP 0, 70	B320, P80	2017	3 Apr–25 Aug	15.5	257	42
(1 × 1)	7°33′59″ E	WF 0, 70	W440, F40	2018	23 Apr–9 Aug	18.8	118	20
Denmark (Taastrup)	56°40′7″ N	BP 20, 60	B528, P60	2017	7 Apr–6 Sep [1]	13.5	351	49
(10 × 1.25)	12°18′20″ E	WF 20, 60	W432, F96	2018	19 Apr–15 Sep [2]	17.3	67	17
United Kingdom (Dundee)	56°48′17″ N	BP 0	B360, P80	2017	29 Mar–2 Aug [3]	12.8	325	51
(6 × 1.55)	3°11′17″ E	WF n/a	WF n/a	2018	2 Apr–5 Sep [4]	13	211	36
Sweden (Uppsala)	59°50′6″ N	BP 0, 90	B400, P90	2017	5 May–5 Sep	14.4	137	28
(6 × 2)	15°42′0″ E	WF 0, 140	W490, F60	2018	30 Apr–6 Sep	17.8	178	18

* Fertilizers were applied as commercial NPK fertilizers, organic fertilizer (Denmark) or NPK fertilizer as basal dressing and urea (46% N) as top dressing (Spain); ** Values indicate sowing densities in the sole crops, and the corresponding values in the intercrops were 50% of the values in the sole crops using replacement designs in all trials; ¥ Durum wheat (*Triticum durum*); § 2017; [1] period is for WF and corresponding period for BP is 7 Apr–17 Aug; [2] period is for WF and corresponding period for BP is 18 Apr–2 Aug; [3] period is for WF and corresponding period for BP is 29 Mar–30 Aug; [4] period is for WF and corresponding period for BP is 10 Apr–21 Aug.

2.2. Calculation of Expected and Observed Variabilities

We assessed yield stability using three different measures, all of which were based on the calculations of expected and observed yield variabilities. The expected yield variability is the variability of the mixture components grown separately as sole crops, considering the sum of their total yields divided by 2 to adjust to the same area base as in the intercrops; and the observed yield variability is the variability observed in the intercrops, considering the total yield of both components together. Yield variabilities were calculated from expected yields (Y_{exp}) and observed yields (Y_{obs}) assessed for various plant teams in the different locations, treatments (where applicable) and years, according to the following equations:

$$Y_{exp} = (Y_{Csole} + Y_{Lsole})/2 \quad (1)$$

where Y_{Csole} is the yield of the cereal component grown as sole crop and Y_{Lsole} is the yield of the legume component grown as sole crop for comparison with a 50:50 mixture (intercrop) of both components in a replacement design:

$$Y_{obs} = Y_{Cmix} + Y_{Lmix} \quad (2)$$

where Y_{Cmix} is the yield of the cereal component and Y_{Lmix} is the yield of the legume component grown in a 50:50 mixture (intercrop) of both components in a replacement design.

All trials used four replicate plots, whilst the means from all replicate plots per year, plant team and, where applicable, fertilizer level were calculated for each trial location and used to compute the corresponding values of Y_{exp} and Y_{obs}.

2.3. Crop Stand-Based Analysis of Grain Yield Stability

Three stability measures were calculated using the corresponding Y_{exp} and Y_{obs} values to obtain estimates of variability and stability of grain yields.

2.3.1. Stability Index$_{aCV}$ (SI$_{aCV}$)

Adjusted coefficients of variance (aCV) were calculated separately for each location and species combination (barley-pea and wheat-faba bean), according to [22]. Adjustment of the slope *b* of the TPL log-log regression to a value of 2 was done based on the TPL regression statistics from a data set of cereal and legume crops grown in long-term experiments across Europe [24] similar to the crops and conditions in our data set, because our data were insufficient to generate a robust value of *b* according to a procedure suggested by [25]. Expected and observed aCV based on the corresponding Y_{exp} and Y_{obs} values from the different locations, plant teams, fertilizer levels and years were computed to obtain overall estimates of stability of grain yields in intercrops vs. sole crops according to the following stability index (SI) equation:

$$SI_{aCV} = \text{Expected aCV}/\text{Observed aCV} \quad (3)$$

with values > 1 indicating greater stability (or smaller variability) of the intercrops, and values < 1 indicating greater stability of the sole crops grown separately. Thus, the SI_{aCV} reflects the comparison of yield variability between the cases of growing the same crop cultivars and plant teams under the otherwise same conditions either as sole crops (expected) or intercrops (observed). In addition to the aCV, we also evaluated the corresponding expected and observed variances.

2.3.2. Stability Index$_{Delta}$ (SI$_{Delta}$)

Expected and observed year-to-year (2017 and 2018) absolute yield differences (Delta) were calculated separately for each plant team and fertilizer level (where applicable); and means of the expected (from sole crops) and observed (intercrops) yield differences were computed for all locations and crop types to obtain an index expressing temporal (year-to-year) stability as follows:

$$SI_{Delta} = \text{Mean expected yield difference/mean observed yield difference} \quad (4)$$

with values > 1 indicating greater temporal (year-to-year) stability of the intercrops, and values < 1 indicating greater temporal stability of the sole crops grown separately. Whilst the absolute values of the year-to-year yield differences are strongly sensitive to the year-to-year differences in weather conditions, which were greater in some locations (e.g., Denmark and Italy, albeit in different directions) and weaker in other locations (e.g., United Kingdom, Austria) (Table 1), the SI_{Delta} eliminates the local influence of weather variability and reflects temporal year-to-year yield variability for the cases of growing the same crop cultivars and plant teams as sole crops and intercrops under the otherwise similar local weather conditions.

2.3.3. Slope of Expected vs. Observed Yields

Stability of grain yield was also analyzed by regressing (linear regressions) Y_{exp} against Y_{obs} for each location and crop type (barley-pea and wheat-faba bean), or different fertilizer levels; and comparing the slopes of the regression lines (or regression coefficients), in a similar approach as was applied by others [23].

