Original Research

# Enhancing Soil Physicochemical Properties, Quinoa Yield, and Nutrients through Intercropping of Quinoa with Legumes

Naiwen Xue<sup>1,2,3</sup>, Jianxia Liu<sup>1,2,3</sup>, Sumera Anwar<sup>4\*</sup>

<sup>1</sup>College of Agronomy and Life Sciences, Shanxi Datong University 037009, Datong, China <sup>2</sup>Facility Agriculture Research and Development Center, Shanxi Datong University 037009, Datong, China <sup>3</sup>Key Laboratory of Organic Dry Farming for Special Crops in Datong City, Datong, China <sup>4</sup>Government College Women University Faisalabad, Faisalabad, Pakistan

Received: 4 May 2024 Accepted: 3 August 2024

## **Abstract**

Intercropping of quinoa with legumes has been studied infrequently, despite quinoa's global importance as a nutrient-dense crop with resilience to diverse growing conditions. This study aims to elucidate the benefits of intercropping by comparing quinoa monocropping with intercropping with red bean, mung bean, and black bean, focusing on yield, plant nutrients, and soil physicochemical properties. The land equivalent ratio of quinoa/legumes intercropping consistently exceeded 1, peaking at 1.52 for quinoa/red bean intercropping, indicating higher productivity than monocropping. Quinoa/red bean intercropping increased the nutrient contents of quinoa plants throughout the quinoa growth period and exhibited the highest levels of ammonium nitrogen, available phosphorus, available potassium, and organic matter content, alongside the highest activity of sucrase, alkaline phosphatase, and urease enzymes in the soil during the quinoa seedling stage. At maturity, quinoa/mung bean demonstrated the highest levels of available phosphorus and total nitrogen, while quinoa/red bean displayed the highest sucrase and urease enzyme activity. Significantly positive correlations were found between the nitrogen, phosphorus, and potassium contents of quinoa and most soil nutrients. Regression analysis revealed a positive relationship between soil phosphatase activity and quinoa yield. Intercropping quinoa with legumes improved yield, plant nutrients, soil nutrients, and soil enzyme activity, with quinoa/red bean exhibiting the most remarkable effect.

Keywords: quinoa, legumes, yield, plant nutrients, soil nutrients, soil enzymes

## Introduction

Quinoa (Chenopodium quinoa Willd.) is a historically significant crop with a rich agricultural

heritage spanning 7000 years and a global presence encompassing approximately 250 recognized species [1]. Originally from the Andes, quinoa has expanded its cultivation to regions such as China, Canada, Argentina, and India [2]. Notably, quinoa thrives in challenging environments characterized by high altitudes, low temperatures, and adverse soil conditions, including

<sup>\*</sup>e-mail: anwer sumera@yahoo.com

arid, saline, varying pH soils, and low nitrogen [3, 4]. Unlike cereals and legumes, quinoa is renowned for its exceptional balance of essential amino acids and a wider spectrum of amino acids, making it nutritionally valuable [5]. Quinoa also contains significant amounts of carbohydrates, protein, lipids, dietary fiber, minerals, vitamins, and functional compounds such as polyphenols, flavonoids, and phytosterols [6, 7]. Due to its nutritional composition, quinoa and its derivatives are recognized as health-promoting foods with the potential benefit of preventing various diseases [8].

Intercropping is an ancient agricultural technique that involves cultivating multiple crop species simultaneously within the same space, promoting mixed cropping practices [9]. The primary objective of intercropping is to enhance the yield per unit of land, achieved by optimizing the utilization of soil resources that might otherwise not be properly exploited by a single crop [10]. Furthermore, intercropping is adopted to reduce the problems of continuous monocropping. Intercropping reduces soil erosion by providing a cover crop [11]. Intercropping with legumes balances the nitrogen nutrient in the soil by enhancing microbial diversity related to nitrogen fixation [12, 13, 14]. Different crops have different root structures. Therefore, mixing crops in intercropping promotes the growth of diverse root structures, altering the overall distribution and architecture of roots and providing spatial complementarity [15, 16]. Intercropping improves nutrient use efficiency, as different crops utilize nutrients and water at various soil depths and growth stages. Additionally, intercropping has the potential to improve water use efficiency, under water deficit conditions and in dryland rainfed areas [17].

Cereals [18, 19], vegetables [20], and other crops are usually intercropped with legumes, as including legumes in intercropping enhances nitrogen in soil and nutrient resource utilization, and improves growth performance in low-input farming systems. Intercropping systems, particularly those incorporating legumes like groundnut and common bean, have shown promising results in terms of maize production and soil fertility enhancement [21, 22].

However, intercropping does not always benefit the yield of crops and sometimes could even cause yield reduction in one of the crops because of intra-species competition [23, 20]. Therefore, searching for the best complementary crops that do not reduce the yield of quinoa is a requirement under specific conditions. Very limited studies have evaluated the effect of intercropping of quinoa with legumes. The positive effects of intercropping quinoa with millet [24], clover and medic [11], maize [25], and beans [26] have been observed.

Adzuki bean or red bean (Vigna angularis (Willd.) Ohwi & H. Ohashi) has been grown and utilized in East Asia nations like China, Japan, and Korea for thousands of years [27]. In China, it is traditionally consumed as sprouts and its use is becoming popular due to its nutritional properties. Its grains have 50%

starch, 25% protein, polyunsaturated amino acids, and many minerals [28]. However, despite its nutritional properties, it is an underrated leguminous crop [29, 30]. Black bean (*Phaseolus vulgaris* L.) is the second most utilized bean in the world after soybeans and is famous for its high anthocyanin content in the seed coat [31].

Intercropping with legumes and quinoa has been studied infrequently, despite quinoa's global importance as a nutritive crop with resilience to diverse growing conditions. Furthermore, the effects of intercropping quinoa with legumes on soil properties remain largely unexplored. We hypothesized that the inclusion of legumes in the intercropping treatments would lead to increased yield by affecting soil properties and improving soil nutrient availability to plants. The objectives of this research were: (i) to study the effect of intercropping quinoa with legumes in improving yield, nutrient content of quinoa, and LER, (ii) to investigate how intercropping impacts on soil properties such as pH, soil water content, soil nutrients, and soil enzyme activities, (iii) to explore the relationship of these traits with quinoa yield.

#### **Materials and Methods**

#### **Experiment Site Description**

The experimental site is located in Sunjiadian Village, Tianzhen County, Datong City, Shanxi Province (40°18′47″N, 113°57′58″E). It is a medium-temperature semi-arid area and experiences an average annual precipitation of 410 mm. The average annual air temperature is 6.8°C, with an average frost-free period of 128 days. The area receives annual sunshine for 2836 hours. The soil type at the site is chestnut brown, with a pH of 8.34 in the top 20 cm layer. The soil contains alkaline dissolved nitrogen (AN) at a concentration of 63.35 mg kg<sup>-1</sup>, effective phosphorus (EP) at 13.80 mg kg<sup>-1</sup>, quick-acting potassium (QAP) at 79.04 mg kg<sup>-1</sup>, total nitrogen (TN) at 0.62 g kg<sup>-1</sup>, total phosphorus (TP) at 0.67 g kg<sup>-1</sup> and organic matter at 11.72 g kg<sup>-1</sup>.

