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Differential Phenological Response of Quinoa to the Application of Vermicompost Leachate and Moderate Doses of Fish Hydrolysate under High Andean Rainfed Conditions

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Abstract: The farmers of Andes in rural areas commonly use fish hydrolysate and vermicompost leachate as cost-effective bio-inputs, either due to limited economic resources or a part of organic farming practices. However, their agronomic effectiveness for primary sources of plant nutrition under rainfed conditions remains insufficiently documented. This study evaluated the phenological response of quinoa (*Chenopodium quinoa* Willd.) to the applications of fish hydrolysate (1% and 2%) and vermicompost leachate (10%) at the Illpa Agrarian Experimental Station in Puno, Peru. Morphological parameters and yield components of quinoa and soil chemical properties were assessed at the end of the crop cycle. The results revealed a differentiated response by phenological stage: vermicompost leachate significantly enhanced plant height during the grain-filling stage, while 1% fish hydrolysate increased above ground dry weight without translating into significant yield gains compared to the control. Both biofertilizers improved soil parameters, including pH and cation exchange capacity, indicating a positive effect on soil quality. In conclusion, these inputs exert specific biostimulant effects during the vegetative stages, but do not entirely replace the function of comprehensive fertilization to achieve high yields. Nonetheless, their strategic use at key phenological stages of the cycle may support the development of more sustainable production systems in high Andean rainfed environments.

Key words: Biofertilizer, quinoa, vermicompost-leachate, fish-hydrolysate, soils peoperties.

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Quinoa (*Chenopodium* quinoa Willd.) a grain native to the Andes, is increasingly recognized as a key contributor to global food security due to its exceptional nutritional profile (Alandia *et al*., 2020; Coello, 2016). Its adaptability to diverse environmental conditions and high genetic diversity confer tolerance to multiple type of abiotic stresses making it an agronomically valuable crop (Torresnian *et al*., 2024). In Peru, Andean regions of Puno and Ayacucho are the leading producers, accounting for 41% and 22% of national production, respectively (Becerra Sánchez, 2023). This growth has been driven by the expansion of cultivated areas, favorable climatic conditions, and the active participation of 67,998 small land holding farmers. These developments have strengthened Peru's position in the global quinoa export market (Becerra Sánchez, 2023; Herrada, 2024; Efeagro, 2024).

The area under quinoa cultivation has remained relatively stable over the past decade, fluctuating between 33,296 and 34,167 ha. Over the past decade, quinoa production in Peru has increased significantly, rising from 38,220 t in 2014 to 41,958 t in 2024. This growth is attributed to rising yields, which have ranged between 1,118 and 1,260 $\mathrm{kg \cdot ha^{-2}}$. Currently, 56,322 families are engaged in quinoa cultivation in this region (INEI, 2023).

National per capita quinoa consumption in Peru has exhibited erratic trends, albeit with some fluctuations over the years. Between 2000 and 2013, consumption remained relatively stable at approximately 1.09 kg $\mathrm{person^{-1}}$. In 2014, it rose sharply to 2.54 kg, reaching a peak of 3.2 kg in 2015. However, in 2016 and 2017, consumption declined to 1.6 kg and 1.8 kg, respectively, primarily due to increased prices which reduced its accessibility for consumers and hindered domestic market supply. By 2018, consumption had partially recovered to around 2.05 kg $\mathrm{person^{-1}}$ (Ku Soria, 2019; Ministry of Agriculture and Irrigation Riego, 2015), and by 2024, it is estimated at 2.5 kg $\mathrm{person^{-1}}$ (Efeagro, 2024).

As part of the agricultural modernisation, chemical fertilisers have been indiscriminately used to increase crop yields, including those of quinoa. However, this practice has often neglected the negative consequences on soil quality and the broader environment (Torres-García *et al*., 2024). These adverse effects have led to the search for safer and more sustainable alternatives, such as organic options (Chandini *et al*., 2019; Kosem *et al*., 2022), that are compatible with the natural environment, avoiding any risk or negative impact on soil health, living organisms, animals, and human health (Chandini *et al*., 2019).

A sustainable alternative to chemical fertilizers is the use of biofertilizers or biostimulants, a group of substances derived from natural interactions among plants, microorganisms, and the environment. This category includes seaweed extracts, humic acids, and *Azotobacter* strains, among others. These natural products optimise plant enzymatic and metabolic processes, thereby improving crop quality and productivity. Additionally, they promote plant growth, strengthen root systems, and increase tolerance to both biotic and abiotic stresses. Biofertilizers also enhance nutrient uptake and stimulate the production of beneficial compounds such as phenolics and cytokinins (Kaur *et al*., 2021)

However, the limitations and potential of biostimulants in quinoa cultivation have not yet been thoroughly investigated. Despite biostimulants' positive effects on plant metabolism and growth, their effectiveness may be influenced by environmental factors, the crop's phenological stage, and interactions with other agricultural inputs. Therefore, its application should be regarded as a complementary tool within a broader fertilization programme that incorporates more robust nutrient sources to ensure optimal crop productivity. For example, fish protein hydrolysate has been demonstrated to promote plant growth and enhance soil microbial activity. However, its impact on final crop yield remains uncertain, as its effectiveness may be limited to specific developmental stages, such as the milky grain stage. In this context, its potential as a biostimulant lies in its ability to complement more comprehensive fertilization strategies, enhancing nutrient use efficiency and improving crop response under adverse conditions.

