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Implications of Pea-Barley-Mixed Cropping on Gross Margin, Root Rot Infestation, and Nitrogen Supply—A **Swiss Case Study**

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ABSTRACT

A growing demand for plant-based proteins and renewable nitrogen supplies has necessitated an intensification of legume cultivation in Europe. However, the cultivation of grain legumes is inherently exposed to various risks, including lodging and fungal infections. Mixed cropping of pea (Pisum sativum L.) and barley (Hordeum vulgare L.) presents a promising approach to increase plant-based protein production while also improving yield stability. We performed a multi-environment-mixed cropping comparison with pure pea and pure barley to determine the effects of the cultivation method on root rot resistance, N supply in successive crops, grain yield, and economic performance. While the economic performance was similar for pure pea and mixed cropping, we observed a lower variability of the farmer's gross margin in mixed cropping compared with pure cropping across 3 years and two locations. Especially in seasons with high precipitation, the mixed cropping approach prevented lodging and harvest losses. The N supply in the successive crop was equal or higher in mixed cropping compared with pure pea cropping. No variation in the pea root rot infestation levels was observed between mixed and pure cropping, indicating a general demand for resistance breeding. Our experiment indicated genotype-specific effects on the gross margin in regard to culture (pure pea and mixed cropping). Leafy genotypes in particular demonstrate substantial performance gains when cultivated in mixed cropping systems. The conclusion is that mixed cropping leads to similarly positive crop rotation nitrogen effects as pure pea cropping, with the advantage of reduced gross margin and yield variability.

1 | Introduction

A growing demand for plant-based proteins and renewable nitrogen supplies has necessitated an intensification of legume cultivation in Europe. Enhanced legume cultivation has the potential to contribute to the improvement of ecosystem services, agricultural diversification, and the promotion of healthy diets (Oyarzun 1993; Watson et al. 2013; Abberton 2010; Keller

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et al. 2024; Wang et al. 2023). However, the cultivation of grain legumes is inherently susceptible to various risks, including lodging and fungal infections. The root rot complex is a particular threat in legume production. Yield loss in infested fields can result in total yield losses (Oyarzun 1993; Watson et al. 2013). The repeated cultivation of legumes (pea, chickpea, lentil, soybean, bean, faba bean, lupin, and alfalfa) can result in the accumulation of complex soil-borne pathogens (Levenfors 2003). Among all legumes, peas are the most sensitively to the root rot disease in terms of yield decline (Nayyar et al. 2009; Bainard et al. 2017; Pilet-Nayel et al. 2024). Extended rotation breaks and efficient soil management are currently considered the most effective strategies to mitigate outbreaks of soil-borne diseases up to date (Kumari and Katoch 2020; Naseri 2019; Thaines Bodah 2017) while developing resistant varieties is considered the most effective future strategy for managing these types of diseases in legumes (Rubiales et al. 2015). Currently, rotation breaks of up to 10 years are recommended for pea, which conflicts with initiatives aimed at expanding the cultivation of legumes (Pulse Root Rot Network, o. J.).

One possibility to obstacles could be mixed cropping (MC) with a cereal partner. MC entails the cultivation of two or more spatially mixed crops within one field (Hiddink, Termorshuizen, and van Bruggen 2010). In a mixture of peas and cereals, cereals are less prone to lodging and can provide stability for legumes, preventing harvest losses. Furthermore, they suppress weeds and may serve as a natural barrier, impeding the dissemination of legume pathogens in the field (Hiddink, Termorshuizen, and van Bruggen 2010). MC with legumes gains increasing attention because it represents a viable strategy for achieving sustainable intensification of food production, especially under low nitrogen fertilization conditions (Layek et al. 2018; Pampana et al. 2022; Wang et al. 2023). In addition to the higher combined yields per hectare, previous studies have documented improvements in yield stability, lodging tolerance, and weed suppression in MC compared with pure pea stands (Podgórska-Lesiak and Sobkowicz 2013; Weih et al. 2021; Bailey-Elkin, Carkner, and Entz 2022). It thereby addresses the imperative for agroecological transitions in modern agriculture (Bedoussac et al. 2015; Duchene, Vian, and Celette 2017; Raseduzzaman and Jensen 2017; Layek et al. 2018; Lizarazo et al. 2020; Dowling et al. 2021). Although MC has historically played a central role in traditional agricultural practices worldwide (Jodha 1980), its application in industrialized agriculture is primarily limited to forage and cover crop grass-legume mixtures. Due to agronomic constraints such as sowing and harvesting times and weed control, only a few crop species mixtures could offer benefits in agriculture. A promising MC to increase domestic plant-based protein production is the combination of pea (Pisum sativum L.) and spring barley (Hordeum vulgare L.). Barley, when cultivated as a supporting crop, requires minimal irrigation, exhibits a relatively brief vegetative cycle, reaches maturity at an earlier stage of development than wheat, and thus represents a suitable companion for spring- and autumn-sown peas in Swiss agricultural systems. Pea, a leguminous crop, not only provides a high protein yield for animal feed and human consumption but also has a positive impact on soil structure and mineral nitrogen levels (Horner, Browett, and Antwis 2019; Meena et al. 2018). This can be particularly valuable in subsequent crops with high nutritional demands.

To fully exploit the potential of MC and increase the share of legumes in the crop rotation, selecting or breeding suitable pea

genotypes for MC is vital. One key trait of interest is the leaf type. The leaf type represents a pivotal factor in determining the lodging resistance (Singh and Srivastava 2015). Although semileafless genotypes have the capacity to prevent lodging by providing stability to one another, full-leaf types are significantly more susceptible to lodging in pure stand (PS) cultivation. For future breeding activities, a range of promising approaches, like evolutionary breeding and genomic selection, were reviewed by Annicchiarico et al. 2019 and Haug et al. 2023. The researchers analyzed the general and specific mixing ability of a large set of different spring pea genotypes in combination with barley. Their objective was to utilize indirect selection in PS pea to predict mixed stand (MS) performance.

Reducing yield risks for pea growers is a key challenge. We, therefore, examined various pea genotypes in PS and MC with barley. The goals of this study were to (i) determine the productivity potential of MC and its ability to improve yield stability and gross margin; (ii) assess if MC can reduce the pea root rot disease pressure compared with PS; (iii) evaluate if MC results in reduced nutrient fixation within the crop rotation; and (iv) identify genotype-specific MC abilities to improve future breeding strategies.

