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ARTICLE

Effects of arbuscular mycorrhizal fungi inoculation in wheat–pea intercropping systems on wheat productivity and soil fertility

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Abstract
Intercropping cereals with legumes and the application of beneficial soil microorganisms are promising strategies for improving crop productivity and maintaining soil fertility in sustainable agricultural systems. This study evaluated the effects of arbuscular mycorrhizal fungi (AMF) inoculation on wheat–pea intercropping productivity and soil agrochemical properties under field conditions in Mongolia. Four treatments were compared: wheat monocropping (control), wheat–pea intercropping, wheat–pea intercropping with native AMF inoculation, and wheat–pea intercropping with a commercial AMF inoculum (Kormilitsa).
AMF colonization of wheat roots increased significantly from 6.1% in the control to 29.0% in the wheat–pea intercrop and reached 44.6–47.0% in the AMF-inoculated treatments. Wheat growth and productivity were enhanced by intercropping and AMF inoculation. The highest plant height (70.6 cm), thousand-kernel weight (44.0 g), and grain yield (23.44 q ha^{-1}) were recorded in the wheat–pea + Kormilitsa AMF treatment. Grain yield increased by 15.6–20.8% compared with the control. Grain quality was also improved, with the highest vitreousness (46.4%) and wet gluten content (26.0%) observed in the Kormilitsa treatment. Soil analyses showed increases in humus, organic carbon, total nitrogen, and available phosphorus in the intercropping treatments, whereas soil pH and electrical conductivity remained relatively stable.
The results demonstrate that wheat–pea intercropping combined with AMF inoculation can improve wheat productivity, grain quality, and soil fertility. Among the evaluated treatments, the commercial AMF inoculum (Kormilitsa) produced the most favorable agronomic and soil responses. These findings highlight the potential of integrating cereal–legume intercropping with AMF inoculation as a sustainable biological approach for improving crop production and maintaining soil health in Mongolia.

Keywords: root colonization, grain quality, nutrient-use efficiency, phosphorus uptake, biological nitrogen fixation, sustainable agriculture

Introduction

Arbuscular mycorrhizal fungi (AMF) are among the most widespread and beneficial soil microorganisms associated with agricultural crops. AMF establish mutualistic relationships with plant roots and improve the uptake of relatively immobile nutrients, particularly phosphorus, through extensive hyphal networks [1,2]. Enhanced phosphorus acquisition contributes to improved plant growth, productivity, and nutrient-use efficiency [3–5]. In addition, AMF improve soil structure, stimulate microbial activity, and promote nutrient cycling, thereby contributing to long-term soil fertility and ecosystem functioning [3,5].

Intercropping, the cultivation of two or more crop species simultaneously on the same field, is widely recognized as an effective strategy for improving resource-use efficiency, crop productivity, and agricultural sustainability [6–8]. Compared with monocropping systems, intercropping enhances the utilization of nutrients, water, and light while increasing system resilience under variable environmental conditions [6]. Among different intercropping systems, cereal–legume combinations are particularly important because legumes contribute biologically fixed nitrogen through symbiotic associations with rhizobia, thereby improving soil fertility and reducing dependence on synthetic nitrogen fertilizers [9,10]

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide and serves as a major source of food and feed. Pea (*Pisum sativum* L.) is an important grain legume capable of fixing atmospheric nitrogen and improving soil fertility. Previous studies have shown that wheat–legume intercropping can increase grain yield, improve nutrient-use efficiency, and enhance grain quality compared with sole cropping systems [11,12]. These benefits are primarily attributed to complementary resource use and positive interactions among plants and soil microorganisms within the rhizosphere [9,10].

The integration of cereal–legume intercropping and AMF inoculation may provide complementary benefits for crop production. While legumes contribute nitrogen through biological fixation, AMF enhance phosphorus acquisition and nutrient-use efficiency [1,9]. Previous studies have reported that AMF inoculation can improve crop productivity, grain quality, and nutrient uptake in cereal-based production systems [4,5,13]. Furthermore, long-term intercropping practices have been shown to influence AMF community composition and improve wheat yield through enhanced plant–soil interactions [14]. Recent studies also suggest that phosphorus availability plays a key role in regulating microbial interactions and nutrient dynamics within cereal–legume intercropping systems [15,16].

