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# Biostimulants-induced improvements in pea-barley intercropping systems: A study of biomass and yield optimization under Ukrainian climatic conditions

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# ABSTRACT

The challenge of enhancing crop yields sustainably is critical in modern agriculture. This study investigated the effectiveness of various biostimulants (extracts derived from insect frass, compost, humic material, seaweeds, and fish hydrolysate) on the yield of barley and peas in sole and intercropped systems. Results demonstrated that pea-barley intercrop delivered higher grain yields compared to pea or barley sole crops, with a maximum yield of 7.08 t/ha achieved using compost tea. Barley grain yields in pea-barley intercrop ranged from 4.72 to 5.57 t/ha with biostimulant applications compared to 4.39 t/ha without biostimulant (control) treatment. While pea sole cropping did not show significant yield improvements with biostimulants, intercropped pea yields increased significantly, with humus extract and compost tea enhancing yields by 61.9 % and 45.4 %, respectively. The application of biostimulants also increased the number and weight of seeds per spike in barley and the number of seeds per plant, seed weight per plant, and thousand seed weight in peas. Although intercropping resulted in fewer spikes compared to sole crops, barley produced more grain per spike, with the highest increase observed with seaweed extract. The stimulatory role of biostimulants is associated with their anti-stress action and rhizosphere stimulation for increased N-fixing activity explaining certain increases in productivity, and consequently in the yield of crops, especially in pea-barley intercrop where there is intense competition between species during the generative period of development. Furthermore, the overall land equivalent ratio (LER) for the pea-barley intercropping system was consistently above 1.0, indicating a consistent yield advantage over monoculture throughout the growing season. These findings indicate that biostimulants and intercropping synergistically enhance crop yields and biomass, promoting sustainable agricultural practices by optimizing resource use and minimizing chemical inputs.

#### 1. Introduction

Agriculture stands at a pivotal intersection of global food security and environmental sustainability. Despite advances in agronomic practices, the escalating pressures from increasing global food demands, coupled with the imperative to mitigate environmental impacts, underscore the need for innovative and sustainable agricultural strategies. Among these, intercropping—growing two or more crop species in close proximity is renowned for its potential to enhance resource utilization, crop productivity, and ecological sustainability [1–3]. Intercropping systems, especially those involving legume-cereal combinations, have the potential to enhance yield, improve resource utilization efficiency, and increase nutrient uptake efficiency [4,5]. For instance, pea-barley intercropping systems demonstrate significant agroecological benefits,

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as the nitrogen-fixing capabilities of peas fulfil barley's nitrogen demands, thereby minimizing reliance on chemical fertilizers and enhancing the sustainability of these systems [6,7]. However, while intercropping systems such as pea-barley combinations have shown promising results in terms of resource efficiency and yield optimization [7], the full potential of these systems is often not realized due to suboptimal interactions between the component species and the environmental context.

Usually, the productivity of cereal-legume intercropping systems in organic farming often falls below that observed in conventional agriculture. This disparity can be attributed to several factors, including the reduced availability of inputs such as fertilizers and pesticides, which are extensively used in conventional systems to optimize growth conditions and pest control. In organic intercropping systems, nutrient dynamics are more complex and often less controllable. Nitrogen, which is critical for crop growth, is not readily available, relying instead on the mineralization process in soil and slower process of biological nitrogen fixation by legumes, which may not always meet the immediate demands of cereals under certain soil conditions. Moreover, the resilience and stability of these intercropping systems under stress conditions such as drought or nutrient deficiency are often compromised [8]. This is due to the competitive nature of the cereals, which tend to dominate the growth environment due to their faster initial growth rates and greater biomass production, effectively suppressing the legume partners [9]. This competition for limited resources can lead to reduced legume growth and nodulation, thereby decreasing the overall efficiency of nitrogen fixation within the system and potentially leading to a decrease in system productivity.

One emergent solution to enhance the efficacy of intercropping systems lies in the application of biostimulants-substances that stimulate natural processes to enhance nutrient uptake, growth, and stress tolerance. Biostimulants are known to influence plant physiological processes including nutrient assimilation, hormone activity, and stress response, which can lead to improved growth and productivity [10,11]. For example, humic substances (HSs) have been shown to enhance root growth and nutrient uptake, particularly in phosphorus-limited conditions [12], while seaweed (Ascophyllum nodosum, Ecklonia maxima, Macrocystis pyrifera and Durvillea potatorum) extracts have been documented to improve plant vigor by supplying hormonal stimuli such as cytokinins and auxins [13]. Moreover, biostimulants such as insect frass and fish hydrolysate provide a range of micronutrients and growth-promoting factors that can enhance both soil health and plant resilience [14–16]. Compost tea, known for its microbial diversity, can significantly alter the rhizosphere microbiome, enhancing nutrient solubilization and pathogen suppression [17,18]. These interactions, particularly in an intercropping context, can lead to more robust systems capable of withstanding environmental stresses while maintaining high productivity.

Moreover, biostimulants can modulate the rhizosphere's microbial population, enhancing the symbiotic relationships essential in intercropping systems. By improving rhizobial activity in legumes, biostimulants help enhance nitrogen fixation, directly benefiting cereals intercropped with legumes [19,20]. Additionally, the production of phytohormones such as cytokinins and gibberellins through biostimulant application can lead to better crop growth and higher tolerance to abiotic stresses, thus indirectly contributing to yield stability [21]. While individual studies have reported on the benefits of specific biostimulants like seaweed extracts (Ascophyllum nodosum, Ecklonia maxima) and humic acids on crop growth and yield in monocropping systems [13,22], systematic studies exploring how these biostimulants influence the complex dynamics of intercropped species are sparse. Questions remain about how biostimulants could optimize nutrient sharing and competition, enhance stress mitigation, and improve overall plant health in these systems. Furthermore, there is a paucity of research on how biostimulants might affect the rhizosphere microbiome, a critical component of intercropping success, thereby influencing the

bioavailability of nutrients and the overall sustainability of agricultural practices.