## Plant Material

The seeds of the quinoa (Chenopodium quinoa Willd.) cultivar Jinli 1, characterized by a 130-day fertility period, were provided by the Maize Research Institute of Shanxi Agricultural University. Red adzuki bean (Vigna angularis (Willd.) Ohwi & H.Ohashi) cultivar Hongxiaodou 6, with a fertility period of 112 days, and Mung bean (Vigna radiata (L.) R. Wilczek) seeds of cultivar Jinly 9, with a fertility period of 98 days, were obtained from the Institute of Alpine Crops, Shanxi Agricultural University. Black bean (Phaseolus vulgaris L.) seeds were purchased from the local market of Yanggao County, Datong City, China.

## Cropping Pattern and Management

The soil properties, crop yield, and nutrient status of quinoa and legumes were tested by adopting monocropping of quinoa and three legume crops (red bean, mung bean, and black bean) and intercropping of quinoa with the legumes. The cropping treatments were as follows: 1) quinoa monocrop, 2) red bean monocrop, 3) mung bean monocrop, 4) black bean monocrop, 5) intercropping of quinoa with red bean, 6) intercropping of quinoa with mung bean, and 7) intercropping of quinoa with black bean. The treatments were arranged in a randomized complete block design. There were 3 blocks, and, in each block, the seven cropping methods were randomly assigned. In total, there were 21 plots (7 treatments×3 blocks), with each plot measuring 5 m×6 m

Quinoa as a sole crop was planted with a spacing of 60 cm between rows and 40 cm between plants. For the sole cropping of red beans, mung beans, and black beans were sown at a row spacing of 33 cm and plant spacings of 33 cm. In the intercropping system, one row of quinoa was planted alongside one row of legumes, with a row spacing of 30 cm.

Before sowing, a 750 kg ha<sup>-1</sup> of basal compound fertilizer (N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O) was applied at a ratio of 40:40:20. Quinoa and legume seeds were sown on May 9<sup>th</sup>, 2022. The seed sowing rates for quinoa, red bean, mung bean, and black bean were 3, 30, 22.5, and 45 kg ha<sup>-1</sup>, respectively. On June 10<sup>th</sup>, quinoa seedlings were transplanted within rows of legumes, followed by manual weeding and soil cultivation of quinoa roots on July 10<sup>th</sup>. The legumes were harvested between August 15<sup>th</sup> and 25<sup>th</sup>, while the quinoa was harvested on September 27<sup>th</sup>, 2022.

### Soil Sampling and Analysis

For soil analysis, we randomly selected five points within each plot to collect soil samples from the 0-20 cm soil layer. The soil samples were mixed, dried, and sieved before further analysis.

#### Soil Water Content and pH

We determined the soil's relative water content using the gravimetric or oven-drying method [32]. Soil samples were weighed, dried in an oven at 105°C for 48 hr to evaporate the water content, and then re-weighed to calculate the percentage of water lost taken as the water content. The soil pH was measured with a pH meter using a paste of water-soil of a 2.5:1 ratio.

### Soil Nutrients

The soil's alkaline dissolved nitrogen content was determined using the alkaline dissolved diffusion method [33]. The soil's available phosphorus content was determined using the colorimetric method after

NaHCO<sub>3</sub> leaching by molybdenum antimony and scandium chromatography [34]. The available potassium content was determined using the flame photometric method after ammonium acetate (NH<sub>4</sub>OAc) leaching. For this, 10 mL of 1 N NH<sub>4</sub>OAc, at pH 7 was mixed with 1 g of air-dried soil and shaken for 5 minutes, and the available potassium was measured by analyzing the filtered extract on an atomic absorption spectrometer set on emission mode at 776.5 nm [35].

The total nitrogen content of the soil was determined using concentrated sulphuric acid decoction and a continuous flow analyzer [36]. The total phosphorus content of the soil was determined using concentrated sulfuric acid-perchloric acid digestion and the molybdenum antimony colorimetric method. The soil organic matter content was determined using the potassium dichromate oxidation method [36].

#### Soil Enzymes

We determined the urease activity using the indophenol blue colorimetric assay, with results expressed as  $\mu g$  of NH<sub>3</sub> per g of soil per h (37°C). The sucrase activity was determined using the salicylic acid colorimetric assay, with results expressed as  $\mu g$  of glucose per g of soil per h (37°C). The phosphatase activity was determined using the sodium benzene disodium phosphate colorimetric assay, with results expressed as  $\mu g$  of p-nitrophenol phosphate per g of soil per h (3°C) [37].

## Plant Sampling and Analysis

On June 10<sup>th</sup>, during the quinoa seedling stage, and on September 27<sup>th</sup>, at the maturity stage, we selected 10 representative plants from each plot. These plants were carefully placed in paper bags, heated in an oven at 105°C for 30 minutes, and then dried at 80°C until a constant weight was achieved. The dry plants were pulverized and the resulting powder was stored in self-sealing bags.

#### Plant Nutrients

At the seedling stage, ten quinoa plants were uprooted from each plot and aboveground parts were dried and crushed. At the maturity stage, the quinoa plants were uprooted and separated into stalks+leaves and spikes, and then crushed with scissors after drying them in an oven. After, the crushed samples were extracted using H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub>, and the nitrogen content was determined using Nye's colorimetric method, the phosphorus content was determined using the vanadium-molybdic acid ammonia colorimetric method, and the potassium content using the flame photometric method [36]. After determining the nitrogen, potassium, and phosphorus concentration in quinoa plants (kg kg<sup>-1</sup>), the nutrient content in kg ha<sup>-1</sup> was obtained by multiplying the dry weight (kg ha<sup>-1</sup>) with the nutrient content [38].

#### Seed Yield

At the maturity stage of quinoa, red bean, mung bean, and black bean, we harvested a 1 m<sup>2</sup> area per plot, repeating this process three times. All crops were airdried, and their seed yield was measured.

## Relative Land Equivalent and Land Equivalent Ratio

The relative land equivalent ratio (RLE) was calculated as the ratio of the yield of a crop from a specific area under intercropping to the yield of that crop under monocropping. RLE for quinoa was calculated as:

$$RLE=Y_{qi}/Y_{qm}$$

RLE for specific legume crops was calculated as:

$$RLE=Y_{li}/Y_{lm}$$

The land equivalent ratio (LER), which measures intercropping advantage, was calculated using the formula provided by Bedoussac et al. [39].

$$LER=Y_{qi}/Y_{qm}+Y_{li}/Y_{lm}$$

In these Equations,  $Y_{qi}$  and  $Y_{li}$  refer to the grain yields of quinoa and legume crops on the total intercropping area (kg ha<sup>-1</sup>), respectively;  $Y_{qm}$  and  $Y_{lm}$  refer to the grain yields of quinoa and legume crops in monoculture (kg ha<sup>-1</sup>), respectively; when the LER>1, there is intercropping advantage; and when the LER is <1, there is no intercropping advantage.

### Statistical Analysis

Origin 2022 was used for data organization and charting. Analysis of variance, Pearson correlation analysis, and stepwise regression analysis were performed using SAS 9.2 statistical software. The differences among treatments were compared with Duncan's multiple range test.