Maintaining soil fertility while sustaining crop productivity remains a major challenge for agriculture in Mongolia. The country's continental climate, short growing season, and limited soil fertility constrain agricultural production. In addition, continuous cereal cultivation and traditional wheat–fallow rotations have contributed to soil degradation, declining organic matter content, and nutrient depletion. Therefore, biologically based management strategies that improve nutrient cycling and reduce dependence on mineral fertilizers are increasingly important for sustainable crop production.

Although the benefits of cereal–legume intercropping and AMF inoculation have been widely documented in different agroecosystems, information regarding their combined effects under Mongolian environmental conditions remains limited. In particular, little is known about the influence of AMF inoculation on wheat productivity, grain quality, and soil fertility in wheat–pea intercropping systems. Therefore, the objective of this study was to evaluate the effects of arbuscular mycorrhizal fungi inoculation on wheat–pea intercropping productivity and soil fertility under field conditions in Mongolia.

Materials and methods

Experimental Site and Design

A field experiment was conducted during the 2024 growing season (15 May–3 September) at the Bayangol Experimental Research Station of the Institute of Plant Protection Research, Bornuur soum, Tuv Province, Mongolia (48.628° N, 106.329° E). The site is located within the forest-steppe zone and is characterized by a semi-arid continental climate with low and variable precipitation. Agricultural production in the region is predominantly rainfed.

1. The experiment was arranged in a randomized complete block design (RCBD) with three replications. Four treatments were evaluated: Wheat monocropping (Control);
2. Wheat + pea intercropping;
3. Wheat + pea intercropping + native arbuscular mycorrhizal fungi (AMF);
4. Wheat + pea intercropping + commercial AMF inoculum (Kormilitsa).

Each experimental plot measured 2 m² and plots were separated by 30 cm.

Plant Materials and AMF Inoculation

The wheat cultivar *Darkhan-144* and pea cultivar *Bayalag* were used in the study. Darkhan-144 is a medium-maturing cultivar adapted to Mongolian environmental conditions, whereas Bayalag is a lodging-resistant, medium-maturing pea cultivar suitable for intercropping systems.

The commercial AMF inoculum (Kormilitsa) was produced in the Russian Federation and contained *Rhizophagus irregularis* with a minimum concentration of 2×10^5 spores mL^{-1}. The inoculum was applied at a rate of 100 ml/ha.

Native AMF inoculum was obtained from soils collected from the Bogd Khan Mountain area, Ulaanbaatar, Mongolia (47.856° N, 106.904° E). Soil samples were used to establish trap cultures using onion (*Allium cepa* L.) as a host plant. AMF spores were isolated using the wet-sieving and decanting method of Gerdemann and Nicolson, and fungal identification was conducted according to Schenck and Pérez. Colonized root fragments were applied at a rate of 24.0 g m^{-2}, corresponding to approximately 84 viable spores g^{-1} soil.

Crop Establishment and Field Management

The experimental field had been mechanically cultivated during the previous growing season. Sowing was performed on 15 May 2024 at a depth of 5 cm. Wheat and pea were sown at rates of 120 and 45 kg ha^{-1}, respectively, with a row spacing of 22 cm.

All agronomic operations were performed according to local production practices. Crop growth was monitored throughout the growing season, and harvesting was conducted on 3 September 2024.

Soil Sampling and Analysis

Soil samples were collected before sowing and after harvest from the 0–30 cm soil layer following standard soil sampling procedures [24]. Composite samples were prepared from each experimental plot and air-dried prior to laboratory analysis.

Soil pH and electrical conductivity (EC) were determined using standard potentiometric and conductivity methods, respectively. Humus content was measured using the Tyurin wet oxidation method ($K_2Cr_2O_7$–H_2SO_4 oxidation), and organic carbon content was calculated from humus values using the Van Bemmelen conversion factor (1.724). Total nitrogen (TN) was determined by the semi-micro Kjeldahl method following sulfuric acid digestion. Nitrate nitrogen (NO_3^-–N) was analyzed using the disulphophenol acid colorimetric method, whereas ammonium nitrogen (NH_4^+–N) was determined using Nessler's reagent. Available phosphorus (P_2O_5) was measured by the antimony–molybdenum blue colorimetric method, and exchangeable potassium (K_2O) was quantified by flame photometry.

