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Mixtures of Commercial Lentil Cultivars Show Inconsistent Results on Agronomic Parameters but Positive Effects on Yield Stability

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Abstract: Cultivar mixtures are a useful tool to enhance cultivated biodiversity to buffer crop biotic and abiotic stresses. There are multiple pieces of evidence of mixture advantages in terms of pathogen control and increase in yield amount, stability and quality. Lentil represents a founder crop in the Mediterranean, yet it experiences strong yield fluctuations in the face of abiotic stresses. The present study aims to assess the mixing ability of four Italian commercial lentil lines in terms of yield amount and stability, nodule number, total lentil biomass and sensitivity to weeds. Since there is very limited information on lentil genotype traits, two-, three- and four-cultivar mixtures were designed with a trait-blind approach and compared to sole cultivars. The nodule number was mainly influenced by cultivar and weather; no interaction between cultivars was observed. Treatments were differently sensitive to weeds, but the effect of spatial heterogeneity prevailed over that of the cultivar. The average yield stability of all mixtures was significantly higher than pure stands, but in terms of yield amount, individual mixtures either outperformed or were outperformed by pure stands. Against our expectations, cultivar mixtures showed the most advantages in the most productive year: likely, the reason lies in the supposed low genetic diversity of commercial lentil lines in Italy. We encourage further research, taking into account the diversity of Italian lentil landraces, in order to gain a broader genetic base for the implementation of a trait-based approach, which may lead to better-performing mixtures.

Keywords: agroecology; functional agrobiodiversity; resource use complementarity; grain legumes; pulses; yield stability; low-input agriculture; weeds

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1. Introduction

Nowadays, agriculture is facing a considerable challenge: it is expected to improve its environmental and social impacts while maintaining or even increasing production levels. Unfortunately, there are no easy ways to achieve both targets simultaneously, being those inversely correlated in mainstream industrialised agriculture [1]. An alternative agricultural paradigm focused on the ecological domain offers interesting perspectives to improve synergies and minimise trade-offs through the targeted use of agrobiodiversity [2].

This is what agroecology aims to do, considering that both farming systems and natural ecosystems are mainly governed by ecological laws [2–4]. In this framework, practices that boost biodiversity are known for their positive impact on yield and the environment, thanks to enhanced resource-use efficiency and stability [5]. The increase in biodiversity may be obtained at different levels (genetic, species and habitat) and scales (from field to landscape), requiring different degrees of technical adjustments by the food chain actors [6].

In this study, we have addressed genetic (intra-specific) diversity, namely the diversity of cultivars within a given cropped species. The implementation of intra-specific diversity in cropping systems has minimal technical disadvantages yet maintains the potential to positively affect ecosystems' services and productivity [6]. Genetic diversity can, e.g., be increased through breeding for multi-genomic mixtures: i.e., a certain number of cultivars grown together on the same field [7]. It has been proven that cultivar mixtures could buffer biotic and abiotic stresses [8–10], especially in organic and low-input farming [11].

The mechanisms underlying these effects have not been fully unveiled, but the intrinsic functioning can be related to well-known ecological theories. The main postulate is that of *niche differentiation* [6], implying the emergence of *functional complementarity*: i.e., each different species/strain has a different and complementary contribution to a given function [11,12]. For example, different varieties may have contrasting strategies of nutrient uptake [13], resulting in a higher uptake at the community level compared to monocultures [6]. Alternatively, they may have different responses to pathogens and thus limit the spread and severity of their attack [14]. Generally speaking, however, many uncertainties remain on which functional traits regulate functional complementarity, as phenotypic differences are hardly correlated to community functioning. Indeed, trait-based approaches rarely work due to the trait-correlation phenomenon (non-genetic correlations between traits are broken up by the repeated crosses required in the breeding process) [6,15].

The target crop of this study is lentil (*Lens culinaris* Medik). Lentil represents a founder crop in the Italian and Mediterranean food culture [16]; however, in Italy, after the peak of the 1960s/1970s, its production has been drastically decreasing until the beginning of the millennium [17]. To date, despite the recent consumption expansion (+210% from 2001 to 2015), only 2% of national consumption is domestically produced, suggesting that the market has considerable growth potential [18]. Italian and foreign farmers indeed report difficulties in cultivation and yield fluctuations, probably due to the lack of genetic improvement in the crop, as grain legumes still represent a minor sector in both Italy (1.3% of total Agricultural Area) and Europe (1.5% of total Agricultural Area) [19,20].

The aim of this study is to apply genetic diversity to lentil cultivation in the form of a cultivar mixture, looking for functional complementarity toward increased grain yield stability. The literature shows that mixtures have proven mostly successful, especially in cereal cultivation, but we are aware that unsuccessful mixtures were also observed, e.g., in soybean cultivation, as reported by Grettenberger [6,21,22].

Due to the cited concerns about the community dynamics of functional traits and the lack of germplasm in the Italian seed sector, the design of mixtures was based on a "trait-blind" approach. We identified certain indicators of diversity in order to ensure a minimum degree of heterogeneity within mixtures, but we did not design the mixtures on the basis of functional traits. Rather, we estimated the mixing ability of the target cultivars within the Italian seed sector [11].

Our aim is to study lentil grain yield and yield stability, as well nodulation, total lentil biomass and weed biomass, with the objective of verifying the advantages highlighted in the literature and to identify the possible underlying reasons.

In more detail, we investigate whether yield variability decreases with the increase in diversity, and we focus on mixture overyielding, assessing the actual occurrence of the phenomenon.

2. Materials and Methods

2.1. Soil, Plants and Growing Conditions

A field experiment was conducted in 2019, 2020 and 2021 at San Piero a Grado (43.6628° N, 10.3485° E), ca. 9 km SW of Pisa, Central Italy. The topsoil (0–0.3 m) of the experimental field was sandy, with a composition of 795 g/kg of sand, 129 g/kg of silt and 76 g/kg of clay. The soil had a pH (water) of 7.5, 2% Organic Matter, 1.4 g/kg total Nitrogen, C/N ratio of 8.9, 29.1 mg/kg of P_2O_5 (Olsen method) and 91.5 mg/kg K ($BaCl_2$).

San Piero a Grado is part of the Mediterranean zone, characterised by mild, relatively rainy winters and hot, dry summers. Annual precipitation reaches 910 mm, ranging from 25 mm in the driest month (July) to 145 mm in the wettest (November), with an irregular pattern throughout the year (autumn being the rainiest season).

In 2019, lentil sowing took place on the 12 March, and the trial was terminated, after harvest, on the 1 July. During the study period, we registered total precipitation of 256.6 mm; the average maximum temperature was 21.8 °C (peaking at 37.4 and 11.3 °C, with 13 days >30 °C and no days <10 °C), while the average minimum temperature was 9 °C (peaking at 22 and −3 °C, with 3 days >20 °C and 1 day <0 °C) (Figure 1).

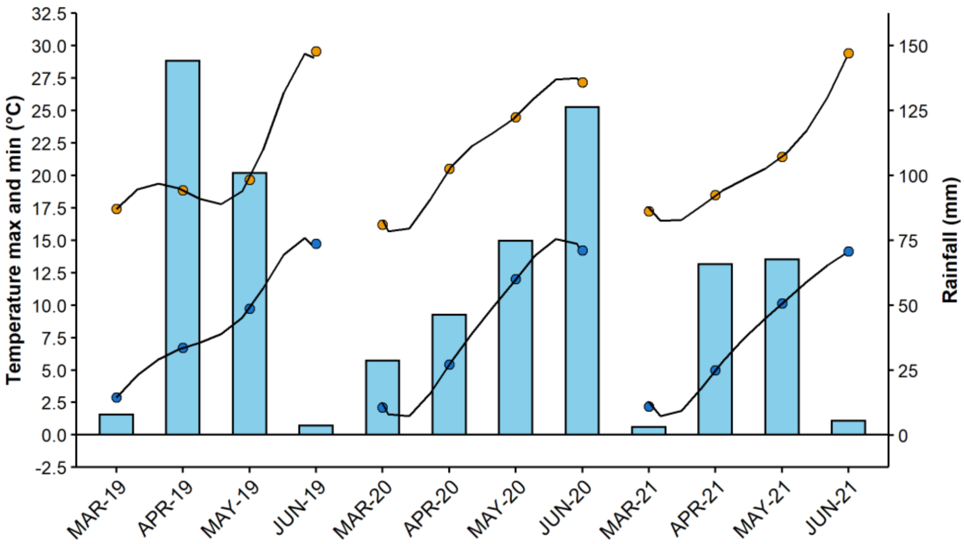

Figure 1. Seasonal climate in San Piero a Grado during the years of the experiment, from March 2019 to June 2021. On the left *y*-axis is the maximum (top lines) and minimum (bottom lines) monthly average temperatures and on the right *y*-axis is the monthly average rainfall.