Given the dynamic interactions between plants in intercropping systems, the role of biostimulants could be pivotal in mediating nutrient competition and optimizing space usage, particularly in the pea-barley combination where nitrogen fixation by peas can significantly benefit the adjoining barley. Ukraine, characterized by temperate continental climatic conditions with distinct seasonal variations, presents unique challenges and opportunities for sustainable agriculture [23,24]. The region experiences cold winters and warm summers, with variable precipitation that affects soil moisture availability [25]. Given these climatic constraints, optimizing biomass production and yield through biostimulant application becomes particularly relevant.

This study evaluated the effects of five biostimulants-insect frass extract, compost tea, humic extract, seaweed extract, and fish hvdrolvsate on the growth and productivity of barley and pea cultivated under sole and intercropping systems in the Ukrainian climate. The research focused on comparing these cropping systems across key agronomic traits, including fresh and dry biomass at 30, 60, and 90 days after sowing, seed yield, yield components and overall system performance assessed using the Land Equivalent Ratio (LER). We hypothesized that biostimulant application would significantly enhance biomass accumulation and seed yield in both cropping systems compared to untreated controls. Additionally, we expected that biostimulant-treated intercropping would improve nitrogen fixation and resource-use efficiency, leading to higher LER values than sole cropping. The findings from this study are expected to guide future agricultural strategies, particularly in regions like Ukraine where resource conservation is crucial and where agriculture must adapt to the challenges of climate change and soil degradation.

# 2. Materials and methods

#### 2.1. Experimental site, soil characteristics and climatic condition

The field trial was conducted as a stationary field experiment by the Department of Plant Science at the National University of Life and Environmental Sciences of Ukraine. The experiment took place at the 'Agronomic Research Station', situated in the village of Pshenychne, Kyiv region. This location is within the northeastern part of the Right-Bank Forest-Steppe, specifically at the coordinates 49°46′ N latitude and 30°44′ E longitude. The soil of the experimental plot is characterized as chernozem, coarse loamy on loess (Table 1). The weather conditions during the research period included a steady rise in temperature in the spring. All plots were sown on March 24, 2023, when the soil temperature was +4 °C, followed by significant rainfall throughout the month. Most of April's rainfall occurred before crop emergence, maintaining soil moisture reserves close to field capacity. Crop emergence

Table	1

Physiochemical characteristics of the experimental soil.

Component	Value
Soil type	Chernozems (CH), according to FAO-UNESCO [26] soil classification
Humus content (0–15 cm, Tyurin)	4.45 %
Porosity	53.9 %
Particle density	2.65 g cm <sup>-3</sup>
Cation Exchange Capacity (CEC)	23.6 cmol kg <sup><math>-1</math></sup>
Bulk density	$1.22 \text{ g cm}^{-3}$
Mineral Nitrogen (N <sub>min</sub> )	1.77 g kg <sup><math>-1</math></sup> of soil
Available Phosphorus (P)	$6.7 \text{ mg } 100 \text{ g}^{-1} \text{ of soil}$
Available Potassium (K)	11.0 mg 100 g <sup>-1</sup> of soil
Magnesium (Mg)	$26 \text{ mg } 100 \text{ g}^{-1} \text{ of soil}$
K:Mg ratio	0.4
Ca	$200 \text{ mg} 100 \text{ g}^{-1} \text{ of soil}$

observed on April 13. Temperature increases were moderate until June, slightly slowing crop development. Despite low rainfall in May, soil moisture was sufficient for normal crop growth. The average monthly temperature in June exceeded 20 °C, but rainfall was adequate for crop development. Most of July's rainfall occurred during the crop's full maturity period, minimally impacting productivity formation. The soil water balance was favourable for the crops. The total soil moisture content in the 0–100 cm layer at the time of sowing was 134 mm, with 88 mm available to the plants. Total precipitation during the vegetation period was 262.9 mm. Residual moisture in the 0–100 cm soil layer after crop harvest was  $62 \pm 16$  mm.

#### 2.2. Field preparation, experimental design

Ploughing was performed in the fall to a depth of 20–22 cm following the harvest of the previous crop. Spring tillage using toothed harrows commenced on March 17, and pre-sowing cultivation to a depth of 6–8 cm was conducted on March 24, just before sowing. The experimental protocol did not include the application of mineral fertilizers or plant protection products. The field trials in 2023 involved the pea variety ORCHESTRA and the barley variety PROSPECT. On the day of sowing, pea seeds were inoculated with a *Rhizobium leguminosarum* bv. *pisum* preparation ( $2 \times 10^9$  *Rhizobium* cells/g) at a rate of 2 L/ton of seeds. The seeds were sown without fungicide treatment.