2.4. Single Crop-Based Analysis of Grain Yield Stability

In addition to the crop stand-based approach, we also assessed yield stability of the individual crops when they were grown as sole crops and intercrops. Thus, grain yield variability was assessed in terms of adjusted coefficients of variance (aCV) and year-to-year variability (Delta) as described above, but for the individual mixture components grown as sole crops and intercrops.

2.5. Statistical Analysis

While all trials used four replicates, the means from all replicate plots at each location were used as the statistical replicates in all analyses done in this study. The three different indicators of grain yield stability were related to each other and to mean grain yields using standard correlation and regression analysis. Levene's test of equal variances performed on $\log_e$-transformed data according to [16] was used to statistically test whether observed variances were different from expected variances. Linear mixed model analysis was performed to test the following effects on the absolute yield differences (Delta, $\log_e$-transformed values) between the two cultivation years 2017 and 2018: Fixed effects of cultivation type (sole crops and intercrops), location, fertilization level (low, high) within location, plant team within location, and the interactions between cultivation type and location, fertilization and plant team, respectively. For the regressions of expected vs. observed yields, we statistically tested whether the slopes of the regression lines (or regression coefficients) were $\neq 1$ with *t*-tests (of slopes) using the $\log_e$ transformed grain yield data. All statistics were calculated using SPSS (version 26).

3. Results

3.1. Mean Yields and Yield Variability across Locations

Mean grain yields were higher in intercrops (observed yield) compared to the mean grain yield of the corresponding two sole crops (expected yield) across all locations (Table 2). Grain yields for barley-pea were 3.8 Mg ha^{-1} and 3.3 Mg ha^{-1} for intercrops and sole crops, respectively; and 4.0 Mg ha^{-1} and 3.7 Mg ha^{-1} for wheat-faba bean intercrops and sole crops, respectively (Table 2). The weather and management conditions, as well as the choice of plant teams, varied widely across the locations (Table 1 and S1), and the highest grain yields (means 2017 and 2018) were achieved in the Danish trial whilst the lowest yields were seen in Germany (barley-pea) and Austria (wheat and faba bean; Table 2).

Table 2. Descriptive statistics for the crop stand-based grain yield data (Mg ha^{-1}) from barley (B) & pea (P) and wheat (W) & faba bean (F) crops grown in seven locations across Europe over two years (2017 & 2018): Expected (from sole crops) and observed (intercrops) means; the corresponding variances and adjusted coefficents of variance (aCV, %); stability indices based on aCV (SI_{aCV}) and the yield differences (Delta) between the two cultivation years 2017 and 2018 (SI_{Delta}); and the slopes for the linear regressions of expected vs. observed yields. Levene's tests of equal variances and *t*-tests of slopes $\neq 1$ were performed on $\log_e$ transformed data (significant: $p < 0.050$ in bold). SP Spain, ITA Italy, AUT Austria, GER Germany, DK Denmark, UK United Kingdom, SWE Sweden, ALL calculations across all sites; *n* indicates the number of individual cultivar combinations (plant teams) and treatments (low and high fertilization) included, for details see Table 1.

Crop Type	Location	*n*	Exp. Mean	Exp. Variance	Exp. aCV *	Obs. Mean	Obs. Variance	Obs. aCV *	SI_{aCV}	*p* for Levene's Test	Exp. Delta	Obs. Delta	SI_{Delta}	Slope Exp. vs. Obs.	*p* for *t*-Test of Slopes
B&P	SP	8	3.15	8.02	89.5	3.48	11.19	99.5	0.90	**0.000**	5.30	6.25	0.85	0.83	**0.000**
B&P	ITA	4	4.23	2.93	42.0	4.36	2.72	39.7	1.06	0.484	2.95	2.84	1.04	1.08	0.204
B&P	GER	8	1.89	0.59	36.1	2.62	1.15	41.1	0.88	0.838	0.43	0.84	0.51	0.67	0.206
B&P	DK	20	5.21	1.98	26.5	5.93	1.85	23.7	1.12	0.262	2.42	2.14	1.13	1.15	0.062
B&P	UK	32	2.47	2.14	52.6	2.94	2.30	49.2	1.07	0.051	2.84	2.82	1.01	1.08	0.350
B&P	SWE	24	3.03	0.50	21.8	3.49	1.57	35.6	0.61	**0.002**	1.22	2.27	0.54	0.58	**0.000**
B&P	ALL	96	3.26	3.17	52.1	3.78	3.86	52.5	0.99	0.958	2.36	2.66	0.89	0.92	**0.011**
W&F	SP	4	2.94	8.22	94.5	3.20	8.94	93.6	1.01	0.322	4.96	5.18	0.96	1.09	0.101
W&F	ITA	8	3.35	1.09	29.6	3.43	1.18	30.4	0.97	0.940	1.88	1.94	0.97	0.96	0.625
W&F	AUT	4	2.25	0.01	2.2	2.78	0.33	20.1	0.11	**0.001**	0.08	0.97	0.09	−0.09	**0.002**
W&F	GER	12	2.97	1.53	43.9	3.06	1.57	43.7	1.00	0.530	0.57	1.11	0.51	0.95	0.642
W&F	DK	14	5.09	3.55	36.0	5.69	1.46	21.6	1.67	0.068	2.99	1.20	2.50	1.51	**0.046**
W&F	SWE	24	3.78	0.31	15.0	4.06	0.62	20.3	0.74	0.210	0.77	1.02	0.75	0.66	**0.000**
W&F	ALL	66	3.72	2.27	40.7	4.02	2.28	38.9	1.05	0.898	1.08	1.55	1.44	1.00	0.943

* The adjusted coefficients of variance (aCV) were calculated according to [22] and based on TPL regressions by [24], further details are found in Table S2.