In 2020, the experiment started on the 5 March and finished on the 9 July. During that time, we registered total precipitation of 276 mm; the average maximum temperature was 22.6 °C (peaking at 32 and 9.5 °C, with 11 days >30 °C and 1 day <10 °C), while the average minimum temperature was 9 °C (peaking at 18 and −6.5 °C, with no days >20 °C and 8 days <0 °C) (Figure 1).

In 2021, the trial was sown on the 25 February, while the harvest took place on the 6 July. During the study period, we registered total precipitation of 142 mm; the average maximum temperature was 21.8 °C (peaking at 33.8 and 13.2 °C, with 18 days >30 °C and no days <10 °C), while the average minimum temperature was 8 °C (peaking at 18.5 and −4.2 °C, with no days >20 °C and 11 days <0 °C) (Figure 1).

Precipitation occurred between the sowing time and the intermediate sampling, 254 mm in 2019 (sampling on 21 June), 276 mm in 2020 (sampling on 28 May) and 136.5 mm in 2021 (sampling on 25 May). The average maximum and minimum temperatures were, respectively, 20.6 and 8.2 °C in 2019; 22 and 8.3 °C in 2020, and 18.6 and 5.4 °C in 2021.

Four commercial lentil cultivars were chosen jointly with a partner farm to compose the mixtures. The main criteria for cultivar choice were (i) availability on the market, (ii) seed colour and (iii) seed dimension: cultivars with smaller seeds (microsperm) are better appreciated on the market and are less subject to damage during harvest. Within microsperm, we selected cultivars with different seed weights, as seed dimension is known to affect nitrogen uptake through the regulation of nodules number [23], and we opted to include the highest seed colour diversity, being colour genes possibly linked to nodulation genes, as it is the case in chickpea [24]. We managed to meet our criteria, but the cultivars available on the market were very limited: in fact, there are very few options regarding lentil cultivars on the Italian market. The material was provided by the partner farm in Tuscany (cv. *Robin* and cv. *Screziata*, with brown and dotted brown seeds, respectively) and by the Apulian branch of an agricultural consultancy (cv. *Turca* and cv. *Nera*, with dotted red and black coated seeds, respectively).

The 4 lentil cultivars were assembled in 16 treatments, representing all the possible combinations between cultivar pairs and triplets, plus the quartet and the sole cultivars (Table 1). A plot in each block was left unseeded as a control for checking weed development.

Table 1. Increasing levels of diversity within lentil mixture combinations.

Baseline: Pure Stand	First Level: 2 Cultivar Mixture	Second Level: 3 Cultivar Mixture	Third Level: 4 Cultivar Mixture
Robin	Robin + Screziata	Robin + Screziata + Turca	Robin + Screziata + Turca + Nera
Screziata	Robin + Turca	Robin + Screziata + Nera	
Turca	Robin + Nera	Robin + Turca + Nera	
Nera	Screziata + Turca	Screziata + Turca + Nera	
	Screziata + Nera		
	Turca + Nera		

The trial was arranged in a randomised block design with four replicates each year. Irrigation was provided only when rain was lacking in the three weeks after sowing to ensure germination; no fertilizer was applied. Since we were also interested in studying lentil competitiveness, weeds were not removed.

Each year, a few weeks before sowing, the soil was ploughed at a depth of 25 cm, and then the seedbed was prepared with a rotary harrow at a depth of 10 cm. Mixtures and sole crops were established in 6 × 1.5 m plots by sowing 360 germinable lentil seeds m^{-2} with a row spacing of 18 cm (plot seeder from Wintersteiger, OYJORD model). The seed proportion of each lentil cultivar in mixtures was 0.5 in the two-variety mixtures, 0.33 in the three-variety mixtures and 0.25 in the four-variety mixtures.

2.2. Sampling Procedure and Analysis

The first sampling took place at the end of the flowering stage to provide data on nodule numbers (21 June 2019, 28 May 2020 and 25 May 2021). Due to the impossibility of identifying individual cultivars within the mixtures (no phenological differences), the nodule number refers to the whole mixture. Five plants per plot were randomly selected and gently pulled out; when the soil was too coarse, water was applied locally to facilitate plant extraction. Dry and living nodules were counted on a root portion 10 cm long.

The second sampling occurred at the seed maturation stage to provide data on lentil total and useful biomass (grain yield) and on weed biomass (1 July 2019, 9 July 2020, 6 July 2021). Biomass was harvested with a sickle on a 0.5 × 0.5 m surface in each plot. Lentil biomass was divided from weeds biomass and oven-dried at 70 °C until constant weight. Subsequently, lentil pods were passed through a plot thresher; seeds were retrieved, oven-dried (as above) and weighed.

2.3. Statistical Analysys

Data were analysed upon a generalised linear mixed model framework using the maximum likelihood procedure (Laplace approximation) for the following parameters: nodule number, grain yield and weed biomass. The residual analysis identified the Poisson distribution as the most accurate in describing the variables distribution.

Weed biomass was analysed as per cent difference from the control, calculated per block as the difference between weed biomass in each treatment and in the control.

Instead, total lentil biomass was analysed upon a linear mixed model framework, using Restricted Maximum Likelihood (REML) procedures and Satterthwaite's method for the *t*-test.

Treatment, year and treatment × year interaction were set as fixed terms, while block was set as the random term.

Treatment means were compared with the Bonferroni post-hoc test using the R package emmeans, version 1.7.5 (Package "Emmeans" Aka Least-Squares Means. Available online: https://cran.r-project.org/web/packages/emmeans/emmeans.pdf accessed on 1 July

2022). Orthogonal contrasts were built to compare the performance of each mixture with that of its components. This procedure allowed us to study the interaction effect between cultivars: when the contrast highlights better results for pure stand components, it indicates an antagonistic effect. Otherwise, it indicates a synergistic effect among lentil cultivars promoted by the mixture.

Yield variability was studied through the analysis of grain yield standard error (SE)

$$\text{SE} = \frac{(\text{treatment standard deviation})}{\sqrt{\#\ \text{treatment components}}}$$

We then grouped the treatments into "pure stands" (six treatments), "mixtures of two cultivars" (eight treatments), "mixture of three cultivars" (six treatments) and "mixture of four cultivars" (one treatment).

We calculated the standard error for each treatment group (mean standard error of all treatments included in the group) for each year and the average standard error for each group over the three years of the experiment.

We thus performed a General Linear Model (GLM) to study the effect of standard error, treatment and year (Gaussian distribution, degree of freedom = 29).

Finally, we used the post hoc result to build orthogonal contrasts between each diversity level in order to test the variability of the increasing levels of diversity against the pure stands.

Contrasts were performed with the emmeans package (Bonferroni-based method).

All data analyses were performed in R for Windows, version 4.0.3 [25]. Data visualisation was performed using the R packages ggplot2, version 3.3.5 (Package "ggplot2" based on "The Grammar of Graphics". Available online at https://cran.r-project.org/web/packages/ggplot2/index.html accessed on 1 July 2022).

3. Results

3.1. Nodule Number

The analysis of nodule number resulted in a significant effect of treatment (χ^2 = 31.9) and year (χ^2 = 82.86).

The nodule number varied significantly between pure stands, where cv. *Nera* was higher than the other cultivars by 28%. The same dynamics occurred in the mixtures: most of the mixtures containing cv. *Nera* produced significantly more nodules (on average +22% ca.) than the mixtures not containing it (Figure 2).

The average nodule number in 2019 and 2020 was ca. 18 nodules per plant, while in 2021, the value dropped to ca. 10 nodules per plant.