Sowing was performed using the row method with a row width of 12.5 cm at a soil temperature of 4–6 °C at the seed embedding depth. A "Klen-1.5" seeder was employed for this purpose. The sowing depth for peas was 6–8 cm, while for barley, it was 4–6 cm. In intercropping, peas were initially sown at a depth of 6–8 cm, followed by barley in the same rows at a depth of 4–6 cm. To ensure the planned stand density, the sowing rate was adjusted considering seed similarity, expected field emergence, and potential damage during re-sowing in mixed crops. The sowing rate for peas was 880,000 seeds/ha, for barley in monoculture it was 3.75 million seeds/ha, and 1.5 million seeds/ha for barley in intercropping. The field experiment followed a multifactorial split-plot design (Table 2). Each plot measured 15 m<sup>2</sup>, and the experiment was replicated three times. The experimental setup included various intercropping systems and biostimulant treatments.

#### 2.3. Biopreparations of biostimulants and application

Compost tea and insect frass extract were prepared through 48-h aerobic fermentation. This process involved using 10 kg of raw materials to create a 50 L solution, to which purified water was added at a 1:5 (v:v) ratio. Following fermentation, the infiltrate was further diluted to 50 L with water. The humus extract, sourced from a local manufacturer, contained 120 g/L and was diluted at a 1:100 (v:v) ratio for the working solution. Fish hydrolysate (Peptostart®), derived as a by-product of trout production waste processing (Peptostart®, produced by Forel, Ukraine), and the seaweed extract, specifically the YaraVita BIOTRAC preparation, were also utilized. These biopreparations were applied at 30 and 60 days after emergence (DAE). Specifically, the insect frass extract, humus extract, and compost tea were each applied at a rate of 50 L/ha at both 30 and 60 DAE, while the fish hydrolysate and seaweed extract were applied at a rate of 2 L/ha at both 30 and 60 DAE. The

Field trial design and application of biostimulants.

Intercropping systems (A)	Biostimulant treatment (T)
A.1 – Pea sole A.2 – Pea-barley intercrop A.3 – Barley sole	$\begin{array}{l} C - {\rm control \ (without \ biostimulant)} \\ T1 - {\rm insect \ frass \ extract \ (50 \ l/ha \ {}_{(30 \ DAE)} + 50 \ l/ha \ {}_{(60 \ DAE)})} \\ T2 - {\rm Humus \ extract \ (50 \ l/ha \ {}_{(30 \ DAE)} + 50 \ l/ha \ {}_{(60 \ DAE)})} \\ T3 - {\rm Compost \ tea \ (50 \ l/ha \ {}_{(30 \ DAE)} + 50 \ l/ha \ {}_{(60 \ DAE)})} \\ T4 - {\rm Fish \ hydrolysate \ (2 \ l/ha \ {}_{(30 \ DAE)} + 2 \ l/ha \ {}_{(60 \ DAE)})} \\ T5 - {\rm Seaweed \ extract \ (2 \ l/ha \ {}_{(30 \ DAE)} + 2 \ l/ha \ {}_{(60 \ DAE)})} \end{array}$

selected biostimulants were chosen for their synergistic roles in enhancing soil microbiota, nutrient availability, and plant resilience [11,27]. Insect frass [28] and compost tea [29] enrich microbial diversity, while humic and seaweed extracts [13] promote root growth and stress tolerance. Fish hydrolysate [30], rich in amino acids and bioactive compounds, boosts nutrient uptake and biomass accumulation, making these biostimulants ideal for optimizing intercropping productivity.

# 2.4. Data sampling

Samples for determining the fresh and dry biomass of crops were collected by harvesting the entire aboveground biomass at a cutting height of 2 cm from an area of 1 m<sup>2</sup> (comprising 8 rows of 1 m each). Samples for moisture determination using the gravimetric method were collected from the field for each treatment variant. The main crop, components of the intercropping system, and any present weeds were accounted for separately. Fresh biomass was measured at 30 and 60 days after emergence (DAE), while biomass at 90 DAE was adjusted to 14 % moisture content, which was close to the lowest observed value in the study.

Biomass samples were collected at three different growth stages: 30 DAE, 60 DAE, and 90 DAE. For structural element analysis of the crops, aggregate biomass samples were utilized, from which 30 spikes/plants were randomly selected for further examination. For barley, parameters such as ear density, number, and weight of seeds per ear, and thousand-seed weight were determined. For peas, the number and weight of seeds per plant, number of plants, and thousand-seed weight were recorded. Yield and structural elements of the crop were standardized to a 14 % moisture content.

#### 2.5. Land equivalent ratio (LER)

Land Equivalent Ratio (LER) is the sum of the yield ratios of the components in intercropping compared to conventional cropping. LER was calculated using formulas based on the method of Mead and Willey [31]:

$$LER_{IC} = LER_P + LER_B [31].$$
(1)

$$LER_{p} = \frac{BM_{PealC}}{BM_{PearSole}}$$
(2)

$$LER_{B} = \frac{BM_{BarleyIC}}{BM_{BarleySole}}$$
(3)

 $BM_{Barley}$  denotes the biomass of barley, and  $BM_{Pea}$  denotes the biomass of pea in both intercropping (IC) and sole cropping (sole). LER was calculated for dry biomass.

# 2.6. Statistical analysis

All data were analyzed using R software (version 4.2.1). Normality and homogeneity of variance were tested using the Shapiro–Wilk and Levene's tests, respectively, and data were transformed when necessary to meet ANOVA assumptions. A linear mixed-effects model was used to assess the effects of cropping system, biostimulant treatment, and their interaction, with replication blocks included as a random effect (using the "lme4" package). ANOVA was performed to test the significance of fixed effects, and post-hoc comparisons between treatment means were conducted using Tukey's HSD test via the "emmeans" package. All statistical tests were performed at a significance level of  $\alpha = 0.05$ . Data visualization was carried out using the "ggplot2" package.

