

Agronomic and nutritional performance of lentil–hull-less barley mixtures under temperate Swiss conditions: protein content, protein yield stability, β -glucan content and separation ability

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Abstract

BACKGROUND: Intercropping of legumes with cereals enhances resilience, resource efficiency, and nutritional quality in food systems, yet few studies have integrated yield stability with grain quantity and post-harvest processability. Lentils (*Lens culinaris* Medik.) and hull-less barley (*Hordeum vulgare* L. var. *nudum*) represent a promising mixture for temperate climates. Here, we provide the first multi-year evaluation of protein content, protein yield stability, β -glucan concentration, and grain separation, across 3 years (2022–2024), two sites, and 22 treatments.

RESULTS: Mixed stands increased barley protein content by 18–26 g kg^{−1} dry matter (DM) compared with pure unfertilized and fertilized systems, whereas lentil protein content was marginally higher in pure stands. Mixed systems buffered environmental variability (Finlay–Wilkinson $b_i \approx 1.1$), with variability substantially lower (CV = 38%) than in pure barley stands (63% CV), approaching that of pure lentil stability (29% CV). The lentil cultivar Beluga paired with the barley cultivar AF Cesar achieved the highest protein yield stability, recording a weighted absolute score of 94.5 via the AMMI (additive main effects and multiplicative interaction) yield stability index. β -glucan content (17–76 g kg^{−1} DM) was highest in fertilized pure AF Cesar and was influenced by year and site. Grain separation showed partially high impurity levels (>1%), due to similar seed sizes, but mixtures with contrasting seed sizes (e.g., Beluga–AF Cesar) improved separation ($r = 0.38$).

CONCLUSION: Intercropping of lentils with barley increases barley protein content and yield stability under Swiss conditions, providing the first evidence of effects on β -glucan content and seed-size-driven impurity reduction. Selecting cultivar pairs with contrasting seed sizes and optimizing sowing time can improve both agronomic and post-harvest performance in legume–cereal systems.

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Supporting information may be found in the online version of this article.

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INTRODUCTION

Climate change poses a serious threat to the productivity of agriculture.¹ At the European level, these changes challenge conventional farming systems and highlight the need for resilient, resource-efficient and ecologically sustainable agricultural practices.² In parallel, growing attention is being directed towards nutritional diversity, both in terms of diet composition and the range of nutrients required to sustain a healthy life.^{3,4}

Cropping systems must, therefore, meet several objectives: advancing sustainability goals while ensuring nutritional stability

and market relevance.⁴ Crop diversification offers one pathway to achieve these objectives. There is evidence that crop diversity

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can contribute to human nutrition by including species that provide distinct and complementary nutrients.^{5,6} One example of crop diversification is intercropping or mixed cropping, which is the simultaneous cultivation of two or more species in the same space at the same time.^{7,8}

Legumes have been shown to improve the agronomic, ecological, and nutritional performance in an agricultural system.^{9–12} Furthermore, legumes are well suited to intercropping systems, which are also known to improve yield stability,¹³ reduce chemical inputs,¹⁴ and enhance biodiversity.¹⁵ In fact, legume–cereal mixtures can optimize land use efficiency¹⁶ and contribute to protein-rich, sustainable diets. The local production of plant-based protein food represents a concrete pathway to fulfil the increasing demand for plant-based protein products, as well as reducing import and greenhouse gas emissions. Recent research by Keller et al.¹⁷ suggests that Swiss agriculture has significant potential to shift from livestock- and fodder-intensive systems towards small-grain legumes for food purposes. Such a transition could replace up to 41% of animal-based protein consumption with plant-derived alternatives, thereby substantially reducing the sector's environmental footprint.¹⁷

Lentils (*Lens culinaris* Medik.) and hull-less barley (*Hordeum vulgare* L. var. *nudum*) are two food crops that are highly resilient to rising temperatures associated with climate change,¹⁸ together they represent a promising legume–cereal combination.^{19–22} Lentils offer important components for human nutrition such as high protein content, minerals, and vitamins,^{23,24} while agronomically contributing to soil fertility through biological nitrogen fixation.²² Barley is highly nutritive, given its high content of the soluble fiber β -glucan²⁵ and antioxidants,²⁶ which has driven growing interest in its use as a functional grain in health-oriented food markets.²⁷ Unlike common barley (hulled barley), which is used mostly as feed or in brewing as malt, hull-less barley has the advantage of allowing the whole grain to be used without pearling away the nutritious bran layer, qualifying it for whole-grain labeling.^{28,29} In addition to providing nutritional benefits to consumers, this crop allows farmers to save on pearling costs.

Apart from the well-known improved agronomic aspects, mixing legumes with cereals has demonstrated complementarity in the source of nutrients they provide.^{30–32} Reviews and food development studies have repeatedly demonstrated that legume–cereal mixtures improve amino acid balance, protein quality and, at times, micronutrient density.^{30,31,33}

Studies on lentil–barley intercrops have so far focused on agronomic performance under temperate conditions,^{19–22} and fewer investigations have addressed nutritional quality.^{21,34} To our knowledge, no study has jointly evaluated the effect of mixed cropping on protein content, protein yield stability, β -glucan content, and separation abilities of grains after harvest, comparing responses from pure and mixed cropping systems.

To fill these gaps in a single field-based evaluation, we present here the first multi-year, multi-site assessment on lentil–hull-less barley mixtures in Switzerland, simultaneously addressing protein content, protein yield stability, β -glucan, and post-harvest separability. We hypothesized that mixed cropping between lentil and hull-less barley will:

- (1) increase protein content in hull-less barley and improve yield stability. Earlier studies reported higher cereal protein content under intercropping of legumes,^{21,35} with improved grain³⁶ and protein yield⁹ together with greater dry grain yield stability;³⁷

- (2) enhance β -glucan content. Variation in β -glucan has been linked to genotype, fertilization, and environment,^{38–40} but the effect of mixed systems is not known;
- (3) improve post-harvest separation. Although sorting efficiency is critical for marketable quality, existing work focuses on physical/engineering aspects.⁴¹

MATERIAL AND METHODS

Site and environmental conditions

Experiments were carried out from 2022 to 2024 in three consecutive seasons at two sites: Agroscope Reckenholz (Zürich; 444 m above sea level) and Agroscope Tänikon (Aadorf; 539 m above sea level). Different fields were selected each year at both sites, resulting in a total of six environments. Coordinates, soil type classification (World Reference Base)⁴², previous crops and site-specific field operations for each experiment are provided in the Supporting Information (Table S1). Throughout the experimental years, Reckenholz experienced overall higher temperatures and lower precipitation than Tänikon (Fig. 1; Supporting Information, Tables S2 and S3).

Experimental design and data collection

For all years and sites, a randomized complete block design with four blocks was employed. The study included four lentil (*Lens culinaris*) cultivars and three spring hull-less barley (*Hordeum vulgare* var. *nudum*) cultivars. The lentil cultivars were Anicia, Beluga, Château Linsen, and Grüne Berry; and the early-maturing spring hull-less barley cultivars were AF Cesar, Goljiat, and Oak Ruby. Cultivar properties (seed size, color, and heading time) are summarized in Table 1. Each individual plot measured 9 m² (1.5 m $\times$ 6 m), and a 1.00 $\pm$ 0.05 m buffer zone was mown between plots to reduce inter-plot interference, resulting in an effective harvested area of around 6.8–7.3 m². Agronomic management was identical for all plots, sites and years; year-specific management details are presented in Supporting Information, Table S1. Sowing was carried out simultaneously, in single rows for both crops in mixed cropping, at a depth of 1.5–3 cm, depending on soil conditions. No herbicides, fungicides, or insecticides were applied during the growing season.

Each crop was cultivated both as a pure crop and in mixtures. All stands had a planting density standardized at 240 plants m⁻², with pure cropping representing 100% of the species and mixtures sown at a 3:1 lentil-to-barley ratio (Table 1), as previously adopted by Rehak et al.⁴⁴ Pure crop barley treatments were established with and without fertilizers. Fertilized treatments received a single application of mineral nitrogen fertilizer (N = 27%; Mg = 2.5%; LANDOR, fenaco Genossenschaft; Birsfelden, Switzerland), following Swiss fertilization guidelines to achieve a target of 90 kg ha⁻¹ N. The amount added per plot ranged between 12 and 16 g m⁻².