Concerning contrasts between mixtures and their components, a marginal significance emerged (p = 0.06): mixture TuNe produced more nodules than the average of its components (TuNe = 17.3, Ne = 17.6 and Tu = 12.3).

3.2. Lentil Biomass

The analysis of total lentil biomass resulted in a nearly significant effect of treatment (p = 0.07, χ^2 = 21.8) and in a significant effect of year (χ^2 = 132.3).

Significant differences indeed emerged among pure stands and two-cultivar mixtures: cv. *Nera* was associated with higher biomass, partially when grown with cv. *Robin*. However, biomass differences were diluted in the three-cultivar mixtures, which did not show any significant differences (Figure 3).

The yearly differences tended towards a general biomass decrease in 2021: lentils produced a total biomass of ca. 360 g m^{-2} in 2019 and 2020 and only 130 g m^{-2} in 2021 (65% reduction).

Concerning contrasts between mixtures and their components, we could not detect any significant differences, implying the existence of no interactions between cultivars.

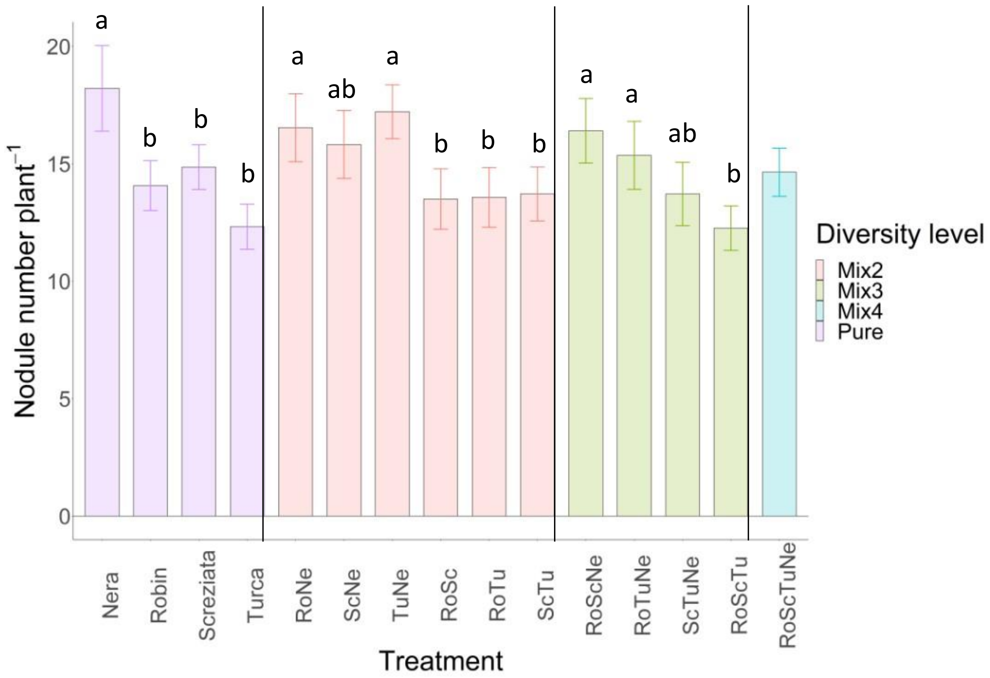

Figure 2. Nodule number per plant in pure lentil stands, two-, three- and four-cultivar mixtures, averaged over the three years of the experiment. Different letters indicate significant differences among treatments at $p \leq 0.05$ (Bonferroni-based method). Ne = cv. Nera, Ro = cv. Robin, Sc = cv. Screziata, Tu = cv. Turca.

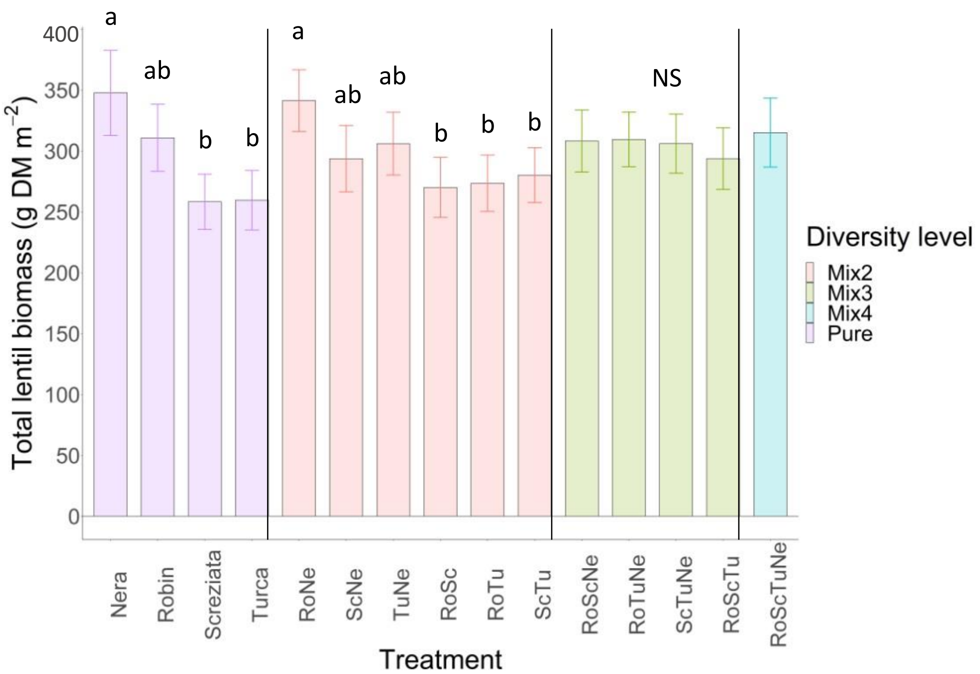

Figure 3. Lentil biomass differences among pure stands, mixtures of two cultivars, mixtures of three cultivars and mixtures of four cultivars: average of the three years of experiment. Values are the above-ground biomass dry weight, expressed as g/m^2. Different letters indicate statistical significance at the $p \leq 0.05$ level (Bonferroni-based method) among treatments. NS means no statistical significance with $p \leq 0.05$. Ne = Nera, Ro = Robin, Sc = Screziata, Tu = Turca.

3.3. Weed Biomass

The analysis of weed biomass resulted in a significant effect of treatment (χ^2 = 291,2), a nearly significant effect of year (p = 0.06, χ^2 = 5.4) and a significant effect of their interaction (χ^2 = 516.8).

We observed a general increase in weed pressure in the third year when lentil biomass production was the poorest: mean weed biomass in lentil plots was ca. 60 g m^{-2} in 2019 and 2020 and more than double in 2021 (125 g m^{-2}). Conversely, the unweeded controls did not follow the same pattern, producing 211, 295 and 239 g m^{-2} of weed biomass in 2019, 2020 and 2021, respectively: the weed biomass in 2021 was closer to that of the control compared to the previous years (ca. 30% in 2019 and 2020, and ca. 50% in 2021).

Weed biomass showed very high variability among treatments and levels of diversity, and a clear trend could not be identified. In general, cv. *Screziata* emerged as the genotype most sensitive to weed pressure when grown alone but not when grown in mixtures (Figure 4).