The present research examines the effect of fish hydrolysate and vermicompost leachate on quinoa production, with a focus on their benefits and limitations at various stages of crop development. The objective of this study is to generate insights that support the optimization

Table 1. Physico-chemical characterization of soil used in the experiment under rainfed conditions at the Illpa Agrarian Experimental Station (EEA), Salcedo Annex, Peru

Trial	Unit	2022	2023
pH		6.2	6.3
Electrical conductivity	$mS \cdot m^{-1}$	5.44	6.2
Organic matter	%	1.17	2.6
Sand	%	35.60	37.80
Clay	%	21.12	21.64
Silt	%	43.28	40.58
Textural class	-	Loam	Loam
Calcium (Ca) (*)	$cmol(+) \cdot kg^{-1}$	10	12.8
Magnesium (Mg) (*)	$cmol(+) \cdot kg^{-1}$	3.9	2.4
Sodium (Na) (*)	$cmol(+) \cdot kg^{-1}$	0.008	0.38
Potassium (K) (*)	$cmol(+) \cdot kg^{-1}$	0.71	0.4
Total nitrogen (*)	%	0.043	0.08
Available potassium (*)	$mg \cdot kg^{-1}$	547.37	148
Available phosphorus (*)	$mg \cdot kg^{-1}$	12	82
CECe (*)	$cmol(+) \cdot kg^{-1}$	14.8	17

of their use within more sustainable and efficient fertilization programs.

Materials and Methods

The study was conducted from November 2023 to May 2024 under rainfed conditions at the Illpa Agrarian Experimental Station, Salcedo Annex, of the National Institute of Agrarian Innovation (INIA). The site is located in the district of Salcedo, Puno Province, Puno Region, Peru. The experimental plot was situated at 15° 52′ 54.31″ S, and 69° 59′ 59.97″W, at an altitude of 3,854 m above sea level. According to Soil Taxonomy classification system (Soil Science Division Staff, 2017), the soil at the experimental field is classified within the Mollisol order (Mamani Nina and Sardon Nina, 2023).

Soil sampling followed the methodology proposed by Havlin *et al.* (2017). Each composite sample represented one experimental area and consisted of five subsamples collected randomly from a depth of 0-30 cm. The subsamples were thoroughly mixed and homogenized, and a representative sample was obtained using the quartering method. Soil samples were analyzed at the Soil, Water, and Foliar Laboratory Network of the National Institute of Agrarian Innovation (LABSAF-INIA). Prior to analysis, samples were air-dried (<40°C) and sieved to <2 mm in accordance with ISO standards (ISO, 2006).

The analyzed soil variables included texture (sand, silt, and clay; NOM-021-RECNAT-2000, 2002), pH (soil-water ratio 1:1; EPA, US, 2004), electrical conductivity (soil-water ratio 1:5; ISO, 1994), equivalent calcium carbonate content (NOM-021-RECNAT-2000, 2002), organic matter, organic carbon, and total carbon (NOM-021-RECNAT-2000, 2002). Available phosphorus was determined using the AS-11 method for acidic to neutral soils (NOM-021-RECNAT-2000, 2002). Exchangeable bases (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) were extracted with ammonium acetate, while exchangeable acidity and Al^{3+} were determined using potassium chloride (NOM-021-RECNAT-2000, 2002). The physical and chemical properties of the soil prior to the establishment of the experiment (2021-2022 and 2022-2023 growing seasons) are presented in Table 1.

According to Köppen’s climate classification, the experimental area is characterized by a cold semi-arid steppe climate (BSk) (Köppen, 1936; Peel *et al.*, 2007). Based on data from the Puno weather station (15°49′34″S; 70°0′43″W; 3,812 m.a.s.l.), operated by the National Service of Meteorology and Hydrology of Peru (SENAMHI), the historical averages indicate an annual rainfall of 725 mm, an average minimum

Table 2. Chemical characteristics of the biofertilizers used

Biofertilizer	pH	CE	MO	N	P	Ca	Mg	Na	K
		(mS·m^{-1})	%	(mg·mL^{-1})			(mg·L^{-1})		
Vermicompost leachate	8	121,25	0,3	0,19	43,95	160	35	550	2540
Fish hydrolysate	4,90	430,00	11,40	27,26	69,86	3200,00	600,00	1620,00	8750,00
Biofertilizer	**Cu**	**Fe**	**Pb**	**Zn**	**Co**	**Cr**	**Mn**	**Mo**	**Al**
					(mg·L^{-1})				
Vermicompost leachate	0,3	2,825	0,05	0,175	0,05	0,05	0,55	0,25	0,6
Fish hydrolysate	1,00	37,95	0,00	3,20	0,00	0,35	0,80	0,00	1,25

Source: Soil, Water, and Foliar Laboratory, Illpa Agrarian Experimental Station (EEA Illpa), 2024.

temperature of 1.3°C, and an average maximum temperature of 15.17°C.