The 22 resulting treatment combinations are listed in Table 1. To minimize border effects, all treatments were established in triplicate adjacent plots, with only the central plot used for data collection. Harvesting was performed on the central plots using a small-scale experimental combine (Wintersteiger, Ried, Austria) and grains were dried to a moisture content of 12%. Seeds were then cleaned using a gravity separator and, for mixed cropping treatments, further separated using a small-scale indented cylinder separator (model LA-T, Westrup, Slagelse, Denmark) with 5.5 mm pockets. Total yield (t ha⁻¹), thousand-kernel weight (TKW) and seed volume were determined using an automatic

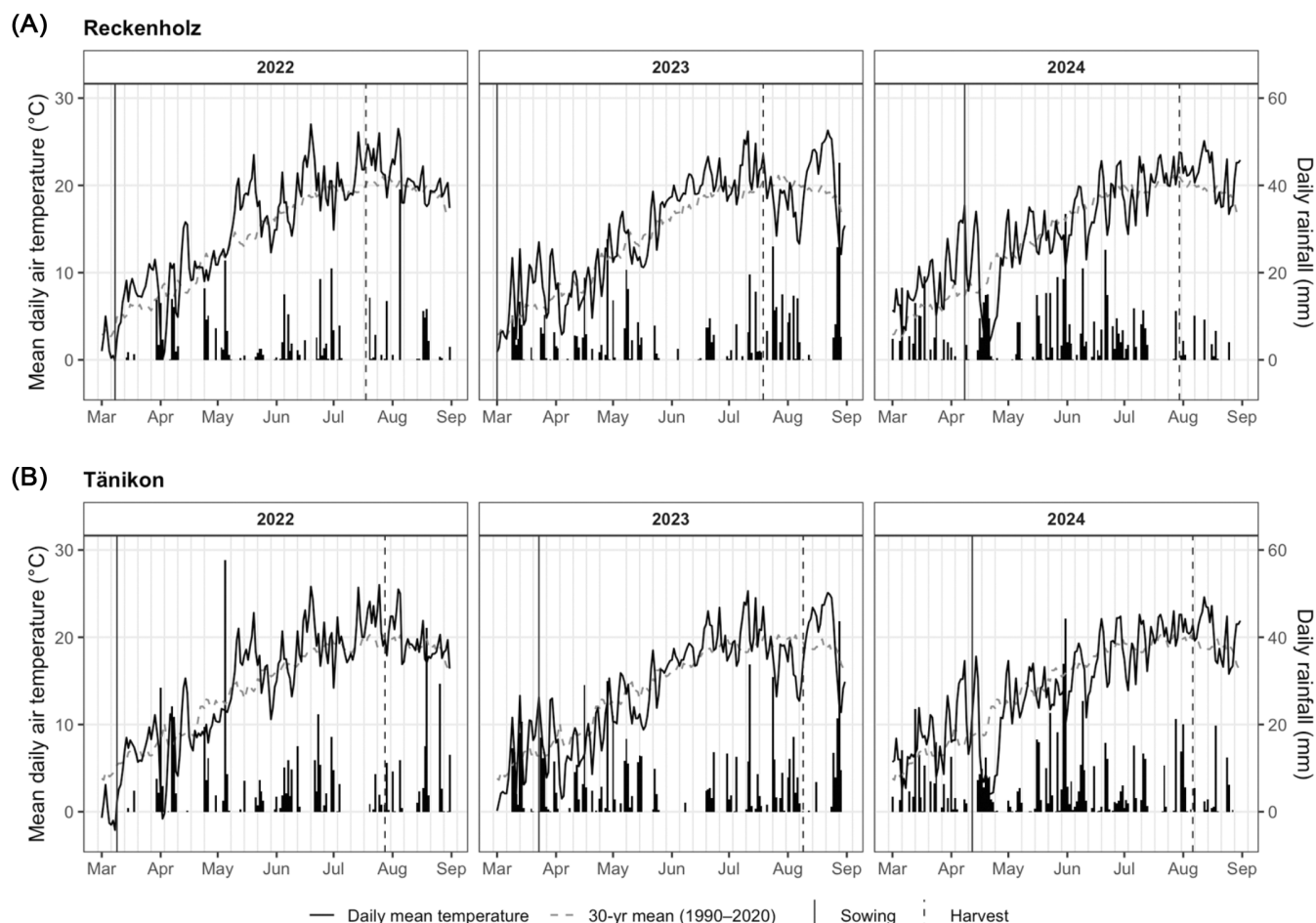


Figure 1. Mean daily air temperature at 5 cm and sum of daily rainfall recorded in 2022, 2023, and 2024 at the site of Reckenholz (A) and Tänikon (B).⁴³ The black continuous lines represent the temperature; the black bars correspond to rainfall for the respective cropping season; and the grey dashed lines represent the mean daily air temperature over a 30-year (1990–2020) period. The solid vertical line represents sowing days, while the dashed lines represent the harvesting dates. The grey grid corresponds to weeks.

seed counter and volumetric analyzer (GTA Saatkorntzählgerät, Sensorik GmbH, Neubrandenburg, Germany) with the software MARViN 5.0 (MARViTECH GbH, Wittenburg, Germany), following the manufacturers' guidelines. Data on TKW, seed volume, and size ratio are reported in Supporting Information, Table S4.

Protein content was predicted by near-infrared transmittance spectroscopy on a dry-matter basis with Kjeldahl ($N \times 6.25$) as the reference method (FOSS Infratec Nova, model 91 892 826, FOSS Analytical A/S, Hillerød, Denmark). Barley was analyzed with WBPR0044 calibration (Application Note 5498, Rev. 2), developed using an artificial neural network (ANN) based on 26316 reference samples (calibration range 6.7–23.6% protein); independent validation ($n = 362$) resulted in a bias-corrected standard error of prediction (SEP) of 0.28% and an R^2 of 0.98. Lentils were analyzed with the FOSS pulse calibration FBPR0012 (Application Note 5387, Rev. 9; chickpea/field and bean/lentil package, 30 mm path length), developed using an ANN from 1849 reference samples (calibration range 19.0–36.4% protein); the lentil-specific validation (range 22.0–26.2% protein) resulted in a SEP of 0.45% and an R^2 of 0.92. Reference analyses were performed by accredited laboratories, with multiple laboratories used to minimize reference-laboratory variation.

β -glucan content in hull-less barley

Representative subsamples of 150 g were obtained using a Riffle seed divider (Schieritz & Hauenstein AG, Arlesheim, Switzerland) and subsequently milled (1 mm sieve size; CT 293 Cyclotec sample mill, Foss, Sweden).

β -glucan content was determined using the mixed-linkage β -glucan kit (Megazyme, Bray, Ireland), specific for mixed-linkage [(1 $\rightarrow$ 3)(1 $\rightarrow$ 4)]- β -D-glucan and validated under AOAC 995.16 and ICC 166.⁴⁵ The protocol was adapted from Hu and Burton.⁴⁶

As an adjustment, plastic Falcon tubes (15 mL) were replaced with glass tubes; this substitution necessitated slightly longer heating times in both the water bath and incubator. Enzymatic incubation was performed in IKA Shakers KS 3000 i control (IKA-Werke, Staufen im Breisgau, Germany) at $0.45 \times g$ (200 rpm) and 50 °C with air-based heating, rather than immersing the samples in water at a controlled temperature. β -glucans were extracted only once per sample instead of twice, due to time constraints. Preliminary tests on a subset of samples showed that duplicate and single extractions yielded identical β -glucan values, confirming that the assay was robust enough to allow single extraction without loss of accuracy. A certified reference flour sample (β -glucan content $41 \text{ g kg}^{-1} \text{ DM}$) was included in