3.2. Crop Stand-Based Yield Stability Indices as Measures of Yield Variability in Sole Crops and Intercrops

Across all locations, grain yield variability in terms of variance, aCV and year-to-year differences (Delta) was greater in barley-pea intercrops than in wheat-faba bean intercrops (Table 2). The yield SI_{aCV}, reflecting the overall yield stability across fertilizer levels and years, was around 1 for both crop combinations when assessed across all trial locations and years, indicating similar stability in intercrops and sole crops (Table 2). When assessed separately for the seven trial locations, SI_{aCV} values of 1 or <1 were found in most locations for both barley-pea and wheat-faba bean crop combinations, indicating similar yield stability in intercrops and sole crops or greater stability in the sole crops (Figure 1A). Only the Danish and UK trials showed SI_{aCV} figures > 1, but Levene's test indicated equal variances for the expected and observed yields in those two locations (Table 2). Significantly different variances for expected and observed yields were found only for a few locations (Spain, Austria, Sweden) with SI_{aCV} figures clearly < 1.

The SI_{Delta}, reflecting the temporal year-to-year stability, showed a similar pattern to the SI_{aCV} (Figure 1B). Linear mixed model analysis indicated significant effects of the cultivation type (sole crops and intercrops), trial location and the interaction between the two on the year-to-year yield differences (Table 3). Thus, year-to-year differences were generally greater in the intercrops (Obs. Delta in Table 2) than sole crops (Exp. Delta), but the pattern varied between the various trial locations (Table 2). Fertilization level significantly affected the year-to-year yield differences only in the barley-pea cultures, with greater variability in the zero or low fertilized (yield difference 2.6 Mg ha^{-1}) plots than the high fertilized plots (2.3 Mg ha^{-1}). Plant team identity had no significant effect on the crop stand-based year-to-year yield differences, but statistical power to detect these effects was low because only 8 out of the 44 plant teams in total were grown in more than one location.

Across all locations and fertilizer levels, the slope of the linear regression line (or regression coefficient) for expected vs. observed yield was significantly <1 (Figure 2A). Separate regressions for the seven locations showed a similar pattern of regression coefficients compared to the two different stability indices (Figure 1C), and the three different yield stability measures were highly correlated with each other (Figure 2B, Table 4). The slopes for expected vs. observed yields were significantly <1 in some locations, and significantly >1 only for the wheat-faba bean grown in Denmark (Figure 1C, Table 2). Overall, year-to-year yield differences increased with increasing mean grain yields albeit with diminishing returns (Figure 2C), and the SI_{Delta} increased with increasing grain yields (Figure 2D).

