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Nitrogen Fertilizer Effects on Pea–Barley Intercrop Productivity Compared to Sole Crops in Denmark

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Abstract: Cereal–legume intercropping increases the nitrogen (N) input from biological nitrogen fixation (BNF) and improves the exploitation of fertilizer and soil N, often leading to higher grain N content and higher productivity per unit land area compared to monocrops. Previous studies have found that these effects are more tangible under low soil and fertilizer N conditions compared to high N availability, and there is a need to assess the N uptake at critical crop development stages in order to time the N application for maximum uptake and use efficiency. The objective of this study was to assess the productivity of pea–barley intercropping compared to monocropping under 0 kg N ha^{-1} (0 N) and 100 kg N ha^{-1} (100 N). In 2017, a split plot experimental design was implemented with pea (*Pisum sativum)* sole crop (SC pea), barley (*Hordeum vulgare*) sole crop (SC barley), and pea–barley intercrop (IC total) as the main plots and 100 N applications in two 50 kg N ha^{-1} splits at 30 and 60 days after emergence as subplots within the main plots. The Land Equivalent Ratio (LER), based on grain dry matter (GDM) yields in the pea–barley intercrop (IC total), was higher (1.14 at 0 N and 1.10 at 100 N), indicating 10–14% greater radiation, nutrient, and water use efficiency compared to the sole crops and 4% greater resource use efficiency at 0 N compared to the 100 N; this illustrated greater total intercrop productivity compared to sole crops. The 100 N treatment decreased the SC pea and pea in intercrop (IC pea) GDM and grain dry matter N (GDMN) and increased the GDM and GDMN in SC barley and barley in the intercrop (IC barley). Intercropping increased the grain N content and therefore the protein content of the grains in 0 N and 100 N treatments. The highest fertilizer N yield, % nitrogen derived from fertilizer (%NDFF), and % nitrogen use efficiency (%NUE) were achieved in SC barley followed by IC total, indicating that intercropping improved the soil and fertilizer N use compared to SC pea. The IC pea increased the % nitrogen derived from atmosphere (%NDFA) from 67.9% in SC pea to 70.1% in IC pea. IC total increased the share of %NDFF, %NDFS, and %NDFA compared to the SC pea, which indicated a significant advantage of intercropping due to the complementarity of the component species under limited N supply in the field.

Keywords: biological nitrogen fixation; ^{15}N stable isotope; land equivalent ratio; grain N content

1. Introduction

Intercropping is the concurrent growing of at least two or more crops on the same land to improve the yield and resource use efficiency compared to sole cropping practices [1,2]. Numerous field studies have documented higher productivity in cereal–legume intercropping: e.g., in pea–barley [3], faba bean–barley [4], lupin–barley [5], pea–wheat [6], maize-soybean [7], and faba bean–wheat combinations [8]. Cereals and legumes are complementary in their acquisition of resources, particularly N sources, and intercropped legumes derive more of their N from the atmosphere compared with legumes grown as a sole crop [7,9–11]. This is largely due to the ability of legumes to obtain atmospheric N through biological nitrogen fixation (BNF) [12] while cereals are particularly good at acquiring N from soil and fertilizer sources [13]. This complementarity predisposes cereal acquisition of nitrogen from the soil, thereby depleting it and forcing the legume to fix atmospheric nitrogen sources for its use. In general, cereals grow more quickly in the early season and are often more competitive for available N in the soil. Therefore, nitrogen fertilization generally decreases the legume proportion in the intercrop, as it favours cereal N acquisition and dominance over legumes [14]. When intercropped, the legumes are therefore forced to rely more on BNF to meet their N needs due to the competition from cereal components [11,13]. Multiple recent meta-analyses confirmed that intercropping consistently increases BNF in legumes and increases the soil N uptake in cereals [13,14].

In further support of this, several studies on cereal–legume intercropping in Denmark [6,15–22] have also reported enhanced yields and resource use efficiency in intercropping systems. Studies in other countries have also shown this trend, for example Dordas et al. [23] in France reported enhanced yields with higher crude protein in pea–oat intercrops, Sobkowicz and Śniady [24] in Poland showed enhanced protein content from intercrops, and Danso et al. [2] in Iceland reported that N uptake per oat plant was higher in the pea–oat intercrop than in the oat monocrop. Furthermore, the timing and amount of fertilizer application influences the utilization dynamics that contribute to improvements in use efficiency and productivity in intercrops [25–28]; it is therefore of the utmost significance to evaluate the N uptake at different fertilizer application timings for intercrop and sole crops to improve fertilizer use efficiency.

Nitrogen (N) is the main factor limiting the achievement of higher yields in organic and conventional production systems [29]. This has led to excessive application of N fertilizer, which increases leaching losses into the groundwater and releases reactive forms of N derivatives into the atmosphere, causing adverse environmental impacts beyond the field and farm boundaries. This inefficiency calls for management practices that reduce the application of N fertilizer and enhances plant N use efficiency to develop sustainable farming systems while minimizing environmental and economic consequences. In order to optimize the amount of N applied in production systems, an assessment of the N uptake at critical crop development stages is necessitated to target the N application amount and timing for maximum uptake and use efficiency.

In the present study, our hypothesis is that intercropping, both under normal (100 kg ha^{-1}) and low (0 kg ha^{-1}) fertilizer N conditions, will produce greater Total Dry Matter (TDM), % nitrogen derived from atmosphere (%NDFA), grain N%, and grain yield compared to monocropping systems because of the interspecific complementarity at work between barley and peas. The objective was to evaluate the productivity and nitrogen use in the pea–barley intercrop in comparison to pea and barley sole crops under 0 kg N ha^{-1} and 100 kg N ha^{-1} treatments applied at 30 and 60 days after emergence (DAE).