Table 1. List of treatments (treatment number), cultivars used, and sowing ratio

Treatment number	Cultivar (hull-less barley)	Cultivar (lentil)	Ratio
1	Oak Ruby		100
2	Goljiat		100
3	AF Cesar		100
4	Oak Ruby + N		100
5	Goljiat + N		100
6	AF Cesar + N		100
7		Anicia	100
8		Beluga	100
9		Château Linsen	100
10		Grüne Berry	100
11	Oak Ruby	Anicia	25:75
12	Oak Ruby	Beluga	25:75
13	Oak Ruby	Château Linsen	25:75
14	Oak Ruby	Grüne Berry	25:75
15	Goljiat	Anicia	25:75
16	Goljiat	Beluga	25:75
17	Goljiat	Château Linsen	25:75
18	Goljiat	Grüne Berry	25:75
19	AF Cesar	Anicia	25:75
20	AF Cesar	Beluga	25:75
21	AF Cesar	Château Linsen	25:75
22	AF Cesar	Grüne Berry	25:75

N represents treatments with mineral nitrogen fertilization. The lentil cultivars were Anicia (3.5–5 mm early- to late-maturing green lentil), Beluga (2–3 mm, early-maturing black lentil), Château Linsen (4–5 mm, late-maturing with red interior color), and Grüne Berry (3.5–5 mm early- to late-maturing green lentil). The early-maturing spring hull-less barley cultivars were AF Cesar (two-rowed, short, late heading), Goljiat (two-rowed, medium-tall, early heading) and Oak Ruby (two-rowed, short, early heading).

each analysis to monitor accuracy. Absorbance was measured at 510 nm with a Sunrise Remote microplate reader (Tecan, Austria) and analyzed with RIDAWIN software (R-Biopharm AG, Darmstadt, Germany).

The β -glucan content was calculated using the following formula:

$$\beta\text{-glucan (g kg}^{-1}\text{ DM)} = \left(\Delta A \times F \times FV \times 0.9 \times \frac{D}{W} \right) \times 10 \# \quad (1)$$

where ΔA represents the absorbance after β -glucosidase treatment minus reaction blank absorbance; F is the factor for the conversion of absorbance values to μg of glucose (100 μg of D-glucose); FV is the final volume (9.6 mL); D is the dilution factor (equal to 1); W is the calculated dry weight of the sample (mg); and 10 is the conversion factor for $\text{g kg}^{-1}\text{ DM}$.

$$\text{Fresh weight} \times \frac{100 - \text{Moisture content (\%)}}{100} \# \quad (2)$$

The factor 0.9 was applied to convert the amount of free glucose measured into its β -glucan equivalent.

Statistical analysis

All statistical analyses were conducted using R version 4.3.3 (R Core Team 2024-02-29) with the *tidyverse* package suite.⁴⁷ Derived variables included total impurities (sum of impurities in lentil and barley fractions) and the seed size ratio (lentil seed volume divided by barley seed volume). Treatments were categorized as unfertilized pure, fertilized pure, or mixed systems; the

experimental environment was defined as the combination of year and site.

Linear mixed-effects models were fitted using the *lme4*⁴⁸ and *lmerTest*⁴⁹ packages to evaluate the effects of cropping system, year, site, cultivar, and their interactions on quantitative variables, including protein concentration, protein yield, impurity percentage, and β -glucan concentration. The random effects structure included *block_ID*, which represented blocks nested within the year-by-site interactions to account for the experimental design. All models were fitted by restricted maximum likelihood and refitted using the *bobyqa* optimizer in the event of convergence issues. Significance of fixed effects was tested using Type III ANOVA with Satterthwaite's approximation for denominator degrees of freedom. The ANOVA outputs are reported in the Supporting Information (Tables S5–S9, S12, and S13). Estimated marginal means (EMMs) and pairwise comparisons were obtained with the *emmeans*⁵⁰ package using Tukey's adjustment.

Model assumptions of normality, homoscedasticity, and independence were verified through residual diagnostics and Q–Q plots. Trait relationships – including between seed size ratio and total impurities – were evaluated using Pearson's correlation and polynomial mixed models.

Stability of protein yield across environments was assessed using the Finlay–Wilkinson regression⁵¹ and the weighted average of absolute scores (WAAS) approach implemented in the *metan* package.⁵² The Finlay–Wilkinson model provided regression slopes (b_i), coefficients of variation (CV), root mean square error (RMSE), and R^2 to characterize treatment responsiveness and stability, while the WAAS method integrated yield (Y) across

environments (WAASY).^{52,53} Graphical outputs were produced using *ggplot2*⁵⁴ and *ggpubr*,⁵⁵ with results considered statistically significant at $P < 0.05$.

RESULTS

Protein content

The range of protein content in lentils varied between 204 and 315 g kg⁻¹ DM, while in hull-less barley it ranged between 99 and 203 g kg⁻¹ DM.

The lentil protein content varied significantly ($P < 0.001$) among stands, years, sites, and lentil cultivars (Table 2). The protein content was slightly but significantly higher in pure lentil stands than when grown in mixtures with hull-less barley; the difference was <10 g kg⁻¹ DM ($\Delta = 7.6$ g kg⁻¹ DM). Across sites and years, protein content in pure stands ranged between 251 and 258 g kg⁻¹ DM, while in mixed stands it was between 245 and 249 g kg⁻¹ DM. However, when comparing different stands grouped by year and site, significant differences between stands were observed in only one case, with significantly higher values in pure stands compared to mixed stands in 2023 at Tänikon (Table 3, Fig. 2(A)).

The largest differences were observed between years. Protein content across sites, treatments, and cultivars was lowest in 2022 (220–227 g kg⁻¹ DM), increasing significantly in 2023

(261–269 g kg⁻¹ DM; +5.6%) and 2024 (260–267 g kg⁻¹ DM; +5.1%) (Fig. 2). The protein content in 2022 was significantly ($P < 0.0001$) lower than in 2023 and 2024, which did not differ from each other ($P > 0.05$). In addition, lentil protein content at Reckenholz (across years, treatment, and cultivars) was marginally lower than at Tänikon (249 vs. 253 g kg⁻¹ DM; $P = 0.04$). Among cultivars and across years and cropping systems, Château Linsen had the highest protein content (254 g kg⁻¹ DM) overall, with significant year-by-cultivar interactions. For instance, Château Linsen reached 264 g kg⁻¹ DM in 2023 but only 247 g kg⁻¹ DM in 2022. Finally, Pearson's correlation analysis revealed a negative relationship between protein content and dried grain yield ($r = -0.34$, $P < 0.001$), indicating a trade-off between yield and protein content.

Similarly, hull-less barley had significant variation ($P < 0.001$) among stands, years, sites and hull-less barley cultivars, as well for the interaction between stand and year (Supporting Information, Table S2). The protein content was significantly higher in mixed stands compared to both pure fertilized (-18 g kg⁻¹ DM) and unfertilized treatments (-26 g kg⁻¹ DM). Furthermore, protein contents of fertilized treatments were significantly higher than the unfertilized ones ($+7.6$ g kg⁻¹ DM; $P < 0.001$). Contrary to lentil, the year effect on protein content of hull-less barley was marginal but consistent across sites; protein contents were

Table 2. Type III ANOVA: significance of fixed effects across all response variables

Source	Lentil protein	Barley protein	Total protein yield	Lentil protein yield	β -glucan	Impurities
Stand ^a	***	***	***	***	***	**
Year	***	***	***	***	***	***
Site	*	***	n.s.	***	**	n.s.
Cultivar	***	***	—	—	***	—
Stand ^a × Year	n.s.	*	*	***	*	***
Stand ^a × Site	n.s.	n.s.	*	n.s.	n.s.	n.s.
Year × Site	n.s.	n.s.	**	n.s.	n.s.	*
Stand ^a × Year × Site	n.s.	n.s.	*	n.s.	*	***

^a 'Stand' denotes the growing system (mixed or pure) for all responses except impurities, where it represents the individual treatments.

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; n.s. = not significant; values are shown in Tables S5–S9; S12, S13; '—', not applicable.