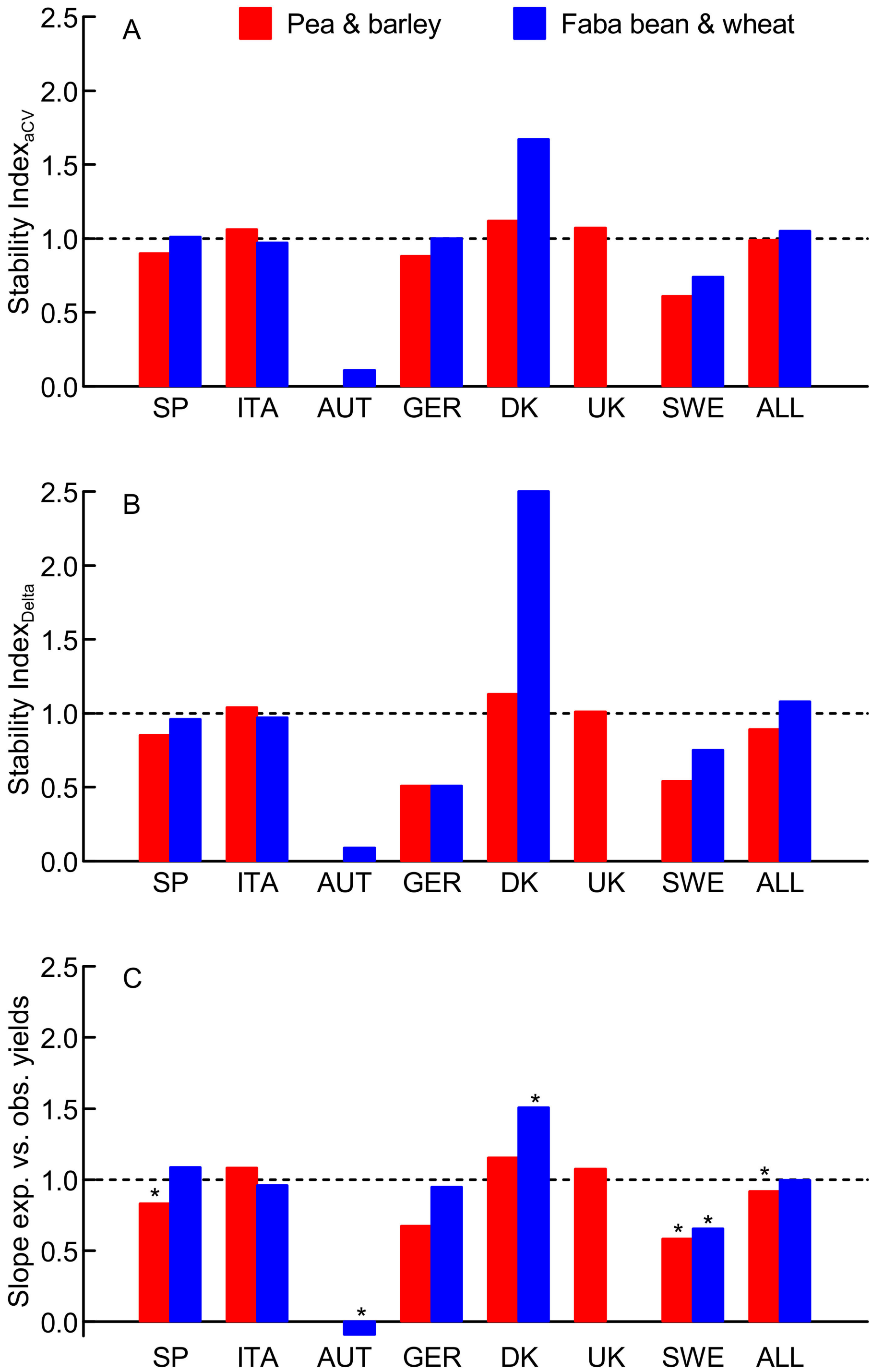

Figure 1. Crop stand-based yield stability indices calculated on basis of adjusted coefficients of variance (**A**), the yield difference (Delta) between the two cultivation years 2017 and 2018 (**B**), and the slopes for the linear regressions of expected vs. observed yields (**C**), across various barley-pea and wheat-faba bean trials cultivated in seven sites across Europe. SP Spain, ITA Italy, AUT Austria, GER Germany, DK Denmark, UK United Kingdom, SWE Sweden, ALL calculations across all sites. Asterisks indicate significant (*t*-test of slopes, 95% CI, $p \leq 0.050$) differences from slope = 1, based on the $\log_e$ transformed yield data. For barley-pea: $n = 4$ (ITA), 8 (SP, GER), 20 (DK), 24 (SWE), 32 (UK) or 96 (ALL). For wheat-faba bean: $n = 4$ (SP, AUT), 8 (ITA), 12 (GER), 14 (UCPH), 24 (SWE) or 66 (ALL). The indices are based on ratios of mean aCV or mean yield differences, and no standard errors can be associated with the ratios and slopes presented here.

Table 3. Linear Mixed Model analysis results for fixed effects of cultivation type (sole crop and intercrop, *df* = 1), location (*df* = 5), fertilization level (low, high) within location (*df* = 5 for pea-barley and *df* = 4 for faba bean-wheat), plant team within location (*df* = 27 for pea-barley and *df* = 14 for faba bean-wheat), and the interactions between cultivation type and location, fertilization and plant team, respectively, on the yield difference (Delta; $\log_e$ transformed values) between the two cultivation years 2017 and 2018. Separate analyses were done for crop stand-based data (barley-pea, wheat-faba bean) and single crop-based data (barley, pea wheat and faba bean grown as sole crops and their respective intercrops). *F*—critical value for F-statistics, *p*—significance (significant: $p < 0.050$ in bold), *df* degrees of freedom.

Source of Variation	**Barley & Pea**		**Wheat & Faba Bean**		**Barley**		**Pea**		**Wheat**		**Faba Bean**	
	F	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
Cultivation type	6.75	**0.017**	10.20	**0.005**	1.95	0.178	127.85	**0.000**	26.22	**0.000**	0.45	0.512
Location	34.54	**0.000**	32.08	**0.000**	93.96	**0.000**	80.98	**0.000**	16.46	**0.000**	36.09	**0.000**
Fertilization (Location)	2.79	**0.045**	12.98	**0.000**	1.45	0.250	2.32	0.082	5.59	**0.004**	1.56	0.228
Plant team (Location)	0.71	0.803	1.28	0.306	1.54	0.161	4.63	**0.000**	2.08	0.073	0.37	0.967
Cultivation type by Location	3.69	**0.016**	2.62	0.070	8.91	**0.000**	5.48	**0.002**	9.57	**0.000**	12.16	**0.000**
Cultivation type by Fert. (Location)	0.69	0.637	0.75	0.706	5.12	**0.004**	5.52	**0.002**	4.67	**0.009**	0.95	0.461
Cultivation type by Plant team (Location)	0.31	0.997	0.74	0.575	1.52	0.167	0.89	0.617	0.85	0.612	0.57	0.854

Table 4. Pearson correlation statistics for expected (from sole crops) and observed (intercrops) mean grain yield values, stability indices based on adjusted coefficients of variance (SI_{aCV}) and the yield differences (delta) between the two cultivation years 2017 and 2018 (SI_{Delta}), and the slopes for the linear regressions of expected vs. observed yields. *r*—Pearson correlation coefficient, *p*—significance (significant: $p < 0.050$ in bold), $n = 12$ for all correlations.

	Exp. Mean		**Obs. Mean**		$\mathbf{SI_{aCV}}$		$\mathbf{SI_{Delta}}$	
	r	***p***	***r***	***p***	***r***	***p***	***r***	***p***
Obs. mean	0.98	**0.000**						
SI_{aCV}	0.62	**0.033**	0.60	**0.039**				
SI_{Delta}	0.73	**0.008**	0.73	**0.007**	0.89	**0.000**		
Slope	0.63	**0.028**	0.59	**0.043**	0.97	**0.000**	0.83	**0.001**