2. Materials and Methods

2.1. Experimental Site

The field trial was carried out during 2017 at the Experimental Farms of the University of Copenhagen (Taastrup, Denmark). The year before the trial, the experimental field site was cultivated with malting barley and conventional management practices were followed. Soil was sampled from 0 to 75 cm depths in four replicates and analysed for available N, using KCl extraction (1 M: 370 g per 5 L water) and flow injection analysis (OK Laboratorium for Jordbrug). The soil samples were collected on the day of sowing: 7 April 2017. The average N mineralization was estimated as 18 kg N ha^{-1} with 1.57 mg NO_3–N and 0.1 mg NH_4–N kg^{-1} soil. Prior to sowing, an organic fertilizer (Biogrow, NPK 10-3-1) was applied at a rate of 12 kg N ha^{-1} to increase the soil background N to 30 kg N ha^{-1}.

2.2. Experimental Design and Management

The experiment was established as a split-plot design in three replicates, where the three cropping systems viz. the pea–barley intercrop (IC total), barley sole crop (SC barley), and pea sole crop (SC pea) were main plots and the subplots were the 100 N treatments with ^{15}N labelled N application in two equal amounts (50 kg N ha^{-1} each) at 30 and 60 days after emergence (DAE). In an adjacent plot, the 0 N treatment was applied within the main plots to determine grain yields for comparison with 100 N plots.

On the 7th of April, 2017, field pea (cv. Mythic), a medium-early maturing cultivar, and spring barley (cv. Salome), a medium maturing cultivar, were established as sole crops and in a 50:50 ratio for the intercrop where barley and pea seeds were mixed and sown within the same rows at a row spacing of 12.5 cm. The SC barley was sown at a seeding density of 528 plants m^2, and the SC pea was sown at 96 plants m^2. The IC total thus consisted of 264 plants and 48 plants m^2 of spring barley and pea, respectively. The germination count for assessment of successful field establishment was carried out in a 1 m^2 area in each experimental plot one month after sowing. The mean germination count was 320 plants and 95 plants m^2 in the SC barley and SC pea plots, respectively. In the IC plots, the mean germination count was 209 and 47 plants m^2 in IC barley and IC pea, respectively.

2.3. ^{15}N Solution Preparation and Application in Subplots

At each application date, labelled ^{15}N was applied to different subplots within the 100 N treatment plots. Plastic frames were inserted approximately 10 cm into the ground to delimit each subplot and to prevent cross-contamination between subplots. Subplot I received 50 kg N ha^{-1} labelled with ^{15}N urea at 30 DAE, and subplot II received 50 kg N ha^{-1} labelled with ^{15}N urea at 60 DAE. This was done at 30 and 60 DAE to quantify the N fertilizer uptake over time. To achieve and maintain the same dosage of 100 kg N ha^{-1} applied per subplot, the remaining area outside the subplots received 50 kg N ha^{-1} standard urea fertilizer at 30 and 60 DAE. The ^{15}N labelled-urea ($H_2{}^{15}NCO^{15}NH_2$) at 5% atom excess was procured from Sigma-Aldrich (sigmaaldritch.com) and diluted with ^{14}N Urea to 2% atom excess. For the fertilizer application, 2.17 g of ^{15}N labelled urea at 5% atom excess and 3.26 g of commercially available urea were mixed to provide 50 kg N ha^{-1} at 2% atom excess. The mixture was diluted with 1-L Milli-Q (deionized water) in plastic bottles, and the ^{15}N labelled-urea solution was applied uniformly within the subplot using 250 mL (volume) squeeze bottles.

2.4. Biomass Harvest

Three biomass samplings were implemented at 30 DAE (early vegetative growth stage), 60 DAE (full bloom/flat pod growth stage in pea and anthesis (heading) in spring barley), and 90 DAE (physiological maturity) for both species. The first biomass sampling was made prior to the first application of fertilizer. Plants were harvested at 2 cm above the soil surface, using manual and electric scissors. Biomass sampling areas were 1 m × 0.4 m (0.4 m^2), corresponding to 8 rows of 0.4 m length. Sampled biomass materials were used to calculate the aboveground biomass and nitrogen accumulation over the growing period and to estimate grain yield at maturity. After each sampling, intercropped pea and barley biomass were separated and the fresh weights were measured. The samples were then dried in an oven at 65 °C for 48 h, followed by dry weight measurements. The samples from 30 and 60 DAE were ground into powder and analysed for carbon and nitrogen content, whereas samples from 90 DAE were first separated into grain and straw and then ground and analysed for carbon and nitrogen content.

At crop maturity (90 DAE), 2 central rows of 30 cm length (2 × 30 cm) were sampled within each labelled ^{15}N subplot while keeping a minimum 10 cm distance from all sides to minimize contamination from the surrounding unlabelled areas. The harvested material was fractionated into pea and spring barley, weighed, and dried in an oven at 65 °C for 48 h. The samples were threshed for further separation of seeds and straw and then milled into powder for determination of total N and ^{15}N contents.

2.5. Nitrogen and ^{15}N Analysis

To assess the N content in the plants, 3–4-mg powdered plant samples (oven dried) were weighed using an analytical micro-balance (sensitivity ± 0.0001 mg) and encapsulated as small round airtight balls with forceps for favourable "flash combustion" in the total C and N analyser. The accurate weight of each sample was recorded with a PC program called "Mettler Toledo", and the samples list (each sample with unique ID) was organized according to the loading of samples into the 96-well tray. Total C and N were analysed by the CHNS/O Elemental Analyzer. The ^{15}N content was determined with an ANCA-SL Elemental Analyzer coupled to a 20–20 Tracermass Mass Spectrometer (Europa Scientific Ltd. Crewe, UK) using the Dumas combustion method.