Table 3. Protein content of pure lentil and mixed systems across two sites (Reckenholz and Tänikon) and 3 years (2022–2024)

		Stand	Protein content (g kg ⁻¹ DM)	SE	Difference (Δ , g kg ⁻¹ DM)	P value
Reckenholz	2022	Pure lentil	224	0.5	0.4	0.3
		Mixed	218	0.3		
	2023	Pure lentil	262	0.5	0.7	0.5
		Mixed	258	0.3		
	2024	Pure lentil	267	0.5	0.6	0.3
		Mixed	261	0.3		
Tänikon	2022	Pure lentil	230	0.5	0.7	0.2
		Mixed	223	0.3		
	2023	Pure lentil	277	0.5	1.4	0.009
		Mixed	263	0.3		
	2024	Pure lentil	267	0.5	0.9	0.1
		Mixed	258	0.3		

SE, standard error of the mean; estimate (Δ), pairwise contrasts.

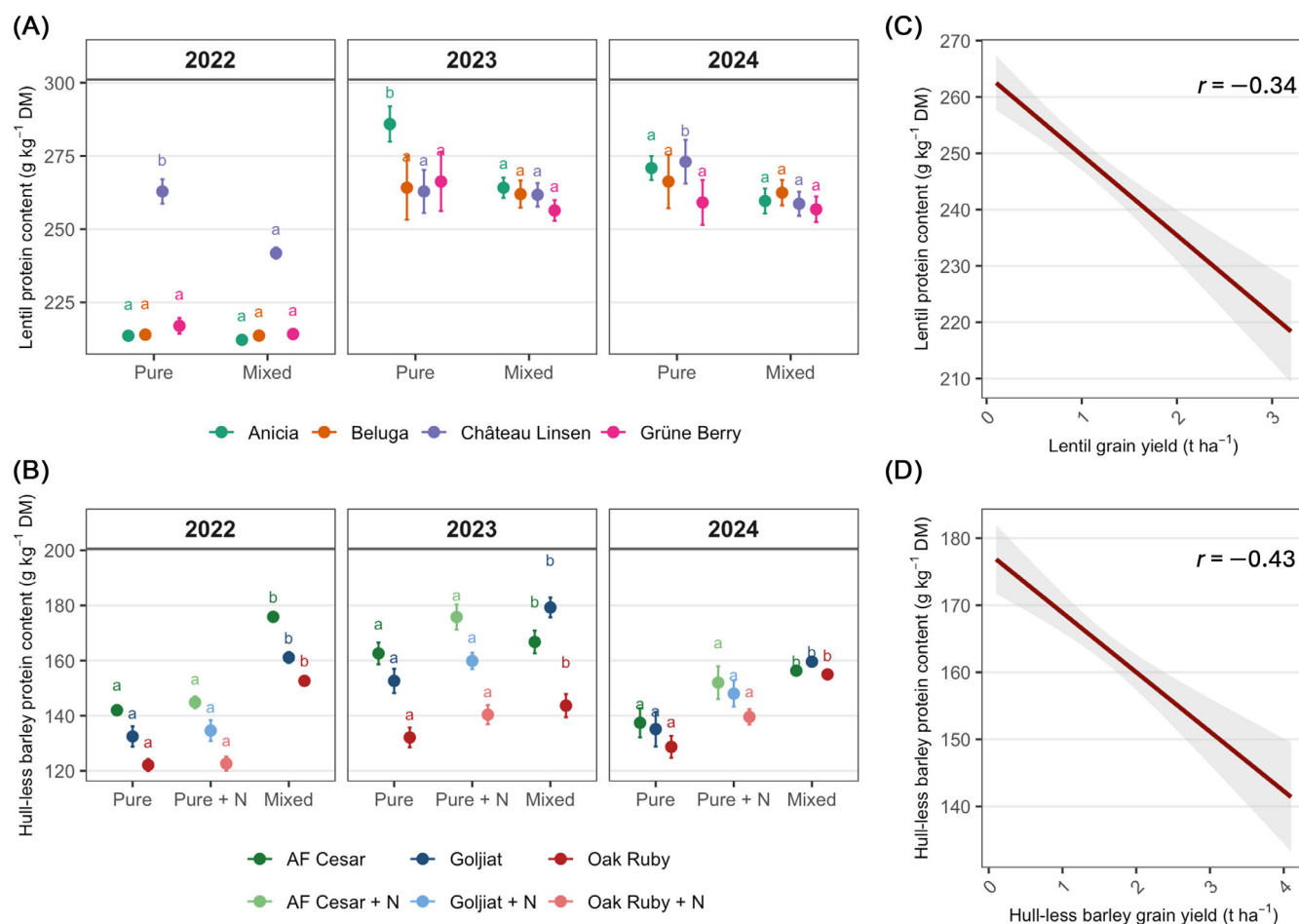


Figure 2. Protein content (g kg^{-1} DM) per year and stand: (A) lentil protein content; (B) hull-less barley protein content; (C) Pearson's correlation between lentil protein content and lentil grain yield and (D) between hull-less barley protein content and hull-less barley grain yield.

lowest in 2024 ($127\text{--}152 \text{ g kg}^{-1}$ DM), slightly higher in 2022 ($127\text{--}167 \text{ g kg}^{-1}$ DM), and highest in 2023 ($143\text{--}180 \text{ g kg}^{-1}$ DM). Among cultivars and across years, sites, and cropping systems, AF Cesar had the highest average protein content ($163 \pm 1.4 \text{ g kg}^{-1}$ DM), followed by Goljiat ($156 \pm 1.3 \text{ g kg}^{-1}$ DM), while Oak Ruby had the lowest content ($140 \pm 1.4 \text{ g kg}^{-1}$ DM) ($P < 0.001$). Similar to lentils, protein content in hull-less barley was negatively correlated with dry grain yield ($r = -0.43$; Fig. 2), confirming also for the companion cereal a trade-off between yield and protein content.

Protein yield

Protein yield ranged between 251 kg ha^{-1} DM in pure unfertilized hull-less barley up to 386 kg ha^{-1} DM in mixed stands, averaged by year, site, and cultivar (Fig. 3). Protein yield significantly varied among stands, years and in the two-way interaction stand by year, stand by site, site by year, and in the three-way interaction of stand, year, and site (Table 2). Mixed and pure lentils had significantly higher values compared to pure unfertilized barley (Table 4), while no differences were found between pure lentil and mixed stands, neither between pure lentils nor pure fertilized barley, despite the large difference (-42.9 kg ha^{-1} DM; Table 4).

Total protein yield varied significantly among years (Table 2). Averaged across treatments, cropping systems, and sites, protein

yield declined from $426\text{--}488 \text{ kg ha}^{-1}$ DM in 2022 to $299\text{--}362 \text{ kg ha}^{-1}$ DM in 2023, and $175\text{--}239 \text{ kg ha}^{-1}$ DM in 2024, with all pairwise year-to-year contrasts being significant ($P < 0.001$). Site did not have a significant main effect but showed significant two- and three-way interactions with other experimental factors.

Considering protein yield from lentils (Fig. 3), significant variation was observed by year, stand, and site and for the two-way interaction year by site (Table 2). Significantly higher values ($P < 0.001$) were observed in pure lentil stands, with means ranging between 335 and 384 kg ha^{-1} DM; whereas lentil protein yield values in mixtures ranged between 212 and 243 kg ha^{-1} DM. In 2022, lentil protein yield peaked with a range between 330 and 381 kg ha^{-1} DM, which was significantly different from 2023 ($-126.6 \text{ kg ha}^{-1}$ DM; $P < 0.001$) and 2024 ($+59.2 \text{ kg ha}^{-1}$ DM; $P = 0.007$). Significant differences were also found for lentil protein yields between 2023 and 2024 (-67.5 kg ha^{-1} DM; $P = 0.002$), with means ranging between 202 and 255 kg ha^{-1} DM and 270 and 322 kg ha^{-1} DM in 2023 and 2024, respectively.