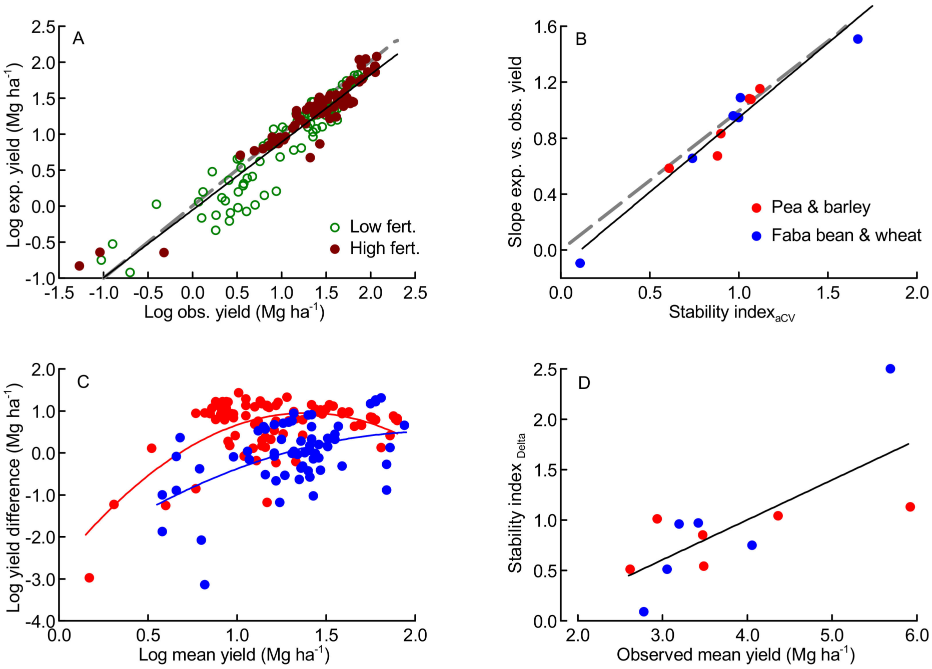

Figure 2. Regressions of (**A**) expected (from sole crops) vs. observed (intercrops) grain yields for various barley-pea and wheat-faba bean crops grown in seven locations across Europe under low and high fertilization levels during two years; the gray broken line indicates slope = 1, and the slope of the regression line (solid line) significantly differed from 1 (*t*-test of slopes, $p = 0.023$, $n = 162$); (**B**) slopes for the regressions between expected and observed grain yields calculated separately for each location vs. the corresponding yield stability indices on basis of coefficients of variance; the broken line indicates slope = 1, and the slope of the regression line (solid line) did not significantly differ from 1 (*t*-test of slopes, $p = 0.432$, $n = 12$); (**C**) mean grain yields vs. yield differences (Delta) between the two cultivation years 2017 and 2018 (Spanish trial was here excluded); and (**D**) stability indices based on the yield differences (Delta) between the two cultivation years vs. the observed (intercrops) mean grain yields. Linear regressions: (**A**) $y = 0.942x - 0.053$, $R^2 = 0.90$, $p = 0.000$, $n = 162$; (**B**) $y = 1.064x - 0.117$, $R^2 = 0.95$, $p = 0.000$, $n = 12$; (**C**) $y = -1.890x^2 + 5.251x - 2.695$, $R^2 = 0.38$, $p = 0.000$, $n = 88$ (barley-pea) and $y = -0.706x^2 + 2.999x - 2.672$, $R^2 = 0.26$, $p = 0.000$, $n = 62$ (wheat-faba bean); (**D**) $y = 0.396x - 0.582$, $R^2 = 0.54$, $p = 0.007$, $n = 12$. The data points represent individual crop cultivar combinations (plant teams) grown at a given location, separately averaged for two fertilization levels (**A**); means for all intercrops grown at a given location, separately averaged for the corresponding barley-pea and wheat-faba bean intercrops (**B**,**D**); individual plant teams from all locations, separately averaged for the corresponding barley-pea and wheat-faba bean plant teams (**C**).

3.3. Single Crop-Based Assessments of Yield Variability for Individual Crops Grown in Sole and Mixed Culture

In general (sensu all locations), yield variability was greater in barley than wheat, but similar in pea and faba bean (Figure 3). The effect of cultivation type (sole crop or intercrop) on aCV and year-to-year variability of the individual cereal and legume crops greatly varied between the locations (Figure 3, Table 3). For example, the intercropped wheat showed increased yield variability in the Danish and Swedish trials, but showed decreased yield variability in the Spanish trial (Figure 3). A significant and general (sensu all locations) yield-stabilizing effect of intercropping was found for pea (with a similar trend in barley); and an overall yield-stabilizing effect of sole cropping was found for wheat (Figure 3; Table 3, main effects cultivation type). In the pea-barley plant teams, some pea cultivars appeared to support lower (Clara, Hardy) or higher (Mythic) year-to-year variability (main effect plant team for pea in Table 3; Supplementary Table S3); whilst no evidence was found for any other significant effects of individual mixture components (cultivars) on the year-to-year variability of the respective crop. High year-to-year yield variation in pea was associated with low yield variation in barley, with a similar pattern in sole crops and intercrops; whilst no such relationship was found for faba bean vs. wheat

(Figure 4). The asynchronous yield response pattern across the two years observed in pea and barley coincided with a greater yield-stabilizing effect of intercropping when compared to the faba bean and wheat crops (Figure 3C,D). Single crop-based SI_{aCV} values were highest in pea and lowest in wheat, and varied greatly between different locations (Table 5). Single crop-based mean grain yields were uncorrelated with the corresponding SI_{Delta} values (Pearson correlations $p > 0.05$, not shown).