#### 3. Results

#### 3.1. Plant biomass dynamics in cropping systems

### 3.1.1. Plant fresh biomass at different days after emergence (DAE)

Fresh biomass dynamics are a key indicator of crop growth and dry matter accumulation efficiency. Significant differences were observed among cropping systems at 30, 60, and 90 days after emergence (DAE) (Fig. 1). At 30 DAE, fresh biomass was relatively low across all systems. However, the pea-barley intercrop showed significantly higher biomass than either pea or barley grown alone (p < 0.05).

By 60 DAE, biomass peaked in all systems. The pea-barley intercrop again recorded the highest biomass, followed by pea sole and barley sole. The differences were statistically significant (p < 0.05), with the intercrop outperforming both monocultures. At 90 DAE, fresh biomass significantly declined in all cropping systems. The pea sole exhibited the sharpest reduction, whereas the barley sole retained the highest fresh biomass compared to the pea sole. Overall, the pea-barley intercrop consistently outperformed the monocultures at the early and midgrowth stages (30 and 60 DAE), while barley sole showed superior biomass retention at the end of the season (90 DAE). In summary, the intercrop system enhanced early biomass accumulation, while barley sole demonstrated better late-season performance.

# 3.1.2. Plant dry biomass at different days after emergence (DAE)

Dry biomass accumulation varied significantly across cropping systems over the three sampling periods (30, 60, and 90 DAE, as illustrated in Fig. 2.

At 30 DAE, the pea-barley intercrop system showed a significantly higher dry biomass compared to the pea sole and barley sole systems. The pea-barley intercrop maintained a slight but consistent advantage over barley sole, with both systems significantly outperforming the pea sole (p < 0.05). At 90 DAE, the pea-barley intercrop and barley sole systems remained dominant, with dry biomass values markedly higher than those of the pea sole (p < 0.05). Although the difference between the intercrop and barley sole narrowed, the intercrop still retained the highest biomass overall.

In summary, the pea-barley intercrop demonstrated consistently superior dry biomass production across all growth stages, particularly in early and mid-season, while the barley sole system emerged as a strong performer at later stages. In contrast, the pea sole system showed significantly lower biomass throughout the growing period.

#### 3.2. Effects of biostimulants on plant biomass at different DAE

The application of biostimulants has the potential to alter plant growth processes, particularly in intercropping systems, where it can either enhance or diminish the competitive interactions between different crop components. In this study, biostimulants were applied at 30 days after emergence (DAE) and 60 DAE. Consequently, the effects of biostimulants on fresh and dry biomass were evaluated at 60 DAE and 90 DAE for each cropping system.

# 3.2.1. Crop fresh biomass at 60 DAE

The fresh biomass significantly differed (p < 0.05) by cultivation system and applied biostimulants at 60 DAE (Fig. 3). Among sole crops, pea produced higher fresh biomass than barley, which may be attributed to its higher moisture content. The pea sole system (PS) maintained relatively consistent biomass across treatments, suggesting stable performance under the tested conditions. The intercropping system (IC) exhibited greater variations in biomass. Treatment T3 (compost tea) resulted in the highest biomass within the IC system, indicating a potential positive interaction between this biostimulant and the crop combination. Notably, in IC plots, pea biomass was lower than in the PS system due to the dilution effect, while barley biomass exceeded that observed in the barley sole (BS) system. Although the BS system generally had the lowest biomass, treatments T3 and T5 (seaweed extract) substantially improved its performance, suggesting a positive response to specific biostimulants. In summary, biostimulant application significantly affected fresh biomass at 60 DAE, with the greatest improvements observed under treatment T3, particularly in the intercropping system.

#### 3.2.2. Crop dry biomass at 60 DAE

Dry biomass accumulation at 60 days after emergence (DAE) varied significantly across cropping systems and biostimulant treatments (Fig. 4). In the pea sole system (PS), dry biomass ranged from 393 to 470 g/m<sup>2</sup> across treatments, with no significant differences observed. In contrast, the barley sole system (BS) produced consistently higher dry biomass, particularly under treatment T3 (compost tea), T4 (fish hydrolysate), and T5 (seaweed extract). The barley sole system (BS) consistently yielded higher dry biomass compared to the pea system, particularly under treatments T3, T4, and T5. Among all treatments, T4



Fig. 1. Plant fresh biomass dynamics in cropping systems at different DAE. Asterisk indicates statistically significant differences between treatments at p < 0.05.



Fig. 2. Plant dry biomass dynamics in cropping systems at different DAE. Asterisk indicates statistically significant differences between treatments at p < 0.05.



Fig. 3. Effects of biostimulants application on the fresh biomass of crops under different cropping systems at 60 DAE. The data represent the mean values  $\pm$  standard error. Different letters denote significant differences at  $p \le 0.05$ . Here, PS - pea sole, IC - intercropping, BS - barley sole.



Fig. 4. Effects of biostimulants application on the dry biomass of crops under different cropping systems at 60 DAE. The data represent the mean values  $\pm$  standard error. Different letters denote significant differences at  $p \le 0.05$ . Here, PS - pea sole, IC - intercropping, BS - barley sole.

(fish hydrolysate) led to the highest biomass accumulation, reaching  $654 \text{ g/m}^2$ , indicating a strong growth-promoting effect.

systems, highlighting its effectiveness in promoting vegetative growth.