Significant variation in lentil protein yield was found among treatment, year and site (Table 2). The treatment with the highest protein yield was pure Anicia, with yields ranging between 417 and 479 kg ha^{-1} DM, followed by pure Grüne Berry ($395\text{--}456 \text{ kg ha}^{-1}$ DM). Among mixed treatments, the highest protein yield recorded was for AF Cesar-Beluga ($300\text{--}362 \text{ kg ha}^{-1}$ DM) and AF Cesar-Anicia

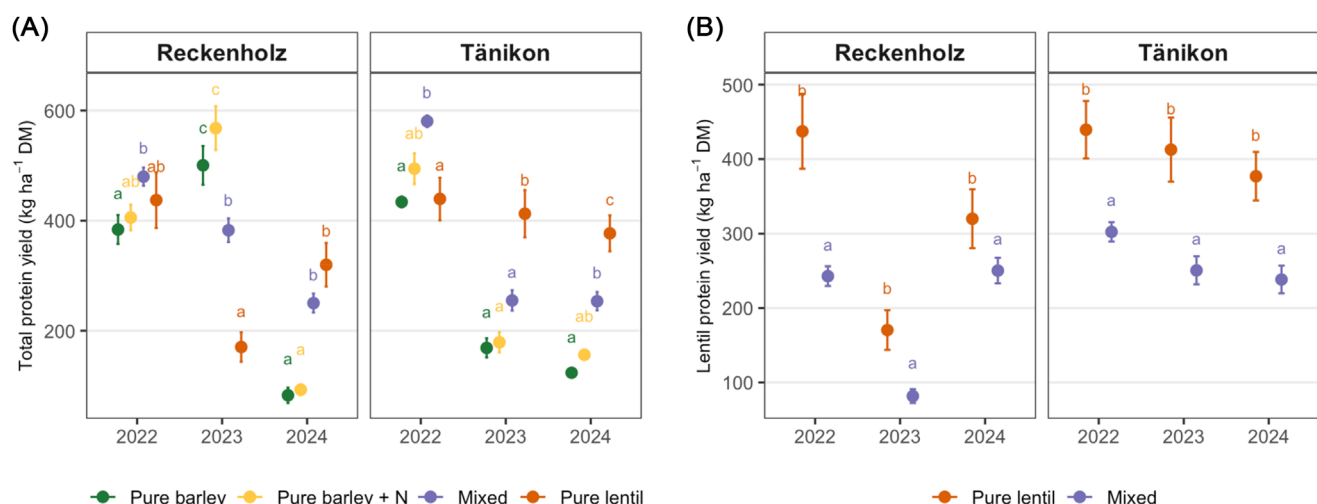


Figure 3. (A) Total protein yield (kg ha^{−1} DM) per year and site. (B) Lentil protein yield per year and site, dependent on the cropping system (pure barley; pure barley + N; mixed and pure lentil).

Table 4. Pairwise contrasts (Δ) for total protein yield (kg ha^{−1} DM) among cropping systems (pure hull-less barley, fertilized hull-less barley (+ N), mixed stands, and pure lentils), averaged across years and sites

	Estimate (Δ)	SE	df	t-ratio	P-value	
Pure barley / Pure barley + N	−35.4	20.1	458	−1.8	0.30	n.s.
Pure barley / Mixed	−85.7	15.7	458	−5.5	< 0.0001	***
Pure barley / Pure lentil	−78.3	18.3	458	−4.3	0.0001	***
Pure barley + N / Mixed	−50.3	15.9	458	−3.2	0.009	**
Pure barley + N / Pure lentil	−42.9	18.5	458	−2.3	0.095	n.s.
Mixed / Pure lentil	7.4	13.5	457	0.5	0.94	n.s.

SE, standard error of the mean; df, degrees of freedom; t-ratio, ratio of the estimated difference to its standard error (Δ /SE); positive values indicate higher yield in the first-listed system. Significant differences are indicated by P-values: ***P < 0.001; **P < 0.01; *P < 0.05; n.s., not significant.

Table 5. Yield stability between cropping systems

	Mean	SD	CV	b_i	pval ($b_i = 1$)	RMSE	R^2
Pure barley	282	178	63	1.4	0.08	81	0.75
Pure barley + N	316	198	63	1.5*	0.02	94	0.73
Mixed	367	139	38	1.1	0.7	16	0.99
Pure lentil	359	102	29	0.07***	< 0.0001	92	0.05

Mean, mean for total protein yield (kg ha^{−1}); SD, standard deviation; CV, coefficient of variation (%); pval, significance of the test that b_i differs from 1 ($H_0: b_i = 1$); b_i , Finlay–Wilkinson regression coefficient; RMSE, root mean square error; R^2 , robustness. ***P < 0.001; **P < 0.01; *P < 0.05; n.s., not significant.

(262–324 kg ha^{−1} DM). The pure stand of Château Linsen showed the lowest protein yield (140–201 kg ha^{−1} DM).

Among the lentil cultivars, Anicia, Beluga, and Grüne Berry had significantly ($P < 0.001$) lower protein yields under mixed stands compared to pure stands, while no significant difference was found for Château Linsen.

Protein yield stability

CV and Finlay–Wilkinson stability analysis revealed notable differences among the four stands (Table 5). The mixed stand had superior performance, combining the highest mean protein yield with

the lowest RMSE, moderate CV and a b_i coefficient close to 1. This profiles a system both highly productive and of consistent stability across environments. Pure hull-less barley systems (fertilized and unfertilized) had contrasting behavior; both showed high responsiveness to environmental variation ($b_i > 1.2$) and large yield variability (CV > 60%), suggesting better performance in favorable conditions but reduced stability. Pure lentil displayed an opposite pattern: a flat environmental response ($b_i = 0.07$) and low variability (CV = 29%) but considerably lower productivity. Neither fertilized barley nor mixed-stand environmental response deviated from $b_i = 1$ ($P > 0.05$). Overall, the mixed stand