2.6. Calculations and Statistics

The determination of nitrogen derived from atmospheric fixation (%NDFA) in the 100 kg N ha^{-1} plots was calculated with the ^{15}N isotope dilution equation (Equation (1) from [30]. The SC barley was used as the reference crop for calculating N fixation in sole and intercrop pea.

$$\%\text{NDFF} = \left(\frac{\text{atom\% }^{15}N\text{ excess in test crop}}{\text{atom\% }^{15}N\text{excess in fertiliser }N}\right) \times 100 \quad (1)$$

where atom %^{15}N excess = atom %^{15}N in the sample—0.3663 (^{15}N composition in atmospheric N_2). In order to compare %NDFA under fertilized and non-fertilized conditions, samples were also taken in unfertilized plots. In unfertilized plots, the natural abundance technique, $\delta^{15}N$‰ (parts per thousand; $\%_0$) of the samples was used to determine %NDFA [31] and calculated as follows:

$$\delta^{15}N‰ = 1000 \times \left(\frac{\frac{^{15}N}{^{14}N}\text{sample}}{\frac{^{15}N}{^{14}N}\text{standard}} - 1\right) \quad (2)$$

where $^{15}N/^{14}N$ sample is the measured $\delta^{15}N$‰ value from the samples and $^{15}N/^{14}N$ standard is the $\delta^{15}N$‰ composition of the standard used in the analysis of samples.

The $\delta^{15}N$‰ of samples were then used to calculate %NDFA in unfertilized plots as follows:

$$\%\text{NDFA} = 100 \times \left(1 - \frac{\delta^{15}N‰\text{ pea} - \delta^{15}N‰\text{ air}}{\delta^{15}N‰\text{ barley} - \delta^{15}N‰\text{ air}}\right) \quad (3)$$

where $\delta^{15}N$‰ denotes the enrichment expressed as parts per thousand in pea and barley. The calculations were based on the assumption that the ^{15}N content of the reference plants (sole cropped barley) is a measure of the ^{15}N level in the soil mineral N that is available for pea [31].

The N derived from soil (%NDFS), N derived from atmosphere (%NDFA) for fertilized plots, and nitrogen use efficiency (%NUE) were computed as follows:

$$\%\text{NDFS}_{\text{barley}} = 100\% - \%\text{NDFF}_{\text{barley}} \quad (4)$$

$$\%\text{NDFS}_{\text{pea}} = 100\% - \left(\%\text{NDFF}_{\text{pea}} + \%\text{NDFA}_{\text{pea}}\right) \quad (5)$$

$$\%\text{NDFA} = \left[1 - \frac{\text{atom \% }^{15}N\text{ excess in pea}}{\text{atom \% }^{15}N\text{excess in barley}}\right] \times 100 \quad (6)$$

$$\%\text{NUE} = \frac{\text{Fertilizer }N\text{ uptake by plant }(\text{kg N ha} - 1)}{100\text{ kg ha} - 1(\text{total fertilizer }N\text{ applied})} \times 100 \quad (7)$$

Land Equivalent Ratio (LER) is the sum of the fractions of intercrop yields divided by monocrop yields. The LER value is an indicator of intercropping benefits compared to growing crops separately; it is calculated as follows [32]:

$$LER_B = \frac{Y_{barleyIC}}{Y_{barleySC}} \tag{8}$$

$$LER_P = \frac{Y_{peaIC}}{Y_{peaSC}} \tag{9}$$

$$LER_{IC} = LER_B + LER_P \tag{10}$$

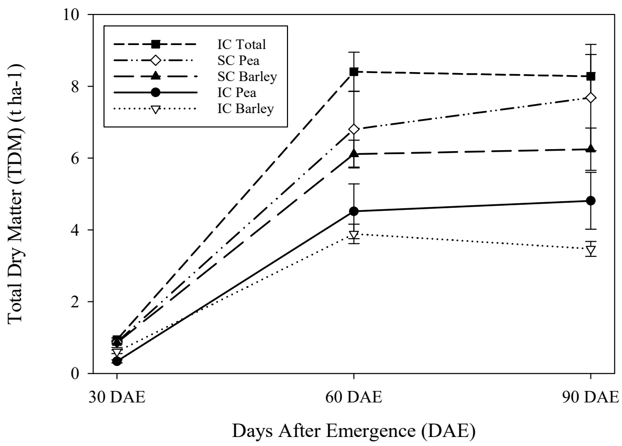
where $Y_{barley\ IC}$ and $Y_{pea\ IC}$ denote intercrop yields while $Y_{barley\ SC}$ and $Y_{pea\ SC}$ represent sole crop yields of barley and pea, respectively. LER_B and LER_P are the partial LER values for the barley and pea components, respectively, under intercropping.

The data was analysed in split-plot design, and analysis of variance (ANOVA) was carried out for Total Dry Matter (TDM), Total N acquisition (TN), Grain Dry Matter (GDM), Grain Dry Matter N (GDMN), Grain N content (%), Fertilizer N yield, %NDFF, and %NUE, and LER. Least significant difference (LSD) values are used to determine the significant effects of cropping systems and time of application. Superscript letters shown in the tables indicate whether there are significant differences between any two means. Differences were considered significant if $p \leq 0.05$. Data were analysed with the Genstat software package (Genstat 20th edition, 2020).

3. Results

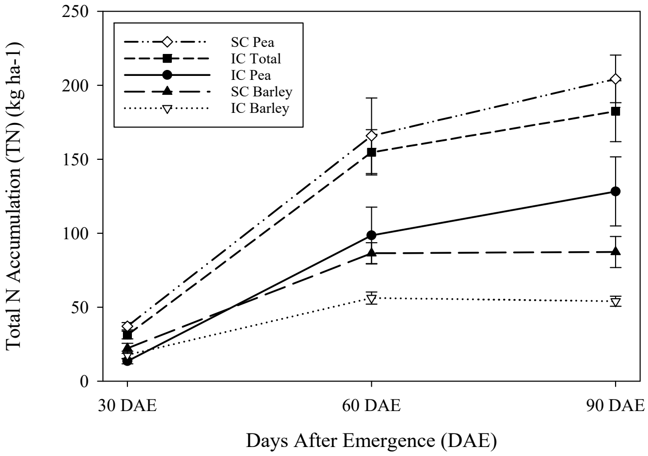
3.1. Total Dry Matter (TDM) Yield and Total Nitrogen Accumulation (TN) in 100 N Treatments