3.2.3. Crop biomass at 90 DAE (harvest) At 90 DAE, corresponding to the harvest stage, dry biomass

Overall, fish hydrolysate (T4) showed the greatest enhancement in dry biomass production, particularly in barley and intercropping accumulation differed significantly across cropping systems and treatments (Fig. 5). In the pea sole system (PS), dry biomass ranged from 393 to 470 g/m<sup>2</sup> across treatments, with no significant differences observed. In contrast, the barley sole system (BS) produced consistently higher dry biomass, particularly under treatment T3 (compost tea), T4 (fish hydrolysate), and T5 (seaweed extract). The intercropping system (IC) demonstrated productivity levels that were comparable to or exceeded those of BS under the same biostimulant treatments. Among all treatments, T4 (fish hydrolysate) led to the highest biomass accumulation, reaching 654 g/m<sup>2</sup>, indicating a strong growth-promoting effect. In summary, fish hydrolysate (T4) showed the greatest enhancement in dry biomass production, particularly in barley and intercropping systems, highlighting its effectiveness in promoting vegetative growth.

#### 3.3. Yield components

#### 3.3.1. Barley yield components

Yield component formation varied significantly between sole and intercropped barley systems, with each biostimulant exerting distinct effects on individual traits (Fig. 6).

All biostimulant treatments significantly increased the number of spikes/m<sup>2</sup> in the barley sole cropping system, ranging from 531 to 542 spikes/m<sup>2</sup>, compared to 503 spikes/m<sup>2</sup> in the control. In contrast, the intercropping system produced significantly fewer spikes, except under treatment T3 (compost tea), which resulted in 453 spikes/m<sup>2</sup>. Other treatments in the intercropped plots ranged from 394 to 413 spikes/m<sup>2</sup>.

In the sole barley system, grain mass per spike increased significantly with T4 (fish hydrolysate, 1.08 g) and T5 (seaweed extract, 1.06 g) compared to the control (1.01 g). Other treatments did not show significant differences. In the intercropped system, all biostimulants except T2 (humic extract) significantly improved grain mass per spike. The highest value was observed under T5 (seaweed extract), reaching 1.30 g.

A reduction in the thousand seed weight was observed when biostimulants were applied to solo crops, compared to the control, although these differences were not statistically significant. The thousand seed weight with T4 (fish hydrolysate) treatment (44.5 g) showed the largest decrease of up to 2.2 g compared to the control. In intercropped plots, deviations in thousand seed weight due to biostimulant treatments were insignificant. Additionally, seeds in intercropped plots were larger (47.2–49.3 g) compared to those in solo crop plots (43.0–45.3 g).

The number of seeds per spike increased significantly with biostimulant treatments in both intercropped and solo barley. In the control variant of solo barley, plants formed an average of 22.4 seeds per spike. Biostimulant treatments increased this value to 23.5–24.6 seeds per spike, with the highest increase observed with T5 (seaweed extract) treatment. In intercropped barley, control plants formed an average of 23.6 seeds per spike. Seaweed extract treatment resulted in the highest seed count increase per spike, with an additional 2.8 seeds (26.4 seeds per spike). Fish hydrolysate and humic extract treatments had slightly lesser effects (25.5 and 25.3 seeds per spike, respectively), but these differences were not significantly different from the best-performing variant.

In summary, biostimulant applications significantly improved key yield components in barley, with seaweed extract (T5) and fish hydrolysate (T4) showing the most consistent and positive effects across both cropping systems.

# 3.3.2. Pea yield components

The number of pea plants per square meter tended to be lower in intercropped systems than in sole cropping, although these differences were not statistically significant (Fig. 7). Plant density in monocrop plots ranged from 69 to 71 plants/m<sup>2</sup>, compared to 56 to 62 plants/m<sup>2</sup> in intercropped plots. While treatment effects within each system were not significant, a slight increase in plant number was observed under insect frass extract (T1) in the intercropping system. However, the number of seeds per plant in monocrop plots was significantly higher than in intercropped systems (Fig. 7). The average number of seeds per plant was 23.1 in sole crop peas. The application of humic extract, fish hydrolysate, and seaweed extract significantly increased this parameter, with the maximum value observed for humic extract treatment (25.5 seeds per plant). In intercropped systems, pea plants in the control plots produced 10.7 seeds per plant, whereas the application of the aforementioned biostimulants significantly increased the seed count to 13.5-14.8 seeds per plant. Additionally, compost tea had a similar effect, resulting in 14.6 seeds per plant.

The thousand seed weight (TSW) varied significantly depending on the biostimulant treatment and production system (Fig. 7). The TSW in sole crop peas was on average higher than in intercropped systems. In sole crop control plots, TSW did not increase significantly with the application of T1 (insect frass extract) or T2 (humic extract). However, significant improvements were observed with T3 (compost tea), T4 (fish hydrolysate), and T5 (seaweed extract). The most effective treatment was fish hydrolysate, resulting in a TSW of 210.3 g. In intercropped systems, the TSW in control plots was 163.3 g, which significantly increased with the application of biostimulants, except for insect frass. The highest TSW of 192.4 g was observed with the humic extract treatment in intercropped plots. The seed weight per pea plant exhibited similar trends in both production systems. In the control plots of monocrop peas, the seed weight per plant was 4.22 g, while in intercropping, it was 1.75 g. Treatment with insect frass resulted in a non-significant increase compared to the control. In summary, biostimulants had a more pronounced positive effect on seed development in sole pea cropping, with fish hydrolysate and humic extract emerging as the most effective treatments across both systems.