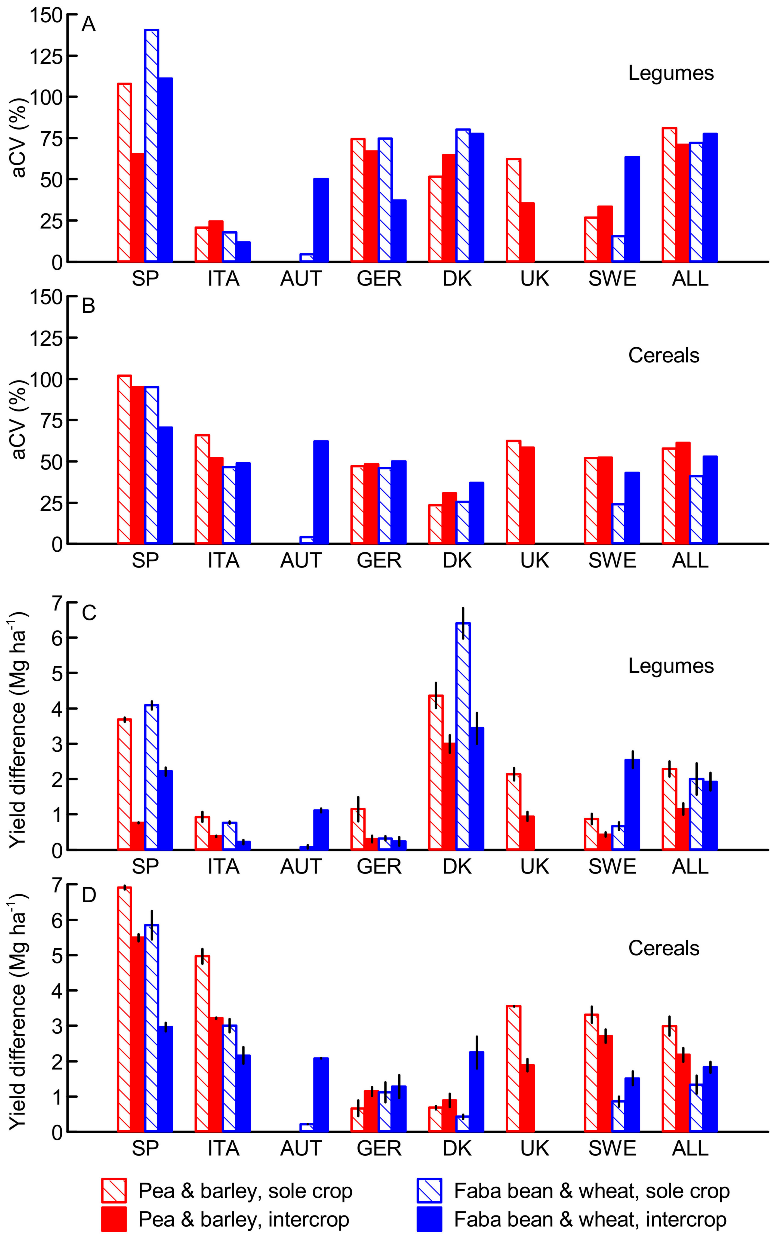

Figure 3. Single crop-based yield stability indices calculated on basis of adjusted coefficients of variance (aCV) (**A**,**B**), and yield differences (Delta, means ± SE) between the two cultivation years 2017 and 2018 (**C**,**D**), for various barley-pea and wheat-faba bean trials cultivated in seven sites across Europe. The bars in (**A**,**C**) represent either pea (red) or faba bean (blue) grown as sole crops or in the intercrop; and the bars in (**B**,**D**) represent either barley (red) or wheat (blue) grown as sole crops or in the intercrop. SP Spain, ITA Italy, AUT Austria, GER Germany, DK Denmark, UK United Kingdom, SWE Sweden, ALL calculations across all sites.

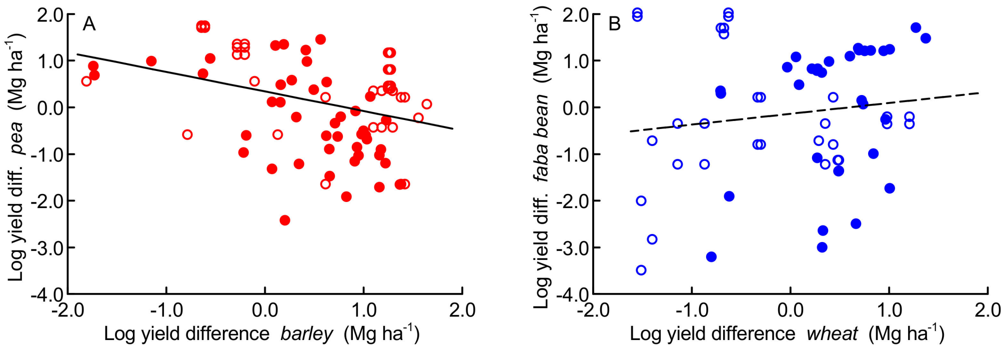

Figure 4. Regressions of single crop-based year-to-year grain yield differences for pea vs. barley (**A**), and faba bean vs. wheat (**B**) crops grown as sole crops (open symbols) and intercrops (closed symbols) in six locations across Europe in 2017 and 2018. The data from Spain were not included, see Section 2.1 for an explanation. Linear regressions: (**A**) y = −0.419x + 0.341, R^2 = 0.12, p = 0.001, n = 88; (**B**) y = 0.232x − 0.138, R^2 = 0.02, p = 0.301, n = 62. The data points represent individual intercrop components grown as sole crops and intercrops in each of six trial locations.

Table 5. Single crop-based estimates of yield stability indices calculated from adjusted coefficents of variance (aCV, %) from grain yield data assessed on barley (B) & pea (P) and wheat (W) & faba bean (F) sole crops and intercrops grown in seven locations across Europe. The stability indices were calculated as ratios between the aCV in the sole crops and the corresponding aCV when the same crop was grown as intercrop. SP Spain, ITA Italy, AUT Austria, GER Germany, DK Denmark, UK United Kingdom, SWE Sweden, ALL calculations across all sites; *n* indicates the number of individual cultivar combinations (plant teams) and treatments (low and high fertilization) included, for details see Table 1. n/a not applicable.