The Total Dry Matter (TDM) and Total Nitrogen (TN) data from the 100 N plots of IC total, SC pea, and SC barley are provided in Figure 1a,b, respectively. For TDM, there were significant differences between cropping systems (p = 0.027) and DAE (p < 0.001). For example, there were significant differences between the cropping systems at 60 DAE (p = 0.04). The TDM was highest in the IC total (both species combined) at 60 and 90 DAE (8.41 and 8.28 Mg ha^{-1}, respectively) compared to sole crops (Figure 1a). At 60 DAE, TDM was higher in the SC pea (6.81 Mg ha^{-1}) than in the SC barley (6.11 Mg ha^{-1}). Again, at 90 DAE, SC pea TDM was higher (7.54 Mg ha^{-1}) than that of SC barley (6.25 Mg ha^{-1}). In the IC total, TDM of the pea component was higher at 60 DAE (4.52 Mg ha^{-1}) and 90 DAE (4.82 Mg ha^{-1}) compared to the barley component (3.89 Mg ha^{-1} and 3.47 Mg ha^{-1}, respectively). The higher total productivity of the intercrop (at 90 DAE) can be explained by IC pea producing 64% of SC pea TDM with only 50% of the plant population, whereas IC barley produced 55% of the SC barley TDM.



(a)

Figure 1. *Cont.*



(**b**)

Figure 1. (**a**) Total dry matter (TDM) (Mg ha^{-1}) and (**b**) total nitrogen accumulation (TN) (kg ha^{-1}) at 30, 60, and 90 days after emergence (DAE) with 100 kg N ha^{-1} in sole crop (SC) pea, SC barley, intercrop (IC) pea, IC barley, and IC total: values are the means (n = 4) ± S.E.

For TN, there were significant interactions for cropping system by DAE (p = 0.01). The TN therefore varied based on the cropping system over time. Among the three cropping systems, TN at 60 DAE was highest in the IC total (155 kg N ha^{-1}), whereas at 90 DAE, TN was highest in the SC pea (204.32 kg N ha^{-1}) (Table 1). The lowest levels of TN at 60 and 90 DAE were found in SC barley and IC barley. At 90 DAE, IC pea contributed 70% of the TN (128.41 Mg ha^{-1}) in the IC total.

Table 1. Total Dry Matter (TDM) and Total Nitrogen Accumulation (TN) at 30, 60, and 90 days after emergence (DAE) in 100 kg N ha^{-1} treatment for the different cropping systems SC pea, SC barley, and IC total: Least significant difference ($LSD_{0.05}$) is for the comparison between SC pea, SC barley, and IC total. CV% is coefficient of variation. Superscript letters indicate significant ($p < 0.05$) and non-significant differences among cropping systems. Superscript letters indicate significant ($p < 0.05$) and non-significant differences between the means. Means sharing the common letters are not significantly different from one another and vice versa.

	TDM 30 (*t* ha^{-1})	**TDM 60 (*t* ha^{-1})**	**TDM 90 (*t* ha^{-1})**	**TN 30 (kg ha^{-1})**	**TN 60 (kg ha^{-1})**	**TN 90 (kg ha^{-1})**
IC pea	0.34 ± 0.04	4.52 ± 0.76	4.81 ± 0.79	17.7 ± 2.59	98.6 ± 19.09	128.4 ± 23.49
IC barley	0.60 ± 0.05	3.89 ± 0.27	3.47 ± 0.21	13.5 ± 1.84	56.2 ± 4.09	54.1 ± 3.31
IC total	0.94 ± 0.05 ^{a}	8.41 ± 0.55 ^{a}	8.28 ± 0.61 ^{a}	31.20 ± 2.56 ^{a}	154.80 ± 15.33 ^{a}	182.50 ± 20.60 ^{a}
SC pea	0.87 ± 0.05 ^{a}	6.81 ± 1.06 ^{a}	7.54 ± 1.12 ^{a}	37.1 ± 3.42 ^{a}	165.9 ± 25.49 ^{a}	204.3 ± 16.06 ^{a}
SC barley	0.86 ± 0.13 ^{a}	6.11 ± 0.39 ^{b}	6.25 ± 0.59 ^{a}	22.2 ± 2.45 ^{b}	86.4 ± 7.15 ^{b}	87.3 ± 10.49 ^{b}
$LSD_{0.05}$	0.27	1.64	2.15	7.04	54.25	47.61
CV%	17.60	13.30	16.20	13.50	23.10	17.40

3.2. Land Equivalent Ratios (LERs) in 100 N Treatments

Based on IC total TDM accumulation, the highest land equivalent ratio (LER) was recorded at 60 DAE (1.30), followed by 90 DAE (1.14) and 30 DAE (1.13) (Figure 2). This indicated that the intercrop had a 13–30% (mean 21.5%) greater productivity than SC pea and SC barley. In other words, growing SC pea and SC barley alone would require an additional 21.5% more land on average to produce the same TDM as the IC total under the same management practice, depending on the harvest time.

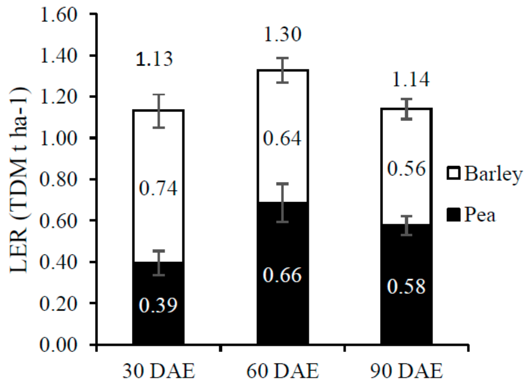


Figure 2. TDM land equivalent ratios (LER) at 30, 60, and 90 DAE in the pea–barley intercrop with 100 kg N ha^{-1} application: the partial LER for pea (black bars) and barley (white bars) are shown in the stacked bar diagrams. Values are the means (n = 4) ± S.E.