Fig. 5. Effects of biostimulants application on the dry biomass of crops under different cropping systems at 90 DAE. The data represent the mean values  $\pm$  standard error. Different letters denote significant differences at  $p \le 0.05$ . Here, PS - pea sole, IC - intercropping, BS - barley sole.



Fig. 6. Effects of biostimulants application on the yield contributing attributes of sole barley (left) and intercropped barley (right) at harvest. The data represent the mean values  $\pm$  standard error. Different letters denote significant differences at  $p \le 0.05$ . TSW: Thousands seed weight (g).

# 3.4. Seed yield

The effects of biostimulants on yield under different cropping systems are shown in Fig. 8 and summarized in Table 3. In barley sole cropping, all biostimulant treatments significantly increased the grain yield compared to the control, which yielded 4.8 t/ha. The highest yields were observed with T2 and T4 treatments, resulting in increases of up to 14.6 % (5.5 t/ha) and 10.4 % (5.3 t/ha), respectively. In the intercropped system, barley yield was generally higher, with the control yielding 5.8 t/ha. The most notable increase was seen with the T3 treatment, achieving 6.8 t/ha, a 17.2 % increase over the control. Other treatments also showed significant yield improvements, with T1, T2, T4, and T5 yielding up to 13.8 % higher than the control. Overall, biostimulant application significantly improved barley yield across both

cropping systems, with compost tea and humic extract showing the most consistent enhancements.

For pea sole cropping, no significant differences were observed across treatments, with the control yielding 3.68 t/ha and the highest yield recorded with fish hydrolysate treatment at 3.91 t/ha, a 6.3 % increase. In intercropped systems, pea yields were significantly enhanced by biostimulant treatments. The control yielded 0.97 t/ha, while the humus extract treatment achieved the highest yield at 1.57 t/ ha, a 61.9 % increase. Compost tea and fish hydrolysate also significantly increased pea yields to 1.51 t/ha and 1.44 t/ha, respectively. Similarly, in intercropped barley, the control yield was 4.39 t/ha. The compost tea treatment resulted in the highest yield of 5.57 t/ha, a 26.9 % increase. Significant yield improvements were also noted with insect frass (5.02 t/ha), fish hydrolysate (5.17 t/ha), and seaweed extract



**Fig. 7.** Effects of biostimulants application on the yield contributing attributes of sole pea (left) and intercropped pea (right) at harvest. The data represent the mean values  $\pm$  standard error. Different letters denote significant differences at  $p \le 0.05$ . TSW: Thousands seed weight (g).

(5.18 t/ha). Biostimulant treatments significantly enhanced barley yields, particularly in intercropping systems, while their effect on pea yields in sole cropping was not statistically significant.

#### 3.5. Total biomass and yield (t/ha) in cropping systems

Pea sole cropping resulted in an average total dry biomass of 6.5 t/ha and a grain yield of 3.8 t/ha (Fig. 9). In comparison, the pea-barley intercropping system significantly outperformed the pea sole cropping, with an average total dry biomass of 8.8 t/ha and a total grain yield of 5.3 t/ha, representing increases of 35.4 % and 39.5 %, respectively. Barley sole cropping also demonstrated notable yield improvements over pea sole cropping, with an average total dry biomass of 7.5 t/ha and a total grain yield of 5.1 t/ha, reflecting increases of 15.4 % and 34.2 %, respectively. Compared to the pea-barley intercropping system, barley sole cropping exhibited a 14.8 % lower total dry biomass and a 3.8 % lower total grain yield. These findings underscore the superior productivity of intercropping systems, particularly when combined with biostimulant applications, in enhancing both biomass accumulation and grain yield.

# 3.6. Land equivalent ratio (LER) of pea-barley intercropping system at different DAE

At 30 DAE, the total LER is around 1.6, indicating a significant yield advantage for the intercropping system at this early growth stage. Both



Fig. 8. Effects of biostimulants application on the seed yield of pea and barley under different cropping systems at harvest. The data represent the mean values  $\pm$  standard error. Different letters denote significant differences at  $p \le 0.05$ .

Table 3Effect of biostimulants on seed yield of pea and barley (moisture content 14 %).

Treatments	Cropping syste	ems	
	Pea sole	Barley sole	Intercropping
Control	3.68 a	5.10 b	5.36 bc
Insect frass	3.88 a	5.60 bc	6.28 de
Humus extract	3.58 a	5.51 bc	6.30 de
Compost tea	3.88 a	5.60 bc	7.0 8f
Fish hydrolysate	3.91 a	5.77 cd	6.61 ef
Seaweed extract	3.78 a	5.71 c	6.66 ef
Average	3.78	5.55	6.38
SD	0.35	0.24	0.65
CV (%)	9.20	4.30	10.20

**Note:** The data represent the mean values  $\pm$  standard error. Different letters denote significant differences at  $p \leq 0.05$ . Values not sharing a common letter are significantly different form others.

pea and barley contributed positively to the LER, with pea contributing slightly more than barley. At 60 DAE, the total LER decreased to around 1.2, still indicating a yield advantage, although less pronounced than at 30 DAE. The relative contribution of barley has increased, equalling that of pea, which suggests that barley is becoming more dominant in resource use or growth. By 90 DAE, the total LER remains above 1.0, indicating a continued but smaller yield advantage of the intercropping system. At this stage, barley contributed more substantially to the LER than pea, reflecting that barley has become the dominant species in resource use or growth within the intercropping system (Fig. 10). Overall, the LER dynamics indicate that intercropping provides a consistent productivity advantage throughout the growing season, with pea dominating early growth and barley contributing more substantially to later stages.