2 | Material and Methods

2.1 | Experimental Site Description

The experiments were conducted at two locations: one in Fislisbach (FIS, Canton of Aargau 47°25′24″ N, 8° 18′17″ E) and another in Kirchlindach (KIR, Canton of Bern 47°00′08"N, 7°24'45" E) in Switzerland over three consecutive years (2020-2022). Both sites were under certified organic management, where Fislisbach was selected for low soil nitrogen availability at sowing time and with one or no grain legume in the crop rotation in the past 10 years. Kirchlindach was chosen because of extensive root rot problems caused by a complex of different pathogens, resulting from a narrow legume crop rotation in previous years (Fuchs et al. 2014; Wille et al. 2020, 2021). The trials were conducted in subsequent years in nearby fields with the same soil type, respecting the mandatory crop rotation for organic-certified farms. Soil analyses were conducted for each experimental site, including mineralized nitrogen (N_{min}) and the soil fraction at sowing (Table S1). Weather data was collected from nearby weather stations (MeteoSwiss and Meteoblue). The weather station located in close proximity to the Kirchlindach field trial is located at 7°28'/46°59' and provided daily measurements of the air temperature 2m above the ground, total precipitation, and the evapotranspiration above grassland (ET_0) according to FAO. The weather station near to the Fislisbach site located at 47°39′/8°25′ provided daily temperature and precipitation measurements relevant to the location.

2.2 | Genetic Material

Across all 3 years and locations, a total of 35 different spring pea varieties, including one mixture of five different spring pea genotypes and two spring barley varieties, were included in the experiment (Table S2). This set of 35 genotypes consisted of a

core set of 12 pea genotypes tested every year and an extra set of genotypes. The remaining 23 of 35 were selected based on seed availability on an annual base. Each genotype was replicated three times per cropping method (PS vs. MC), site, and year. The core set represents mainly commercially available pea varieties. The extended set of 23 genotypes included both commercial varieties and unadopted genetic resources. The set contained full-leaf and semi-leafless pea line types. The included barley genotypes were the released varieties KWS Atrika (Germany) and Rubaszek (Poland).

2.3 | Experimental Design and Trail Management

The experiment was set up in a randomized complete block design. Each genotype × culture method (culture method = pure or mixed cropping) was replicated three times per cropping method, location, and year. Sowing occurred between March 16 and 31, and harvest between July 6 and 23. The sowing density of PS was 100 germinable seeds per square meter for peas and 400 germinable seeds per square meter for barley. MS comprised 80% pea plus 40% barley of their respective PS seed density (according to recommendations of Dierauer et al. 2017). For MS seeds of pea and barley were mixed before sowing. No supplementary fertilization or mechanical weed control was applied.

In order to assess the supply of plant available nitrogen in the different cropping systems (PS pea, PS barley vs. MC), mustard (*Synapis alba* L.) was sown within 1–2 weeks after the harvest of the main trial at Fislisbach (mid of October). After removing the

plant residues, minimal soil preparation was carried out before sowing to minimize possible N transfer on the field.

2.4 | System Productivity Assessment

The total grain and protein yield of the three cropping methods PS pea, PS barley, and the MC pea and barley combination, was measured at both locations in all 3 years. The harvested seeds were separated for the MC cultivation method to assess the pea ratio of the total MC yield. The separation is relevant to calculate the premium (a subsidy for growing peas, Table 1), seed weight, and water content. According to farmers' practice, the water content was corrected to 15% in pea and 16% in barley to calculate yield in deciton per hectare (dt/ha). A non-destructive NIRS measurement assessed the protein content according to the producers' suggestions (Foss Infratec 1241 ANALYZER ["Infratec-NIR Grain Analyser," o. J.]). Further, we calculated the gross margin relative to the Swiss market and agronomic regulations to have a comparable unit (Ofori and Stern 1987). The production income and cost information was collected from the 2023 Agridea gross margin catalogue (Table 1, [agridea and FiBL 2023; "Deckungsbeiträge DBKAT-Software-Betrieb, Familie, Diversifizierung-AGRIDEA," o. J.]).

All 35 genotypes were used to identify significant variations between the cropping methods (PS pea, PS barley, and MC pea and barley), locations, and annual effects regarding grain yield, gross margin, land equivalent ratio (LER), and protein yield. Additionally, the core set of 12 from these 35 genotypes was used to calculate the gross margin according

TABLE 1 | base values to calculate the gross margin for pea, barley and the intercropped culture method.

		Pea	Mixed cropping	Barley
Production income	Grain Yield (CHF/dt)	98	Pea = 98; barley = 78	78
	Area bonus (CHF/ha)	1000	1000 (if more than 30% of yield in MC is pea, else 120)	120
	Straw value (CHF/dt)	0	0	7
	Organic bonus (CHF/ha)	1100	1100	1100
	Supply security bonus (CHF/ha)	1200	1200	1200
	Extensive bonus (CHF/ha)	400	400	400
Production costs	Seed (CHF/ha)	495	515	263
	Fertilizer (CHF/ha)	0	0	240
	Working costs (CHF/ha)	495	440	440
	Drying (CHF/dt)	1.45	Peas = 1.45 ; barley = 1.23	1.23
	Cleaning (CHF/dt)	4.65	Peas = 4.65 ; barley = 3.5	3.5
	Separation (CHF/dt)	0	3.5	0
	Insurance (%)	3.4	3.4	2.2
	Machine costs (CHF/ha)	328	279	421
	Other (CHF/ha)	1.12	1.12	1.12

Note: 1 CHF=1.18 US\$, 1dt/ha=10 g m⁻². Source: Deckungsbeiträge catalouge agridea 2023. to genotype. For the gross margin and protein yield, outliers were identified and removed by the interquartile range. Any data point observed beyond the 1.5× interquartile range above or below the 0.25 and 0.75 quartiles were removed (Tukey 1981; Walfish 2006). Subsequently, normality was tested by a Shapiro–Wilk normality test and a mixed linear model (R package lme4; Bates et al. 2015) with the replication within location and year as a random factor and the culture method, interaction of culture method with year and location as the fixed effect was calculated (Equation 1; Bates and DebRoy 2004; Gurka and Edwards 2011).

Gross
$$margin_{ian} = m_n + m_n x z_a + m_n x l_j + (r)_{g_{minj}}$$
 (1)

Adjusted means were calculated from the described model, where m_n is the culture method, z_a is the year, l_j is the location and r_{giaj} is the replicate of the ith genotype in nth culture method, the jth location and the ath year. Any pairwise comparisons were conducted using an honest significant differences (HSD) Tukey test (Steel, Torrie, and Dickey 1997). The same procedure was applied to estimate variations in the total seed protein content between the culture methods.