There were no significant differences over time for the IC total LERs (p = 0.21). However, the LER based on TDM changed over the growing period from 30 DAE to 90 DAE (Figure 2). This can be explained by the partial LER values of pea (LER_P) and barley (LER_B) shifting over time as a result of interactions between component species. At 30 DAE, LER_B was 0.74 compared to LER_P of 0.39, showing that the barley component had more vigorous growth during the early vegetative phase compared to the pea component in the intercrop. At 60 DAE, LER_B decreased to 0.64 and LER_P increased to 0.66; at 90 DAE, LER_B decreased further to 0.56 and LER_P decreased to 0.58. Compared to barley, the pea component shows slower growth during the early period, but as the growing season progresses, pea becomes the predominant component.

3.3. LER_{IC} of Grain Yield in 0 N and 100 N Treatments

The LER_{IC} based on grain dry matter (GDM) recorded 10–14% greater grain yields for IC total compared to sole crops for both treatments (Table 2). There was no significant difference between the 0 N and 100 N treatments (p = 0.17). However, the LER_{IC} for GDM was on average 4% higher for the 0 N (1.14) compared to the 100 N (1.10), indicating 4% greater production of grain/pea yield in 0 N treatments per unit area compared to 100 N treatments for the IC total (Table 2). The greater GDM in the 0 N treatments can be explained by the pea component producing 61% of the IC total yield in 0 N compared to 56% in 100 N, whereas the barley component produced similar GDM in 0 N (0.53) and 100 N (0.54) treatments.

Table 2. Partial and total land equivalent ratios in IC pea (LER_P), IC barley (LER_B), IC total (LER_{IC}) based on grain dry matter (GDM) with 0 kg N ha^{-1} (0 N) and 100 kg N ha^{-1} (100 N) treatments.

Treatment	0 N	100 N
LER_P	0.61 ± 0.05	0.56 ± 0.09
LER_B	0.53 ± 0.14	0.54 ± 0.04
LER_{IC}	1.14 ± 0.16	1.10 ± 0.08

3.4. Grain Dry Matter (GDM) and Grain Dry Matter Nitrogen (GDMN)

Among the N treatments, the SC pea GDM was highest at 5.39 ± 0.52 Mg ha^{-1} for 0 N and 5.18 ± 0.17 Mg ha^{-1} for 100 N followed by the IC total GDM of 4.93 ± 0.14 Mg ha^{-1} and 5.03 ± 0.36 Mg ha^{-1} under 0 N and 100 N, respectively (Table 3). There were significant differences between the cropping systems within both the 0 N and 100 N treatments. The same trend was recorded in GDMN; SC pea accumulated the highest GDMN, measuring 187.57 ± 8.22 kg ha^{-1} and 169.73 ± 6.10 kg ha^{-1} under 0 N and 100 N, respectively. The second highest GDMN was measured in IC total for both 0 N and 100 N treatments. Conversely, SC barley and IC barley recorded the lowest

GDM and GDMN in 0 N and 100 N treatments. For GDMN, SC barley was significantly different compared to the other cropping systems for both 0 N (p = 0.001) and 100 N (p = 0.002) (Table 3).

Table 3. Grain dry matter (GDM) yield (Mg ha^{-1}) and grain dry matter nitrogen (GDMN) accumulation (kg ha^{-1}) in sole crops (SC) and intercrops (IC) of pea and barley with 0 (0 N) and 100 kg N ha^{-1} (100 N): values are the mean (n = 4) ± S.E. The $LSD_{0.05}$ is calculated for comparison between IC total, SC pea, and SC barley. CV% is coefficient of variation. Superscript letters indicate significant ($p < 0.05$) and nonsignificant differences among cropping systems. Superscript letters indicate significant ($p < 0.05$) and non-significant differences between the means. Means sharing the common letters are not significantly different from one another and vice versa.

Grain Yield	Crop Treatment	Nitrogen Application (kg N ha^{-1})		Yield Change with N Application (%)
		0 N	100 N	
GDM (Mg ha^{-1})	SC pea	5.39 ± 0.52 [a]	5.18 ± 0.17 [a]	−3.9
	SC barley	3.05 ± 0.52 [b]	3.95 ± 0.32 [b]	29.5
	IC pea	3.31 ± 0.25	2.92 ± 0.49	−11.8
	IC barley	1.62 ± 0.25	2.11 ± 0.13	30.2
	IC total	4.93 ± 0.14 [a]	5.03 ± 0.36 [a]	2.0
	$LSD_{0.05}$	1.28	1.10	
	CV%	16	13.4	
GDMN (kg ha^{-1})	SC pea	187.57 ± 8.22 [a]	169.73 ± 6.10 [a]	−17.8
	SC barley	37.25 ± 8.45 [b]	61.12 ± 7.0 [b]	63.8
	IC pea	120.5 ± 9.70	106.41±19.16	−11.7
	IC barley	23.90 ± 2.42	38.54 ± 1.94	61.1
	IC total	144.4 ± 8.21 [a]	145.0 ± 17.24 [a]	0.4
	$LSD_{0.05}$	61.30	42.76	
	CV%	26.9	19.7	

The 100 kg N ha^{-1} treatment had contrasting effects on the GDM and GDMN, resulting in increased values for SC barley and IC barley and decreased values in SC pea and IC pea due to the vigorous growth response of barley to fertilizer application (Table 3). For the 100 N treatment compared to the 0 N, GDM and GDMN of SC barley increased by 29.5 and 63.8%, respectively, while GDM and GDMN of IC barley increased by 30.2 and 61.1%, respectively. In contrast, GDM and GDMN of SC pea decreased by 3.9% and 17.8%, respectively, while GDM and GDMN of IC pea decreased by 11.8 and 11.7%, respectively, compared to the corresponding values at 0 N (Table 3).

3.5. Grain N Content of Sole Crops and Intercrops

Intercropping increased grain N content of pea and barley in both the 0 N and 100 N treatments (Table 4). In 0 N and 100 N, there were significant differences between cropping systems ($p < 0.001$). Under 0 N, intercropping increased the grain N content of barley by 28.6% and increased the grain N content of pea by 4.6% compared to sole cropping (Table 4). Under 100 N, intercropping increased the grain N content of barley by 17.4% and the grain N content of pea by 10.7%. The 0 N treatment therefore produced a greater average % increase in grain N content compared to 100 N (average increase of 16.6% for 0 N and 14.02% for 100 N).