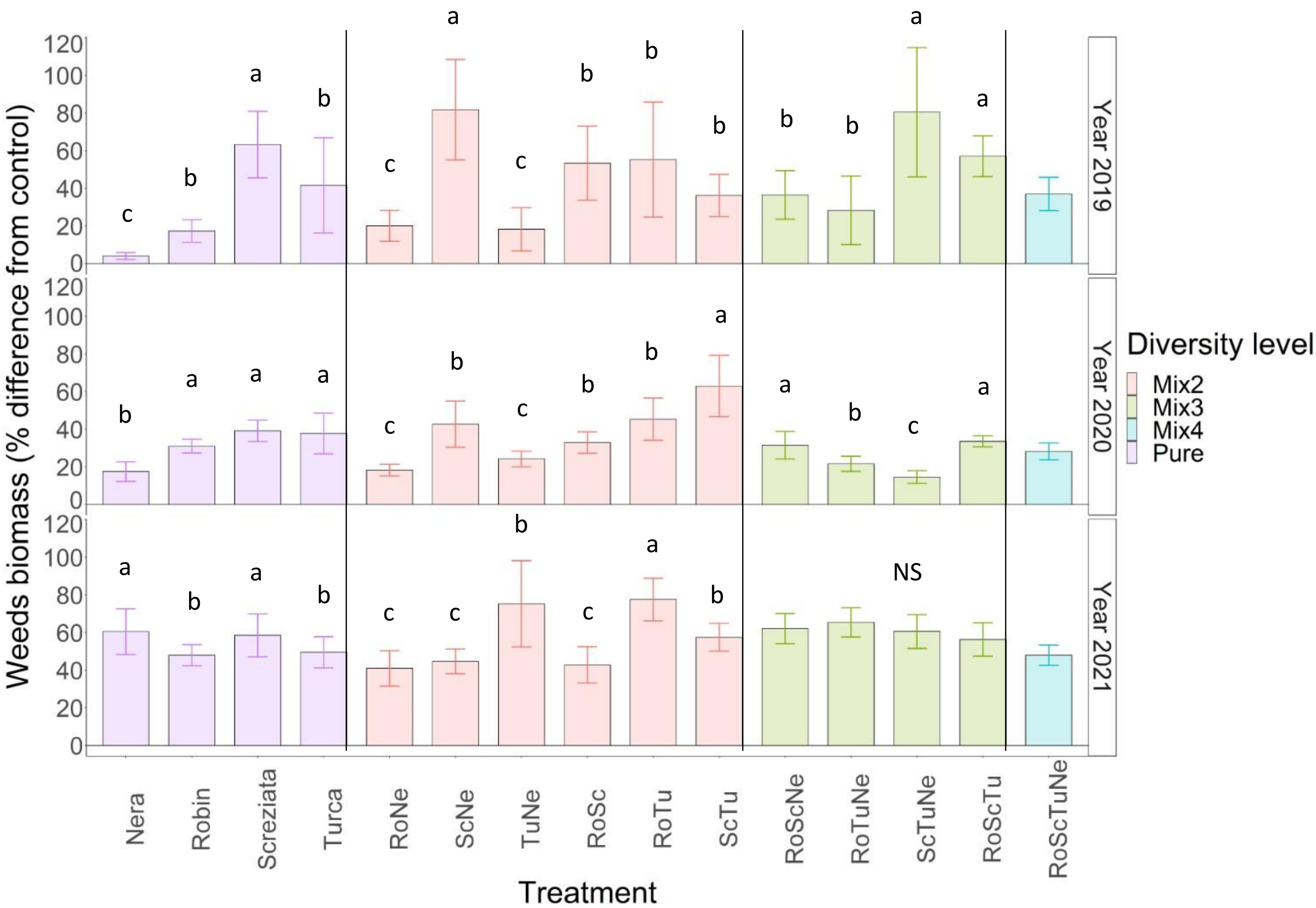

Figure 4. Weed biomass per cent differences from control in pure stands, mixtures of two cultivars, mixtures of three cultivars and mixture of four cultivars. Values are per cent differences from the control, calculated from the above-ground biomass dry weight: a value of 60% means that the weed biomass represents 60% of the yearly control. Different letters indicate statistical significance at the $p \leq 0.05$ level (Bonferroni-based method) among treatments. NS means no statistical significance with $p \leq 0.05$. Ne = Nera, Ro = Robin, Sc = Screziata, Tu = Turca.

Concerning contrasts, significant interactions emerged, but without a consistent pattern across years: the NeRo mixture had significantly more weeds than its components in 2019 and less in 2020 and 2021 (Table 2); while the NeSc mixture had more weed pressure than its components in 2019 and 2020, but less in 2021. The RoTu mixture instead showed an overall increase in weed pressure compared to its components every year. Finally, the NeRoTu mixture had more weeds than its components in 2019 and 2021 but less in 2020.

Table 2. Results of orthogonal contrasts for weed biomass for all lentil mixtures in the three experimental years; expressed as sensitivity ratios.

	Year 1			Year 2			Year 3		
Contrast	**Sensitivity Ratio**			**Sensitivity Ratio**			**Sensitivity Ratio**		
NeRo vs. 1/2 Ne + Ro	2.49	(0.45)	***	0.77	(0.11)	*	0.76	(0.07)	*
NeSc vs. 1/2 Ne + Sc	4.62	(0.67)	***	1.56	(0.17)	***	0.75	(0.07)	**
NeTu vs. 1/2 Ne + Tu	2.29	(0.42)	***	0.96	(0.12)		1.14	(0.09)	
RoSc vs. 1/2 Ro + Sc	1.50	(0.15)	***	0.94	(0.10)		0.81	(0.07)	*
RoTu vs. 1/2 Ro + Tu	2.47	(0.28)	***	1.34	(0.13)	*	1.59	(0.12)	***
ScTu vs. 1/2 Sc + Tu	1.11	(0.12)		1.60	(0.14)	***	1.07	(0.09)	
NeRoSc vs. 1/3 Ne + Ro + Sc	2.46	(0.32)	***	1.13	(0.12)		1.12	(0.08)	
NeRoTu vs. 1/3 Ne + Ro + Tu	2.76	(0.38)	***	0.79	(0.10)	*	1.25	(0.10)	*
NeScTu vs. 1/3 Ne + Sc + Tu	3.29	(0.39)	***	0.46	(0.07)	***	1.08	(0.08)	
RoScTu vs. 1/3 Ro + Sc + Tu	2.16	(0.19)	***	0.94	(0.09)		1.08	(0.08)	
RoScTuNe vs. 1/4 Ro + Sc + Tu + Ne	2.28	(0.26)	***	0.89	(0.10)		0.90	(0.07)	

The values represent the ratio between the weed sensitivity of the mixture and the weed sensitivity of its components (S.E.) Weed sensitivity is calculated by normalising the weed biomass of each treatment over the weed biomass of the control (without lentils). If the ratio is <1, the mixture has less weeds than its components, and if it is >1, the mixture has more weeds than its components. * indicates statistical significance at $p \leq 0.05$ level, ** at $p \leq 0.01$, *** at $p \leq 0.001$ (Estimated Marginal Means post hoc test) between the mixture yield and the average of its components. Ne = cv. Nera, Ro cv. = Robin, Sc = cv. Screziata, Tu = cv. Turca.

3.4. Grain Yield

The analysis of lentil grain yield resulted in a significant effect of treatment (χ^2 = 225.8), year (χ^2 = 61.4) and their interaction (χ^2 = 198.6); thus, we analysed each year separately.

Concerning yearly difference, 2021 showed a strong yield decrease for all treatments (−61% and −68% of the total yield average compared to years 1 and 2, respectively, Figure 5): this may be due to the drier season compared to the previous ones (−44% and −55% of precipitation compared to years 1 and 2, respectively (Figure 1).

Concerning the pure stands, certain differences between treatments were confirmed across years: cv. *Nera* yielded significantly less from cv. *Robin* and cv. *Turca* (average −30%, $p \leq 0.05$); also cv. *Screziata* produced less than cv. Robin and cv. Turca in 2019 and 2021 (average −40%, $p \leq 0.05$), with no differences from cv. *Nera*.

For two-cultivar mixtures, there were no clear trends in grain yield among treatments, especially due to the performances in the third year, which flattened the differences previously observed (Figure 5).

Concerning the interactions between mixtures and their components, we observed some effects of certain combinations, which, however, did not prove consistent across the years: the only consistent interaction effect was represented by the combination of cv. *Robin* and cv. *Turca*. When grown together, these cultivars performed either better or worse than the pure stands, according to environmental differences: worse in the first and third year and better in the second year (Table 3).

For three-cultivar mixtures, the yearly grain yield trend was comparable to that of two-cultivar mixtures, in that, in the third year, most of the differences were diluted. The only exception, with a slightly significant difference also in the third year (p = 0.071), is the combination of cv. *Robin*, cv. *Screziata* and cv. *Nera* against the combination of cv. Robin, cv. Screziata and cv. Turca, which had a better performance (Figure 5). We observed no interaction effects in three-cultivar mixtures (Table 3).