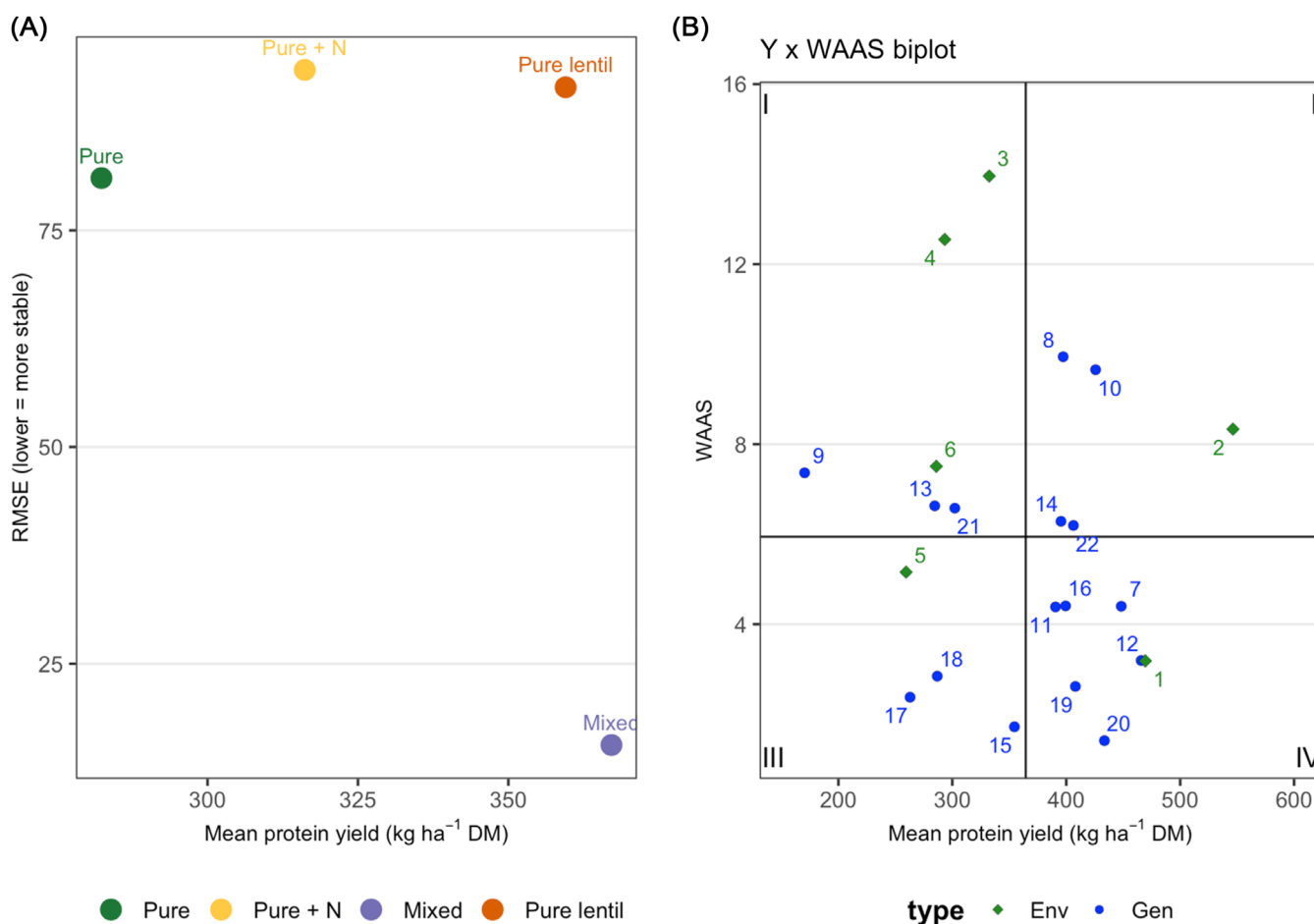


Figure 4. (A) Combination between root mean square error (RMSE) and mean protein yield. (B) Bipartite plot between weighted average of absolute scores (WAAS) and mean protein yield. Env = environment (site × year); Gen = treatment (barley cultivar × lentil cultivar). Each number corresponds to the treatment number (Table 1), while X with numbers correspond to the environments (unique combinations between year and site). The full list of coefficients can be found in Supporting Information, Table S11.

provided the best balance between performance and stability (Fig. 4).

The additive main effects and multiplicative interaction (AMMI) analysis indicated significant variation in both environments (defined as unique combination between year and site), cropping system, and their interaction (Supporting Information, Table S10).

Among the lentil treatments, mixtures with Beluga were the most favorable combinations for high yield and stability coefficient (WAAS; Supporting Information, Table S11). The Beluga–AF Cesar mixture showed the optimal balance (433.5 kg ha⁻¹; WAAS = 3.1; WAASY = 94.5; OrWAASY = 1), combining high productivity and lowest stability coefficient (WAAS). Two additional combinations of Beluga using Oak Ruby (465.8 kg ha⁻¹; WAAS = 3.4; WAASY = 87.3; OrWAASY = 7) and Goljiat (448.3 kg ha⁻¹; WAAS = 2.9; WAASY = 79.6; OrWAASY = 4) as companion cultivars showed strong performance. Beluga–Oak Ruby produced the highest absolute yield, while its slightly higher WAAS indicated marginally reduced stability compared to the other two combinations with Goljiat and AF Cesar.

β-glucan content

The β-glucan content ranged between 17 and 76 g kg⁻¹ DM across all samples. A significant effect was detected for different years, sites, cultivars, and cropping systems, as well as in two-way (stand-by-year)

and three-way (stand-by-year-by-site) interactions (Table 2). Differences between years were pronounced; β-glucan content was significantly higher in 2022 (48.3 ± 0.7 g kg⁻¹ DM) than in 2024 (39.7 ± 0.7 g kg⁻¹ DM; *P* < 0.001). Site-level differences were also found to be significant (*P* = 0.007), with Tännikon leading to higher β-glucan contents (44.9 ± 0.7 g kg⁻¹ DM) than Reckenholz (39.7 ± 0.7 g kg⁻¹ DM; Fig. 5). Cropping systems significantly influenced β-glucan accumulation (Fig. 5). Pure fertilized barley had the highest content (48.5 ± 1.1 g kg⁻¹ DM), significantly exceeding that of mixed stands (42.5 ± 0.6 g kg⁻¹ DM; *P* < 0.001) and pure unfertilized barley stands (44.0 ± 1.1 g kg⁻¹ DM; *P* = 0.01), while no significant difference was detected between pure unfertilized barley and mixed stands (*P* > 0.05). Cultivar effects were substantial: AF Cesar had the highest β-glucan content (49.2 ± 0.8 g kg⁻¹ DM), followed by Goljiat (43.7 ± 0.8 g kg⁻¹ DM) and Oak Ruby (38.4 ± 0.8 g kg⁻¹ DM); all pairwise contrasts were significant (*P* < 0.001).

Post-harvest separation

The proportion of grains from the other crop species (defined as impurities remaining after separation) ranged between 4.9% and 30.4% (Fig. 6). All treatment combinations exceeded both thresholds of 1% and 0.5%, dictated respectively by the Codex Alimentarius (Codex STAN 171-1989) and the Swiss grain grading standards.⁴³ Significant effects of treatment (*P* < 0.01), year (*P* < 0.0001), and their

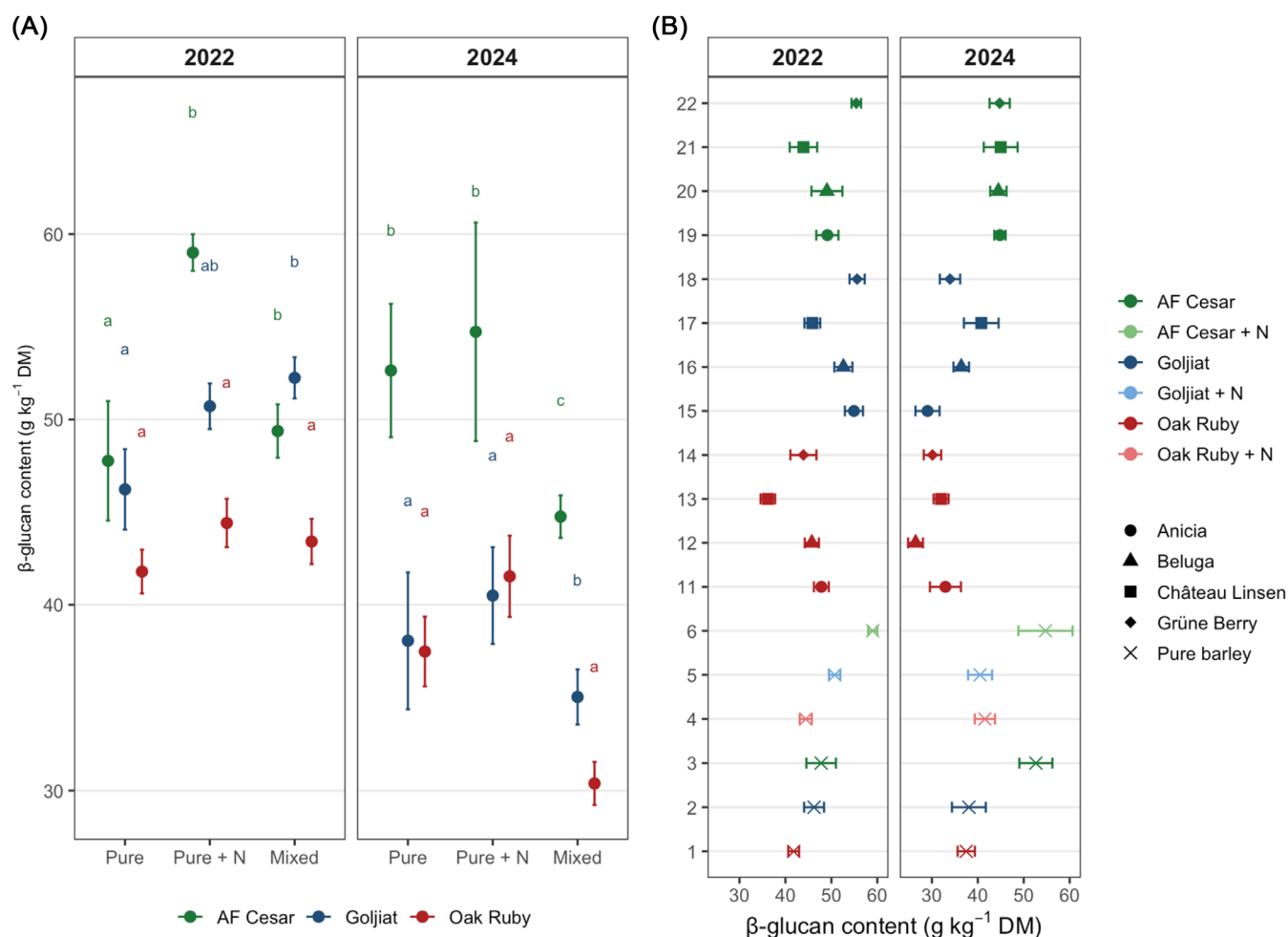


Figure 5. (A) β -glucan content (g kg^{-1} DM) in hull-less barley grain from different stands (pure barley = Pure; pure fertilized barley = Pure + N; mixed barley = Mixed). (B) β -Glucan content (g kg^{-1} DM) among different treatments. Treatments sharing the same letter are not significantly different from each other ($P > 0.05$). In the Supporting Information, the values for β -glucan as percent dry matter (DM) among different stands (Table S14) and contrasts with P -values between stands (Table S15) are shown.