A randomized complete block design (RCBD) was used with four treatments: fish hydrolysate at 2% (T1); fish hydrolysate at 1% (T2) (provided by the Instituto del Mar del Perú - IMARPE, Peru); vermicompost leachate at 10% (T3); and an untreated control plot (T0). Each treatment was replicated four times. Each experimental unit consisted of a gross plot measuring 4 m × 8 m, with five furrows spaced 0.6 m apart. Within each gross plot, two net plots were established for data collection. Each net plot measured 1 m × 1 m and was located at the center of the gross plot.

Treatments were applied at four phenological stages: branch formation (53 days after sowing, DAS), panicle formation (84 DAS), flowering (102 DAS), and milky grain formation (120 DAS). Treatment solutions were prepared as follows: for fish hydrolysate at 2%, 200 ml of fish hydrolysate was diluted with water in a 20 L backpack sprayer; for fish hydrolysate at 1%, 100 mL of fish hydrolysate was diluted with water in a 20 L backpack sprayer; and for vermicompost leachate at 10%, 2 L of vermicompost leachate was added and the volume was brought to 20 L with water in a 20 L manual backpack sprayer. All solutions were prepared shortly before application to ensure effectiveness. Treatments were applied by uniformly spraying the leaves and stems of plants within the plots. A 20 L mixture volume was sufficient to treat all four blocks of each respective treatment. All applications were conducted in the late afternoon to minimize exposure to direct sunlight.

Fish hydrolysate is an innovative biofertilizer produced from waste and by-products generated during fish processing (Zapata and Castañeda, 2017). Its production involves the addition of a carbohydrate source, as well as enzymes and lactic acid bacteria, which enable fermentation and maturation over 7 to 10 days. The final product is a liquid with a pH of 4.6, rich in peptides and amino acids, and functions as a 100% organic fertilizer. Its versatility allows for application via both fertigation and foliar spraying, benefiting a wide range of crops. This product is manufactured by the Association of Biofertilizer Producers San Pedro de la Caleta de Carquín (Quispe Argumedo, 2023). For the present study, the fish hydrolysate was provided by the Instituto del Mar del Perú (IMARPE).

Vermicompost leachate is a liquid biofertilizer derived from the irrigation of beds containing *Eisenia foetida*, a worm species highly efficient at decomposing organic matter (FAO, 2024). It contains essential macro- and micronutrients, as well as enzymes, amino acids, and beneficial microorganisms, making it ideal for application on crops via irrigation or foliar spraying (Mupambwa *et al.*, 2024; Singh *et al.*, 2024). In this study, the leachate were collected from the fertilizer production facility at the EEA Illpa. It had a pH of 8.0 and an electrical conductivity (EC) of 121.20 mS·cm^{-1}. To prevent salt-induced phytotoxicity, it was diluted to 10% with water before foliar application (Garcia-Gomez *et al.*, 2008; Gutiérrez-Miceli *et al.*, 2017; Zamora *et al.*, 2017).

Both biofertilizers (fish hydrolysate and vermicompost leachate) used in the experiment were analyzed at the Soil, Water and Foliar Laboratory (LABSAF) of EEA Illpa - INIA (Table 2), where their macro- and micronutrient contents were determined using appropriate analytical methods.

Table 3. Procedure for variable evaluation

Variable	Measuring instrument	Procedure[a]
Plant height (cm)[b]	Stainless steel flexible measuring tape (0-100 cm, accuracy ±1 mm)	Plant height was measured on five plants per treatment, from the plant collar to the apex, at the following phenological stages: branching (53 days DAS), panicle formation (84 DAS), flowering (102 DAS), milky grain (120 DAS), dough grain (132 DAS), and final dough grain (144 DAS). The average of the obtained values was calculated for each stage.
Panicle length (cm)[b]	Stainless steel flexible measuring tape (0-100 cm, accuracy ±1 mm)	Five plants were selected from each treatment, and the length was measured using a measuring tape from the base of the panicle to its apex. The average of the obtained values was calculated.
Panicle width (cm)[b]	Stainless steel flexible measuring tape (0-100 cm, accuracy ±1 mm)	Five plants were selected from each treatment, and the maximum panicle width was measured using a measuring tape. The average of the obtained values was calculated.
Grain size (mm)[b]	Makawa Professional Mechanical Vernier Caliper MK-0035, 0-6 inches - Accuracy: 8x1 / 1000 in, made in China.	Ten quinoa grains were randomly selected, measured using the instrument, and each measurement was recorded. The average of the obtained values was then calculated.
1000-grain weight (g)[b]	Bel Engineering Balance BL0246, 2200 g capacity, 0.1 g resolution, made in Italy.	One thousand quinoa grains were counted and separated, with three replicates per treatment. The grains were weighed on a digital balance, and the total weight in grams was recorded.
Yield (t ha^{-1})[b]	Axis Balance AKA-4200, 4200 g capacity, 0.01 g precision, manufactured in Gdansk, Poland	The total weight of quinoa grains was obtained for each treatment, and the following calculation was performed: $Yield\ (t \cdot ha^{-1}) = \frac{grain\ weight\ (kg)}{area\ (m^2)} \times \frac{10000\ m^2}{1\ ha} \times \frac{1\ t}{1000kg}$