The core set was utilized to undertake a detailed examination of any genotype-specific PS and MC abilities within the core set of genotypes. We, therefore, estimated the adjusted means for each of the 12 genotypes from the core set in the PS and MC method using the simplified mixed model (Equation 2)

Gross margin_{ianj} =
$$m_n x g_{pi} + (z_a) + (l_j) + (r)_{g_{niaj}}$$
 (2)

where we omitted genotype interaction effects with the site (l_j) and year (z_a) to simplify the model (round brackets indicate random effects). A subsequent pairwise comparison of the genotype's adjusted means to each other and of the cropping method per genotype should indicate genotypes with significant advantages compared with the other genotypes. Further, a pairwise comparison was conducted to identify genotypes with significantly different gross margins comparing MC with pure cropping.

The total grain yield was examined using all 35 genotypes tested to determine variations between the cropping methods. For the grain yield of the pea and barley components, the pea genotype was included as a fixed cofactor in the model. The measured yield was normally distributed after a square root transformation and no outliers were removed. Two identical models were created to estimate adjusted means for the pea and barley yield separately (Equation 3).

Pea | Barley grain yield_{ian} =
$$m_n x g_{pi} x z_a x l_j + (r)_{g_{piai}}$$
 (3)

Based on the grain yield calculations, the LER of grain yield per crop (fraction LER_{pea} , fraction LER_{barley}) was calculated by dividing the yield of pea (barley) in the MC method by the yield in the PS cropping approach within the same year and site per genotype. Afterward, the total LER (= fraction LER_{pea} + fraction LER_{barley}) was calculated and an ANOVA was conducted on these values to identify relevant site × year interactions affecting the LER. In the same manner, the grain yield per culture was determined by separating the barley

and pea components in the mixed model and merging them afterward.

Finally, we investigated in the core set of 12 genotypes if the barley protein content was elevated in the mixed cropped compared with the pure barley cultivation (Equation 4).

ProteinContent_{ian} =
$$m_n + m_n x z_a + m_n x l_j + (r)_{g_{nigi}}$$
 (4)

 m_n is the culture method, z_a is the year, l_j is the location, and r_{niaj} is the replicate of the *i*th genotype in *n*th culture method, the *j*th location, and the *a*th year.

2.5 | Pea Root Rot Complex (PRRC) Depending on the Cropping System

The symptoms of pea root rot complex (PRRC) were assessed in the years 2020, 2021, and 2022 for PS and MC at the field location Kirchlindach for a subset of 18 pea genotypes and one mixture of genotypes. The emergence of four genotypes was insufficient in 2021. We, therefore, excluded them for the respective year. The pea lines were selected based on contrasting root rot resistance and seed availability, with the full-leaf winter pea "EFB.33" considered as the resistant standard (Baćanović-Šišić et al. 2018) and the semi leaf-less "Respect" as susceptible standard (Wille et al. 2020, 2021).

Root rot symptoms were scored on 15 randomly selected plants per plot in three replicates on a scale from 1 to 9, as described by Wille et al. 2020 at a time point with good differentiation of the disease expression among the pea lines (BBCH stages 51–65; equal to flower development to 50% of all flowers fully emerged). A score of one is associated with no visible infections, while nine is related to a dead plant. An average infection score was calculated subsequently from these 15 measurements. Outliers were removed if the values were >1.5 times the inter-quantile distance beyond the 0.25 or 0.75 quantiles. Subsequently, the normality of the residuals was determined by normal quantile-quantile (QQ) plots of the residuals of the linear model

$$y_{ina} = g_{pi} x m_n + z_a + r_{g_{ia}},$$
 (5)

where g_{pi} presents the pea genotype, m_n the culture method, z_a the year, and r_{gia} the replicate of each genotype within the respective year (Equation 5). The same model with the replication as a random factor was used to calculate adjusted means to investigate if the culture method (PS pea, PS barley, and MC) had an impact on the PRRC intensity and if genotype-specific variations between these two methods can be identified.

The explained variance of all experimental parameters was assessed by a simple linear model, including the parameters culture method m_n , pea genotype g_{pi} , barley genotype g_{bi} , year z_a , replication r_{gia} , and interactions, as indicated in Equation (6) (Bates and DebRoy 2004; Gurka and Edwards 2011).

$$y_{p/bia} = m_n + g_{pi} + g_{pi} x g_{bi} + g_{bi} x z_a + g_{pi} x z_a + r_{g_{ia}}$$
 (6)

The difference among the genotypes in regard to the PRRC symptom level was determined by adjusted means and a

subsequent Tukey HSD test separately for the MC and PS on the mixed model (Steel, Torrie, and Dickey 1997)

$$y_{ia} = g_i + z_a + (r)_{g_{ia}}. (7)$$

where g is the genotype, z the year, and r the replicate as a random factor.

2.6 | Soil Nitrogen Balance Affected by the Cropping System

Soil mineral nitrogen (nitrate and ammonium) measurements were conducted in Fislisbach in 2022 in two rhizosphere horizons (0-30 cm and 30-60 cm). Measurements were performed at the flowering stage of pea (BBCH 65, only 0-30 cm), full ripening of pea barley MC (BBCH 91, harvestable), and the flowering stage of the following cover crop mustard (BBCH 65). The sampling was conducted by drilling a drill stick into the soil at three random locations in the plot. The soil was bulked, and the NO₂ and NH₄ concentrations were then measured with the 0.01-M CaCl₂ extraction method (according to Wey et al. 2021). Soil sensors (Plant Root Simulators [PRS] from Western AG, Canada) were placed in the plot to measure the anion and cation flux in the soil matrix of the top 30 cm in a period of 10 days. Four pairs of anion and cation probes were placed in the plots during flowering and harvest time points of pea, barley, or the mixed-cropped variant. Additionally, the sensors were placed in the flowing catch crop mustard for 10 days (BBCH 65). The measured sum of NO2 anions passing the membranes in the duration of field exposure was converted to nitrogen per hectare by the formula:

> NO_3 in mg/areacovered $(0.00015 \, \text{m}^3)$ \times soilmassinthe top 30 cm in one hectare $(3000 \, \text{m}^3)/1$ $\times 10^6$ (conversion mg to kg)/daysinthesoil \times the Molarweight of N in NO₃ (1/4.5)

Given the arduous nature of this task, we selected the genotypes exhibiting the most pronounced phenotypic differences. In total, 175 soil samples were collected in all three cultures (PS barley, PS pea, and MC), with 33, 82, and 60 measurements in barley, pea, and MC, respectively. Five different pea cultivars ("Astronaute," "Impuls," "Milwa," "Protecta," and "Respect") and one barley cultivar ("Atrika") were used for the soil mineral nitrogen analysis. Each genotype×cropping method was replicated three times. For each sample, the nitrate and ammonium nitrogen were separately measured and combined to obtain a mineral nitrogen ($N_{\rm min}$) measurement in kilogram per hectare. Further, the total dry biomass in the following catch crop mustard was measured at the time of flowering by harvesting the biomass of a randomly selected 0.5-m² area in the plot and dividing it into the fractions mustard and weeds.