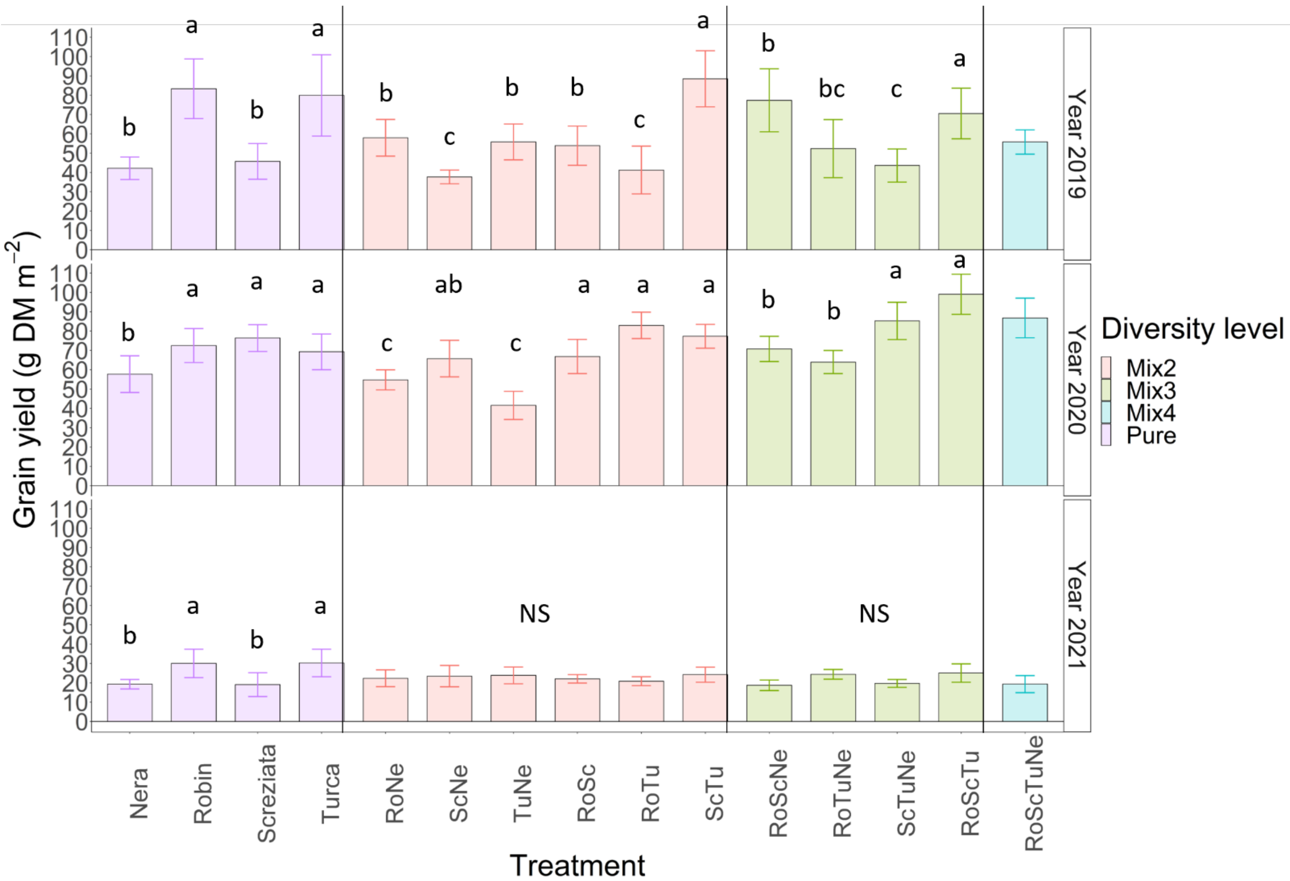

Figure 5. Average DW grain production in g/m2 for pure stands, mixtures of two cultivars, mixtures of three cultivars and mixture of four cultivars (Ne = Nera, Ro = Robin, Sc = Screziata, Tu = Turca). Different letters within each year indicate statistical significance at $p \leq 0.05$ level (Bonferroni-based method) among all treatments. Error bars represent the standard error of the mean (S.E.).

Table 3. The results of orthogonal contrasts for lentils grain yield for all lentil mixtures in the three experimental years; expressed as yield ratios.

	Year1			Year2			Year3		
Contrast	**Yield Ratio**			**Yield Ratio**			**Yield Ratio**		
NeRo vs. 1/2 Ne + Ro	0.98	(0.08)		0.83	(0.07)	*	0.93	(0.12)	
NeSc vs. 1/2 Ne + Sc	0.86	(0.08)		1.00	(0.08)		1.24	(0.16)	
NeTu vs. 1/2 Ne + Tu	1.02	(0.08)		0.64	(0.06)	***	0.99	(0.13)	
RoSc vs. 1/2 Ro + Sc	0.96	(0.08)		0.91	(0.07)		0.92	(0.12)	
RoTu vs. 1/2 Ro + Tu	0.54	(0.05)	***	1.14	(0.08)	*	0.71	(0.09)	**
ScTu vs. 1/2 Sc + Tu	1.56	(0.11)	***	1.09	(0.08)		1.01	(0.13)	
NeRoSc vs. 1/3 Ne + Ro + Sc	1.06	(0.08)		1.02	(0.07)		0.86	(0.11)	
NeRoTu vs. 1/3 Ne + Ro + Tu	0.83	(0.07)	*	0.93	(0.07)		0.91	(0.11)	
NeScTu vs. 1/3 Ne + Sc + Tu	0.89	(0.08)		1.32	(0.08)	***	0.88	(0.11)	
RoScTu vs. 1/3 Ro + Sc + Tu	1.09	(0.08)		1.37	(0.08)	***	0.97	(0.11)	
RoScTuNe vs. 1/4 Ro + Sc + Tu + Ne	0.96	(0.07)		1.28	(0.08)	***	0.82	(0.10)	

Values represent the ratio between the mixture yield and the average yield of the mixture components (S.E.). If the ratio is <1, the mixture performs worse than its components, and if it is >1, the mixture performs better than its components. * indicates statistical significance at $p \leq 0.05$ level, ** at $p \leq 0.01$, *** at $p \leq 0.001$ (Estimated Marginal Means post hoc test) between the mixture yield and the average of its components. Ne = cv. Nera, Ro cv. = Robin, Sc = cv. Screziata, Tu = cv. Turca.

Finally, concerning the four-cultivar mixture, we observed no consistency in the degree and type of interaction: not significant in the first year, strongly positive in the second ($p < 0.001$) and slightly negative in the third year ($p = 0.10$).

3.5. Yield Variability

The grain yield variability in lentils was studied in terms of standard error. Due to the unbalanced design, we could not use the values of the standard error per se; thus, with the terms "variability" and "standard error", we refer to the output of the linear model.

Grain yield variability in the two-cultivar mixtures and in all mixtures was significantly lower (at $p \leq 0.10$) than in pure stands, whereas no significant differences were found in any other contrast (Table 4).

Table 4. Results of orthogonal contrasts between grain yield variability in the different types of lentil canopies (pure stands or mixture types), expressed in terms of the standard error of the mean (see text for further explanation).

Contrasts	SE Means	*p*-Value
Two-cultivar mixtures vs. pure stands	7.37–9.55	0.0629 (*)
Three-cultivar mixtures vs. pure stands	8.53–9.55	0.4137
Four-cultivar mixture vs. pure stands	7.46–9.55	0.2928
Two- vs. three-cultivar mixtures	7.37–8.53	0.3133
Any mixtures vs. pure stands	7.78–9.55	0.0964 (*)

(*) indicates a significant difference at $p \leq 0.10$ (Estimated Marginal Means post hoc test).

Further considerations of the variability of the standard error within each diversity level are displayed in Appendix A (Figure A1).

4. Discussion

4.1. Nodules

Studies on the effect of cultivar mixtures on nodulation are lacking: mixtures of non-nodulating and nodulating soyabeans were observed in terms of nutrient acquisition but not in terms of nodule development [26]. However, the influence of legume cultivars on nodulation and nitrogen fixation is widely recognised [27–31], even though the underlying mechanisms are not clear. Due to the lack of literature and the structure of the trial (non-specific mixture design [6]), we could not select lentil cultivars with different rhizobia affinity, but such an approach may be adopted in a specific experiment. Our trial focused on agronomic traits, and the nodule number was recorded only to identify possible correlations with agronomic performances.