Land preparation was initiated in August 2023 with disc plowing to a depth of 20 to 30 cm. In October, two passes with a disc harrow were carried out at the same depth to break up soil clods and refine the soil structure. Sowing was carried out in early November using a mechanical furrower that opened furrows 15 cm deep, spaced 60 cm apart. Seeds were continuously distributed along the bottom of the furrows. Seed covering was conducted by dragging a shrub branch over the surface, ensuring a covering depth of 2 to 3 cm. Certified quinoa seed of the Salcedo INIA variety (Illpa-Puno Agrarian Experimental Station, 2013) was used, sourced from the 2022-2023 agricultural season at the Tahuaco-Yunguyo Experimental Station of INIA. Soil moisture depended solely on rainfall, as the crop was grown under a rainfed agricultural system with no irrigation applied. Likewise, no additional fertilizers were used throughout the crop cycle. For plant health management, a preventive strategy was implemented through the application of the agricultural fungicide Metalaxyl (Fitoklin) at a dose of 0.15 kg per 200 L of water. This treatment aimed to prevent downy mildew (*Peronospora farinosa*), a common foliar disease in quinoa crops. Weed control was done at growth stage (up to four true leaves) and at panicle formation stage. Bird damage was observed during the emergence and vegetative stage (two true leaves), as well as during the dough stage (grain ripening) was mitigated through daily monitoring carried out by a field caretaker.

The harvest was done on April 15, 2024, following continuous monitoring to determine grain maturity. Maturity was indicated by the yellowing and abscission of basal leaves, as well as the hardening of the grains. Panicle-bearing branches were cut using a sickle, with care taken to minimize grain loss. Subsequently, the grains were piled up to form a mound, with the cut stems positioned in an ‘A’ shape and the quinoa panicles facing upward to promote pre-drying over 15 days. Threshing was performed manually to prevent wasting or mixing the grains. Finally, the seeds were winnowed and cleaned to remove impurities such as leaf and stem debris.

Evaluated variables

The evaluated variables and the data collection procedures are presented in Table 3.

Statistical análisis

Field data were initially processed using Microsoft Excel, which was used for statistical analysis. The results were subsequently analyzed in R software (version 4.3.1; Lucent Technologies, Murray Hill, NJ, USA) through analysis of variance (ANOVA) at a significance level of 0.05, following verification of the data's normality assumptions (Shapiro-Wilk test) and homogeneity of variances (Bartlett's test). Means were compared using Tukey's test with a significance level of $p \leq 0.05$.

Results and Discussion

Effect on the vegetative phenological stage

The effects of treatments on plant height (cm) at panicle formation, flowering, milky grain formation, and doughy grain across different phenological stages in table. The mean values ± standard deviation (n = 4) are reported each treatment and phenological stage. Statistical comparison was performed using analysis of variance (ANOVA), followed by Tukey's test for mean separation. Different letters in the 'Tukey Group' column indicate statistically significant differences between treatments ($p < 0.05$). The coefficient of variation (CV, %), F-value, and p-value for each evaluated stage are also provided.

Table 4 presents the mean plant height values for the different treatments at each phenological stage. No significant differences were observed between treatments during the branching, panicle formation, flowering, and milky grain formation stages ($p > 0.05$).

Table 4. Effect of treatments on plant height (cm) at different phenological stages

Phenological stage and treatment	Mean ± SD (cm)	n	Tukey group	CV (%)	F value	p-value	Significance
Branching							
Control	50.70 ± 5.85	4	a	7,07	0,932	0,4644	p > 0.05
Fish hydrolysate 2%	53.55 ± 5.29	4	a				
Fish hydrolysate 1%	50.05 ± 5.02	4	a				
Vermicompost leachate	53.25 ± 5.24	4	a				
Panicle formation							
Control	94.40 ± 11.35	4	a	6,99	0,76	0,5418	p > 0.05
Fish hydrolysate 2%	99.80 ± 10.67	4	a				
Fish hydrolysate 1%	93.65 ± 8.83	4	a				
Vermicompost leachate	98.15 ± 7.98	4	a				
Flowering							
Control	123.10 ± 14.49	4	a	6,71	0,66	0,597	p > 0.05
Fish hydrolysate 2%	128.50 ± 13.61	4	a				
Fish hydrolysate 1%	121.75 ± 10.87	4	a				
Vermicompost leachate	128.00 ± 10.29	4	a				
Milky grain formation							
Control	137.45 ± 5.12	4	a	7,46	0,97	0,4465	p > 0.05
Fish hydrolysate 2%	132.85 ± 9.13	4	a				
Fish hydrolysate 1%	128.70 ± 7.16	4	a				
Vermicompost leachate	139.90 ± 13.75	4	a				
Formation of pasty grain							
Control	127.60 ± 7.33	4	ab	4,24	4,1799	0,0413	* (p < 0.05)
Fish hydrolysate 2%	132.85 ± 9.38	4	ab				
Fish hydrolysate 1%	126.35 ± 6.28	4	b				
Vermicompost leachate	138.85 ± 6.55	4	a				

[*]Different letters in the 'Tukey group' column indicate statistically significant differences between treatments for the same phenological stage ($p < 0.05$).

However, significant differences were detected at the milky grain stage ($p = 0.0413$), where treatment Vermicompost leachate showed higher values compared to the other treatments, as determined by Tukey's test. The coefficients of variation were low to moderate, indicating adequate experimental homogeneity. These results suggest that the impact of the treatments on plant growth depends on the phenological stage evaluated, being more pronounced during the grain ripening phase.