three-way interaction with site ($P < 0.0001$) on impurity levels were detected. The treatment-by-year interaction was also significant ($P < 0.001$; Table 2). In 2022, impurities ranged between 2% and 9%, but they were higher in 2023 (35–42%) and in 2024 (12–19%).

Separation efficiency was strongly correlated with seed-size ratio ($r = 0.38$, $P < 0.001$; Fig. 6). Lentil seed volumes averaged from the smallest volume for Beluga ($90.9 \pm 1.8 \text{ mm}^3$) to largest for Grüne Berry ($179 \pm 4.4 \text{ mm}^3$); in barley, smallest volumes were recorded in Goljiat ($222 \pm 4.1 \text{ mm}^3$) and largest volumes in AF Cesar ($342 \pm 3.9 \text{ mm}^3$). Among the mixtures, the total impurities were lowest in Beluga–Oak Ruby (10.6%) and Beluga–AF Cesar (11.1%) – about 9% below the overall mean of $19.6 \pm 1.3\%$ ($P = 0.02$).

DISCUSSION

To address the critical gap in integrating yield stability with grain quality and post-harvest processability in sustainable food systems, this study provided the first multi-year evaluation of lentil and hull-less barley mixed cropping across varying temperate environments and management treatments.

Our values for lentil protein content align well with the global mean reported in the review of Dhull *et al.*⁵⁶ on the nutritional profile of lentils. Our overall mean was 248 g kg^{-1} DM, while

Dhull *et al.*⁵⁶ reported an average of 246 g kg^{-1} DM. Similarly, the mean protein content observed in barley (153 g kg^{-1} DM) reflected the values reported by Oscarson *et al.*⁵⁷ (151 g kg^{-1} DM). We observed a marginal increase in lentil protein content in pure stands compared with mixed stands – an increase that became negligible when stratified by year and site, suggesting that year was the main source of variation. Our findings are consistent with those of Wang *et al.*,²¹ who, in a lentil–barley intercropping system with the same mixing ratio, reported values of 274 g kg^{-1} DM in pure stands and 277 g kg^{-1} DM in mixture at a 240 seeds m^{-2} sowing density and a 75% lentil ratio. However, in our study, mean protein contents were overall slightly lower, with pure lentil stands averaging 255 g kg^{-1} DM and mixed stands 247 g kg^{-1} DM.

Most of the protein variation was driven by year rather than stand type. Environmental conditions – including precipitation, growing season length and temperature – alongside agronomic factors such as plant density, weeds, or soil fertility, are factors influencing protein content, mainly by modulating nitrogen availability and its incorporation into grain amino acids.^{58–60}

Year-to-year differences in precipitation and sowing date likely affected nitrogen uptake and assimilation across the three growing seasons, as observed by Tang *et al.*⁶¹

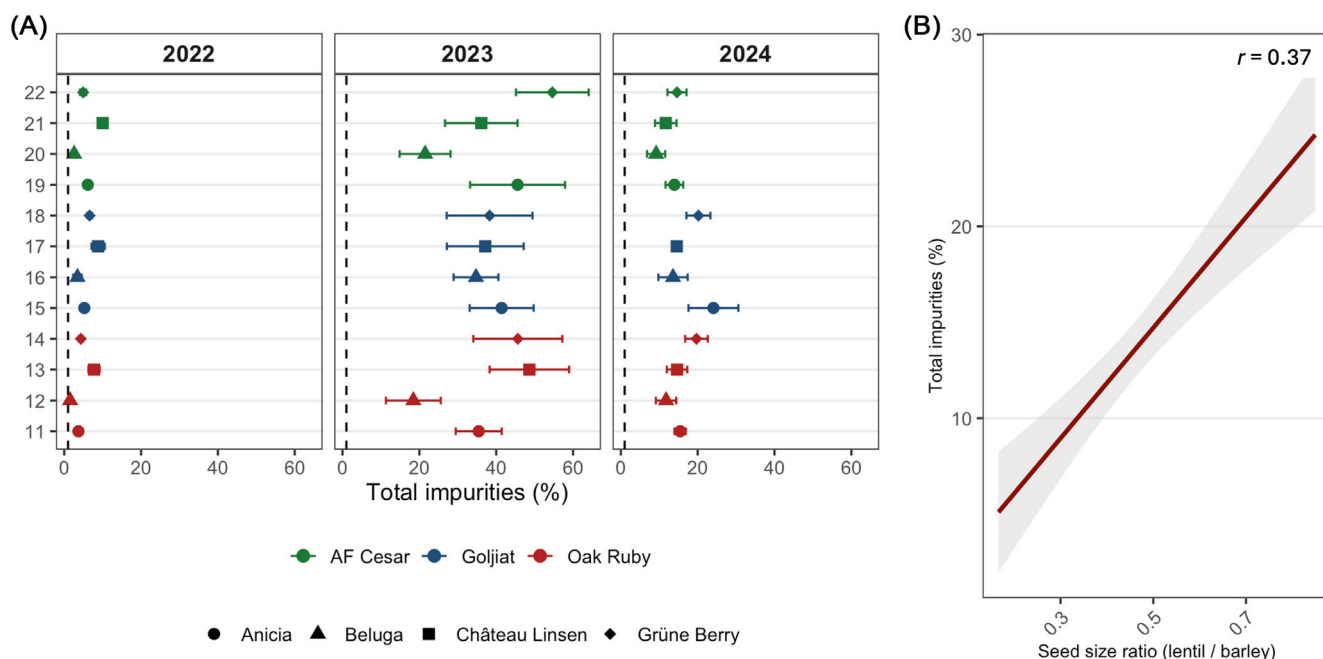


Figure 6. (A) Total percentage of impurities among treatments over three years at two sites (Reckenholz and Tänikon). The dashed line is set at 1% of total impurities as the threshold limit. (B) Pearson's correlation between total impurities and seed size ratio between lentils and hull-less barley.

In 2022, favorable conditions (earlier sowing, drier weather) produced the highest lentil grain yields but the lowest protein content, consistent with a dilution effect when nitrogen is shared across a larger biomass.^{62,63} Competition between carbon and nitrogen during grain filling may have further amplified this trade-off.

A well-documented negative correlation between dry grain yield and protein concentration in cereals^{7,64,65} and in legumes^{66,67} was confirmed in this study. We observed higher protein levels in fertilized barley as well as from mixed systems with lentils. The latter can be explained by nitrogen supplied through lentil N₂ fixation, together with lower competition for barley in the mixture, where conspecific neighbors were partly replaced by lentils, which compete less for soil nitrogen (reduced intraspecific and interspecific competition relative to the pure stand), as was previously proposed by Schmidke et al.,²² Gooding et al.,⁶³ and Bedoussac and Justes.⁶⁸ Under a similar setting, Wang et al.²¹ reported barley crude protein between 145 and 158 g kg⁻¹ DM, compared to 137 g kg⁻¹ DM from barley in pure cropping.