The collected data were analyzed by a linear mixed model, where the nitrogen component was dependent on the cropping method, depth of measurement (30 or 60 cm), time point of measurement, and genotype (Bates and DebRoy 2004; Bates et al. 2015; Henderson 1982). The replicate in block was considered a random effect. A subsequent ANOVA was performed

to identify which variable significantly affects the nitrogen concentration of the soil. Pairwise comparisons were conducted on adjusted means using an HSD Tukey test from the "emmeans" R package. Besides the total $N_{\rm min}$, we also examined the two subfractions:nitrate (NO $_{\rm 3}$) and ammonium (NH $_{\rm 4}$). If the measurements were not normally distributed, these were transformed to a normal distribution by either a log or a square root function. The normality of the residuals was examined by a Shapiro–Wilk normality test (Shapiro and Wilk 1965). Any transformed data were reconverted for illustrations or tables.

2.7 | Prediction of MC Ability

Lastly, we assessed multiple phenotypes at different developmental stages. The assessed traits include emergence, early vigor, soil coverage, PRRC (see above), biomass estimation, flowering time, canopy height, SPAD, lodging, and stipule length (Table 2).

The adjusted mean was calculated per genotype. A subsequent Pearson correlation of the traits to the gross margin was estimated to identify phenotypes suitable to predict the gross margin. Significantly correlated phenotypic traits were subsequently correlated to each other to identify and remove redundant traits (limit: r = 0.7). The remaining phenotypic traits were implemented in a linear mixed model (replicate as random, all others as fixed factors), where these phenotypic parameters were used to model and predict the gross margin. The goal was to identify a suitable combination of two easily measurable phenotypic traits in PS pea to predict the gross margin in MC approaches. Subsequently, the predicted values were correlated with the actual gross margin in order to ascertain the optimal model. In addition to the aforementioned correlation, the root mean square error (RMSE) was employed as a further evaluative measure (Chai and Draxler 2014). The objective was to achieve a high degree of correlation between the predicted and actual gross margins, as well as to minimize the RMSE.

2.8 | Statistical Analysis

All statistical analysis were conducted with R 4.3.1 and the packages ggpubr (v0.6.0), lme4 (v1.1.35.1), emmeans (v1.8.9), ggplot2 (v3.4.4), lmerTest (v3.1.3), gridExtra (v2.3), ggpattern (v1.0.1), ggsignif (v0.6.4), kableExtra (v1.3.4), stringr (v1.5.0), tidyverse (v2.0.0), formattable (v0.2.1), and agricolae (v1.3-7) with the details of the models for the various data sets as given above. The heritability was estimated using the Cullis approximation (Cullis, Smith, and Coombes 2006).

3 | Results

3.1 | System Productivity Assessment

Across the 3 years, we see a significant environmental (site, year) effect (p < 0.001). The heritability of the pea grain yield is $H^2 = 0.757$, and the total MC yield of $H^2 = 0.71$ across all years and locations. The pea yield was significantly lower in Kirchlindach (KIR) compared with Fislisbach

TABLE 2 | Traits assessed on two cropping methods pure pea and mixed cropped pea barely crop at both location in all 3 years (except PRRC was assessed in KIR only).

Trait Unit of assessment		Time point	
Emergence	Plant count 2×1 m per plot	BBCH 09-13	
Early vigor	Score 1–9, 9 given for highly vigorous plants, estimation on plot level	BBCH 17-19/32-35	
Soil coverage	Estimation of pea, barely, weed and uncovered area in % (total 100%) of a representative part of the plot, view from above	BBCH 39-51 and BBCH 73-78	
Pea root rot complex	Score 1–9 on 15 randomly selected plants per plot, 1 given for no visible infection, while 9 is related to a total decay of the root system or stem (according to Wille et al. 2020	BBCH 65/66	
Biomass	Score 1–9, 9 being plots with the highest biomass, estimation on plot level	BBCH 65	
Onset of flowering	Day after 1st of January when 50% of the plants of a plot had started flowering	BBCH 65	
Stipule length	Measurement of the two most distinct tips of the 2nd topmost fully developed stipule, on four representative plants per plot.	BBCH 75	
Canopy height	Measurement on plot level. Pea and barley separately assessed	BBCH 17-19/30-38 BBCH 62-69 and BBCH 85-95	
SPAD	Measurement of two leaves from 4 representative plants per plot ($n = 8$, 2-3rd highest leave) (Chlorophyll Meter Spad-502PLUS, Konica Minolta)		
Lodging	Score 1–9, 5 was a plot lodging in a 45° angle and 9 was a fully lodged plots, estimation on plot level	BBCH 74-79 and BBCH 85-87	

(FIS)—especially for PS pea method (p < 0.001, Figure 1A). The yield of peas was lower in 2021 than in both 2020 and 2022, with statistical significance (p < 0.001) observed in both the MC and PS pea approaches.

The LER was significantly higher in FIS (1.18) compared with KIR (1.12) (p=0.05) in the years 2021 and 2022 (p<0.005), with a heritability of 0.543. The years 2020 and 2022 deviated from the year 2021. Year 2021 was different from the other years as we had extremely high precipitation during the whole growing season (Figure S1) and a stronger side effect, as the humidity promoted the PRRC symptoms in KIR. Although the LER was 1.33 (FIS) and 1.10 (KIR) which is above or close to the interannual average, the pea share in the LER was significantly lower at the KIR site in 2021, reaching only a value of 0.31, less than half the observed value compared with FIS (0.67) (Figure S1B).

The heritability for the pea protein content was 0.542, which presents a significant effect for the culture, year, location, and interaction of culture with year and site (p<0.001) for the entire set of 35 genotypes. The genotype component was significant as well (Table 3). The average protein yield across all years and locations was 3.61 dt/ha in PS barley, 5.82 in PS pea, and 5.35 in MC (Figure 1C). All of these three culture methods were significantly different from each other (p<0.001). Similarly, the protein yield differed between the years, ranging from 3.62 dt/ha (average across all cultures and locations)

in 2021 to 6.35 dt/ha in 2022. Generally, we observed a significantly higher protein yield in PS pea and MC compared with PS barley across all years and sites. The year had the biggest impact on the protein yield, explaining more than 58% of the total variance. In the context of the culture and year interaction, we observed a 3.52 times higher variance in the PS pea protein yield compared with that of the MC (7%, Table 3). For the culture-site interaction, we observed a higher protein yield in PS peas in the not-infested field (FIS; no PRRC present, 0.96 dt/ha higher in pea, p < 0.001) while the difference between MC and PS vanished in the infested field (KIR; p = 1). A comparison of PS pea and MC revealed that protein yield was higher in 2022 at both sites in pea (p < 0.001) and at FIS in 2020. Conversely, protein yield was higher in 2021 at both locations for MC (Figure 1C). The protein content of barley was another subject of interest for us. Although, on average, the protein concentration in the seed was 10.9% in PS barley, the protein content of the barley grains increased significantly to 12.6% by MC (p < 0.001, data not shown).