Effect on the reproductive phenological stage

Table 5 presents the effects of the treatments on key agronomic variables, i.e., panicle width, panicle length, thousand-grain weight, grain size, and yield. For each variable, mean values ± standard deviation ($n = 4$) are reported. Statistical comparison was conducted using one-way analysis of variance (ANOVA), followed by Tukey's test for mean separation. Different letters in the 'Tukey Group' column indicate statistically significant differences between treatments ($p < 0.05$). However, for all evaluated variables, the treatments belong to the same statistical group (a), indicating no significant differences.

No statistically significant differences were observed between treatments for any of the analyzed variables ($p > 0.05$ in all cases). The F-values and coefficients of variation (CV%) were within acceptable ranges, indicating adequate experimental precision. These results suggest that, under the conditions of the present study, the application of fish hydrolysate (at 1% and 2%) and vermicompost leachate did not significantly affect yield components or the morphological characteristics of the panicle and grain compared to the control treatment.

Table 5. Effect of the treatments during the crop yield stage

Yield stage of the crop and treatment	Mean ± SD (cm)	n	Tukey group	CV (%)	F value	p-value	Significance
Panicle width (cm)							
Control	6.20 ± 0.98	4	a	7	1,75	0,2262	$p > 0.05$
Fish hydrolysate 2%	5.80 ± 0.73	4	a				
Fish hydrolysate 1%	5.95 ± 0.47	4	a				
Vermicompost leachate	5.55 ± 0.31	4	a				
Panicle length (cm)							
Control	38.85 ± 3.39	4	a	8,86	0,74	0,5497	$p > 0.05$
Fish hydrolysate 2%	38.80 ± 6.52	4	a				
Fish hydrolysate 1%	41.27 ± 4.25	4	a				
Vermicompost leachate	41.67 ± 5.67	4	a				
Thousand-grain weight (g)							
Control	2.73 ± 0.19	4	a	5,77	0,32	0,8064	$p > 0.05$
Fish hydrolysate 2%	2.63 ± 0.22	4	a				
Fish hydrolysate 1%	2.63 ± 0.12	4	a				
Vermicompost leachate	2.69 ± 0.14	4	a				
Grain size (mm)							
Control	2.12 ± 0.04	4	a	3,07	0,89	0,4834	$p > 0.05$
Fish hydrolysate 2%	2.09 ± 0.10	4	a				
Fish hydrolysate 1%	2.05 ± 0.06	4	a				
Vermicompost leachate	2.06 ± 0.06	4	a				
Yield ($t \cdot ha^{-1}$)							
Control	3551.91 ± 606.29	4	a	12,37	1,96	0,1912	$p > 0.05$
Fish hydrolysate 2%	3473.42 ± 635.46	4	a				
Fish hydrolysate 1%	4101.03 ± 532.04	4	a				
Vermicompost leachate	4054.38 ± 561.68	4	a				

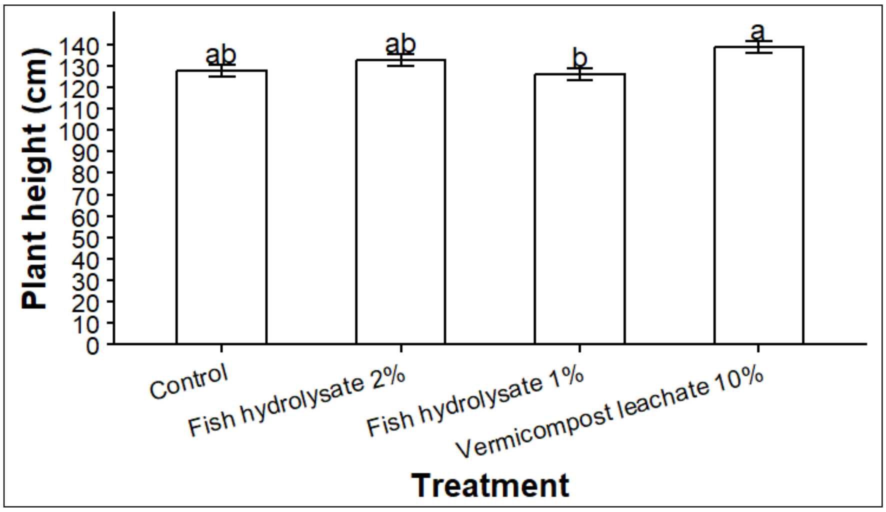

Fig. 1. Plant height (cm) at the milky grain formation phenological stage (135 DAS). Control; 2% fish hydrolysate; 1% fish hydrolysate; 10% vermicompost leachate. A Type I ANOVA (a = 0.05) revealed significant differences between treatments (p-value = 0.0413).

Effect of biofertilizer application on the soil

Soil samples collected just after the harvest to analyze both crop response and changes in soil quality and fertility. The obtained results show a heterogeneous variation in the chemical components of the soil, which are described below.