#### Results

## Crop Yield under Different Intercropping Patterns

The crop yields of quinoa, red bean, mung bean, and black bean were found to be higher in monoculture compared to intercropping (P<0.05) (Table 1). Specifically, when comparing the three intercropping methods, the yield of quinoa in intercropping ranged from 60% to 65% of the yield in monoculture, while the yield of beans ranged from 80% to 87% of the yield in monoculture. Additionally, the land equivalent ratios (LERs) for the intercropping methods ranged from 1.40 to 1.52, indicating yield advantages per unit area. Notably, intercropping quinoa with red bean demonstrated the highest LER of 1.52.

## Effect of Different Intercropping Patterns on the Nutrient Content of Quinoa Plants

The intercropping of quinoa with red bean showed a tendency to increase the nitrogen content of the quinoa plant during the seedling stage, as well as the nitrogen content in the stem and leaves during the maturity stage,

Table 1. Comparison of crop grain yield under different planting modes.

Cropping pattern	Crop	Yield (kg ha <sup>-1</sup> )	RLE	LER	
Monoculture	Quinoa	2162.50ª	-		
	Red bean 1717.5		-	-	
Intercropping	Quinoa	1405.63 <sup>b</sup>	0.65	1.52	
	Red bean	1500.04 <sup>b</sup> 0		1.52	
Monoculture	Quinoa	2162.50ª	-	-	
Monoculture	Mung bean	1330.07ª	-		
Intercropping	Quinoa	1340.75 <sup>b</sup>	0.62	1.45	
	Mung bean	1103.96 <sup>b</sup>	0.83		
M	Quinoa	2162.50ª	-		
Monoculture	Black bean	2660.04ª	-	-	
Intonomonino	Quinoa	1297.50 <sup>b</sup>	0.60	1.40	
Intercropping	Black bean	2128.03 <sup>b</sup>	0.80		

Note: RLE: relative land equivalent ratio; LER: land equivalent ratio. The different alphabets following numbers indicate significant differences between yield under intercropping and monocropping using Duncan's multiple range test.

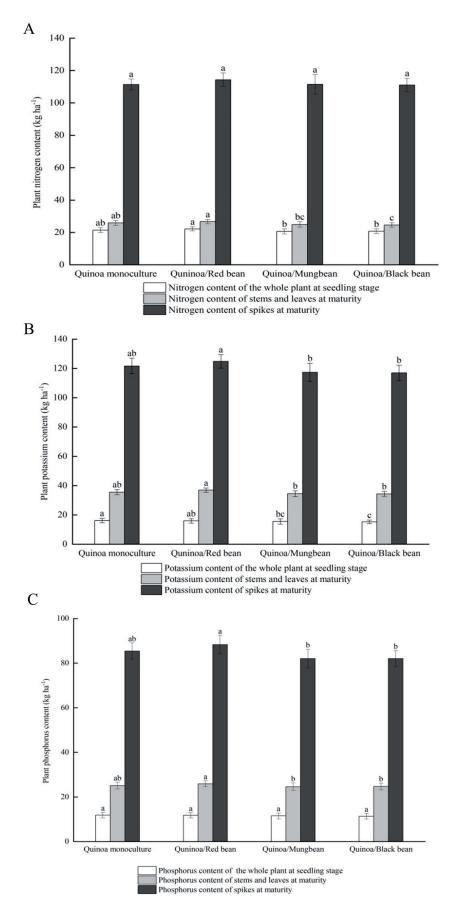


Fig. 1. (a) Nitrogen, (b) potassium, and (c) phosphorous content in the whole plant at seedling, in stem and leaves at maturity, and in spikes at maturity of quinoa under monocropping and intercropping with red bean, mung bean, and black bean. Different alphabets indicate significant differences among cropping systems and crop type at a specific plant organ and growth stage using Duncan's multiple range test (p<0.05). Vertical bars correspond to standard error.

compared to the quinoa monoculture treatment (Fig. 1a). Furthermore, compared to the quinoa monoculture treatment, the quinoa/red bean treatment tended to increase the potassium content in the stem, leaves, and spikes at maturity (Fig. 1b). Additionally, the quinoa/red bean treatment showed a tendency to increase phosphorus content in the stems, leaves, and spikes at maturity, compared to the quinoa monoculture treatment (Fig. 1c).

## Effect of Different Intercropping Patterns on Soil pH and Water Content

During the seedling stage, the monoculture of red beans and intercropping of quinoa with red beans and black beans showed lower soil pH values in comparison to the quinoa monoculture. Moreover, at maturity, the black bean monoculture exhibited a lower pH level than the quinoa monoculture treatment (Fig. 2a). The soil water content of quinoa monocropping was significantly similar to other treatments at the seedling stage. The only significant difference in soil water content was between red bean monoculture and intercropping of quinoa with red beans. The quinoa/black bean intercropping displayed a lower water content than the quinoa monoculture at the seedling stage (Fig. 2b). At maturity, the water content under red beans monoculture was significantly higher than mung beans monoculture and quinoa black beans intercropping.

## Effect of Different Intercropping Patterns on Soil Nutrients

In comparison to the quinoa monoculture treatment, all other treatments exhibited higher soil alkaline dissolved nitrogen (ADN) content at both the seedling and maturity stages (Fig. 3a). Specifically, the quinoa/red bean intercropping showed the highest ADN content during the seedling stage, while the red bean monoculture treatment had the highest ADN content during the maturity stage.

At the seedling stage, the intercropping of quinoa with red bean and black bean demonstrated improved soil available phosphorus content compared to the quinoa monoculture (Fig. 3b). Similarly, at the maturity stage, all treatments significantly increased soil available phosphorus content compared to the quinoa monoculture treatment, with the quinoa/red bean intercropping showing the highest enhancement.

During the seedling stage, the quinoa/red bean intercropping exhibited the highest soil available potassium content (Fig. 3c). Among all the intercropping treatments, the quinoa/red bean intercropping had the highest soil available potassium content during the maturity stage.

Except for the quinoa/mung bean intercropping, all other treatments significantly increased soil organic matter content at the seedling stage compared to the quinoa monoculture, with the quinoa/red bean

intercropping showing the highest increase (Fig. 3d). At the maturity stage, the mung bean monoculture treatment had the highest soil organic matter content.

The quinoa/red bean and quinoa/mung bean intercropping exhibited higher soil total nitrogen content compared to the quinoa monoculture during the seedling stage (Fig. 3e). Similarly, at the maturity stage, the quinoa/mung bean intercropping had higher soil total nitrogen content compared to the quinoa monoculture treatment.

During the seedling stage, the quinoa monoculture treatment had lower soil total phosphorus content compared to all the intercropping treatments (Fig. 3f). However, at the maturity stage, all treatments, except for the quinoa monoculture treatment, significantly enhanced soil total phosphorus content, with the quinoa/red bean treatment showing the highest enhancement among the intercropping treatments.

## Soil Enzyme Activities under Different Intercropping Patterns

Mung bean monoculture, black bean monoculture, quinoa/red bean, and quinoa/black bean treatments had significantly higher soil sucrase activity at seedling and maturity stages than quinoa monoculture, and the highest soil sucrase activity at both stages was found at quinoa/red bean intercropping (Fig. 4a).

Quinoa/red bean and quinoa/mung bean treatments showed higher soil phosphatase activity than quinoa monoculture, with quinoa/red bean having the highest soil phosphatase activity at the seedling stage (Fig. 4b). At the maturity stage, all other treatments had better soil phosphatase activity than quinoa monoculture, with quinoa/mung bean having the highest soil phosphatase activity among the intercropping treatments.