Location	Barley (*n*)	Pea (*n*)	Wheat (*n*)	Faba Bean (*n*)
SP	1.07 (8)	1.66 (8)	0.56 (4)	1.27 (4)
ITA	1.27 (4)	0.84 (4)	0.95 (8)	1.51 (8)
AUT	n/a	n/a	0.07 (4)	0.09 (4)
GER	0.97 (8)	1.11 (8)	0.92 (12)	2.01 (12)
DK	0.76 (20)	0.80 (20)	0.68 (14)	1.03 (14)
UK	1.07 (32)	1.75 (32)	n/a	n/a
SWE	0.99 (24)	0.80 (24)	0.56 (24)	0.24 (24)
ALL	0.95 (96)	1.14 (96)	0.78 (66)	0.93 (66)

4. Discussion

Using data from seven experimental field trials across Europe under various environmental conditions, this study found evidence for increased productivity in two-species (cereal-legume) intercrops compared to the intercrop components grown as sole crops, but limited evidence for a general yield-stabilizing effect of these intercrops except when productivity was high. Thus, a yield-stabilizing effect of intercrops was found in some cereal-legume combinations and locations, especially in more productive conditions, highlighting the importance of the environmental context for the species-specific mechanisms that apparently have contributed to yield stability in our study. The findings provide a sound basis for further studies to test whether diversity-stability benefits can be achieved in agricultural fields, for example by increasing the species diversity to higher levels than the two-species intercrops evaluated in this study; and investigating the external factors (temperature, water and fertilizer levels) that influence productivity and the diversity-stability relationships. A limitation of this study is the availability of yield data from only two years (albeit with contrasting weather conditions), and the lack of additional information on, for example, the main growth-limiting factors that would allow identification of the mechanisms responsible for these patterns. Thus, the growth and yield limiting factors probably varied widely between the locations and years assessed in this study, but the lack of detailed information on the most limiting factors and resources hindered our ability to understand the large differences in yield variability that were observed between the locations. In addition, grain yields were assessed partly with different methodology (manual or combine harvester) and based on different plot sizes and dimensions. For example, small plot sizes such as the ones used in the German trial could have caused disadvantages for the legumes (sown at lower plant densities) and the intercrops compared to the cereals, especially in sole crops [26]. While the direct comparison of grain yields between trials should be done with care, the main focus of this investigation was on the comparison of yield stability in sole crops vs. intercrops which were grown under the same management conditions and assessed in the same way in both years in a given trial. This implies that the partly different plot sizes and/or methods of yield assessment in the different locations likely did not affect the results strongly.

4.1. Measuring Yield Variability and Stability of Intercrops vs. Sole Crops

Different approaches for measuring biomass and yield stability have been discussed for decades in community ecology and intercropping research, but there is still no generally agreed methodology [3,15,16,18,19,21]. In this study we used three different crop stand-based measures, which all quantify the expected yield variation in the sole crops of the admixed components in relation to the observed yield variation in the intercrops. Thus, the SI_{Delta} was calculated based on the year-to-year yield differences to capture temporal stability; the SI_{aCV} was computed on the basis of adjusted coefficients of variation to accommodate both temporal and spatial variability and avoiding the disadvantages of the standard coefficient of variation being dependent on the mean yield [22]; and the regression coefficient describing the relationship between expected (in sole crops) and observed (intercrops) yields was used as an alternative measure accommodating both temporal and spatial variability, similar to the approach used also by others [23]. One of the stability indicators (regression coefficient) is amendable to sound statistical testing (i.e., *T*-tests of slopes). Whilst two of the indicators (i.e., SI_{Delta} and SI_{aCV}) are ratios which are not directly amendable to sound statistical testing, an indirect method (Levene's test) could be applied to test whether the expected variances were significantly different from observed variances, which are the essential components of the SI_{aCV} (eq 3). In addition, and most importantly, all three crop stand-based stability measures revealed largely consistent patterns and were highly correlated with each other, which allowed us to produce robust results. Furthermore, all three measures adopt a farm perspective in the sense of risk and cropping security [15]: we assume that a farmer's interest in growing intercrops would increase only if a farming system relying on intercrops supplied that farmer with more stable yields in adverse conditions compared to growing both intercrop components separately in sole crops; i.e., from that perspective, the farmer does not mind if the crops are produced as intercrops or sole crops on the same farm, provided that grain sorting is not an issue. When we used our aCV data to compare the yield stability of the intercrops with the stability of a single sole crop alone, as also others have done using standard CV [17], the comparison intercrop vs. sole crop strongly depended on the single sole crop of focus and on the environmental conditions i.e., the different locations; with no obvious overall pattern (Supplementary Table S4). However, this kind of comparison needs to be interpreted with caution, not least due to statistical averaging effects that may lead to misleading conclusions [19,20]; and we believe that our approach provides a better basis for decisions at farm level than the comparison of the stability of an intercrop vs. (any) single sole crop. As a complement to the crop stand-based analysis, we also considered the possibility of specific farming interests for particular crops grown either in sole cropping or intercropping farming systems. For that purpose, we also calculated the single crop-based variability and stability measures for individual crops grown as sole crops and intercrops, in addition to the crop stand-based measures. The single crop-based analysis revealed a general yield-stabilizing effect of intercropped pea along with a general yield-stabilizing effect of sole cropped wheat, but also that the effect of cropping type (sole crop or intercrop) on the stability of individual crops varied significantly between the different locations investigated here. This pattern highlights the importance of the environmental context for the species-specific mechanisms contributing to yield stability [4], and therefore the need to evaluate the stability of individual species and cultivars grown as sole crops or intercrops at local or regional scales.