A descriptive comparison between treatments is presented for illustrative purposes based on an exploratory analysis of means, as shown in Fig. 2. The results should not be interpreted as conclusive evidence of statistical significance. The primary objective of this visualization is to provide a general overview of the observed

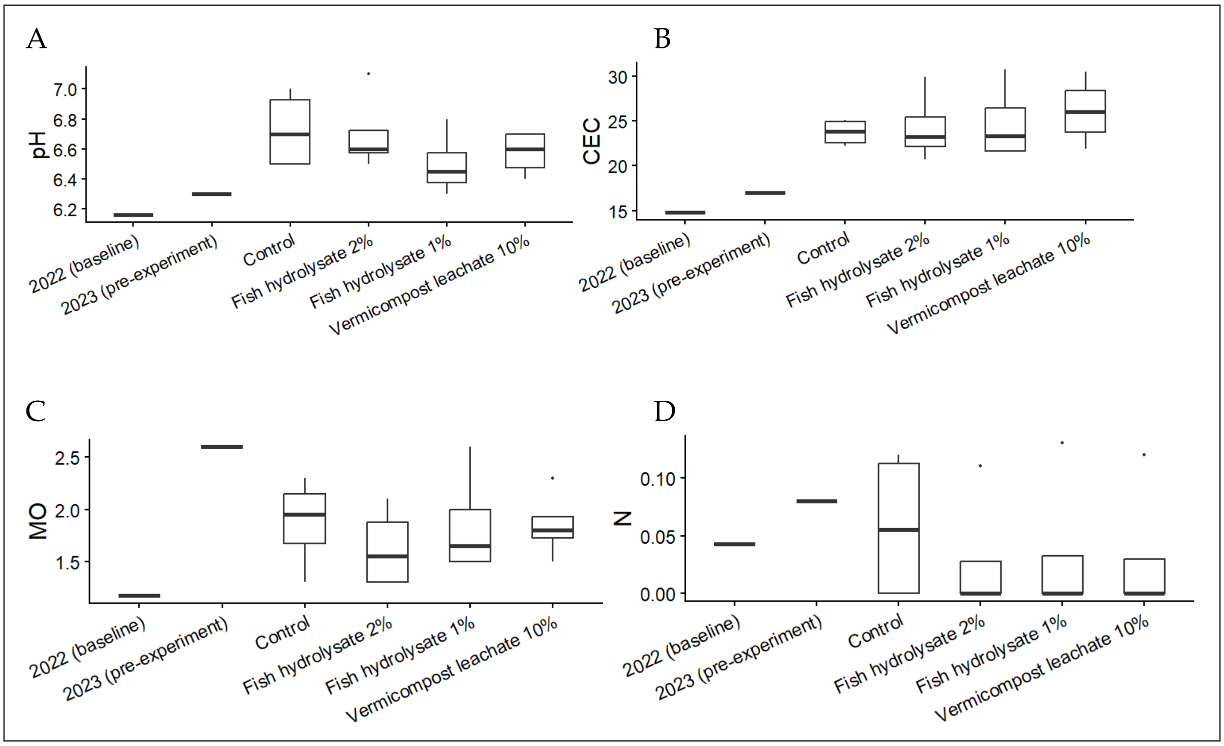

Fig. 2. Distribution of soil chemical properties across experimental stages and post-harvest treatments: (A) pH; (B) cation exchange capacity (CEC); (C) organic matter (OM); and (D) total nitrogen (N). Soil properties were evaluated at three stages: (i) 2022 (baseline year), (ii) 2023 prior to treatment application, and (iii) post-harvest following treatment application. Post-harvest treatments included control (T0), 2% fish hydrolysate (T1), 1% fish hydrolysate (T2), and 10% vermicompost leachate (T3).

Table 6. Variation in soil chemical parameters across experimental stages and post-harvest treatments

Property	Unit	Initial	Pre-treatment	2024			
				Control	Fish hydrolysate 2%	Fish hydrolysate 1%	Vermicompost 10%
pH		6.16	6.30	6.73	6.70	6.50	6.58
CE	(mS m^{-1})	5.44	6.20	5.13	5.03	6.70	6.03
Organic matter	(%)	1.17	2.60	1.88	1.63	1.85	1.85
Exchangeable bases							
Calcium (Ca)	(cmol (+) kg^{-1})	10.00	12.80	17.53	18.08	18.03	19.20
Magnesium (Mg)	(cmol (+) kg^{-1})	3.90	2.40	2.80	2.78	2.90	3.15
Sodium (Na)	(cmol (+) kg^{-1})	0.01	0.38	3.13	3.13	3.53	3.40
Potassium (K)	(cmol (+) kg^{-1})	0.71	0.40	0.28	0.30	0.30	0.38
CECI	(cmol (+) kg^{-1})	14.80	17.00	23.73	24.28	24.75	26.13
Total nitrogen	(%)	0.04	0.08	0.06	0.03	0.03	0.03
Available phosphorus	(mg kg^{-1})	12.00	82.00	89.08	92.50	117.90	108.70
Available potassium	(ppm)	547.37	148.00	110.00	117.00	126.00	143.00

variability in the conducted analysis over the two years preceding the experiment, in comparison with the results obtained at the end of the field trials.

Soil chemical components

The analysis of soil chemical components after harvest reveals that the treatments have significantly affected certain variables, while others have shown no substantial changes.