All intercropping treatments and red bean monoculture had higher soil urease activity than quinoa monoculture at the seedling stage, with quinoa/mung bean intercropping keeping the highest urease activity (Fig. 4c). Quinoa/mung bean had the highest soil urease activity among all the treatments at the maturity stage.

Regression Analysis of Quinoa Yield with Plant Nutrients, Soil Physicochemical Properties, and Soil Enzyme Activities at the Seedling Stage under Different Intercropping Patterns

Multiple linear stepwise regression analyses were performed on quinoa yield (Y), plant nutrients, soil physicochemical properties, soil nutrients, soil organic matter, and soil enzyme activities, and the insignificant variables were excluded. The P-value of the optimal regression model was less than 0.0001, and the coefficient of determination was 0.9484, indicating that the optimal regression model was extremely significant and had a high fitting accuracy (Table 2). The regression coefficients had significant P-values (P<0.0001), and the optimal regression equation was Y=1399.1360-

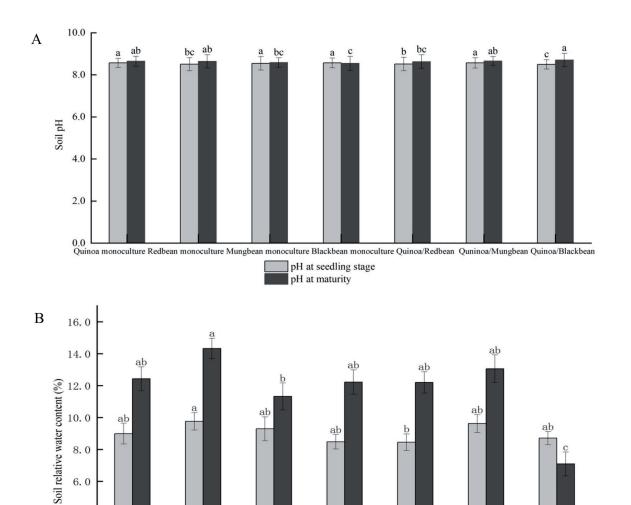


Fig. 2. (a) Soil pH and (b) relative water content at the seedling stage and maturity stage under different planting modes. Different alphabets indicate significant differences among cropping systems and crop type at a specific plant organ and growth stage using Duncan's multiple range test (p<0.05). Vertical bars correspond to standard error.

O. Quinoa monoculture Redbean monoculture Mungbean monoculture Blackbean monoculture Quinoa/Redbean Quinoa/Mungbean Quinoa/Blackbean

Soil relative water content at seedling stage
Soil relative water content at mature stage

40.2478X6+10.2075X13 (Table 3). It indicated that the soil's alkaline nitrogen content and alkaline phosphatase activity at the seedling stage had the greatest effect on yield, and alkaline phosphatase activity was significantly positively correlated with yield.

4.0

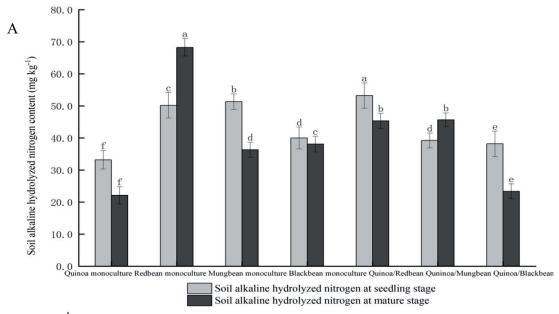
2.0

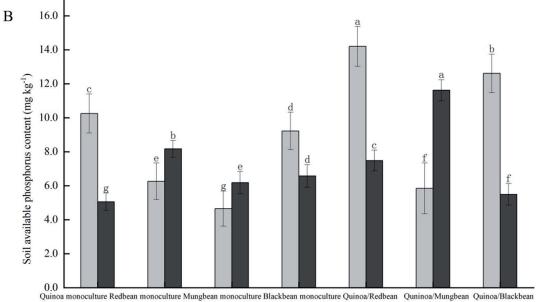
Correlation Analysis between Quinoa Plant Nutrients and Soil Physicochemical Properties under Different Intercropping Patterns

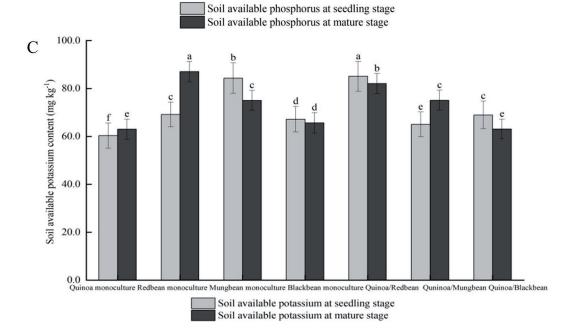
At the maturity stage, the nitrogen content of the stem and leaf was significantly positively correlated with soil water content, available phosphorus content, organic matter content, and total nitrogen content (Fig. 5). The nitrogen content of the stem and leaf significantly negatively correlated with soil pH. The potassium content of the spike significantly positively correlated with soil water content, available potassium content, and organic matter content, and it significantly negatively correlated with soil pH.

## **Discussion**

This study investigated the effect of different intercropping patterns on crop yield. The yield indices indicate that the yield of quinoa, red beans, mung beans, and black beans is lower under intercropping compared to monocrop. Previous studies have also reported reduced yields of the individual crops under







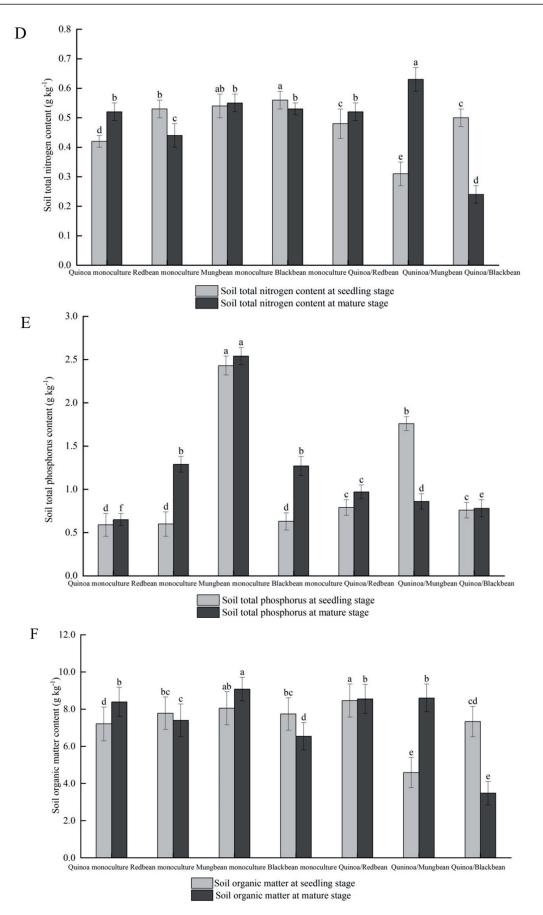


Fig. 3. Comparison of soil nutrients at the seedling stage and mature stage under different planting modes. (a) soil alkaline hydrolyzed nitrogen content, (b) soil available phosphorus content, (c) soil available potassium content, (d) soil total nitrogen content, (e) soil total phosphorus content, (f) soil organic matter content. Different alphabets indicate significant differences among cropping systems and crop type at a specific plant organ and growth stage using Duncan's multiple range test (p<0.05). Vertical bars correspond to standard error.