Determination of AMF Root Colonization

Root samples were collected during the wheat tillering stage. Five plants were randomly sampled from each replication. Roots were carefully washed, cleared in 10% KOH, and stained with 0.05% trypan blue. The percentage of root colonization by AMF was determined using the gridline-intersect method of Giovannetti and Mosse:

$$\text{Colonization (\%)} = \frac{Nm}{Nt} * 100$$

where (N_m) is the number of colonized intersections and (N_t) is the total number of observed intersections.

Measurement of Agronomic Traits

Plant height was measured at the heading stage from the soil surface to the tip of the spike, excluding awns. Five plants were randomly selected from each replicate and measured using a ruler.

At harvest, wheat grain yield was determined from each plot and converted to q ha^{-1}. Thousand-kernel weight (TKW) was determined according to MNS 0487:2001. Grain samples were air-dried, and 1000 kernels were counted and weighed using an electronic balance with a precision of 0.01 g.

Grain quality parameters, including protein, starch, vitreousness, and wet gluten content, were analyzed using a Perten IM9500 grain analyzer at an MNS/ISO/IEC 17025-accredited laboratory.

Statistical Analysis

Data were analyzed using R statistical software. Treatment effects were evaluated using one-way analysis of variance (ANOVA). When significant differences were detected, means were separated using Tukey's Honest Significant Difference (HSD) test at the 5% probability level ($p < 0.05$).

For each measured variable, replicate means were used as the experimental units for statistical analysis. Results are presented as mean ± standard error (SE).

Results

Root Mycorrhizal Colonization

Root colonization by arbuscular mycorrhizal fungi (AMF) differed significantly among treatments (Figure 1). The lowest colonization rate was observed in the wheat monocrop (control), where only 6.1% of root intersections were colonized. Intercropping wheat with pea significantly increased colonization to 29.0%, representing nearly a fivefold increase compared with the control.

The highest colonization levels were recorded in the AMF-inoculated treatments. Colonization reached 47.0% in the Wheat + Pea + Kormilitsa AMF treatment and 44.6% in the Wheat + Pea + Native AMF treatment. Both treatments showed significantly greater colonization than the control and the non-inoculated wheat–pea intercrop ($p < 0.05$). However, no significant difference was detected between the Kormilitsa and Native AMF treatments, indicating that both inocula established successful symbiotic associations with wheat roots.