4.2. No Evidence for a General Yield-Stabilizing Effect of Intercrops

We explored the yield stability hypothesis in a crop stand-based approach by using yield data from different environments (e.g., locations) and years (two years with contrasting weather), and we were therefore able to assess both temporal and spatial yield stability aspects [13,16]. In our analysis, we found that only the case of faba bean and wheat grown in Denmark conferred statistical evidence for greater yield stability in the intercrops compared to sole cropping of the intercrop components. Based on our data, we therefore found little support for a general yield-stabilizing effect of intercrops compared to sole crop cultures as was proposed by others [13,17,27]. The discrepancy of results is likely in part caused by the use of different methods, and we believe that our approach is reliable and promising because it avoids the problems associated with both the use of unadjusted coefficients of variance [18] and statistical averaging effects [19,20]. However, our data also showed that any yield-stabilizing effect of intercrops is strongly dependent on the environmental conditions, the type of growth-limiting factors and the magnitude of yield levels achieved. Contrary to our hypothesis, the yield-stabilizing effect of intercrops was more pronounced in the more productive conditions of the locations assessed here. Our hypothesis was based on the results from global analyses mostly of natural vegetation, where authors concluded that positive plant-plant interactions increase with the intensity of environmental stress and thus from more productive to less productive environments [7,8]; and that more diverse plant stands will be more resistant or resilient to perturbations such as extreme climate events [9]. In contrast to natural vegetation, the vegetation in agricultural fields is generally less diverse and the interactions with plants other than crops, i.e., weeds, is reduced through appropriate management actions. The stronger yield-stabilizing effect of intercrops seen in our more productive locations thus might simply be an effect of more intense plant-plant interaction accomplished in the more productive compared to the less productive sites, which is in line with the observation of greater yield stability in fertilized as compared to unfertilized crops seen in a long-term experiment performed in Germany [28]. Based on the crop stand-based assessments of our study, the intercropping of legumes and cereals on the same piece of land alone is probably not a safe option for stabilizing yields in many locations across Europe, but might well be a promising alternative especially in productive environments and together with other appropriate management actions such as the diversification over time in crop rotations [29,30].

4.3. Crops with Asynchronous Yield Response Pattern Show Increased Stability When Admixed

Functional asynchrony could be an important property of intercrop components explaining why a given combination of crops supports increased stability when they are grown in intercrops [6]. Functional asynchrony refers here to the asynchronous production trends of crops over time, in our study reflected by asynchronous year-to-year variability of the two intercrop components in a given plant team. We found indications for functional asynchrony in pea vs. barley, but not wheat vs. faba bean. However, overall yield variability at crop stand basis was indeed larger in pea-barley as compared to faba bean-wheat, and the greater yield variability in pea-barley was caused by the cereal (barley) component in some locations (Sweden, Italy), and by the legume (pea) component in other locations (Denmark, Germany). This suggests that the functional asynchrony of pea vs. barley apparently did not increase yield stability at a crop stand basis; and that the stabilizing component varied depending on the specific environmental conditions, highlighting the importance of the environmental context for the stabilizing effect of the components in diverse crop stands such as intercrops [4]. Thus, we found indeed indications for functional asynchrony, but those plant teams with greater asynchrony (i.e., pea-barley) showed greater yield variability and thus lower stability, which contradicts our hypothesis. Larger yield variability is often interpreted in terms of reduced stability and poorer stress tolerance, but yield formation is also an adaptive trait, because plants may adapt to different environmental conditions by increasing or decreasing growth rate and biomass allocation to reproductive organs such as grains [10]. For example, the larger variability of the pea seen in our study could reflect a higher capacity to adapt to changes in environmental conditions through altered allocation to grain yield, whilst the lower variability of the wheat could reflect enhanced stress tolerance. Indeed, high stress tolerance along with high yield stability was found in wheat in a comparison of different crop species grown in four long-term experiments [24]. Apart from the individual functional characteristics of the crop species combined in an intercrop, also the characteristics of the specific crop cultivars could affect the plant team characteristics and yield stability. In our study, we found little evidence for individual plant teams (cultivar combinations) to affect yield stability in both the crop stand-based and single crop-based analyses, but statistical power to detect those effects was low because only a few plant teams were grown in more than one location. In the future, a detailed characterization of the cultivar-specific functional traits relevant for stabilizing yields in intercrops should enable us to design desirable intercrops of cereal and legume cultivars that support greater yield stability.

5. Conclusions

From a farmer's perspective in terms of risk evaluation and cropping security, their interest in intercrops would increase if these supplied more stable yields under adverse conditions compared to growing the components separately as sole crops and provided that grain sorting is not an issue. The yield stability indices applied here recognize the farm perspective, because they reflect spatial and temporal (year-to-year) yield variability seen in the sole cropping of the intercrop components in relation to the corresponding yield variability observed in the intercrops. In that perspective, our results indicate that a yield-stabilizing effect of intercrops is less likely to occur in low-yielding locations and appears to become more likely as the growth conditions become more favorable for high yields. The yield-stabilizing effects of intercrops can probably be further enhanced by designing locally adapted crop/cultivar combinations. Even in those situations in which a yield-stabilizing effect of intercrops is less likely to occur, intercrops may frequently provide additional agronomic and environmental values that can motivate their use instead of sole crops. Such additional values can include increased soil fertility, yield productivity and quality, and biodiversity.

Supplementary Materials: The following are available online at https://www.mdpi.com/2077-0472/11/3/255/s1, Table S1: Overview of the crop cultivars and cultivar combinations (i.e., plant teams) grown as sole crops and intercrops across Europe in both 2017 and 2018 and used in the analyses. Table S2: Calculation of crop stand-based adjusted coefficients of variance (aCV) for expected (from sole crops) and observed (intercrops) mean grain yield values computed across all field trial locations, fertilizer levels and years. Table S3: Single crop-based year-to-year mean grain yield differences for various pea varieties grown as sole crops and as pea-barley intercrops (plant teams) grown across Europe in both 2017 and 2018. Table S4: Comparison of standardized coefficients of variance (aCV, %) for intercrops (crop stand-based analysis) and their corresponding component sole crops (single crop-based analysis) for barley & pea and wheat and faba bean crops grown in seven locations across Europe.

Author Contributions: Conceptualization, M.W.; methodology, M.W. and M.R.; software, M.W. and M.R.; formal analysis, M.W.; investigation, J.A., A.C.N., A.V.-F.; data curation, J.B., S.P., L.P.K., C.S.; writing—original draft preparation, M.W.; writing—review and editing, A.J.K., A.C.N., D.R., J.B., L.P.K., M.R., S.P., C.S.; visualization, M.W.; project administration and funding acquisition, A.J.K.,

A.C.N., C.S., D.R., E.A., L.P.K., M.W., S.T. All authors have read and agreed to the published version of the manuscript.

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