## 4. Discussion

# 4.1. Biomass, yield, and resource dynamics in intercropping systems

The integration of biostimulants into cereal-legume intercropping systems represents a progressive approach to sustainable agriculture, enhancing not only biomass accumulation and yield performance but also overall system efficiency. The present study revealed that peabarley intercropping significantly improved biomass production compared to monocultures, especially when combined with biostimulant applications. This supports the concept that intercropping leverages ecological synergies, optimizing nutrient dynamics and resource partitioning.

The increased productivity in intercropping systems can be attributed to interspecific facilitation, particularly nitrogen transfer from legumes to cereals, thereby improving nutrient use efficiency [32–34]. In this study, pea-barley intercropping exhibited a notable increase in biomass production compared to monoculture systems. The improved performance can be attributed to the facilitation of nitrogen transfer from legumes to companion cereal crops, optimizing nutrient acquisition and promoting superior growth [35,36]. The complementary use of spatial and temporal resources (light, water, nutrients) allows each crop to occupy distinct ecological niches, enhancing system-level productivity [32,37,38].

However, Cowden et al. [39] reported a significantly higher proportion of peas in the pea-barley intercropping yield than obtained in the current study. However, variability in performance between sites or seasons such as the dominance of barley observed in this study is likely influenced by environmental factors, particularly soil fertility and moisture availability [40]. It is well established that cereals, under nutrient-rich conditions, can outcompete legumes for light and water, while in nutrient-limited environments, legumes may gain a relative advantage due to their capacity for symbiotic nitrogen fixation [41,42]. The shift in dominance observed in this study, with barley outperforming pea later in the season, reinforces earlier observations



Fig. 9. Effects of biostimulants application on the total biomass and yield of pea and barley under different cropping systems. The data represent the mean values  $\pm$  standard error.



Fig. 10. LER of pea-barley intercropping system at different DAE.

regarding competitive dynamics in legume-cereal systems [37,42,43].

In cereal-legume intercropping systems, plant reproductive allometry (PRA) improves significantly under specific management conditions, particularly benefiting legumes in unfertilized mixtures and cereals under resource-limiting conditions, with hierarchical competition (biomass differences) strongly influencing reproductive output [44, 45]. However, due to the more efficient uptake of soil nitrogen by cereals [46], leguminous plants become more dependent on symbiotic nitrogen fixation. At the same time, the dry biomass of intercropped systems may exceed that of pure stands under favourable conditions [47]. Even with equal biomass indicators of monocrop barley and intercropped systems, intercropped systems with legumes are prioritized because they contain more digestible protein and accumulate more nitrogen overall [48,49].

Furthermore, competition for life factors increases in the later stages of development. The overall decrease in the proportion of pea biomass in intercropping until the end of the vegetation period is likely associated with competition for life factors, primarily light and moisture. Overall, the obtained results in the current study are consistent with previous results [50], except for the tendency of dry biomass distribution during the vegetation period towards bias in favour of barley, which may be related to higher natural soil fertility and accordingly better conditions for the cereal component.

#### 4.2. Biostimulant effects on crop growth dynamics and productivity

Biostimulants offer a promising route to enhancing crop productivity through mechanisms that transcend nutrient supplementation [11,51]. In this study, the application of biostimulants such as seaweed extract and fish hydrolysate in barley monoculture significantly enhanced grain yield, dry matter accumulation compared to the control treatments. These effects were primarily driven by an increase in spike density and improved grain-filling efficiency, as evidenced by higher thousand seed weight. The ability of seaweed extracts to modulate hormonal responses and stress tolerance in barley likely contributed to the improved grain weight per spike, aligning with previous studies that highlight the role of bioactive compounds in mitigating abiotic stress [51,52]. This aligns with previous studies demonstrating that biostimulants can significantly enhance key yield-contributing attributes through mechanisms such as improved nutrient uptake, enhanced photosynthetic efficiency, and increased resistance to abiotic stresses [52,53].

Biostimulants further amplified these benefits in intercropping systems, particularly in barley-pea combinations, leading to even greater yield improvements. For instance, the application of compost tea in these systems resulted in significantly higher yield increase over the control, highlighting the synergistic effects of intercropping and biostimulants. Biostimulants can improve nutrient uptake by enhancing root growth and increasing root surface area, thereby facilitating greater access to soil nutrients. Additionally, biostimulants can enhance the activity of soil microorganisms, which play a crucial role in nutrient cycling and availability. For instance, humus extracts can stimulate microbial activity and improve soil structure, leading to better water retention and nutrient availability [27]. This study's findings underscore the potential of combining intercropping with biostimulants to optimize resource use and improve agricultural sustainability [35,54, 55].

The current study findings further demonstrated that the application of biostimulants significantly enhanced biomass accumulation and yield in pea-barley intercropping, reinforcing their role in mitigating climateinduced stress factors. The Ukrainian climate, with its periodic drought stress and fluctuating seasonal temperatures, often limits crop productivity [24,25]. In this context, the observed improvements in nutrient uptake and stress tolerance suggest that biostimulant application can serve as an adaptive strategy for improving agricultural resilience. Compared to other regions with similar climatic profiles, our results highlight the potential of integrating biostimulants into intercropping systems to optimize productivity and sustainability under Ukrainian environmental conditions.