As the total grain yield, LER and protein content might not be conclusive for a comparative analysis of the three cropping methods from the perspective of a farmer, we also calculated the gross margin per hectare for the Swiss market situation in 2023 (Figure 1D). In each year and site combination, the PS pea and MC resulted in a significantly higher gross margin than PS barley (p < 0.001, H²=0.662). While a farmer could earn, on average, 4800 US dollars (US\$) per hectare growing barley, the monetary

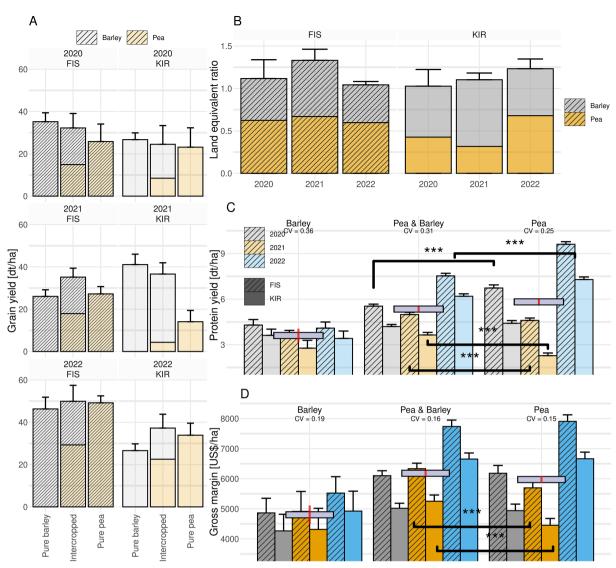


FIGURE 1 | System productivity assessment by (A) the total grain yield of pea and barley in the six environments for the three cropping systems pure (PS) pea, mixed cropping (MC) pea barley, and pure barley. The three rows differentiate years (2020–2022), columns and bar pattern the two sites (FIS=Fislisbach, KIR=Kirchlindach). Gray bars indicate the barley yield, while yellow ones show the pea yield. The error bar signals the standard deviation. The grain yield is shown in decitons per hectare (1 hectare=10,000 m²); (B) Land equivalent ratio (LER) for grain yield for the MC pea barley culture per year and site. The error bar describes the standard deviation. The partial LER of pea (yellow) and the partial LER of barley (gray) in MC are illustrated by color. (C) Similar to subpart (D), only for the total protein yield per hectare in decitons. (D) The gross margin to be earned per hectare in the respective year in US\$ (gray, orange and blue bars indicate 2020, 2021, and 2022, respectively) and site (plain bars (Kirchlindach, dashed bars [Fislisbach] in Switzerland [color legend analog to subpart C]). Error bars indicate the standard error. The horizontal gray squares indicate the average gross margin across years and environments per culture method. The red vertical lines describe the 95% confidence interval related to the gray square. Stars and brackets indicate significant variations between pure pea and intercropped pea and barley combinations (significant differences to barley not shown). CV, coefficient of variation.

yield is 1200 and 1360 US\$ higher when growing PS pea or MC pea barley, respectively (p<0.001). Across the 3 years and all 35 pea genotypes, the MC resulted in the highest gross margin with the lowest standard error—significantly higher than the PS barley and PS pea yields (p=0.005, Figure 1D, gray bars). When we investigated the difference between MC and PS pea at the year level, we distinguished similar gross margins in 2020 and 2022 but a significantly higher gross margin in 2021 in MC compared with PS pea (p<0.001, +720 US\$/ha, Figure 1D, black brackets)

Further, we investigated whether a genotype effect on the gross margin exists by examining the core set of 12 genotypes in all 6year \times site combinations. We observed both the genotype and genotype \times culture method interaction to be significant (ANOVA, genotype p<0.001, genotype \times culture p=0.005). Except "Volt," all genotypes had higher gross margins in the MC than in PS pea, calculated across all sites and years (Figure 2). The best-overall performing genotype in MC was "Protecta" while under PS pea cropping, it was "Karioka" (Figure 2). The two genotypes "Protecta" and "S199" showed a significant gross margin increase under MC compared with PS ($p_{Protecta}=0.05$, +685 US\$; $p_{S199}=0.003$, +1690 US\$) (Figure 2). A leafy karyotype characterizes both genotypes. Genotypes with a leafy morphology had, on average, a significantly higher (p=0.03)

TABLE 3 | Explained variance (R^2) and the p value of the linear model variables effecting the gross margin (US\$/ha) and the protein yield (kg/ha) in the core genotype set (12 pea genotypes and one barley genotype) at Kirchlindach and Fislisbach from 2020 to 2022.

	Gross margin		Protein yield	
	Expl. var.	р	Expl. var.	р
Genotype	0.164	< 0.001	0.119	< 0.001
Culture method	0.005	< 0.001	< 0.001	0.247
Year	0.391	< 0.001	0.554	< 0.001
Site	0.184	< 0.001	0.112	< 0.001
Genotype×cult. method	0.017	< 0.001	0.014	< 0.001
Genotype×year	0.035	< 0.001	0.021	< 0.001
Genotype×site	0.015	< 0.001	0.013	< 0.001
Cult. $method \times year$	0.054	< 0.001	0.067	< 0.001
Cult. $method \times site$	0.001	0.191	0.019	< 0.001
Replication in site×year	0.006	0.004	0.003	0.041
Residuals	0.127	_	0.077	_

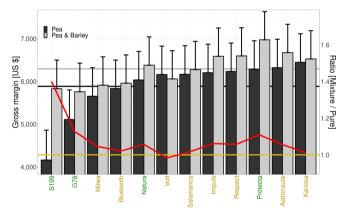


FIGURE 2 | Mixed cropping ability, correlation of phenotypic traits to the gross margin and the gross margin prediction ability in mixed cropping by selected traits. The gross margin per genotype (12 genotypes present in all 3 years and both sites) in the pure pea cropping (black) and the mixed cropping (gray) scenario. The error bars indicate the standard error. The horizontal black line shows the average gross margin across all genotypes in pure pea cropping. The gray horizontal line illustrates the similar information for the mixed cropping. The yellow line refers to the second y-axis (right side), and shows gross margin ratio of mixed cropping to pure pea cropping of 1—where the same gross margin was achieved in mixed cropping and in pure pea cropping. The red line is the actual ratio of the mixed cropping to pure cropped gross margin for each pea genotype. Values above 1 indicate a higher gross margin in the mixed cropping scenario (e.g., S199 has a ratio value of > 1.4, indicating a 40% higher gross margin in the mixed cropping compared with the pure pea cropping approach; Volt has a ratio value <1, indicating a higher monetary gain in pure than mixed cropping). Colors of the xaxis genotype names refer to leafy (green) and semi-leafless (golden) karyotypes of pea genotypes.