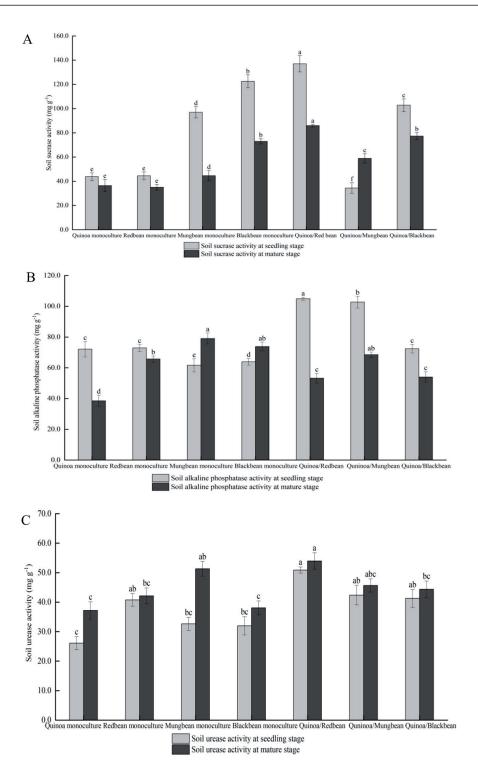


Fig. 4. Soil enzyme activities at the seedling and maturity stage under different planting modes. (a) soil sucrase activity, (b) soil alkaline phosphatase activity, and (c) soil urease activity. Different alphabets indicate significant differences among cropping systems and crop type at a specific plant organ and growth stage using Duncan's multiple range test (p<0.05). Vertical bars correspond to standard error.

Table 2. Stepwise regression table.

Source	Sum of squares	DF	Mean square	F Value	Pr> F
Model	1496936	2	748468	82.79	< 0.0001
Error	81369	9	9040.9534		
Sum	1578305	11		R <sup>2</sup> =0.9484	

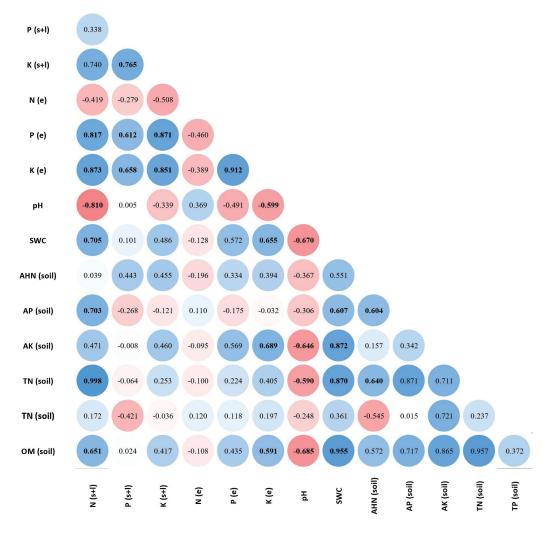


Fig. 5. Correlation coefficients between plant nutrients and soil physical and chemical properties at the maturity stage of quinoa. s+l: stems and leaves, e: ears, SWC: soil water content, AHN: alkaline hydrolyzed nitrogen content, AK: available phosphorus content, AK: available potassium content, TN: total nitrogen content, OM: organic matter content. Bold values represent significance at p<0.05 by Pearson correlation.

Table 3. Parameter estimation and testing for stepwise regression.

Variable	DF	Parameter estimate	Standard error	t Value	Pr> t	Squared partial
Intercept	1	1399.1360	187.2879	7.47	< 0.0001	
$X_6$	1	-40.2478	3.9268	-10.25	< 0.0001	0.9211
X <sub>13</sub>	1	10.2075	0.9359	10.91	< 0.0001	0.9297

Note: X<sub>6</sub>: soil alkaline dissolved nitrogen, X<sub>13</sub>: alkaline phosphatase

intercropping [24, 26]. Specifically, quinoa yields were 35%, 38%, and 40% lower when intercropped with red bean, mung bean, and black bean, respectively, compared to monocropping. This decrease is attributed to higher intra-specific competition for nutrients and water [26, 40]. The yields of red beans, mung beans, and black beans were 12.7%, 17%, and 20% lower under intercropping compared to monocropping. The reduced yield of legume crops under intercropping is likely due

to the reduced density rate, as legumes were sown in the spaces between quinoa rows. Despite the reduced density of quinoa crops, the significant reduction in quinoa yield (35-40%) under intercropping highlights the highly competitive ability of legumes.

Despite the lower crop yields, the land equivalent ratio (LER), which is the ratio of the sole cropping area to the intercropping area, was increased by intercropping. For desirable and effective intercropping,

LER is considered an effective measure of yield advantages under the intercropping system [41]. Our study revealed that intercropping of quinoa with red bean, mung bean, and black bean resulted in LERs of 1.52, 1.45, and 1.40, respectively. This indicates higher yield advantages of quinoa intercropping with all legumes with the maximum LER at quinoa/red beans intercropping with a 52% yield advantage. This indicates that 52% extra land would be required for the sole cropping of quinoa and red beans as compared to their intercropping [26, 42]. Higher LER values than 1 indicate yield advantages under all intercropping systems compared to monocropping. The higher LER with red beans and less yield reduction is attributed to more resistance under intercropping [26], faster growth rate, and good leaf area of red beans [43]. The observed yield advantage in our study may be attributed to the border row effects of intercrop strips [44]. Previous research has shown that intercropping systems generally provide a yield benefit [45]. For example, intercropping legumes with maize has been found to have a significant positive effect on maize grain yield compared to sole maize [46]. Similarly, the yields of maize and peanut intercropping treatments were better than those of monoculture treatments, with LERs greater than 1 [47]. The higher LER under quinoa/red bean intercropping indicates higher spatial complementarity [16].

The improvement of yield on a per area base under quinoa/legume intercropping indicates better availability of nutrients, especially nitrogen [48] and phosphorus [49]. In our study, intercropping quinoa with legumes, particularly the quinoa/red bean treatment, significantly improved the content of alkali-hydrolyzable nitrogen. Legumes enrich the soil with nitrogen by fixing nitrogen in root nodules [50]. While some experiments have shown an increasing amount of nitrogen transferred from legumes to other crops over time [51], others have failed to reveal significant nitrogen transfer in field conditions [52]. This inconsistency may be attributed to factors such as limitations in experimental techniques or the variability of nutrient transfers in field environments. The amount of nitrogen released through rhizodeposition is influenced by factors such as total nitrogen assimilation by legumes, total root production, and the age of the plant. Despite these challenges, estimates suggest that legumes can release substantial amounts of nitrogen into the soil during their growth cycle, with a significant portion contributed through rhizodeposition. Nitrogen accumulation increases with time with the turnover of leguminous crops with cropping seasons. For example, pea plants have been estimated to release about 129 kg N/ha during their growth cycle, including 56 kg from rhizodeposits, whereas wheat releases only 26 kg N/ha as rhizodeposits [50].