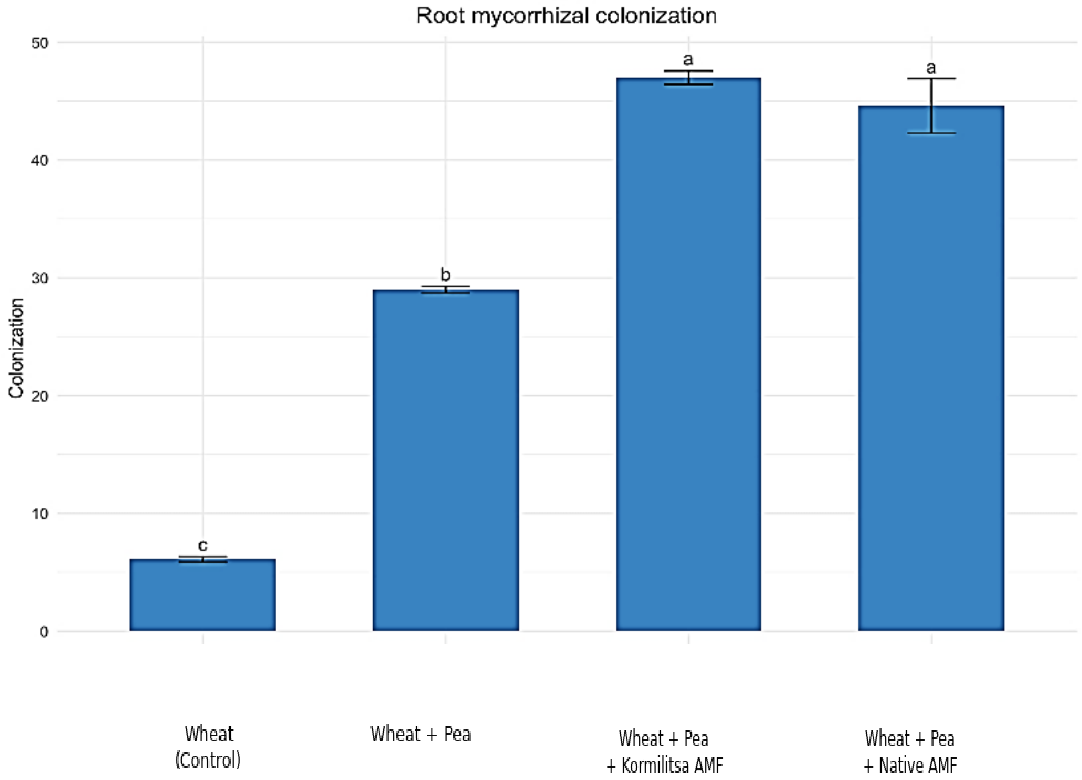

Figure 1. Root colonization (%) of wheat by arbuscular mycorrhizal fungi (AMF) under different treatments. Bars represent mean ± standard error (SE). Different letters indicate significant differences among treatments according to Tukey's HSD test at $p < 0.05$.

Effect of AMF Inoculation on Wheat Plant Height

Significant differences in plant height were observed among treatments (Figure 2). Wheat grown as a monocrop produced the shortest plants, with an average height of 58.0 cm. Intercropping with pea increased plant height to 66.5 cm. The tallest plants were recorded in the Wheat + Pea + Kormilitsa AMF treatment (70.6 cm), followed by the Wheat + Pea + Native AMF treatment (68.3 cm). Both AMF treatments produced significantly taller plants than the control ($p < 0.05$).

All intercropping treatments produced significantly taller plants than the control ($p < 0.05$). In addition, both AMF-inoculated treatments resulted in significantly greater plant height than the non-inoculated wheat–pea intercrop. The Kormilitsa AMF treatment produced the tallest plants and differed significantly from all other treatments, whereas the Native AMF treatment showed intermediate values.

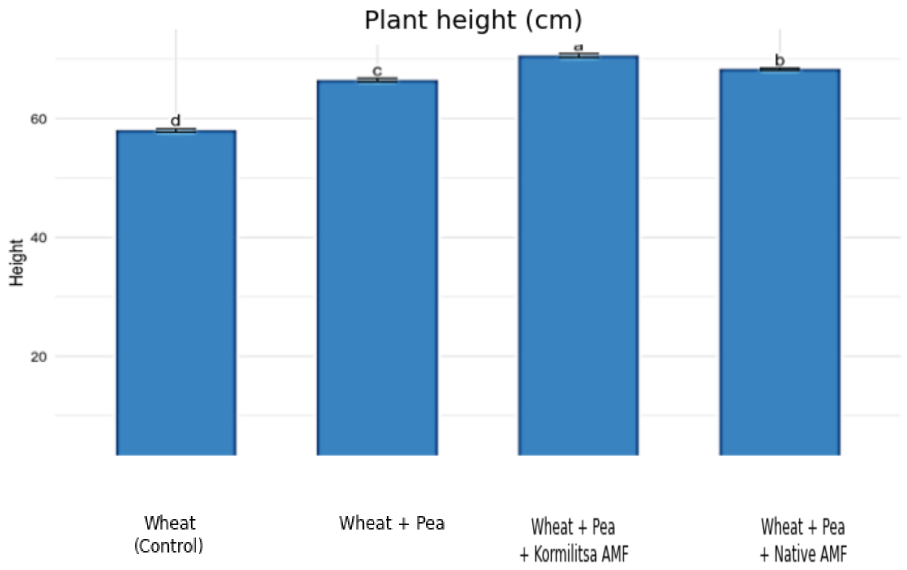

Figure 2. Plant height (cm) of wheat under different intercropping and AMF treatments. Bars represent mean ± standard error (SE). Different letters indicate significant differences among treatments according to Tukey's HSD test ($p < 0.05$).