income increase under MC over PS (837 US\$) compared with the semi-leafless karyotype (195 US\$). Under the pure pea cropping, the variety S199 had a low gross margin and significantly differed from 10 of 11 other genotypes (p<0.01). The best-performing genotypes with respect to gross margin in PS pea were "Karioka," "Astronaute," and "Protecta." The rank correlation (Table S3, columns 3 and 4) of the genotypes under PS pea and MC was r=0.72, indicating a general, equal gross margin pattern for PS pea and MC across the genotypes.

3.2 | PRRC

The root rot infestation significantly affected the gross margin (p < 0.001). Across all 3 years, the observed disease symptoms of PRRC ranged from 3 to 6 on a scale from 1 to 9. More than 50% of the root rot's variation can be explained by the pea genotype (29%), year (9%), pea genotype and year interaction (7%), pea and barley genotype interaction (3%), and the culture method (3%) (Figure 3A). The highest disease pressure was observed in 2021, with an average of 5 and 5.33 in the MC and PS pea culture methods, respectively (Figure 3B). This observation was made in the context of total precipitation in 2021, summed up to 657 mm in KIR and 570 mm in FIS, which equals 2.05 and 2.11 times the other 2 years' precipitation sum (Figure S1). A Pearson correlation revealed a substantial variation of the PRRC across the years (Figure 3C), which had a heritability 0.49 across all years for the PRRC. Although significant variations between the genotypes within the culture methods exist (Figure 3D), no significant interaction between the genotypes and the culture method was observed. None of the tested pea genotypes showed a significant change in symptoms of PRRC between the different culture methods (PS pea and MC). Genotypes with a low root rot index in the MC scenario were also found among the genotypes with a low root rot index in the pure culture and vice versa.

3.3 | Mineral Nitrogen Content in the Soil

At the harvest time points of (I) pea and barley and (II) the follow-on mustard at development stage BBCH 65 (50% of all flowers emerged), measurements were made of the soil mineral nitrogen content. Significant variations were detected for the cropping method, the sampling depth, and the time point (p < 0.001). Pea genotype and replicate had no significant effect. Across both time points, the concentration of available nitrogen (NH₄+NO₂) was significantly higher in the topsoil horizon (0-30 cm) compared with the lower horizon (30-60 cm) (p < 0.001). The N_{min} measurement at the harvest time point differed significantly between PS barley, PS pea, and the MC (p < 0.001, Figure 4A). Both PS pea (41 kg/ha) and MC (47 kg/ha) divagated significantly from the PS barley (31 kg/ ha) (p < 0.001). When the N_{\min} was divided into a nitrate and ammonium fraction, almost no ammonium was observed, regardless of soil horizon, cropping method, and measurement timepoint (Table 4). Although the soil N_{min} concentrations were similar in the follow-up crop mustard (comparing PS to MC to Barley, data not shown), the produced mustard biomass was 2.5 times higher on plots with previous PS pea and MC of pea and barley compared with PS barley (p = 0.1 & p = 0.05,

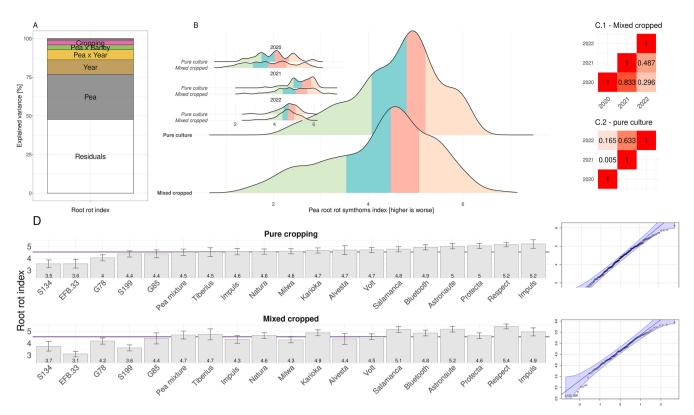


FIGURE 3 | Pea root rot complex (PRRC) symptoms (1 = no symptoms, to 9 = severe symptoms/dead plants) levels of 18 pea genotypes and one mixture of five genotypes grown in pure (pea) and mixed cropping (pea-barley) method in three consecutive years. (A) Explained variance of PRRC by the experimental variables (cropping = pure or mixed cropping); (B) Distribution of the PRRC scores for each year (small subfigures) across years for both cropping methods (mixed and pure pea cropping). Colors differentiate the 0.25, 0.5, and 0.75 quantiles; (C) Pearson correlation of PRRC scores between the years for mixed and pure pea cropping; (D) adjusted mean infestation scores on genotype level in mixed and pure pea cropping methods. No genotype was significantly different for the cropping method. The blue (mixed) and red (pure pea) lines indicate the average mean across the entire set of genotypes. Pea line EFB.33 is considered as resistant and "respect" as susceptible standard. The qqnorm plots illustrate the distribution of the residuals.

Figure 4B). The biomass development of the mustard catch crop mustard indicates the plant's available nitrogen content in the soil after the harvest of pea and barley. Further, the harvested biomass of weeds was almost doubled in the mustard plots following PS barley compared with the plots following PS pea and the MC (p = 0.09, p = 0.04). This indicates that the overall productivity of mustard following PS barley was significantly reduced compared with PS pea and MC, while there is no difference between PS pea and MC.

As the mentioned $N_{\rm min}$ measurements are limited to a single time point and soil mineral properties are subject to change over time, we additionally assessed the NO₃-derived $N_{\rm min}$ concentrations in the soil for 10 days during flowering, harvest time point of PS and MC as well as in the follow-on mustard catch crop mustard at development stage BBCH65 stage with soil sensors (plant root simulators, PRS). The results of the PRS (Figure 4C) indicate a peak of $N_{\rm min}$ concentration in the top soil at the harvest time point for the MC, which was significantly higher than the $N_{\rm min}$ concentration of PS pea and PS barley (Figure 4C). The $N_{\rm min}$ concentration of MC and PS pea was also significantly higher at the flowering time compared with PS barley (p<0.01), indicating the high nitrogen uptake of barley until the flowering stage. The $N_{\rm min}$ concentrations in the soil equalize to similar values across all three cropping methods in

the follow-on mustard catch crop at BBCH 65 measurement, which can be partly attributed to higher N uptake by mustard weed biomass in the plots with previous PS barley cultivation. There is a general trend that the $N_{\rm min}$ concentration in the soil of PS barley plots increases from flowering to harvesting and the catch crop, whereas the opposite trend was observed for PS pea method.