Phosphorous is the second most deficient nutrient because of less availability in most of the soil [53]. Our results indicate the positive effect of intercropping on soil available phosphorous content. At maturity, the soil phosphorous content under the intercropping of quinoa with legumes was significantly higher than under the sole cropping of quinoa. The higher available phosphorous is because of the higher alkaline phosphatase activity [54] as also depicted by the present results. Furthermore, the positive regression of quinoa yield with soil alkaline phosphatase in the present study indicates high phosphorous availability under intercropping. Intercropping with legumes plays a significant role in improving phosphorus uptake efficiency by affecting specific inorganic phosphorus pools in the soil. Cu et al. [55] demonstrated that different crops have a preference for utilizing either citric acid-leachable phosphorus or a water-leachable soil phosphorus pool. This preference supports the hypothesis of resource partitioning for soil phosphorus. Although there are some other factors such as alterations in soil pH or enzymatic activity involved in phosphorus solubilization, the previous and present findings suggest that intercropping enhances phosphorus uptake efficiency by enhanced phosphatase enzyme activity in the soil [56].

Similar to nitrogen and phosphorus, a significant increase in soil-available potassium was observed under intercropping. Overall, significant improvement in alkali-hydrolyzable nitrogen, available phosphorus, available potassium, and organic matter in the soil by intercropping quinoa with legumes, particularly the quinoa/red bean treatment, aligns with previous research and may be attributed to the root exudates of legumes and microbial activity in soil as evident by urease, alkaline phosphatase and sucrase activities in soil [56, 57].

In our study, the intercropping of quinoa with legumes, especially the quinoa/red bean treatment, significantly improved the activities of soil sucrase, alkaline phosphatase, and urease during the quinoa growing period. This is consistent with previous studies showing that intercropping can influence microbial biomass and enzymatic activities in the soil [58, 59] and may be related to the impact of cereal/legume intercropping systems on the composition of soil rhizosphere bacterial communities [60].

The higher soil enzyme activities and higher available forms of nutrients in the soil resulted in a higher uptake of nutrients by quinoa plants at the seedling and maturity stages. Results showed that compared to quinoa monoculture, the quinoa/red bean treatment showed a trend toward improving the nitrogen, potassium, and phosphorus content in the stem and leaves at the maturity stage. This may be due to the ability of legumes in intercropping systems to fix biological nitrogen and facilitate its transfer to quinoa crops, as well as the production of root exudates that enhance the availability of limited soil nutrients such as phosphorus [61, 15]. Previous research by Qiu et al. [62] found that intercropping increased aboveground nitrogen, phosphorus, and potassium concentrations and contents at maturity.

The impact of different intercropping patterns on soil physicochemical properties was also assessed.

Quinoa/black bean treatment tended to increase soil pH compared to quinoa monoculture treatment, which is consistent with findings by Nwite et al. [46] who observed improved soil pH when legumes were intercropped with maize. The effect of intercropping treatments on soil water content varied, possibly due to differences in above-ground biomass. Intercropping legumes have shown promise in diversifying crops, enhancing soil quality, and increasing soil organic carbon through the fixation of biologically fixed nitrogen [63, 64].

#### **Conclusions**

Intercropping quinoa with three leguminous crops showed a land equivalent ratio greater than 1, with the quinoa/red bean treatment having the highest ratio of 1.52. This treatment also led to increased uptake of nitrogen, phosphorus, and potassium nutrients by the quinoa plant compared to quinoa grown alone. At the quinoa seedling stage, the quinoa/red bean treatment had the highest content of soil nutrients and organic matter. Sucrase and urease activities were also highest under the quinoa/red bean treatment during the growing stage of quinoa. The study suggests that intercropping quinoa with red beans can improve yield, nutrient uptake, soil nutrients, and enzyme activities in the future.

#### Acknowledgments

This research project is supported by a Doctoral research start-up funding project of Shanxi Datong University (2018-B-30), Shanxi Scholarship Council of China (2021-145), and Shanxi Basic Research Program (20210302124068).

## **Conflict of Interest**

The authors declare no conflict of interest.

#### References

- LOPEZ-MORENO M., SABATER-MU<sup>\*</sup>NOZ B., IGLESIAS-LOPEZ M., MIGUEL-CASTRO M., GARC'ESRIM'ON M. Red Quinoa hydrolysates with antioxidant bioactive properties on oxidative stressinduced Saccharomyces cerevisiae. LWT-Food Science and Technology. 184, 115038, 2023.
- KIBAR H., SONMEZ F., TEMEL S. Effect of storage conditions on nutritional quality and color characteristics of quinoa varieties. Journal of Stored Products Research 91, 101761, 2021.
- SONG J.X., SHAO Y., YAN Y.M., LI X.H., PENG J., GUO L. Characterization of volatile profiles of three colored quinoas based on GC-IMS and PCA. LWT-Food Science and Technology. 146, 111292, 2021.

- 4. DENG Y., SUN X., ZHANG Q., ANWAR S., LU J., GUO H., QIN L., ZHANG L., WANG C. Comprehensive evaluation and physiological response of quinoa genotypes to low nitrogen. Agronomy. 13 (6), 1597, 2023.
- WANG X.W., ZHAO R.Y., YUAN W.Q. Composition and secondary structure of proteins isolated from six different quinoa varieties from China. Journal of Cereal Science. 95, 103036, 2020.
- SONG J.X., YAN Y.M., WANG X.D., LI X.H., CHEN Y., LI L. Characterisation of fatty acids, amino acids and organic acids in three colored quinoas based on untargeted and targeted metabolomics. LWT-Food Science and Technology. 140, 110690, 2021.
- LAN Y., ZHANG W., LIU F., WANG L., YANG X., MA S. Recent advances in physiochemical changes, nutritional value, bioactivities, and food applications of germinated quinoa: a comprehensive review. Food Chemistry. 426, 136390, 2023.
- JIANG F., DU C.W., GUO Y., FU J.Y., JIANG W.Q., DU S.K. Physicochemical and structural properties of starches isolated from quinoa varieties. Food Hydrocolloids. 101, 105515, 2020.
- DAI J., QIU W., WANG N., WANG T., NAKANISHI H., ZUO Y. From Leguminosae/Gramineae intercropping systems to see benefits of intercropping on iron nutrition. Frontiers in Plant Science. 10, 605, 2019.
- VLAICULESCU A., VARRONE C. Sustainable and eco-friendly alternatives to reduce the use of pesticides. In: Pesticides in the Natural Environment. Elsevier, 329, 2022.
- 11. WALTERS H., CARPENTER-BOGGS L., DESTA K., YAN L., MATANGUIHAN J., MURPHY K. Effect of irrigation, intercrop, and cultivar on agronomic and nutritional characteristics of quinoa. Agroecology and Sustainable Food Systems. 40 (8), 783, 2016.
- 12. YIN W., CHAI Q., ZHAO C., YU A., FAN Z., HU F., FAN H., GUO Y., COULTER J.A. Water utilization in intercropping: a review. Agricultural Water Management. 241, 106335, 2020.
- ZHANG N.N., SUN Y.M., LI L., WANG E.T., CHEN W.X., YUAN H.L. Effects of intercropping and Rhizobium inoculation on yield and rhizosphere bacterial community of faba bean (*Vicia faba* L.). Biology and Fertility of Soils. 46, 625, 2010.
- 14. MALVIYA M.K., SOLANKI M.K., LI C.N., WANG Z., ZENG Y., VERMA K.K., SINGH R.K., SINGH P., HUANG H.R., YANG L.T., SONG X.P. Sugarcane-legume intercropping can enrich the soil microbiome and plant growth. Frontiers in Sustainable Food System.s 5, p.606595, 2021.
- 15. LI L., TILMAN D., LAMBERS H., ZHANG F.S. Plant diversity and overyielding: insights from belowground facilitation of intercropping in agriculture. New Phytologist. 203 (1), 63, 2014.
- 16. DUCHENE O., VIAN J.F., CELETTE F. Intercropping with legume for agroecological cropping systems: Complementarity and facilitation processes and the importance of soil microorganisms. A review. Agriculture, Ecosystems & Environment. 240, 148, 2017.
- 17. XIE J., WANG L., LI L., ANWAR S., LUO Z., ZECHARIAH E., KWAMI FUDJOE S. Yield, economic benefit, soil water balance, and water use efficiency of intercropped maize/potato in responses to mulching practices on the semiarid loess plateau. Agriculture. 11 (11), 1100, 2021.
- 18. TSUBO M., WALKER S., OGINDO H.O. A simulation