Table 4. Grain N content (%) in sole crops and pea–barley intercrops with 0 (0 N) and 100 kg N ha^{-1} (100 N): $LSD_{0.05}$ is calculated for comparison within 0 N and 100 N treatments and between cropping systems components. CV% is coefficient of variation. Superscript letters indicate significant ($p < 0.05$) and nonsignificant differences among cropping systems. Superscript letters indicate significant ($p < 0.05$) and non-significant differences between the means. Means sharing the common letters are not significantly different from one another and vice versa.

Treatment	0 N	100 N
SC pea	3.48 ± 0.00 ^{a}	3.28 ± 0.09 ^{a}
SC barley	1.19 ± 0.05 ^{b}	1.54 ± 0.07 ^{b}
IC pea	3.64 ± 0.03 ^{a}	3.63 ± 0.07 ^{c}
IC barley	1.53 ± 0.14 ^{c}	1.83 ± 0.03 ^{d}
$LSD_{0.05}$	0.29	0.29
CV%	4.9	5.1

3.6. Nitrogen Fertilizer Uptake and Use Efficiency, and Nitrogen Fixation

There were significant interactions between cropping systems and time of fertilizer application on fertilizer N yield ($p = 0.001$), %NDFF ($p < 0.001$), and NUE ($p = 0.001$). This indicated that the differences in N uptake and utilization dynamics are a product of both cropping systems and time of fertilizer application.

Of the 100 kg N ha^{-1} applied in total (30 + 60 DAE), SC barley accumulated the highest quantity (44.15 kg N ha^{-1}), followed by IC total (42.06 kg N ha^{-1}) and finally by SC pea (32.51 kg N ha^{-1}) (Table 5). As the N uptake in different cropping system treatments is based on the application of 100 kg N ha^{-1}, the amount of N uptake demonstrates the N use efficiency, which means that the highest N use efficiency was recorded in SC barley and the lowest was recorded in SC pea.

At 30 DAE, fertilizer N yield was highest in the IC total (28.77 kg ha^{-1}), which is equivalent to 15.76% NDFF, followed by SC barley and SC pea (Table 5). The highest NUE (57.54%) was attained in the IC total, followed by SC barley and then SC pea at 30 DAE. At 60 DAE, the highest fertilizer N yield was measured in SC barley (18.11 kg N ha^{-1}), constituting 20.75% NDFF and a NUE of 36.22%, followed by a similar fertilizer N yield in SC pea (13.85 kg N ha^{-1}) and IC total (13.29 kg N ha^{-1}). Therefore, the highest NUE at 30 DAE was recorded in IC total, whereas at 60 DAE, SC barley recorded the highest NUE.

There were significant differences between cropping systems for %NDFF ($p < 0.001$) and %NDFA ($p = 0.003$) (Table 6). For example, in SC barley, 50.6% of the TN was %NDFF, whereas in SC pea, it was only 15.1% (Table 6). Similarly, 49.4% of the TN was %NDFS in SC barley, whereas in SC pea, it was only 17%. IC total enhanced the %NDFF and %NDFS to 28.8% and 36.1% respectively, which was significantly higher compared to the SC pea (Table 6). Because of interspecific complementarity, the IC obtained 35% of its TN through BNF. Intercropping enhanced the BNF (%NDFA) from 67.9 to 70.1% due to the fact that more N was taken up by the fast-growing barley, leaving less N for the pea component and stimulating its BNF.

Table 5. Fertilizer N yield, percentage N derived from fertilizer (%NDFF), and nitrogen use efficiency (%NUE) in sole crops and intercrops with application of 50 kg N ha^{-1} each at 30 and 60 days after emergence (DAE): $LSD_{0.05}$ is calculated for comparison between IC total, SC pea, and SC barley. CV% is coefficient of variation. Superscript letters indicate significant ($p < 0.05$) and non-significant differences between the means. Means sharing the common letters are not significantly different from one another and vice versa.

Crop System	Fertilizer N Yield (kg ha^{-1})			%NDFF			%NUE		
	30 DAE	60 DAE	Total	30 DAE	60 DAE	Total	30 DAE	60 DAE	Total
IC pea	12.09 ± 2.29	6.74 ± 2.00	18.83 ± 3.94	9.42 ± 0.91	5.25 ± 0.62	14.67 ± 3.07	24.18 ± 4.59	13.48 ± 4.01	37.66 ± 7.88
IC barley	16.68 ± 1.01	6.55 ± 0.82	23.23 ± 1.61	30.84 ± 3.28	12.12 ± 1.34	42.96 ± 2.98	33.35 ± 2.02	13.11 ± 1.65	46.46 ± 3.22
IC total	28.77 ± 2.67 ^{a}	13.29 ± 1.63 ^{a}	42.06 ± 4.11 ^{a}	15.76 ± 1.73 ^{a}	7.28 ± 0.79 ^{a}	23.05 ± 2.38 ^{a}	57.54 ± 5.34 ^{a}	26.59 ± 3.27 ^{a}	42.06 ± 8.21 ^{a}
SC pea	18.67 ± 6.89 ^{b}	13.85 ± 3.47 ^{a}	32.51 ± 4.64 ^{a}	8.67 ± 3.06 ^{a}	6.43 ± 1.41 ^{a}	15.10 ± 1.98 ^{a}	37.33 ± 13.78 ^{b}	27.70 ± 6.94 ^{a}	32.51 ± 9.28 ^{a}
SC barley	26.04 ± 1.23 ^{a}	18.11 ± 2.35 ^{a}	44.15 ± 1.67 ^{a}	29.83 ± 2.83 ^{b}	20.75 ± 2.80 ^{b}	50.58 ± 2.52 ^{b}	52.08 ± 2.47 ^{a}	36.22 ± 4.71 ^{a}	44.15 ± 3.39 ^{a}
$LSD_{0.05}$	5.267	9.48	13.22	14.09	12.17	21.29	10.53	18.96	26.45
CV%	13.6	35.7	20.3	15.0	24.7	14.9	13.6	35.7	20.3

Table 6. Fractional contribution of total dry matter N (TDMN) derived from fertilizer (%NDFF), soil (%NDFS), and atmosphere (%NDFA) in sole crops and intercrops with 100 kg N ha^{-1} application: $LSD_{0.05}$ is calculated for comparison between IC total, SC pea, and SC barley. CV% is coefficient of variation. Superscript letters indicate significant ($p < 0.05$) and non-significant differences between the means. Means sharing the common letters are not significantly different from one another and vice versa.