3.4 | Prediction of MC Ability

Another aspect of interest was identifying physiological or phenotypic traits measured in PS pea, which could be useful as predictors for the gross margin in MC scenarios for Swiss farmers. The canopy height, early vigor, SPAD, and LER had the highest Pearson correlations with the gross margins among all tested parameters ranging from 0.35 to 0.61 (Figure S2). We further used the phenotypes plant height, SPAD, and early vigor (H^2 =0.791; 0.903; 0.873) to forecast the gross margin in MC. The models based on the height and SPAD, and vigor and SPAD assessed in PS pea resulted in the best correlations (r=0.78, Figure S3A). Although the correlation was equal, the RMSE was 120 US\$ lower in the vigor & SPAD model, making it the better model with the lower residual deviation (Figure S3B)

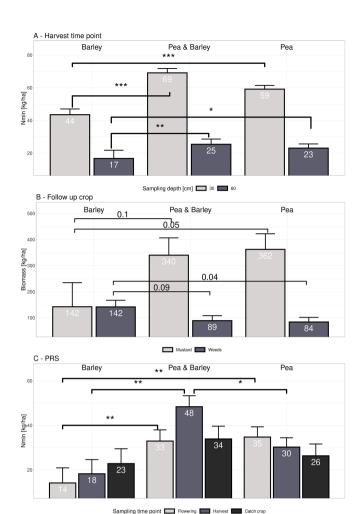


FIGURE 4 | (A) Mineral Nitrogen content ($N_{\rm min}$ kg/ha) in the soil measured in 2022 at the harvest of pea and barley and Error bars show the standard error. Brackets highlight (marginally) significant variations (*p<0.05, **p<0.01, ***p<0.001) between cropping methods by sampling depth. The color differentiates the sampling depth (light 0–30 cm, dark 30–60 cm) (B) biomass of the mustard catch crop at flowering in plots with previous pure pea, barley, or mixed pea–barley cropping. Error bars, colors, and brackets similar to subfigure A. (C) $N_{\rm min}$ estimation of topsoil (0–30 cm) by the PRS soil sensors for a period of 10 days at three time periods. Reported values are equivalent to the sum across the measured time horizon. Sampling time points (flowering, harvest, and flowering of the catch crop at flowering stage) are separated by color. Error bars show the standard error. Brackets indicate significant variations (*p<0.05, **p<0.01, and ***p<0.001) between cropping methods by sampling time.

4 | Discussion

Farming is becoming more and more challenging due to the increased demand for affordable food produced on the same or reduced farmland, the need to mitigate adverse impacts (e.g., loss of soil fertility, biodiversity decline, contamination of air, water, and soil, and CO_2 emissions) accelerated by climate change, and increasingly extreme weather scenarios ranging from extensive drought periods to intense rainfall events. In this context, cropping methods with the potential to reduce the risks of yield losses and increase production per area should be tested

to provide valuable information to farmers and strengthen food production and security. Our case study focused on the MC of pea and barley in Switzerland and how this can enable farmers to produce more ecologically and economically sustainable manner. Legume production is challenging, and mills usually do not buy crop mixtures. Therefore, cost-efficient sorting techniques are needed. In Switzerland, the promotion of MC started 15–20 years ago, and today, modern sorting techniques make the separation of seeds from MC possible in Switzerland (Bedoussac et al. 2021). Now, in-depth investigations of different cropping systems under agronomically relevant conditions are required to deliver evidence-based decision support for farmers.

4.1 | Economic Aspects

The LER is a popular measurement emphasizing the value of MC systems. Nevertheless, it is difficult to translate the LER into a definitive pro or con for MC cropping. Therefore, we calculated the gross margin under Swiss conditions to provide farmers with an easy-to-interpret value. The gross margin calculation allows us to untangle MC's advantages and disadvantages easily (Khanal et al. 2021). Analogous to the findings of (Bedoussac et al. 2015), our experiment has shown a lower inter-annual fluctuation of the gross margin in MC compared with pure pea, with a non-significantly different average yield across the years. In fact, the gross margin across the 3 years was highest in MC. This indicates that MC poses a valid option to minimize yield losses due to unfavorable weather conditions in the cropping season. For example, in the 2021 season, which was characterized by extremely high rainfall during harvest, all pea varieties grown in PS were severely affected by lodging (especially leafy karyotypes), resulting in significant harvest losses. Compared with the pure pea cropping, the MC did not show similar problems during harvest because the barley plants prevent the pea from lodging. In line with our findings, Podgórska-Lesiak and Sobkowicz 2013 have also highlighted this positive aspect of improved lodging resistance. What could be seen in this experiment as a substantial source of an experimental error can also be considered an ordinary risk farmers have to deal with these days—the risk of harvest losses due to low harvestability and growing out in a wet harvest season.

To determine the suitability of genotypes for MC, we used a subset of 12 genotypes present in all tested years and sites. Although the genetic diversity was reduced in the core set, we observed genotypes that performed better under MC, like S199. The Protecta (leafy karyotype) and Astronaute (semi-leafless) genotypes performed well under both pure and MC conditions. Another well performer for pure pea cropping is Karioka. For MC, it is Impuls. Further, testing genotypes in MC yield trials is costly, so we also tried to identify predictor traits that could help breeders to select genotypes that were likely to perform well in MC. The early vigor (similar to Haug et al. 2023) and the SPAD measurements provided a reasonably good estimate of the MC yield in pure pea-cropped genotypes. Incorporating these two phenotypic measurements in a simple linear model could enable breeders to unselect genotypes with a poor gross margin prediction.

TABLE 4 Ammonium and nitrate concentration (kg/ha) in the soil of plots for the three culture methods PS barley, PS pea, and MC (Pea Barley), differentiated by sampling depth (top 0–30 cm, 30–60 cm) and time point (harvest of pea and barley, flowering of following mustard catch crop BBCH 65).