model of cereal-legume intercropping systems for semiarid regions. Field Crops Research. 93, 10, 2005.

- 19. LAYEK J., DAS A., MITRAN T., NATH C., MEENA R.S., YADAV G.S., SHIVAKUMAR B.G., KUMAR S., LAL R. Cereal+Legume Intercropping: an option for improving productivity and sustaining soil health. In: Meena, R.S., Das, A., Yadav, G.S., Lal, R. (Eds.), Legumes for Soil Health and Sustainable Management. Springer: Singapore, Singapore, pp. 347, 2018.
- 20. NISA NU., SHAFIQ F., ANWAR S., MAHMOOD A., IQBAL M., ULLAH K., ZULQARNAIN M., HAIDER I., ASHRAF M., ZHANG L. Physiological effects of some engineered nanomaterials on radish (*Raphanus sativus* L.) intercropped with pea (*Pisum sativum* L.). Environmental Science and Pollution Research. 1, 2023.
- MUPANGWA W., NYAGUMBO I., LIBEN F., CHIPINDU L., CRAUFURD P., MKUHLANI S. Maize yields from rotation and intercropping systems with different legumes conservation agriculture in contrasting agro-ecologies. Agriculture Ecosystems & Environment. 306, 107170, 2021.
- 22. BI Y., ZHOU P., LI S., WEI Y., XIONG X., SHI Y., LIU N., ZHANG Y. Interspecific interactions contribute to higher forage yield and are affected by phosphorus application in a fully-mixed perennial legume and grass intercropping system. Field Crops Research. 244 (1), 107636, 2019.
- 23. ERDOĞAN H., KOCA Y.O. Effect of quinoa-corn intercropping production system on yield and quality of mixture silage. Turkish Journal of Range and Forage Science. 1 (2), 57, 2020.
- 24. VAHIDI H., MAHMOODI S., PARSA S., FALLAHI H.R. Evaluation the Yield and Intercropping Indices of Millet (*Panicum miliaceaum* L.) and Quinoa (*Chenopodium quinoa* Willd.) under Effect of Plant Density and Cultivation Ratios in Birjand Region. Journal of Agroecology. 13 (3), 471, 2021.
- 25. KOCA Y.O. Determination of the forage yield and growth parameters of maize (*Zea mays* L.) with quinoa (*Chenopodium quinoa*) intercropping at different plant mixtures. Turkish Journal of Field Crops. 26 (1), 44, 2021.
- 26. ABDI S. Evaluation of yield, yield components and competitive indices in different patterns of intercropping on quinoa (*Chenopodium quinoa* Willd) and bean (*Phaseolus vulgaris* L.). Isfahan University of Technology-Journal of Crop Production and Processing. 13 (3), 31, 2003.
- 27. WANG L.X., CHENG X.Z., WANG S.H., JING T.I.A.N. Analysis of an applied core collection of adzuki bean germplasm by using SSR markers. Journal of Integrative Agriculture. 11 (10), 1601, 2012.
- LI L., LIU B., ZHENG X. Bioactive ingredients in adzuki bean sprouts. Journal of Medicinal Plants Research. 5 (24), 5894, 2011.
- 29. DESTA K.T., CHOI Y.M., YI J.Y., LEE S., SHIN M.J., WANG X.H., YOON H. Agro-morphological Characterization of Korean, Chinese, and Japanese Adzuki Bean (Vigna angularis (Willd.) Ohwi & Ohashi) Genotypes. The Korean Journal of Crop Science. 68 (1), 8, 2023.
- 30. SINGH N., KHARWAL N., BHARDWAJ N., SINGH S. Adzuki bean [Vigna angularis (Willd.) Ohwi & Ohashi]. In: Neglected and Underutilized Crops, Eds Farooq, M. Siddique, K.H.M. Academic Press, pp. 539, 2023.
- 31. TAKEOKA G.R., DAO L.T., FULL G.H., WONG R.Y., HARDEN L.A., EDWARDS R.H., BERRIOS J.D.J. Characterization of black bean (*Phaseolus vulgaris*

- L.) anthocyanins. Journal of Agricultural and Food Chemistry. **45** (9), 3395, **1997**.
- 32. FAO. Standard operating procedure for soil moisture content by gravimetric method. Rome. Global Soil Laboratory Network Glosolon, pp. 1, 2023. https://www.fao.org/3/cc483len/cc483len.pdf.
- MULVANEY R.L., KHAN S.A. Diffusion methods to determine different forms of nitrogen in soil hydrolysates. Soil Science Society of America Journal. 65 (4), 1284, 2001
- 34. Soil Testing-Part 7. Method for determination of available phosphorus in soil. Beijing, China. Ministry of Agriculture of the People's Republic of China, 2014.
- 35. DU Y.J., HAYASHI S., XU Y.F. Some factors controlling the adsorption of potassium ions on clayey soils. Applied Clay Science. **27** (3-4), 209, **2004**.
- BAO S.D. Analysis on Soil and Agricultural Chemistry (in Chinese), China Agricultural Press, Beijing, China, 2005.
- 37. MANDRI B., DREVON J.J., BARGAZ A., OUFDOU K., FAGHIRE M., PLASSARD C., PAYRE H. GHOULAM C. Interactions between common bean genotypes and rhizobia strains isolated from Moroccan soils for growth, phosphatase and phytase activities under phosphorus deficiency conditions. Journal of Plant Nutrition. 35 (10), 1477, 2012.
- 38. PANDEY A., ELDRIDGE S.M., WEATHERLEY A., WILLETT I.R., MYINT A.K., OO A.N., NGWE K., MANG Z.T., CHEN D. High fertilizer nitrogen input increases nitrogen mining in sandy paddy soils. Nutrient Cycling in Agroecosystems. 125 (1), 77, 2023.
- 39. BEDOUSSAC L., JOURNET E.P., HAUGGAARD-NIELSEN H., NAUDIN C., CORRE-HELLOU G., JENSEN E.S., PRIEUR L., JUSTES E. Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming. Agronomy for Sustainable Development. 35, 911, 2015.
- 40. LIANG B., MA Y., SHI K., CHEN G., CHEN H., HU Y., CHEN P., PU T., WU Y., SUN X., YONG T. Appropriate bandwidth achieves a high yield by reducing maize intraspecific competition in additive maize—soybean strip intercropping. European Journal of Agronomy. 142, 126658, 2023.
- 41. DEB D., DUTTA S. The robustness of land equivalent ratio as a measure of yield advantage of multi-crop systems over monocultures. Experimental Results. 3, e2, 2022.
- 42. ATABO J.A., UMARU T.M. Assessing the land equivalent ratio (LER) and stability of yield of two cultivars of sorghum (Sorghum bicolor L. Moench)-soybean (Glycine max L. Merr) to row intercropping system. Journal of Biology, Agriculture and Healthcare. 5 (18), 144, 2015.
- 43. RAMESHJAN Y., KOOCHEKI A., MAHALLATI M.N., KHORRAMDEL S. Effect of different intercropping ratios of three bean ecotypes as replacement series on their physiological indices. Iranian Journal of Field Crops Research. 18 (4), 385, 2021.
- 44. GOU F., VAN ITTERSUM M.K., WANG G., VAN DER PUTTEN P.E.L., VAN DER WERF W. Yield and yield components of wheat and maize in wheat-maize intercropping in the Netherlands. European Journal of Agronomy. 76, 17, 2016.
- RASEDUZZAMAN M.D., JENSEN E.S. Does intercropping enhance yield stability in arable crop production? A meta-analysis. European Journal of Agronomy. 91, 25, 2017.
- 46. NWITE J.N., NJOKU C., ALU M.O. Effects of