Effect of Treatments on Thousand-Kernel Weight

The thousand-kernel weight (TKW) of wheat differed among treatments (Figure 3). The control treatment produced a TKW of 41.4 g. Similar values were recorded in the Wheat + Pea treatment (41.6 g) and the Wheat + Pea + Native AMF treatment (41.3 g), with no significant differences among these treatments ($p > 0.05$).

The highest TKW was observed in the Wheat + Pea + Kormilitsa AMF treatment, reaching 44.0 g. This value was significantly greater than those of the control, Wheat + Pea, and Wheat + Pea + Native AMF treatments ($p < 0.05$). Compared with the control, the Kormilitsa AMF treatment increased TKW by approximately 6.3%.

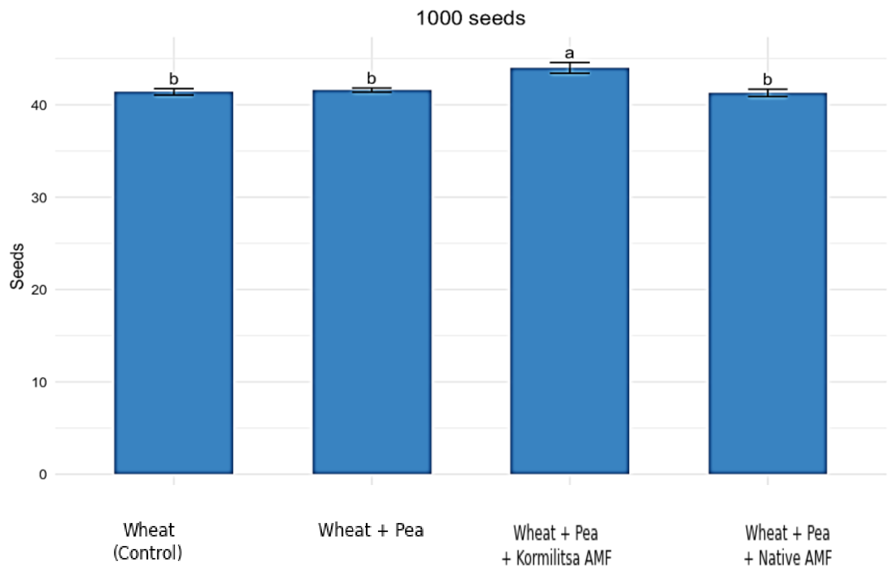

Figure 3. Thousand-kernel weight (g) of wheat under different intercropping and AMF treatments. Bars represent mean ± standard error (SE). Different letters indicate significant differences among treatments according to Tukey's HSD test ($p < 0.05$).

Effect of Treatments on Wheat Grain Yield

Wheat grain yield differed significantly among treatments (Figure 4). The control treatment produced the lowest grain yield, averaging 19.4 q The Wheat + Pea treatment yielded 22.8 q ha⁻¹, while the Wheat + Pea + Native AMF treatment produced 22.42 q ha⁻¹. The highest grain yield was recorded in the Wheat + Pea + Kormilitsa AMF treatment (23.44 q ha⁻¹). Compared with the control, grain yield increased by 17.5%, 16.0%, and 20.8% in the Wheat + Pea, Wheat + Pea + Native AMF, ha⁻¹. All intercropping treatments significantly increased grain yield compared with the control ($p < 0.05$).

and Wheat + Pea + Kormilitsa AMF treatments, respectively.

Although the Kormilitsa AMF treatment produced the highest numerical yield, no significant differences were detected among the three intercropping treatments.