		Ammonium		Nitrate	
	Depth	Harvest	Catch crop	Harvest	Catch crop
Barley	30	0.03 ± 0.01	0.10 ± 0.01	12.08 ± 3.47	6.70 ± 1.06
Pea	30	0.13 ± 0.01	0.13 ± 0.00	16.01 ± 2.26	5.70 ± 1.04
Pea Barley	30	0.08 ± 0.01	0.12 ± 0.01	18.82 ± 2.59	6.48 ± 1.05
Barley	60	0.08 ± 0.01	0.40 ± 0.00	5.24 ± 1.14	2.56 ± 1.13
Pea	60	0.31 ± 0.00	0.31 ± 0.00	7.05 ± 1.07	2.04 ± 1.08
Pea Barley	60	0.24 ± 0.00	0.38 ± 0.00	7.85 ± 1.09	2.42 ± 1.11

4.2 | Soil-Borne Disease

Another aspect that affects both grain yields and the gross margin is infestation with soil-borne diseases from the PRRC. This complex can consist of pathogens like Aphanomyces euteiches, Fusarium solani, and F. oxysporum, which can cause severe root damage and can lead to plant death (Hossain et al. 2021; Persson, Bødker, and Larsson-Wikström 1997). These reports indicate that the compositions of the root and rhizosphere microbiomes are closely linked to plant health. Similar phenotypic characteristics were observed at the site Kirchlindach, which was chosen for its severe soil fatigue due to previous iterative legume cultivation. Across the 3 years, we observed a negative correlation between the root rot severity with the grain yield and gross margin. Regardless of the culture method (MC and pure cropping), the root rotinfested field resulted in a yield reduction. This reduction did not indicate significant variations in PRRC symptom levels between MC and PS, other than what we hypothesized. The idea was that barley plants in the mixed system could act as a physical barrier to prevent the rapid spread of the disease in the canopy. However, Cadot et al. (2024) reported equal or increased infestation levels in mixtures of pea and barley. Another hypothesis was that an increase in the barley sowing density might have a reductive effect on the infestation pressure due to a potentially higher border effect. However, that would have a negative effect on the pea yield in the mixture, as previously reported by (Tosti, Falcinelli, and Guiducci 2023). The barley yield was reduced in two of the 3 years (comparing KIR to FIS, Figure 1A), which might indicate that barley itself got infected by some of the pathogens present in the PRRC. As Xiong et al. (2023) have reported, the soil-borne pathogen problem is becoming more prevalent for cereal crops.

4.3 | Resource-Efficient Agricultural Production

Pea, as a legume crop, has a high value in crop rotation, especially in organically managed farms, due to its potential to fixate atmospheric nitrogen. The comparison of the mineral nitrogen content in barley, pea, and the MC alternative indicated equal or higher soil mineral nitrogen contents in the MC compared with the pure pea cropping, as previously also shown by Jensen, Carlsson, and Hauggaard-Nielsen 2020 and

Salinas-Roco et al. 2024. As reported earlier, N-fertilizer and higher N-levels in the soil can inhibit the N2-fixation by pea (Pampana et al. 2022, 2018). Therefore, the barley component in the MC might have stimulated the biological nitrogen fixation. Barley consumes the soil's available mineral N, which triggers peas to intensify their nitrogen fixation. Although soil N_{min} measurements indicated a higher total nitrogen concentration after MC, it did not translate into higher biomass in the following catch crop. Both pure pea and MC lead to a high production level in the follow-on catch crop. Additionally, the N_{min} levels measured in the mustard catch crop were statistically not different from each other anymore (Figure 4C). The reason for this unproductive N_{min} decline, which did not translate into higher mustard biomass, might be associated with the high C:N ratio in the barley residuals (root and shoot tissue) left on the field. This carbon-rich residual biomass on the field increased N immobilization and reduced nitrifying microbial activity, as described among others by Wang et al. (2023). It can be postulated that a proportion of the N_{min} in MC was employed to increase the total soil carbon pool (Hu et al. 2023).

Further, mustard after barley was much more prone to severe weed infestations of the field plots, indicating elevated sustainability of MC compared with pure barley cropping regarding herbicide management. Similar observations have been reported by Tavoletti, Cocco, and Corti (2023).

As the high weed density was accompanied by low productivity in mustard after barley, an unproductive allocation of soil mineral nitrogen can be assumed. This contrasts with expectations made by Hauggaard-Nielsen and Jensen (2001) but might be associated with the high N-demand of mustard or a lower leaf area index (LAI) in barley compared with pea. The lower LAI might have allowed the weed seeds to spread into the barley plots more easily than in the pea plots.

Similar to the reports from Kadziulienė, Sarūnaitė, and Deveikytė (2011), the grain protein content in barley when grown in MC was significantly increased compared with PS barely, indicating a positive effect of the MC on the barley component, too. More available mineral nitrogen during the seed filling phase in MC compared with PS barley might have caused this variation. This increase can be useful when using the MC harvest of both barley and pea as alternative

protein-rich farmed-based feed and for protein-rich cereal flakes. We could confirm the results of Haug et al. (2023). Across two examined barley varieties, we did not observe any MC-based genotype variations. We therefore have concentrated mainly on one barley variety in the later stages of the experiment.

5 | Conclusions

This study, conducted in Switzerland, aimed to compare both the agronomic and financial benefits of a MC system consisting of barley and peas with that of pure barley or pure peas. It was deemed relevant given the fact that the demand for plant-based proteins in Europe is growing, necessitating the intensification of legume production, while at the same time less predictable weather patterns and plant disease risks are hampering production. Therefore, there is a need for new solutions for growing these leguminous crops to ensure both yields and stability for farmers and consumers. MC systems could be one solution. The study produced some interesting results. First, it found a clear financial benefit for the MC system. The MC systems had the highest gross margins and lowest variability in both locations over the 3-year study period. MC systems were also shown to have either a comparable or positive effect on nitrogen mineralization than that found in pure pea, highlighting their value in crop rotation with regard to nutrient availability and soil structure. MC systems were also shown to have an equivalent prevalence of pathogens from the root rot complex as those found in pure pea, meaning that MC systems did not increase the incidence of disease. Finally, the study found that pea genotypes with a leafy karyotype tend to benefit more from MC, as they have a greater propensity for lodging than semi-leafless peas. This is a valuable finding, as lodging represents a significant issue that results in considerable harvest losses. Overall, the study found that the MC approach could potentially maintain or improve the soil nutrient balance, while stabilizing yields and profits.

Author Contributions

Seraina Vonzun: data curation, investigation, methodology, project administration, writing – original draft. Michael Schneider: data curation, formal analysis, visualization, writing – original draft. Valentin Gfeller: investigation. Pierre Hohmann: conceptualization, funding acquisition. Benedikt Haug: investigation. Matthias Meyer: investigation. Monika M. Messmer: conceptualization, funding acquisition, methodology, project administration, supervision.

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Ethics Statement

Not relevant for this research.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The raw data of the study will be made available as additional supporting information Excel file.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.