- intercropped legumes with maize (*Zea mays* L.) on chemical properties of soil and grain yield of maize in Abakaliki, Nigeria. Nigeria Agricultural Journal. **48** (2), 105, **2017**.
- 47. LI Q., CHEN J., WU L., LUO X., LI N., ARAFAT Y., LIN S., LIN W. Belowground interactions impact the soil bacterial community, soil fertility, and crop yield in maize/peanut intercropping systems. International Journal of Molecular Sciences. 19, 622, 2018.
- 48. MORALES E.B, ALCONADA M.M., ASIMBAYA B.L., PANTOJA J.L. Impact of the Association Quinoa (Chenopodium quinoa Willd.) Bean (Vicia faba L.) on Agricultural Production, Biological Fixation and Recycling of Nitrogen. In International Conference on Applied Technologies, Cham: Springer Nature Switzerland, pp. 447, 2022.
- 49. TANG X., ZHANG C., YU Y., SHEN J., VAN DER WERF W., ZHANG F. Intercropping legumes and cereals increases phosphorus use efficiency; a meta-analysis. Plant and Soil. 460, 89, 2021.
- 50. GENG S., LI L., MIAO Y., ZHANG Y., YU X., Duo ZHANG D., YANG Q., ZHANG X., WANG Y. Nitrogen rhizodeposition from corn and soybean, and its contribution to the subsequent wheat crops. Journal of Integrative Agriculture. 2023.
- 51. SALINAS-ROCO S., MORALES-GONZÁLEZ A., ESPINOZA S., PÉREZ-DÍAZ R., CARRASCO B., DEL POZO A., CABEZA R.A. N<sub>2</sub> Fixation, N Transfer, and Land Equivalent Ratio (LER) in Grain Legume–Wheat Intercropping: Impact of N Supply and Plant Density. Plants. 13 (7), p.991, 2024.
- 52. HAUGGAARD-NIELSEN H., AMBUS P., JENSEN E.S. Interspecific competition, N use and interference with weeds in pea-barley intercropping. Field Crops Research. 70 (2), 101, 2001.
- CONG W.F., SURIYAGODA L.D., LAMBERS H. Tightening the phosphorus cycle through phosphorusefficient crop genotypes. Trends in Plant Science. 25 (10), 967. 2020.
- 54. MNDZEBELE B., NCUBE B., FESSEHAZION M., MABHAUDHI T., AMOO S., DU PLOOY C., VENTER S., MODI A. Effects of cowpea-amaranth intercropping and fertiliser application on soil phosphatase activities, available soil phosphorus, and crop growth response. Agronomy. 10 (1), p.79, 2020.
- 55. CU S.T., HUTSON J., SCHULLER K.A. Mixed culture of wheat (*Triticum aestivum* L.) with white lupin (*Lupinus*

- *albus* L.) improves the growth and phosphorus nutrition of the wheat. Plant and Soil. **272**, 143, **2005**.
- 56. LO PRESTI E., BADAGLIACCA G., ROMEO M., MONTI M. Does legume root exudation facilitate itself P uptake in intercropped wheat? Journal of Soil Science and Plant Nutrition. 21 (4), 3269, 2021.
- CHEN X., CHEN J., CAO J. Intercropping increases soil N-targeting enzyme activities: A meta-analysis. Rhizosphere. 26, 100686, 2023.
- 58. SUN Y.M., ZHANG N.N., WANG E.T., YUAN H.L., YANG J.S., CHEN W.X. Influence of intercropping and intercropping plus rhizobial inoculation on microbial activity and community composition in rhizosphere of alfalfa (*Medicago sativa L.*) and Siberian wild rye (*Elymus sibiricus L.*). FEMS Microbiology Ecology. 70, 218, 2009.
- 59. MOURADI M., FARISSI M., MAKOUDI B., BOUIZGAREN A., GHOULAM C. Effect of faba bean (Vicia faba L.)-rhizobia symbiosis on barley's growth, phosphorus uptake and acid phosphatase activity in the intercropping system. Annals of Agrarian Sciences. 16 (3), 297, 2018.
- 60. CHAMKHI I., CHETO S., GEISTLINGER JO., ZEROUAL Y., KOUISNI L., BARGAZ A., GHOULAM C. Legume-based intercropping systems promote beneficial rhizobacterial community and crop yield under stressing conditions. Industrial Crops & Products. 183, 114958, 2022.
- 61. HAUGGAARD-NIELSEN H., GOODING M., AMBUS P., CORRE-HELLOU G., CROZAT Y., DAHLMANN C., DIBET A., VON FRAGSTEIN P., PRISTERI A., MONTI M., JENSEN E.S. Pea-barley intercropping for efficient symbiotic N<sub>2</sub>-fixation, soil N acquisition and use of other nutrients in European organic cropping systems. Field Crops Research. 113 (1), 64, 2009.
- 62. QIU Y., LI X., TANG Y., XION S., HAN Y., WANG Z., FENG L., WANG G., YANG B., LEI Y., DU W., ZHI X., XIN M., JIAO Y., ZHANG S. Directly linking plant N, P and K nutrition to biomass production in cotton-based intercropping systems. European Journal of Agronomy. 151, 126960, 2023.
- 63. RAJI S.G., DORSCH P. Effect of legume intercropping on N<sub>2</sub>O emissions and CH<sub>4</sub> uptake during maize production in the Great Rift Valley, Ethiopia. Biogeosciences. 17, 345, 2020
- 64. TIRADO R., COTTER J. Ecological farming: droughtresistant agriculture. Greenpeace Research Laboratories, Technical Note 02/201, Exeter, UK, 2010.