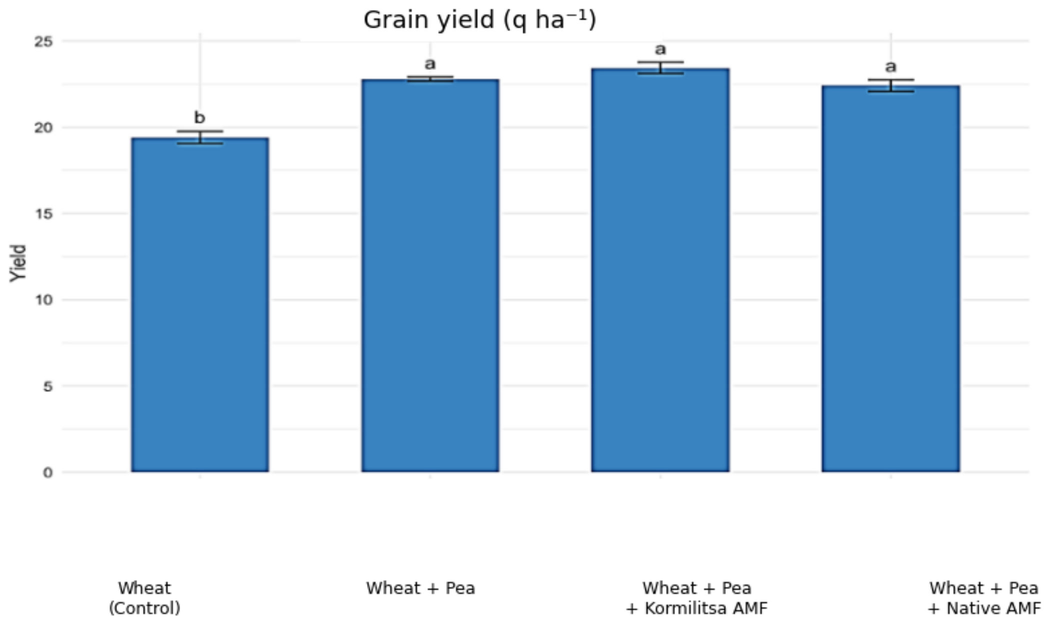

Figure 4. Wheat grain yield (q ha⁻¹) under different intercropping and AMF treatments. Bars represent mean ± standard error (SE). Different letters indicate significant differences among treatments according to Tukey's HSD test ($p < 0.05$).

Effect of Mycorrhizal Inoculation on Wheat Grain Quality

Effect of Treatments on Wheat Grain Quality

Grain quality characteristics of wheat under different treatments are presented in Table 1. Protein content ranged from 11.6% in the control treatment to 13.0% in the Wheat + Pea + Kormilitsa AMF treatment. However, differences among treatments were not statistically significant ($p > 0.05$). Similarly, starch content remained relatively stable, ranging from 69.1% to 69.6%, with no significant treatment effects. Significant differences were observed for vitreousness ($p < 0.05$). The control treatment exhibited the lowest vitreousness (37.6%), whereas the highest value was recorded in the Wheat + Pea + Kormilitsa AMF treatment (46.4%). Intermediate values were observed in the Wheat + Pea (41.1%) and Wheat + Pea + Native AMF (42.9%) treatments. Wet gluten content ranged from 23.4% to 26.0%. Although the highest value was observed in the Wheat + Pea + Kormilitsa AMF treatment, differences among treatments were not statistically significant ($p > 0.05$).

Table 1.
Grain quality characteristics of wheat under different intercropping and AMF treatments

Treatment	Protein (%)	Starch (%)	Vitreousness (%)	Wet gluten (%)
Wheat (Control)	11.6 ± 1.7 a	69.6 ± 0.8 a	37.6 ± 0.6 c	23.4 ± 0.2 a
Wheat + Pea	12.2 ± 0.8 a	69.4 ± 0.9 a	41.1 ± 0.7 b	24.3 ± 0.3 a
Wheat + Pea + Kormilitsa AMF	13.0 ± 0.5 a	69.1 ± 0.7 a	46.4 ± 0.4 a	26.0 ± 0.6 a
Wheat + Pea + Native AMF	12.3 ± 1.4 a	69.4 ± 0.4 a	42.9 ± 0.5 b	24.7 ± 1.2 a

Values are presented as mean ± standard error (SE). Different letters within a column indicate significant differences among treatments according to Tukey's HSD test ($p < 0.05$).