We observed an effect of cultivars on nodulation, but we could not identify any effect related to mixtures, except for the slight significance of the TuNe mixture that surprisingly produced more nodules than its components ($p = 0.07$). This being an isolated case, we cannot drive any general conclusions from this phenomenon: we rather suggest refining observations in further studies, taking into account other parameters related to rhizobia symbiosis efficiency beyond nodule number.

It is likely that the higher nodule number in cv. *Nera* influenced its biomass, or vice versa [32,33]. Additionally, cv. *Nera* registered the largest height, although not significantly different from the other cultivars. It is interesting to note that the correlation between shoot length and nodulation was highlighted in peas [34]. However, apparently, the underlying mechanisms of such behaviour could not influence the companion cultivars, with a slight exception for the case of the TuNe mixture.

It is very likely that weather conditions were the main driver influencing yearly changes in nodule numbers in our trial: 2021 was the driest of the three years as well as the least productive in terms of nodule numbers, in accordance with Sigh et al. and Mwamilima et al. [35,36], who demonstrated the positive correlation between nodulation and soil moisture.

Finally, sandy soil may have inhibited the expression of nodulation capacity [37,38], flattering the performances of the different mixtures; therefore, we suggest deepening the analysis of such dynamics in soils that better sustain nodulation.

We were not able to find any correlation between nodulation and yield, but nodulation dynamics should not be discarded as a possible driver of lentil mixture behaviour: in further studies, we suggest also taking into account nodules' vitality and weight, as well as nutrient content and the amount of biologically-fixed nitrogen.

4.2. Weeds and Lentil Biomass

Weeds have always been a major threat to lentil cultivation due to the low competitive ability of the crop; weed management is, therefore, a crucial practice in lentil production, but herbicides are often toxic to the crop, besides their environmental impact [39–42]. We investigated lentil mixtures as a possible means to increase competition against weeds, but in our study, weed behaviour was erratic: in spite of the detection of several significant differences among treatments, we were not able to identify any clear pattern in weed biomass differences. Apparently, the spatial differences in weed pressure across the field were stronger than the effect of the treatments.

The yearly pattern of weed biomass, though, is quite clear and mirrors that of lentil biomass, grain yield and nodule number: the lowest difference in weed biomass with the control was registered in 2021 when lentil performance was the poorest. To understand whether it was the higher weed pressure to outcompete lentils or the worst lentil establishment that allowed for more weeds to grow, we can observe the yearly weed biomass in the control plots. Weed biomass in control plots (where no lentil was seeded) was on average 211 g m^{-2} in 2019, 295 g m^{-2} in 2020 and 239 g m^{-2} in 2021: we could not see a substantial increase in 2021, the most difficult year. It is then likely that the reason for high weed biomass is the lower vigour and competitiveness of lentils, which are strongly affected by unfavourable weather conditions [43].

To conclude, we could not identify the effect of the cultivar mixtures on weed competition.

4.3. Grain Yield and Variability

From wheat to rice to maize and soybeans, there are several pieces of evidence of mixtures' advantages in terms of pathogen control [44–46] and increase in yield amount, stability and quality [47,48]. However, in some cases, cultivar mixtures were not proven successful due to the lack of knowledge of their performances and/or the underlying mechanisms [6,21].

Concerning cultivar interactions in mixtures, in our experiment, we observed equal tendencies towards antagonism and synergy: across the years, five mixtures significantly outperformed the pure stands, and five mixtures were outperformed by the pure stands. In 2020, we registered the highest occurrence of overyielding: during that cropping season, rainfall and temperature were at optimal levels for lentil development. Only one combination showed a consistent interaction over the three years, RoTu, but the type of interaction effect varied (in 2019, RoTu yielded less than its pure stand components, in 2020, more and, in 2021, less again, challenging our understanding of the phenomenon). The ScTu mixture, even if not always significant, outperformed its pure stands components in each of the three years. Although we cannot draw general conclusions from this behaviour, we may suggest RoTu as a successful lentil mixture for sandy soils in Tuscany.

Regarding total grain yield, again, the highest performances were registered in 2020, probably due to the mild temperature and even rain distribution, especially at pod development [49]. The three-cultivar mixtures produced significantly better than pure stands and two-cultivar mixtures, and the yield of the four-cultivar mixture was even higher. The good performance of the three and four-cultivar mixtures in 2020 is partially in contrast with the literature, where the occurrence of overyielding has been observed, especially under stress conditions [48,50,51]. We will try to explain the phenomenon in the next paragraphs.

Pure stands generally yielded slightly higher than mixtures (even though not significantly), but they also showed a higher overall yield variability in terms of the mean standard error. However, when considered individually, only the two-cultivar mixtures had significantly more stable yields than the pure stands ($p \leq 0.10$), an effect that likely contributed to the significant effect of all mixtures vs. pure stands comparison. At this stage of the study, the result may support the use of lentil cultivar mixtures, but only with a lack of cultivars adapted to specific growing conditions.

In our study, the low number of cultivars may explain the lack of the expected overyielding: it was proven that the higher the number of cultivars composing a mixture, the higher the benefits [6,52]. Similarly, mixtures designed according to specific cultivar characteristics were proven more productive by some authors [48,53,54]. Unfortunately, the Italian seed market experiences a lack of diverse genetic material, the consequence of a lack of breeding programmes and dedicated research for grain legumes compared to cereals [20,55]. Therefore, we had limited ground for designing cultivar mixtures.

Furthermore, against our expectations, cultivar mixtures behaved very similarly under high environmental stress conditions (i.e., the harsh climate in 2021), reinforcing our hypothesis of high genetic homogeneity in the available cultivars. We think that this may be a crucial obstacle to design effective lentil mixtures with a trait-blind approach. To overcome this problem, the use of local landraces may be considered as a source of trait variability to test a trait-based approach to cultivar mixture design.

5. Conclusions

Lentil cultivar mixtures designed upon a trait-blind approach showed inconsistent results. Nodule number was mainly driven by cultivar (in relative terms) and weather (in absolute terms); we did not observe overyielding. Though, nodulation dynamics in mixtures still comprise many unexplored aspects: in future studies, we suggest considering nodules' vitality and weight, as well as nutrient content and biologically-fixed nitrogen. Conversely, weed biomass showed significant differences among treatments, but such differences have been proven to be caused by spatial differences rather than cultivars. The average yield stability of all mixtures was significantly higher than pure stands over the three years, but the individual mixtures equally outperformed or were outperformed by the pure stands. Against our expectations, the mixtures showed the most advantages in the most productive year, flattering their performances under environmental stress. The most likely reason for this lies in the presumed low genetic diversity of commercial lentil lines in Italy. In order to sidestep underperformance and benefit from the higher yield stability in mixtures, we suggest designing mixtures of at least three cultivars. In addition, we encourage further research to take into account the diversity of Italian lentil landraces so that higher genetic diversity could allow the implementation of a trait-based approach.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

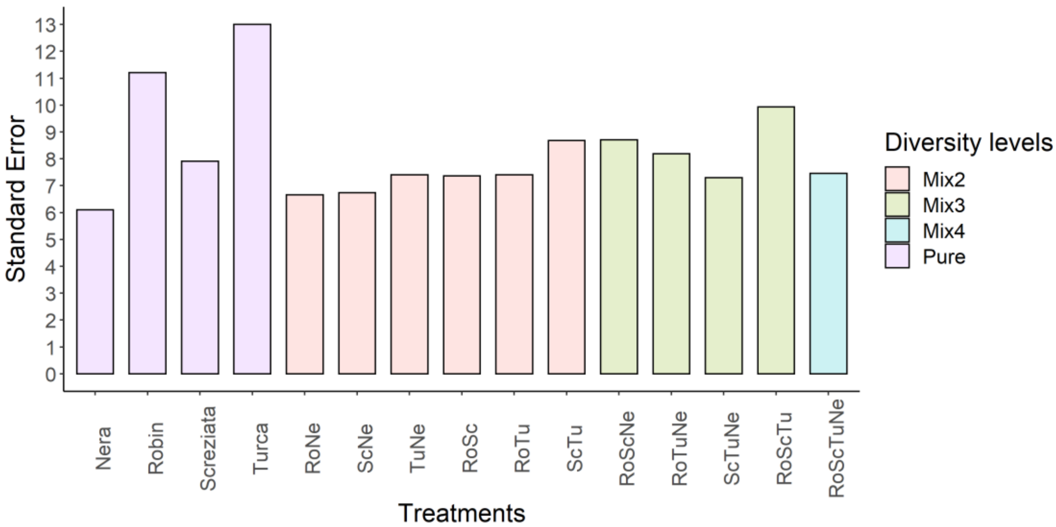

Figure A1. Average standard error (expressed as the output of the linear model) for each treatment over the three years of the experiment (Ne = Nera, Ro = Robin, Sc = Screziata, Tu = Turca).