The soil pH increased significantly throughout the experimental period, shifting from moderately acidic conditions in the baseline years (pH 6.16 in 2022 and 6.30 in 2023) to near-neutral values in 2024 (pH 6.50-6.73), depending on treatment, as shown in Table 6. This change is particularly noteworthy, as a more neutral pH enhances the availability of essential nutrients for plant uptake (Havlin *et al.*, 2023). A substantial increase in available phosphorus (P) was observed across all treatments compared to the initial values. Its levels ranged from 89.08 to 117.00 mg$\cdot$kg^{-1}, indicating improved availability of this essential nutrient, which is critical for root development, as well as flower and fruit formation (Havlin *et al.*, 2023). The Cation Exchange Capacity (CEC), which reflects the soil's capacity to retain nutrients, also showed a significant increase in all treatments. Values ranged from 21.73 to 26.13 cmol(+) $\cdot$kg^{-1}, suggesting an improvement in the soil's capacity to store and release nutrients to plants (Weil and Brady, 2017). A notable increase in exchangeable sodium (Na) was detected among all treatments, with values ranging from 3.13 to 3.53 cmol(+) $\cdot$kg^{-1}. Although sodium is essential in small quantities, its excessive accumulation can negatively affect soil structure and reduce the availability of other nutrients (Rengasamy, 2006).

No significant differences in organic matter (OM) content were observed among treatments. Values ranged from 1.63% to 1.88%, remaining similar to initial levels. This suggests a more extended period may be necessary for biofertilizers to have a significant impact on soil organic matter accumulation. Electrical conductivity (EC), an indicator of soil salt concentration, showed no significant variation among treatments. Values ranged from 5.03 to 6.70 mS$\cdot$m^{-1}, indicating that biofertilizer application did not substantially affect soil salinity, as shown in Figure 3A. Total nitrogen (N) levels did not differ significantly between treatments, with values ranging from 0.03% to 0.06%. These results suggest that biofertilizers did not have an immediate effect on the fixation or mineralization of nitrogen within the soil.

A decrease in available potassium (K) was observed in all post-harvest treatments compared with pre-experimental levels (Fig. 3D). Available K values ranged from 110 to 146 ppm. This reduction may be associated with plant uptake during the cropping cycle, as potassium plays a key role in biomass production and grain filling in quinoa. Additionally, partial removal through harvest and possible leaching under rainfall conditions cannot be ruled out.

Consistent with this trend, exchangeable potassium (K^+) levels also showed a decrease, ranging from 0.28 to 0.38 cmol(+)·kg^{-1}. A similar pattern was observed for exchangeable magnesium (Mg^{2+}), with values between 2.78 and 3.15 cmol(+)·kg^{-1}. These reductions may reflect nutrient uptake by the crop and, to a lesser extent, potential leaching losses, particularly under rainfed high-altitude conditions.

The objective of this study was to evaluate the effect of fish hydrolysate application and vermicompost leachate, on quinoa grain yield in plots cultivated under rainfed conditions. The 2% fish hydrolysate treatment results in the highest quinoa yields (kg·ha^{-1}) compared to the other treatments and the control plot. This expectation is based fact that fish hydrolysate supplies amino acids that contribute to increasing soil organic matter content (%), thereby enhancing nitrogen mineralization capacity and improving nitrogen availability to the crop. Greater nitrogen availability is expected to promote above-ground biomass production (kg·m^{-2}) and inflorescence development, as indicated by increases in their length and width (cm). As an indirect effect, this would lead to a higher number of grains per panicle and an increase in the weight of 1000 grains (g), ultimately resulting in greater total quinoa yield (kg·ha^{-1}).

Although plant height did not exhibit significant differences between treatments during the early growth stages, the 10% vermicompost leachate treatment promoted greater height during the milky grain stage. This finding suggests that the leachate may stimulate vegetative development during this critical phase, possibly due to its content of phytohormones such as cytokinins, auxins, and gibberellins (Aremu *et al*., 2015; Velasco-Jiménez *et al*., 2020). Notably, cytokinins have been reported to influence plant-associated microbia, thereby enhancing plant growth (Velasco-Jiménez *et al*., 2020).

In terms of grain yield, the 1% fish hydrolysate treatment achieved the highest yield, although the difference was not statistically significant. This outcome may be associated with the contribution of essential amino acids present in the hydrolysate, including leucine, lysine, arginine, cysteine, phenylalanine, isoleucine, and tyrosine. These amino acids serve as a valuable source of available nitrogen for plants, which is crucial for key physiological processes such as protein synthesis and photosynthesis (Harikrishna *et al*., 2017; Nelson and Cox, 2017; Yang *et al*., 2023). Additionally, plants possess the capacity to synthesise amino acids through mechanisms such as amination and transamination, further underscoring the importance of these compounds in plant nutrition.

The absence of significant differences in thousand-grain weight and grain size indicates that the treatments did not significantly affect the physical quality of the grain. However, the overall results demonstrate that both fish hydrolysate and vermicompost leachate exhibit high potential to enhance quinoa vegetative growth and yield.

Although biofertilizers may not consistently achieve the yield levels associated with conventional chemical fertilization, they represent a sustainable alternative that can enhance soil quality and promote more environmentally friendly agricultural practices (Čabilovski *et al*., 2023; Velasco-Jiménez *et al*., 2020).