The present study quantified grain protein concentration but did not assess protein quality *sensu stricto* (e.g., amino acid composition, *in vitro* digestibility, or protein digestibility). Nevertheless, the nutritional value of legume–cereal mixtures is not only influenced by protein concentration but also by the complementarity of their amino acid profiles. Pulses such as lentils are rich in lysine – an essential amino acid that is often limited in cereal grains^{69,70} – but are relatively deficient in sulfur-containing amino acids,^{71,72} which are provided in greater amounts by cereals.⁷³ Consequently, combining both species in a single harvest may produce a more balanced amino acid profile than either pure stand, as demonstrated by Gibson et al.⁷⁴ and, more recently, by Sá and House.⁷⁵ The +18 and +26 g kg⁻¹ DM increases in protein concentration observed in mixed crops (relative to fertilized and unfertilized pure barley, respectively) likely reflect improved nitrogen supply through biological N₂ fixation. Whether these gains

were accompanied by changes in the amino acid composition, and thus protein quality, remains unknown. Moreover, our analyses were limited to total protein and β -glucan and did not include ash, minerals (e.g., Fe, Zn, Ca, Mg, and P), other dietary fibers, or phytates, all of which contribute to the overall nutritional value of legume and cereal crops.⁷⁶ These differences in analytical scope may partly explain why protein yields in our study were lower than those reported by Wang et al.²¹ Despite the lower protein yield of pure lentils, mixed cropping exceeded it by 7.4 kg ha⁻¹ DM, highlighting the positive contribution of hull-less barley.

Mixed cropping exhibited higher yield variability than pure lentils (CV 38% vs. 29%), yet its Finlay–Wilkinson regression coefficient was close to unity ($b_i = 1.1$), indicating proportional responsiveness to environmental variation. In contrast, pure lentils showed almost no responsiveness ($b_i = 0.07$), suggesting a more buffered, less environmentally responsive production system. These findings support the hypothesis that mixed cropping enhances yield stability, consistent with previous reports on cereal–legume intercrops.^{13,37} The cultivar choice also influenced the performance: mixtures of Beluga with AF Cesar or Oak Ruby achieved higher protein yields and greater yield stability than their respective pure stands.

Interestingly, the pattern observed for β -glucan content differed from that for protein content. The β -glucan concentrations measured in hull-less barley (17–76 g kg⁻¹ DM) were consistent with the range reported by Geng et al.,⁷⁷ who found values between 12 and 65 g kg⁻¹ DM.

Our study showed significantly higher β -glucan concentrations in 2022 and in fertilized pure stands than in unfertilized and mixed stands. This is consistent with the findings of Geng et al.,²⁷ who reported that barley grains developing under low soil moisture and high air temperature, particularly during grain filling, accumulate higher concentrations of both protein and β -glucan. These conditions were more prevalent in 2022 than in 2024, whereas

2024 was characterized by a shorter growing season and higher rainfall. As β -glucan accumulates during the final stages of grain filling, environmental stresses that shorten this period, such as excess rainfall or heat, can reduce its concentration,⁷⁸ likely explaining the lower levels observed in 2024. The mean β -glucan concentration ($44 \pm 0.6 \text{ g kg}^{-1} \text{ DM}$) indicates that consuming 40–80 g of hull-less barley flour per day would provide the 3 g β -glucan intake required to meet health claims by EFSA and the FDA for maintaining normal blood cholesterol concentrations.^{79,80} Although our protein results align with reports of higher protein concentrations in organic than in conventional systems,⁸¹ the opposite trend observed for β -glucan suggests that management effects are trait specific. Because the pesticide-free, low-fertilization management used in this study resembles Swiss low-input and organic production systems, these findings are likely relevant to such production conditions.

The site effect is consistent with the same grain-filling-duration mechanism. Across all 3 years, Tännikon had a longer growing season than Reckenholz and accumulated greater thermal time during grain filling (2027 °C vs. 1925 °C in 2022; 1828 °C vs. 1778 °C in 2024). Tännikon also received substantially more rainfall and is characterized by well-drained Cambisols and Luvisols, whereas Reckenholz has an endogleyic Cambisol with stagnic properties. The longer grain-filling period and more sustained water supply at Tännikon likely prolonged the late stages of grain development, when β -glucan is deposited in endosperm cell walls, whereas the drier, shorter seasons at Reckenholz likely curtailed this process. Thus, although smaller than the cultivar and year effects, the site effect is consistent with the same underlying mechanism: environmental conditions that extend the grain development favor β -glucan accumulation.

The higher β -glucan content under fertilized conditions agrees with previous reports.^{82–84} Although mixed cropping increased barley protein content, β -glucan concentrations remained lower than in fertilized pure stands, suggesting that protein and β -glucan respond differently to nitrogen supply. This likely reflects the slower, more gradual release of legume-derived nitrogen compared with the readily available mineral nitrogen supplied by fertilizers.

Overall, β -glucan concentration was primarily determined by cultivar, while environmental conditions – mainly growing season duration and thermal accumulation during grain filling – modulated its absolute levels. To maximize grain quality and β -glucan content in hull-less barley, cultivation practices should promote an extended grain-filling period, notably through early spring sowing and adequate nitrogen supply under temperate conditions.

Beyond production and nutritional considerations, post-harvest grain separation remains a major barrier to the adoption of mixed cropping systems⁸⁵: the technical challenges associated with separating grain fractions increase processing costs, potentially offsetting the agronomic and environmental benefits of mixed cropping. In our study, the centrifugal separation method using a rotating mantle did not result in grain fractions with <1% impurities. The greater variability observed in 2023 was likely due to higher grain moisture at harvest, which increased seed breakage of legumes and reduced separation efficiency. Resistance to seed cracking is therefore an important trait in both pure and mixed systems.⁸⁶

Our results further suggest that separation becomes particularly challenging when the component crops produce seeds of similar size and color.⁸⁷ Mixtures of similarly sized seeds exhibited significantly higher impurity levels than those combining large and

small grains. Breeding or selecting cultivars with contrasting seed size and color could therefore improve sorting efficiency, reduce the number of separation cycles, lower processing costs, and enhance product quality.⁸⁵ However, large differences in seed size can be problematic during sowing, as they often cause segregation within the seed hopper and complicate simultaneous planting of mixtures.⁸⁶ Using an indented cylinder separator, mixtures of small, dark-seeded Beluga lentils combined with larger seeds of hull-less barley cultivars (such as AF Cesar or Oak Ruby) achieved the most efficient separation. Furthermore, the distinct black seed coat of Beluga lentils can further enhance separation efficiency when optical sorters are utilized.

CONCLUSIONS

Mixed cropping of lentils with hull-less barley consistently increased barley protein content by 18–26 g kg⁻¹ DM compared with both pure and fertilized systems. Conversely, different cropping systems had no significant effect on lentil protein content. Although total protein yield in mixed stands exceeded pure stands only in 2022, the mixed systems demonstrated greater overall yield stability and environmental responsiveness, suggesting a superior capacity to buffer against environmental fluctuation. Among the mixtures, those involving Beluga lentils exhibited the best combination of yield stability and productivity. The hypothesis that mixed cropping would enhance β -glucan concentrations was not supported, as the highest β -glucan levels were observed in fertilized pure barley stands. β -glucan concentration was strongly genotype dependent, with AF Cesar recording the highest values. Impurity levels in mixed harvests exceeded quality thresholds, primarily due to technical rather than biological constraints. The wet harvest conditions in 2023 likely increased kernel breakage, contributing to high impurity levels. Nevertheless, mixtures of crops with contrasting seed sizes (e.g., Beluga–AF Cesar) achieved substantially better separations. Overall, our findings highlight the potential of lentil–barley mixtures to enhance grain protein quantity and yield stability. For practical implementation, we recommend early sowing of spring hull-less barley (late February under temperate conditions) and the selection of cultivar combinations with contrasting seed sizes to optimize both productivity and post-harvest processing efficiency. These recommendations are consistent with the low-input, pesticide-free management used in this study and are directly applicable to organic and extensive Swiss production systems.

Future studies should evaluate harvest efficiency during field operations by quantifying kernel losses caused by phenological asynchrony, and investigate advanced post-harvest separation technologies, such as optical sorting, to improve grain purity in mixed systems.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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