Effect of Treatments on Soil Agrochemical Properties

The effects of intercropping and AMF inoculation on soil agrochemical properties are presented in Table 2. Soil pH ranged from 7.5 to 7.8, while electrical conductivity (EC) varied between 0.031 and 0.036 dS m^{-1}. No significant differences were observed among treatments for either parameter ($p > 0.05$).

Humus content increased from 2.3% in the control treatment to 2.4% in all intercropping treatments. Similarly, organic carbon content increased from 1.3% in the control to 1.4–1.5% in the intercropped treatments. Total nitrogen content ranged from 0.1% to 0.2%, with significantly greater values recorded in the AMF-inoculated treatments than in the control ($p < 0.05$).

Nitrate nitrogen (NO_3^-–N) showed little variation among treatments, ranging from 0.33 to 0.40 mg 100 g^{-1} soil. Ammonium nitrogen (NH_4^+–N) was highest in the Wheat + Pea treatment (1.0 mg 100 g^{-1} soil) and differed significantly from the control and AMF-inoculated treatments ($p < 0.05$).

Available phosphorus (P_2O_5) exhibited the greatest treatment response. The control treatment contained 1.0 mg 100 g^{-1} soil, whereas values increased to 2.4, 3.3, and 2.1 mg 100 g^{-1} soil in the Wheat + Pea, Wheat + Pea + Kormilitsa AMF, and Wheat + Pea + Native AMF treatments, respectively. Exchangeable potassium (K_2O) ranged from 12 to 14 mg 100 g^{-1} soil and did not differ significantly among treatments.

Table 2.
Soil agrochemical properties under different intercropping and AMF treatments

Treatment	pH	EC (dS $^{-1}$)	Humus (%)	Organic carbon (%)	Total N (%)	NO_3^-–N (mg 100 g^{-1})	NH_4^+–N (mg 100 g^{-1})	P_2O_5 (mg 100 g^{-1})	K_2O (mg 100 g^{-1})
Wheat (Control)	7.8 ± 0.1 a	0.036 ± 0.05 a	2.3 ± 0.4 a	1.3 ± 0.2 a	0.1 ± 0.02 a	0.33 ± 0.00 a	0.59 ± 0.10 a	1.0 ± 0.4 b	12 ± 1.2 a
Wheat + Pea	7.7 ± 0.1 a	0.036 ± 0.01 a	2.4 ± 0.2 a	1.4 ± 0.1 a	0.1 ± 0.11 a	0.40 ± 0.00 a	1.00 ± 0.20 b	2.4 ± 0.4 a	12 ± 1.2 a
Wheat + Pea + Kormilitsa AMF	7.7 ± 0.6 a	0.032 ± 0.01 a	2.4 ± 0.2 a	1.4 ± 0.1 a	0.2 ± 0.23 b	0.40 ± 0.10 a	0.60 ± 0.14 a	3.3 ± 0.4 a	14 ± 0.0 a
Wheat + Pea + Native AMF	7.5 ± 0.2 a	0.031 ± 0.05 a	2.4 ± 0.2 a	1.5 ± 0.1 a	0.2 ± 0.22 b	0.34 ± 0.00 a	0.60 ± 0.11 a	2.1 ± 0.0 a	12 ± 0.6 a

Values are presented as mean ± standard error (SE). Different letters within a column indicate significant differences among treatments according to Tukey's HSD test ($p < 0.05$).

Discussion

The present study demonstrated that wheat–pea intercropping, particularly when combined with AMF inoculation, significantly enhanced root colonization, plant growth, grain yield, grain quality, and selected soil fertility parameters. These findings support the hypothesis that beneficial plant–microbe interactions can improve crop performance and nutrient availability under field conditions.

The substantial increase in root colonization observed in intercropped treatments confirms that legume-based systems create favorable conditions for AMF development. Colonization increased from only 6.1% in monocropped wheat to 29.0% in the wheat–pea intercrop and exceeded 44% in both AMF-inoculated treatments. Similar increases in colonization have been reported in cereal–legume intercropping systems, where legumes stimulate mycorrhizal development through root exudates and improved rhizosphere conditions [25], [29]. The absence of significant differences between the Kormilitsa and native AMF inocula suggests that both inoculants were capable of establishing effective symbiotic associations with wheat roots.