The standard error of the pure stands has a higher volatility than that of the mixtures. In detail, the standard error of the pure stands spans from 6 to 13, whereas that of the mixture of two cultivars is from 6.6 to 8.6, one of the mixtures of three cultivars from 7.3 to 9.9, finally, the mixture of four cultivars shows a value of 7.4.

These values provide information on the reliability of mixtures' performance (in terms of yield stability) compared to the pure stands.

References

1. Bommarco, R.; Kleijn, D.; Potts, S.G. Ecological intensification: Harnessing ecosystem services for food security. *Trends Ecol. Evol.* **2013**, *28*, 230–238. [CrossRef] [PubMed]
2. Bàrberi, P.; Moonen, A.C. Functional biodiversity for the provision of agroecosystem services. In *Reconciling Agricultural Production with Biodiversity Conservation*, 1st ed.; Bàrberi, P., Moonen, A.C., Eds.; Burleigh Dodds Science Publishing: London, UK, 2020; pp. 101–146.
3. Altieri, M.A. *Agroecology: The Science of Sustainable Agriculture*, 2nd ed.; CRC Press: Boca Raton, FL, USA, 2018; ISBN 0-429-96401-3.
4. Gliessman, S.R. Agroecology: Researching the Ecological Basis for Sustainable Agriculture. In *Agroecology. Ecological Studies*; Gliessman, S.R., Ed.; Springer: New York, NY, USA, 1990; Volume 78. [CrossRef]
5. Tamburini, G.; Bommarco, R.; Wanger, T.C.; Kremen, C.; van der Heijden, M.G.A.; Liebman, M.; Hallin, S. Agricultural diversification promotes multiple ecosystem services without compromising yield. *Sci. Adv.* **2020**, *6*, eaba1715. [CrossRef] [PubMed]
6. Wuest, S.E.; Peter, R.; Niklaus, P.A. Ecological and evolutionary approaches to improving crop variety mixtures. *Nat. Ecol. Evol.* **2021**, *5*, 1068–1077. [CrossRef] [PubMed]
7. Hoebe, P.; Hoad, S.; Creissen, H.; Scott, P.; Dawson, I.; Watson, C. Overview SRUC related research on breeding for diversity. In Proceedings of the Symposium on Breeding for Diversification, University of Kassel, Witzenhausen, Germany, 19–21 February 2018.
8. Finckh, M.R.; Mundt, C.C. Stripe rust, yield, and plant competition in wheat cultivar mixtures. *Phytopathology* **1992**, *82*, 905–913. [CrossRef]
9. Wolfe, M.S. The current status and prospects of multiline cultivars and cultivar mixtures for disease resistance. *Annu. Rev. Phytopathol.* **1985**, *23*, 251–273. [CrossRef]

10. Kiær, L.P.; Skovgaard, I.M.; Østergård, H. Effects of inter-varietal diversity, biotic stresses and environmental productivity on grain yield of spring barley cultivar mixtures. *Euphytica* **2012**, *185*, 123–138. [CrossRef]
11. Barot, S.; Allard, V.; Cantarel, A.; Enjalbert, J.; Gauffreteau, A.; Goldringer, I.; Lata, J.-C.; LE Roux, X.; Niboyet, A.; Porcher, E. Designing mixtures of varieties for multifunctional agriculture with the help of ecology. A review. *Agron. Sustain. Dev.* **2017**, *37*, 13. [CrossRef]
12. Garibaldi, L.A.; Andersson, G.K.; Requier, F.; Fijen, T.P.; Hipólito, J.; Kleijn, D.; Pérez-Méndez, N.; Rollin, O. Complementarity and synergisms among ecosystem services supporting crop yield. *Glob. Food Secur.* **2018**, *17*, 38–47. [CrossRef]
13. Zhang, W.; Liu, G.; Sun, J.; Fornara, D.; Zhang, L.; Zhang, F.; Li, L. Temporal dynamics of nutrient uptake by neighbouring plant species: Evidence from intercropping. *Funct. Ecol.* **2016**, *31*, 469–479. [CrossRef]
14. Kristoffersen, R.; Jørgensen, L.N.; Eriksen, L.B.; Nielsen, G.C.; Kiær, L.P. Control of *Septoria tritici* blotch by winter wheat cultivar mixtures: Meta-analysis of 19 years of cultivar trials. *Field Crop. Res.* **2020**, *249*, 107696. [CrossRef]
15. Van Der Plas, F.; Schröder-Georgi, T.; Weigelt, A.; Barry, K.; Meyer, S.; Alzate, A.; Barnard, R.L.; Buchmann, N.; De Kroon, H.; Ebeling, A.; et al. Plant traits alone are poor predictors of ecosystem properties and long-term ecosystem functioning. *Nat. Ecol. Evol.* **2020**, *4*, 1602–1611. [CrossRef] [PubMed]
16. Murphy, D.J. *People, Plants & Genes: The Story of Crops and Humanity*; Oxford University Press on Demand: Oxford, UK, 2007.
17. Laghetti, G.; Piergiovanni, A.R.; Sonnante, G.; Lioi, L.; Pignone, D. The Italian lentil genetic resources: A worthy basic tool for breeders. *Eur. J. Plant. Sci. Biotechnol.* **2008**, *2*, 48–59.
18. Crepaldi, G. Il 98% Delle Lenticchie e il 95% dei Fagioli Secchi è Importato. 2018. Available online: https://ilfattoalimentare.it/legumi-secchi-importazione.html (accessed on 14 July 2022).
19. Watson, C.A.; Reckling, M.; Preissel, S.; Bachinger, J.; Bergkvist, G.; Kuhlman, T.; Lindström, K.; Nemecek, T.; Topp, C.F.; Vanhatalo, A.; et al. Grain Legume Production and Use in European Agricultural Systems. *Adv. Agron.* **2017**, *144*, 235–303.
20. Ballén-Taborda, C.; Samoluck, S.S.; Podio, M. Special Issue "Advances in Research for Legume Breeding and Genetics". 2020. Available online: https://www.mdpi.com/journal/plants/special_issues/legume_breed (accessed on 14 July 2022).
21. Haghshenas, A.; Emam, Y.; Sepaskhah, A.R.; Edalat, M. Can extended phenology in wheat cultivar mixtures mitigate post-anthesis water stress? *Eur. J. Agron.* **2021**, *122*, 126188. [CrossRef]
22. Grettenberger, I.M.; Tooker, J.F. Cultivar mixtures of soybeans have inconsistent effects on herbivore and natural-enemy populations. *Agric. Ecosyst. Environ.* **2020**, *292*, 106835. [CrossRef]
23. Dobert, R.C.; Blevins, D.G. Effect of seed size and plant growth on nodulation and nodule development in lima bean (*Phaseolus lunatus L.*). *Plant Soil* **1993**, *148*, 11–19. [CrossRef]
24. Davis, T.M. Linkage relationships of genes for leaf morphology, flower color, and root nodulation in chickpea. *Euphytica* **1991**, *54*, 117–123. [CrossRef]
25. R Core Team. R. The R Project for Statistical Computing. Available online: https://www.R-project.org/ (accessed on 14 July 2022).
26. Burton, J.W.; Brim, C.A.; Rawlings, J.O. Performance of Non-Nodulating and Nodulating Soybean Isolines in Mixed Culture with Nodulating Cultivars. *Crop Sci.* **1983**, *23*, 469–473. [CrossRef]
27. Payakapong, W.; Tittabutr, P.; Teaumroong, N.; Boonkerd, N. Soybean cultivars affect nodulation competition of Bradyrhizobium japonicum strains. *World J. Microbiol. Biotechnol.* **2004**, *20*, 311–315. [CrossRef]
28. Hafeez, F.Y.; Asad, S.; Malik, K.A. The effect of high temperature on *Vigna radiata* nodulation and growth with different bradyrhizobial strains. *Environ. Exp. Bot.* **1991**, *31*, 285–294. [CrossRef]
29. Abi-Ghanem, R.; Carpenter-Boggs, L.; Smith, J.L. Cultivar effects on nitrogen fixation in peas and lentils. *Biol. Fertil. Soils* **2010**, *47*, 115–120. [CrossRef]
30. Hungria, M.; Bohrer, T.R.J. Variability of nodulation and dinitrogen fixation capacity among soybean cultivars. *Biol. Fertil. Soils* **2000**, *31*, 45–52. [CrossRef]
31. Sharaf, H.; Rodrigues, R.R.; Moon, J.; Zhang, B.; Mills, K.; Williams, M.A. Unprecedented bacterial community richness in soybean nodules vary with cultivar and water status. *Microbiome* **2019**, *7*, 1–18. [CrossRef] [PubMed]
32. Pereyra, G.; Hartmann, H.; Michalzik, B.; Ziegler, W.; Trumbore, S. Influence of Rhizobia Inoculation on Biomass Gain and Tissue Nitrogen Content of *Leucaena leucocephala* Seedlings under Drought. *Forests* **2015**, *6*, 3686–3703. [CrossRef]
33. Hazra, K.K.; Nath, C.P.; Singh, S.S.; Swain, D.K.; Kumar, N.; Das, K.; Lamichaney, A. Categorization of Chickpea Nodules and Their Relation with Plant Growth. *Natl. Acad. Sci. Lett.* **2020**, *44*, 91–95. [CrossRef]
34. Ludidi, N.N.; Pellny, T.K.; Kiddle, G.; Dutilleul, C.; Groten, K.; Van Heerden, P.D.R.; Dutt, S.; Powers, S.J.; Römer, P.; Foyer, C.H. Genetic variation in pea (*Pisum sativum* L.) demonstrates the importance of root but not shoot C/N ratios in the control of plant morphology and reveals a unique relationship between shoot length and nodulation intensity. *Plant Cell Environ.* **2007**, *30*, 1256–1268. [CrossRef]
35. Singh, K.K.; Srinivasarao, C.; Ali, M. Root Growth, Nodulation, Grain Yield, and Phosphorus Use Efficiency of Lentil as Influenced by Phosphorus, Irrigation, and Inoculation. *Commun. Soil Sci. Plant Anal.* **2005**, *36*, 1919–1929. [CrossRef]
36. Mwamlima, L.H.; Ouma, J.P.; Cheruiyot, E.K. Soybean (*Glycine max* (L) Merrill) Root Growth and Nodulation Responses to Different Soil Moisture Regimes. *J. Crop Sci. Biotechnol.* **2019**, *22*, 153–159. [CrossRef]
37. Issa, S.; Wood, M. Multiplication and survival of chickpea and bean rhizobia in dry soils: The influence of strains, matric potential and soil texture. *Soil Biol. Biochem.* **1995**, *27*, 785–792. [CrossRef]