#### 4.3. Species-specific responses to biostimulants application

One of the key findings of this study is the species-specific responses to biostimulants. While barley exhibited yield improvements across both sole and intercropped systems, peas showed significant gains primarily in intercropping conditions. The differential response of peas and barley to biostimulants suggests a species-specific mode of action, where crop physiology dictates the effectiveness of various treatments. This is corroborated by Bulgari et al. [10], who also noted variable responses to biostimulants among different crops.

In pea-barley intercropping systems, pea yields were significantly enhanced by humus extract and compost tea. This suggests that while peas may not respond strongly to biostimulants in monoculture, their growth can be substantially improved in intercropping systems due to complementary interactions between the two crops [32,37]. Moreover, this underscores that intercropping systems may facilitate a synergistic interaction between biostimulants and crop species, enhancing nutrient availability and uptake.

The mechanisms through which biostimulants enhance crop growth and productivity are diverse and complex and may have differential effects on different crop species. Seaweed extracts, for example, contain various bioactive compounds, including hormones, polysaccharides, and amino acids, that stimulate plant growth and improve stress tolerance [11,56,57]. These compounds can enhance nutrient uptake, promote root growth, and increase photosynthetic efficiency, leading to higher yields [52,56]. Fish hydrolysates provide a rich source of amino acids and peptides that can improve soil microbial activity and nutrient availability, enhancing plant growth and resilience to abiotic stresses such as drought and salinity [52]. Humus extracts and compost tea contain a variety of organic compounds that improve soil structure, increase nutrient retention, and promote beneficial microbial activity [11,27,56]. Insect frass in the form of a water solution may not exhibit a visible effect as it does not directly affect changes in crop structure elements, but its positive impact on yield may result from improved growth conditions and increased plant resilience to adverse environmental conditions, especially in competition for life factors, due to the presence of auxin-like components [58,59].

The current study further demonstrated that the parameters of individual productivity are suppressed by the cereal component. The application of biostimulants allows for the formation of more fruits and seeds compared to the control by enhancing the competitiveness of peas compared to the control variant. The compost tea and humic extract had the greatest positive impact on the formation of generative organs in peas in intercropping systems, while in sole pea crops, the fish hydrolysate and seaweed extract had the most significant effect. Collectively, these results underscore the importance of considering both crop identity and cropping context when designing biostimulant-based interventions in diversified farming systems.

#### 4.4. Land-use efficiency in intercropping systems

The LER results clearly demonstrated the superior land-use efficiency of the pea-barley intercropping system compared to monoculture throughout the growing season. The high LER at this stage suggested that the intercropping system effectively utilized available resources, possibly due to complementary interactions between the species. However, the LER declined at 60 and 90 DAE, indicating that competition between pea and barley might have increased, reducing the intercropping advantage as the crops matured.

This temporal decline in LER suggests a shift in interspecies dynamics, with barley becoming increasingly dominant later in the season, likely due to its stronger competitive capacity for resources such as light, water, and nutrients. Despite this, the sustained LER >1.0 across all stages underscores the efficiency of land use in intercropped systems relative to sole cropping. These findings are consistent with.

Chapagain and Riseman [60], who reported that intercropping displayed higher land productivity, with an LER of 1.32, reflecting a 12–32 % increase in productivity over monoculture systems. Overall, this study demonstrated that the integration of biostimulants into intercropping systems represents a viable strategy for enhancing crop yields and biomass production.

### 5. Conclusions

This study highlighted that biostimulant applications substantially modulate crop performance across both sole and intercropping systems, with their efficacy being strongly influenced by species-specific responses and cropping system interactions. Among the tested biostimulants, compost tea and humus extract consistently promoted

biomass accumulation and yield formation, particularly in the peabarley intercropping system. In contrast, seaweed extract and fish hydrolysate exhibited greater benefits in sole-cropped barley, where they increased yield primarily by enhancing spike density and grain weight per spike. Barley exhibited strong adaptability under intercropping conditions, where seaweed extract enhanced grain weight despite reduced spike density, indicating that biostimulants can effectively reinforce the productivity and resource-use efficiency advantages of intercropping systems. The overall land equivalent ratio (LER) for the pea-barley intercropping system remained consistently greater than one, reaching its highest value at the early growth stage, indicating a sustained yield advantage over monoculture throughout the growing season. Thus, intercropping, particularly of barley and peas, combined with targeted biostimulant applications, emerges as a promising strategy to enhance both grain yield and biomass production. This approach not only improves overall crop performance but also contributes to more sustainable agricultural practices by optimizing resource use and reducing the need for chemical inputs. Future research should further explore the long-term benefits and potential environmental impacts of biostimulant applications in diverse cropping systems.

#### CRediT authorship contribution statement

Bohdan Mazurenko: Writing - original draft, Investigation, Formal analysis, Data curation, Conceptualization. Md Nasir Hossain Sani: Writing - review & editing, Writing - original draft, Investigation, Formal analysis, Data curation. Dmytro Litvinov: Writing - review & editing, Supervision, Methodology, Investigation, Data curation. Svitlana Kalenska: Supervision, Resources, Methodology, Investigation, Formal analysis. Vitaliy Kovalenko: Supervision, Resources, Methodology, Investigation, Formal analysis. Iryna Shpakovych: Writing original draft, Methodology, Investigation, Formal analysis, Data curation. Olena Pikovska: Writing - review & editing, Supervision, Resources, Methodology, Investigation, Data curation. Lyudmyla Gordienko: Writing - review & editing, Investigation, Formal analysis, Data curation. Jean Wan Hong Yong: Writing - review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. Bhim Bahadur Ghaley: Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. Oksana Tonkha: Writing - review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

# Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Data availability

Data will be made available on request.

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