The use of biofertilizers not only provides immediate benefits for plant growth but also contributes to long-term soil sustainability. The application of fish hydrolysate improved the availability of key nutrients, while vermicompost leachate enhanced organic matter content. Over the time, these effects may promote greater soil structural stability and support the recovery of degraded soils.

The increase in soil pH is a positive outcome, particularly with the treatment of 2% fish hydrolysate and 10% vermicompost leachate, indicating their beneficial effect on pH regulation. A shift toward a more neutral pH enhances nutrient availability, which is essential for maintaining soil health and supporting optimal plant development.

This effect may be attributed to the alkaline nature of the vermicompost leachate, which had a pH of 7.98. In contrast, although the fish hydrolysate had an acidic pH of 4.86, it contains minerals such as calcium, magnesium, and other basic elements capable of neutralizing soil acidity. These minerals function as alkalizing agents by reacting with soil acids, thereby contributing to an increase in pH. Chipana Mendoza (2022) also reported that fish hydrolysates are associated with pH increases in soil due to their high content of basic compounds.

On the other hand, vermicompost leachate contains humic and fulvic acids, which, although classified as weak organic acids, can enhance the soil's buffering capacity. This buffering effect helps stabilise soil pH by neutralizing both acidic and basic compounds within the soil system (Aremu *et al*., 2015). Additionally, as these organic acids decompose, they can release basic cations such as calcium and magnesium, further contributing to the improvement of the soil's chemical balance.

Studies have also demonstrated that fish hydrolysate contains varying amounts of essential amino acids such as leucine, isoleucine, phenylalanine, and lysine, as well as non-essential amino acids including tyrosine, arginine, and cysteine (Harikrishna *et al*., 2017). Amino acids are organic compounds characterized by the presence of an amino group ($-NH_2$) and a carboxyl group (-COOH) bonded to the same central alpha carbon, along with a unique side chain (R group) that differentiates each amino acid. At physiological pH (approximately 7.4), the carboxyl group typically loses a proton, forming a negatively charged carboxylate ion ($-COO^-$), while the amino group tends to accept a proton, becoming positively charged ammonium group ($-NH_3^+$). This coexistence of opposite charges within the same molecule confers a zwitterionic nature to amino acids, allowing them to behave as amphoteric substances that can function as either acids or bases, depending on the pH of their environment (Nelson and Cox, 2017).

The results of this study demonstrate a significant improvement in cation exchange capacity (CEC), which increased from 17 cmol (+) $\cdot kg^{-1}$ in the initial state to 26.13 cmol (+) $\cdot kg^{-1}$ following the application of fish hydrolysate. This increase in CEC indicates an enhanced capacity of the soil to retain and supply essential nutrients, a key factor for maintaining soil fertility (FAO, 2024). This improvement can be attributed to the addition of labile organic matter from biofertilizers, which promotes the formation of complexes with essential cations, thereby increasing nutrient retention (Haynes and Naidu, 1998).

These results are consistent with previous studies, such as that of Orozco Corrales *et al*. (2016), who reported an 83.05% increase in CEC following the combined application of biofertilizers, chemical fertilizers, vermicompost, and pine sawdust as mulch. Collectively, these findings underscore the importance of biofertilizer use in enhancing soil chemical properties and improving nutrient availability for crops.

The results indicate that the application of fish hydrolysate and vermicompost leachate can positively influence specific chemical properties of the soil, including pH, available phosphorus, and CEC. However, some adverse changes were also observed, such as reductions in available potassium and magnesium, along with an increase in exchangeable sodium, which warrant careful consideration. Overall, these findings suggest that while biofertilizer application can enhance specific aspects of soil fertility, it is essential to closely monitor nutrient dynamics and salinity levels to prevent potential imbalances and to ensure the long-term sustainability of soil health.

Conclusions

The results indicated that the application of 1% fish hydrolysate and 10% vermicompost leachate elicited favourable agronomic responses at specific stages of the quinoa phenological cycle, functioning more as complementary biostimulants rather than as standalone sources of fertilization. Treatment vermicompost leachate significantly enhanced plant height during the grain-filling stage, while 1% fish hydrolysate promoted greater above-ground biomass development. However, these positive physiological responses did not result in statistically significant increases in final grain yield compared to the control treatment.

Both bio-inputs demonstrated positive effects on the chemical properties of the soil, particularly in stabilizing pH and increasing cation exchange capacity (CEC), thereby contributing to the gradual improvement of soil quality.

It can be concluded that the use of organic liquid biofertilizers, such as fish hydrolysate and vermicompost leachate, should be considered complementary tools within an integrated fertilization programme, rather than substitutes for conventional nutrition under high Andean rainfed conditions.

Finally, it is recommended that future trials may evaluate incremental doses or enriched formulations, as well as sequential or combined applications at different phenological stages. Additionally, assessing their medium-term effects on soil health and crop yield under different agroclimatic conditions will be essential. These research efforts will help to validate the potential of these bio-inputs as integral components of sustainable management strategies for Andean cropping systems.

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Author Contributions

Nestor Cuellar-Condori: Investigation, original draft writing, methodology. Cesar Padilla: Formal analysis, software. Sócrates Olivera: Methodology, visualization. Richard Solórzano: Conceptualization, writing - review, and editing.

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.

Data Availability

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

Conflict of Interest

The authors declare that they have no conflicts of interest.

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