Enhanced AMF colonization was associated with improved wheat growth. Plant height increased from 58.0 cm in the control treatment to 70.6 cm in the Wheat + Pea + Kormilitsa AMF treatment. Increased plant growth following AMF inoculation has been widely attributed to improved nutrient acquisition, particularly phosphorus uptake, as AMF hyphae extend beyond the root depletion zone and increase the effective absorptive area of plant roots [1], [2], [30].

Similar responses have been reported by Marrassini et al. [4] and Khalleefah [28], who observed improved wheat growth following AMF inoculation.

The positive effects of intercropping and AMF inoculation were also reflected in yield performance. Grain yield increased by 16–21% relative to the control treatment, while thousand-kernel weight was significantly improved only in the Kormilitsa AMF treatment. The yield advantages observed in this study are consistent with previous reports showing that cereal–legume intercropping improves resource-use efficiency and overall productivity through complementary utilization of nutrients, water, and light [6], [7], [26], [27]. Although the Kormilitsa treatment produced the highest numerical yield and kernel weight, the lack of significant yield differences among intercropped treatments suggests that intercropping itself contributed substantially to the observed yield benefits, whereas AMF inoculation provided additional but more moderate advantages.

Grain quality responses were less pronounced than yield responses. Protein and wet gluten contents showed increasing trends but did not differ significantly among treatments. In contrast, vitreousness increased significantly under intercropping and AMF inoculation, reaching its highest value in the Kormilitsa treatment. Previous studies have reported that AMF can influence grain composition and quality through improved nutrient acquisition and carbohydrate metabolism [30]. The observed increase in vitreousness may therefore reflect improved grain filling and assimilate accumulation during grain development.

Improvements in soil fertility parameters further demonstrate the benefits of combining intercropping with biological inoculation. Intercropped treatments showed greater concentrations of organic carbon, total nitrogen, and available phosphorus than the control treatment. The increase in total nitrogen is likely associated with biological nitrogen fixation by pea, whereas the substantial increase in available phosphorus, particularly in the Kormilitsa treatment, reflects the ability of AMF to mobilize phosphorus from soil fractions that are otherwise poorly accessible to plant roots [1], [29], [31]. Similar improvements in nutrient availability and soil biological activity have been reported in other cereal–legume intercropping systems [13], [21], [29].

Despite these positive responses, soil pH, electrical conductivity, and exchangeable potassium remained unchanged among treatments. This indicates that the improvements observed in crop performance were primarily driven by biological processes rather than major alterations in the soil chemical environment. Maintaining stable soil chemical properties while increasing nutrient availability is an important characteristic of sustainable cropping systems.

The study was conducted during a single growing season at one experimental location. Therefore, further multi-year and multi-location studies are needed to evaluate the consistency of these responses under varying environmental conditions. Additional research should also investigate the long-term effects of AMF inoculation on soil biological activity, nutrient cycling, and crop productivity in cereal–legume production systems.

Conclusion

Wheat–pea intercropping significantly increased wheat productivity and improved selected grain quality and soil fertility parameters compared with monocropped wheat. Intercropping increased grain yield by 16–21%, while the highest thousand-kernel weight and grain quality indicators were observed in the Wheat + Pea + Kormilitsa AMF treatment. Root colonization by arbuscular mycorrhizal fungi increased substantially under intercropping and AMF inoculation, demonstrating successful establishment of symbiotic associations. Intercropping and AMF inoculation also increased soil organic carbon, total nitrogen, and available phosphorus without significantly affecting soil pH, electrical conductivity, or exchangeable potassium. Among the evaluated treatments, Wheat + Pea

+Kormilitsa AMF showed the most favorable overall performance. These findings suggest that combining wheat–pea intercropping with AMF inoculation represents a promising biological approach for improving crop productivity and maintaining soil fertility under Mongolian conditions.

Author Contributions

M.E. conducted the field experiment evaluating the effects of arbuscular mycorrhizal fungi on wheat, analyzed the data, and interpreted the results. D.B. supervised the study and developed the experimental design. B.M. contributed to the implementation of the field experiment and provided expert guidance on mycorrhizal fungi throughout the study. All authors reviewed and approved the final version of the manuscript.

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