38. Uaboi-Egbenni, P.; Okolie, P.; Okafor, C.; Akinyemi, O.; Bisi-Johnson, M.; Teniola, O. Effect of soil types and mixtures on nodulation of some beans and groundnut varieties. *Afr. J. Food Agric. Nutr. Dev.* **2010**, *10*, 2277. [CrossRef]
39. Al-Ahmed, M.J. *Competitive Interrelation and Impact of Weeds on Soil Moisture and Yield of Lentils in Dry Regions*; Arab Center for the Studies of Arid Zones and Dry Lands: Damascus, Syria, 1982.
40. Al Thahabi, S.A. Weed Control in Lentils. 1991. Available online: https://agris.fao.org/agris-search/search.do?recordID=JO9300053 (accessed on 14 July 2022).
41. Ghosheh, H.Z.; El-Shatnawi, M.K. Broadleaf weed control in chickpeas (*Cicer arietinum*), faba beans (*Vicia faba*) and lentils (*Lens culinaris*). *Acta Agron. Hung.* **2003**, *51*, 437–444. [CrossRef]
42. Yang, T.; Liu, K.; Poppy, L.; Mulenga, A.; Gampe, C. Minimizing Lentil Harvest Loss through Improved Agronomic Practices in Sustainable Agro-Systems. *Sustainability* **2021**, *13*, 1896. [CrossRef]
43. Sehgal, A.; Sita, K.; Kumar, J.; Kumar, S.; Singh, S.; Siddique, K.H.M.; Nayyar, H. Effects of Drought, Heat and Their Interaction on the Growth, Yield and Photosynthetic Function of Lentil (*Lens culinaris* Medikus) Genotypes Varying in Heat and Drought Sensitivity. *Front. Plant Sci.* **2017**, *8*, 1776. [CrossRef] [PubMed]
44. McDonald, B.A.; Allard, R.W.; Webster, R.K. Responses of two-, three-, and four-component barley mixtures to a variable pathogen population. *Crop Sci.* **1988**, *28*, 447–452. [CrossRef]
45. Xu, X.-M.; Ridout, M.S. Stochastic simulation of the spread of race-specific and race-nonspecific aerial fungal pathogens in cultivar mixtures. *Plant Pathol.* **2000**, *49*, 207–218. [CrossRef]
46. Mundt, C.C. Use of multiline cultivars and cultivar mixtures for disease management. *Annu. Rev. Phytopathol.* **2002**, *40*, 381–410. [CrossRef]
47. Newton, A.; Begg, G.; Swanston, J. Deployment of diversity for enhanced crop function. *Ann. Appl. Biol.* **2009**, *154*, 309–322. [CrossRef]
48. Reiss, E.R.; Drinkwater, L. Cultivar mixtures: A meta-analysis of the effect of intraspecific diversity on crop yield. *Ecol. Appl.* **2017**, *28*, 62–77. [CrossRef]
49. Shrestha, R.; Turner, N.; Siddique, K.; Turner, D.W.; Speijers, J. A water deficit during pod development in lentils reduces flower and pod numbers but not seed size. *Aust. J. Agric. Res.* **2006**, *57*, 427–438. [CrossRef]
50. Kaut, A.H.E.E.; Mason, H.E.; Navabi, A.; O'Donovan, J.T.; Spaner, D. Performance and stability of performance of spring wheat variety mixtures in organic and conventional management systems in western Canada. *J. Agric. Sci.* **2008**, *147*, 141–153. [CrossRef]
51. Chen, H.; Nguyen, K.; Iqbal, M.; Beres, B.L.; Hucl, P.J.; Spaner, D. The performance of spring wheat cultivar mixtures under conventional and organic management in Western Canada. *Agrosyst. Geosci. Environ.* **2020**, *3*, e20003. [CrossRef]
52. Qin, X.; Li, Y.; Shi, C.; Song, D.; Wen, X.; Liao, Y.; Siddique, K.H.M. The number of cultivars in varietal winter-wheat mixtures influence aboveground biomass and grain yield in North China. *Plant Soil* **2019**, *439*, 131–143. [CrossRef]
53. Fletcher, A.; Ogden, G.; Sharma, D. Mixing it up—Wheat cultivar mixtures can increase yield and buffer the risk of flowering too early or too late. *Eur. J. Agron.* **2019**, *103*, 90–97. [CrossRef]
54. Lowry, C.J.; Bosworth, S.C.; Goslee, S.C.; Kersbergen, R.J.; Pollnac, F.W.; Skinner, R.H.; Warren, N.D.; Smith, R.G. Effects of expanding functional trait diversity on productivity and stability in cultivar mixtures of perennial ryegrass. *Agric. Ecosyst. Environ.* **2019**, *287*, 106691. [CrossRef]
55. Pachico, D. Towards Appraising the Impact of Legume Research: A Synthesis of Evidence. Rome, Italy, Standing Panel on Impact Assessment (SPIA), CGIAR Independent Science and Partnership Council (ISPC) 2014. Available online: https://cas.cgiar.org/sites/default/files/pdf/Legumes_Pachico-2014.pdf (accessed on 26 August 2022).