Treatments	%NDFF	%NDFA	%NDFS
SC pea	15.1 ± 4.31 ^{a}	67.9 ± 5.48 ^{a}	17.0 ± 2.42 ^{a}
SC barley	50.6 ± 3.88 ^{b}		49.4 ± 3.88 ^{b}
IC pea	14.7 ± 3.07	70.1 ± 1.71	15.2 ± 4.28
IC barley	43.0 ± 2.98		57.0 ± 2.98
IC total	28.8 ± 2.03 ^{c}	35.1 ± 0.86 ^{b}	36.1 ± 2.69 ^{b}
$LSD_{0.05}$	7.68	14.545	13.92
CV%	13.00	24.80	9.00

4. Discussion

4.1. Biomass Accumulation and Land Productivity in Sole Crops and Intercrops

There is a general trend when evaluating pea–barley intercropping that is based only on final grain yields. In this study, however, we sampled biomass accumulation three times at 30, 60, and 90 DAE to better assess the dynamics of biomass and nitrogen accumulation during the entire growing season, which is more informative [18]. In our study, pea–barley intercrop (IC total) was consistently more productive for both TDM and GDM per unit area compared to sole crops, which is in line with other studies [6,17,19,20,22,33,34]. For both the 0 N and 100 N treatments, TDM LER results demonstrated a significant advantage of intercropping compared to sole cropping, with a 21.5% average increase in productivity per unit area of land for the 100 N over the growing period. Higher TDM on its own is advantageous if the biomass is harvested for fodder or silage for animals, depending on the production target under a specific farming context. Furthermore, our study found that SC pea yielded the highest TN in consonance with other studies [18,35]. These results correspond with other studies in similar environments that have also found similar results [2,6,17,36].

The repeated sampling in this study enabled further insights into the dynamic relative contribution of pea and barley components to the higher IC total yield. For instance, IC barley had particularly vigorous early growth, constituting 74% of the IC total biomass at 30 DAE, but its relative biomass decreased over the course of the growing season. In contrast, pea constituted only 36% of TDM at 30 DAE, which increased at 60 DAE and decreased slightly at 90 DAE. The initially greater partial LER of barley (LER_B) than that of pea (LER_P) may be explained by IC barley being more effective than IC pea in their early competition for and retrieval of soil N. This has also been reported in other studies [22,37–39]. Hence, the highest productivity in the IC total was attained at 60 DAE with 30% higher TDM yields compared to the monocrops. At final harvest for this study, the TDM LER_{IC} was 1.14, indicating 14% higher TDM production in the IC total compared to their monocrop counterparts. This is in line with a field study in the same experimental farm in Denmark, where pea–barley LER_{IC} based on TDM was 1.15 [18]. Other studies have found this pattern whereby decreased N inputs provide a conducive environment for the N-fixing legume to produce similar or greater biomass outputs [6,22,40]. This illustrates the consistent trend of improved LER values of barley–pea intercropping over sole cropping practices in the context of low-input fertilizer use. Furthermore, the increased TN values in the 0 N and 100 N treatments for the intercrop compared to the sole crops demonstrated the improvement in N use and acquisition due to component species complementarity. These results showed that intercropping pea–barley at a 50:50 ratio can increase TN per unit area compared to the SC barley due to increased N input through BNF stimulation. With only 50% of the plant population, the IC barley component had more than 50% of the TN of the SC barley, illustrating the advantage that was gained by IC barley under intercropping.

4.2. Grain Dry Matter, N Yield, and LER in 0 and 100 N Treatments

SC pea produced the highest GDM yields (Table 3), followed by IC total and then SC barley, which is consistent with results from another field study in Denmark [17]. In our study, use of 100 kg N ha^{-1} suppressed the GDM in SC pea and IC pea. The same trend of lower GDM with fertilizer application was observed in pea–barley field trials in Denmark that were applied with 40 and 50 kg N ha^{-1} [17]. The reduced GDM in IC pea with N application is attributed to the increased competition by the barley component due to vigorous growth and biomass accumulation, adversely affecting pea growth and GDM. The 100 N treatment also resulted in decreased GDMN accumulation in SC pea and IC pea in conformity with other studies [3,17,18]. The greater amount of GDMN in SC pea with 0 N compared to 100 N indicated greater resource use efficiency for SC pea without external N input (Table 3). GDM and GDMN in IC pea were highest with 0 N and were substantially reduced with 100 N due to a decrease in pea component and an increase in the barley component, which has also been reported in other studies [6,17]. Irrespective of N treatments, pea–barley intercropping increased the overall grain N concentration in IC barley and IC pea compared to SC barley and SC pea. The enhanced N accumulation in the harvested grain can be attributed to the complementarity of the intercropped species as they access soil N from different soil profiles and due to the ability of the pea component to increase BNF under intercropping systems. As grain N concentration is the critical factor for determining the protein content of grain, thereby determining its food value and price, high grain N concentrations are regarded as an important advantage of intercropping. This is further supported by other studies in the literature [25,36].

While GDM LERs were above 1.0 for both the 0 N and 100 N plots, the higher LER in 0 N plots (Table 2) indicated that intercropping is especially advantageous in low-input agriculture, as seen in other studies [6,41,42]. In our study, the application of N in the 100 N treatment decreased the relative GDM contribution by pea due to the increased vigour of barley. Without the addition of any fertilizer, the intercrop system can obtain, on average, 14% greater yields per unit area than monocropping practices, which is in conformity with another pea–barley field trial in Denmark where the application of 0, 40, and 50 kg N ha^{-1} resulted in GDM LER_{IC} of 1.18, 1.11, and 1.03 respectively [17]. The LER values from this study demonstrated that the intercrop performance was better under low N supply, which has been also documented in several field studies [6,42]. This decrease in the LER due to N application has also been documented under diverse pedo-climatic zones, illustrating a general trend under diverse management and production regimes (Table 7) [6,15,18,40].

Table 7. Cereal–legume intercrop LER based on GDM in field trials in Denmark and France.

Cereal Species	Legume Species	Location	Fertiliser (kg N ha^{-1})	LER_{Cereal}	LER_{Legume}	Total LER	Intercrop Densities (of % Sole Crop)	Reference
Durum wheat	Winter pea	France	0	0.50	0.64	1.14	50:50	[40]
		France	0	0.58	0.62	1.20		
Spring wheat	Field pea	Denmark	0	1.00	0.34	1.34	50:50	[6]
			40	0.62	0.34	0.96		
Spring barley	Field pea	Denmark	0	0.74	0.37	1.11	50:50	[15]
			50	0.83	0.19	1.02		
Barley	pea	Denmark	5			1.26	33:50	[18]
			40			1.00		

4.3. Nitrogen Recovery and Nitrogen Fixation in Sole and Intercrops

The fertilizer N yield in the crop biomass with 100 kg N ha^{-1} application was highest in SC barley, followed by IC total and SC pea (Table 5), which is in agreement with other studies where N recovery rates were similar to this study [6,22]. For instance, in a pea–wheat intercrop trial under similar environmental conditions in Denmark, Ghaley et al. [6] observed fertilizer N recovery in SC pea of only 15% with the application of 40 kg N ha^{-1} at sowing. In this study, SC pea took up more than double the amount of N fertilizer (32.51 kg N ha^{-1}) compared to the other study [6], likely because in this study the fertilizer N was provided in two applications of 50 kg N ha^{-1} at two critical growth stages, increasing fertilizer N uptake and use efficiency. This indicated that the N applied during the active vegetative growth stage was taken up more efficiently for growth and biomass accumulation in SC pea compared to N application at sowing. Hence, intercropping pea–barley enhances fertilizer N uptake compared to SC pea for better utilization of applied nutrients for crop production while also reducing off-site N run-off losses and mitigating eutrophication downstream. Ghaley et al. [6] found that the N recovery was 37–53% for SC wheat and 30–34% for IC total, which is comparable to our results of 44.15% for SC barley and 42.06% for IC total. In the present study, the 30 DAE %NUE (57.54%) is slightly lower than the previously reported 63% NUE measured in a pea–barley intercrop trial that was fertilized with 50 kg N ha^{-1} and grown under similar conditions in Denmark [22]. However, this is likely because %NUE increases with lower fertilizer levels. For example, increased %NUE with a decrease in fertilizer levels was reported in two other studies on maize-soybean and oat-pea intercrop systems where fertilizer levels were decreased from 240 to 180 kg N ha^{-1} and 120 to 60 kg N ha^{-1}, respectively [43,44].

This study has shown that pea–barley intercropping is beneficial in many ways: for example, by enhancing the % nitrogen derived from atmosphere (%NDFA) and increasing the exploitation of N derived from soil (%NDFS) and N derived from fertilizer N (%NDFF), which has been supported by other studies as well [17,22,42]. Our study also found similar advantages, as intercropping increased the %NDFA to 70.1% in IC pea compared to 67.9%NDFA in SC pea, for example. Intercropping also increased the %NDFF to 28.8% in IC total, which is higher than SC pea due to enhanced N uptake from the applied fertilizer N by the intercropped components. Similarly, the %NDFS for IC pea increased to 36.1%, which is a substantial increase compared to the 17% NDFS in SC pea. Hence, intercropping facilitated the increase in %NDFA, %NDFS, and %NDFF, improving the overall resource use efficiency due to the complementarity of the pea and barley species. Such benefits have been recorded under diverse pedo-climatic and management regimes illustrating how cereal–legume intercropping can improve the resource use uptake and efficiency to reduce the fertilizer N input, mitigate the downstream adverse effects of N fertilization, and decrease carbon emissions [25,26,45,46].

5. Conclusions

Overall, the results of this study are consistent with previous research illustrating the productivity and N use efficiency gains of intercropping compared to sole cropping. Furthermore, these results demonstrated the benefits of crop component synergy and complementarity within intercropping systems under low N inputs, as pea–barley intercropping under 0 N outperformed the 100 kg N ha^{-1} treatment. At physiological maturity (90 DAE), the pea–barley intercrop measured the highest yields per unit area. The TDM LER_{IC} demonstrated a 14% greater efficiency in intercrop resource use efficiency compared to sole cropping. As a further demonstration of the advantage of low-input agriculture and the disadvantage of excessive fertilizer and fossil fuel inputs, the application of a moderate amount of N fertilizer, in this case, 100 kg ha^{-1} N, was shown to decrease the GDM LER_{IC} from 1.14 at 0 N to 1.10 at 100 N. This was likely due to the competition from the barley crop for the available fertilizer N, which decreased pea GDM and GDMN. The 100 N treatment decreased the SC pea and IC pea GDM and GDMN yield and increased the GDM and GDMN in SC barley and IC barley. Intercropping increased the grain N concentration and therefore the protein content in barley and pea compared to sole crops, thereby increasing the value for food and fodder usage. With the application of 100 N in two 50 kg splits at 30 and 60 DAE, the highest %NUE was recorded in SC barley, followed by IC total with the lowest %NUE recorded in SC pea. Compared to SC pea, intercropping enhanced %NDFF, NDFS, and NDFA as a result of complementary species strategies for the acquisition of N. Hence, intercropping is more productive under low N input conditions, thus lowering the input cost of production while also attaining greater yields. This illustrates that intercropping is a viable sustainable approach to lower the carbon footprint of agro-ecological systems while also avoiding the consequences of fertilizer over-application on downstream ecosystems.

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