

## Article

# Comparative Response of Fermented and Non-Fermented Animal Manure Combined with Split Dose of Phosphate Fertilizer Enhances Agronomic Performance and Wheat Productivity through Enhanced P Use Efficiency

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**Abstract:** Low availability of native soil phosphorus (P) is a major constraint limiting sustainable crop production especially in alkaline calcareous soils. Application of organic manure in this regard has gained attention of the scientific community. Yet, the potential of fermented animal manure in improving P use efficiency and subsequent crop yield has not been assessed. This pot experiment was designed to study the performance of wheat under application of non-fermented and fermented animal manure in combination with 0, 45 or 90 kg·ha<sup>−1</sup> phosphorus in the form of diammonium phosphate (DAP). Results show that non-fermented animal manure and split dose of phosphorus fertilizer improved plant quantitative attributes including plant growth, yield and nutrient uptake parameters. However, the placement of fermented animal manure combined with the full amount of P (90 kg·ha<sup>−1</sup>) fertilizer gave the mean highest value of fertile tillers per pot (12) and their grain yield (5.2 g). Moreover, plant physiological parameters were enhanced with fermented animal manure and the recommended rate of P fertilizer compared with the control. Likewise, the biochemical properties of wheat grain such as fat, fiber, ash and protein contents were increased by 1.24, 2.26, 1.47 and 11.2%, respectively, in plants receiving fermented animal manure and P fertilizer (90 kg·ha<sup>−1</sup>). Furthermore, co-application of fermented animal manure with P (90 kg·ha<sup>−1</sup>) into soil improved phosphorus uptake from 0.72 to 1.25 g·pot<sup>−1</sup>, phosphorus usage efficiency from 0.715 to 0.856 mg·pot<sup>−1</sup>, and soil phosphorus extent from 7.58 to 16.1% over controls. It is thus inferred that this new approach resulted in release of P from fermented manure that not only reduced fixation but also enhanced the growth, yield, physiology and nutrient uptake in wheat.

**Keywords:** fermented animal manure; inorganic phosphorus; P use efficiency; wheat yield; diammonium phosphate



## 1. Introduction

The ever-increasing human population is one of the pressing issues in modern agriculture as it has become a global challenge to feed around 9 billion people by 2050 [1]. Moreover, rapid decline in the soil productivity coupled with increased urbanization and human population has reduced the available cropland [2,3]. The scarcity of the existing cropland further threatens the food security and nutrition needs of the growing population. Among all staple food crop, wheat (*Triticum aestivum* L.) is dominant because wheat stores both micro- (calcium and iron) and macro-nutrients (sugars, fat and protein), which helps in the establishment of a healthy society [4]. So, the demand for wheat is globally increasing and there is need to produce sufficient food from the existing land base. Wheat contributes 18.9% to the GDP and accommodates 42.3% of labor force in Pakistan [5].

Currently, 40–60% of cereal production depends on fertilizers and by 2050, almost 110% grain production will have to depend on fertilizers. Phosphorus (P) is one of the most important macronutrients for optimal crop production and its deficiency caused growth reduction and delayed maturity [6–8]. When inorganic phosphorus is applied to the soils, only 10–20% is taken up by the crops [9–12], whereas the rest is precipitated through adsorption and precipitation reactions with cations such as calcium, magnesium, iron and aluminum [13–15]. Thus, over-abundant application of inorganic sources not only causes yield losses but also environmental issues, for example, waterway eutrophication and groundwater contamination [16].

If we look at Western countries, there is an effort to fulfill the crop requirements by application of organic amendments. For that purpose, 10 million animals are being grown annually that can generate manure of about 5–6% of their body weight each day, a dry mass of roughly 5.5 kg per animal per day [17]. Animal manure directly spread on the land can stay for a longer period, which causes greenhouse gases to be released to the atmosphere and the overrunning of nutrients and pathogens to water bodies. Thus, thermochemical and biochemical management practices are unacceptable [18].

In order to address the P fixation in calcareous soil, the use of fermented manures seems a promising technique to reduce P surplus in wheat crops. Manure can undergo anaerobic fermentation naturally that results in the breaking of its components down to produce a stable solid digestate along with biogas. It can produce humic and organic acids that can alter the precipitation and adsorption of P in soil [19]. Through electrostatic competition, organic acids that have low molecular weight can compete with P for adsorption sites [20–22]. Similarly, humic acids that have high molecular weight can form complexes with metal ions such as iron (Fe) which reduces the availability of P due to fixation [23]. A review of past work concluded that adequate decomposition of organic materials is essential for obtaining better quality of organic matter, not only by way of crop nutrition but also to improve soil quality and productivity [24,25].

It also improves the P availability and manure suitability because pH controls the P solubility in manure [26]. Phosphorus availability increases at low pH; therefore, phosphorus content can be altered in manure by the acidification process. Moreover, microorganisms utilize organic matter and increase their population, which helps to change organic and inorganic phosphorus reserves in the root zone, which greatly affects biological processes and root characteristics. Generally, it is accepted that manure phosphorus is 80–100% more effective than mineral P [27]. Similarly, anaerobic fermentation may also reduce ammonia volatilization. Application of manure to *Zai pits* increased nutrient uptake by 43–87% and yield by 35–220% [28]. In Niger, manure application in *Zai pits* resulted in 2–68 times higher grain yields than in non-amended *Zai pits* [29]. Previous studies have focused on the application of manure either alone or combined with mineral fertilizers. However, how fermentation of manure with varying pH ranges with or without a split dose of P fertilizer (DAP in this case) affects soil physio-chemical and crop growth characteristics has remained relatively neglected. Furthermore, the comparison of responses of fermented and non-fermented manure on soil fertility and crop nutrient uptake and use efficiency have been the least explored, which in the present study constitutes the novelty of this work.



It was thus hypothesized that application of fermented animal manure in combination with chemical P fertilizer would improve wheat yield by enhancing P use efficiency, however, their effects on plant nutrients may vary depending upon the type and rate of P fertilizer applied. The objectives of the present study were to assess the effect of anaerobically fermented manure with inorganic P fertilizer on the soil properties, growth, yield and P uptake in wheat crop. Furthermore, we examined the nutrient status of the soil and plants due to temporary changes in pH and analyzed its effect on improving the yield and growth of wheat crop.

## 2. Materials and Methods

### 2.1. Incubation Experiment and Preparation of Fermented Animal Manure

Fermented animal manure was prepared by incubating 7 kg of cattle dung collected from the Directorate of farms, University of Agriculture Faisalabad (UAF), Pakistan. The organic material was transferred to a vessel (500 kg capacity) under controlled temperature and aeration (shaking at  $60 \text{ rev} \cdot \text{min}^{-1}$ ). An optimum moisture level of the organic material ( $v/w$ ) was maintained during the fermentation process, which was carried out at  $25^\circ\text{C}$  for (2–3) weeks. Molasses (as carbon source), rice polish (starch), gram floor (protein) and mustard oil cake (fat) sources were added at the rate of 1% ( $w/w$ ) along with an inoculum of a cellulose-degrading bacterial strain *Bacillus* sp. MN54 ( $10^9 \text{ CFU} \cdot \text{mL}^{-1}$ ) as a decomposer.

For fermentation of manure, nine treatments were arranged as follow: ( $T_1$ ) untreated animal manure, ( $T_2$ ) molasses + animal manure, ( $T_3$ ) molasses + *Bacillus* sp. MN54 + animal manure, ( $T_4$ ) molasses + gram floor + animal manure, ( $T_5$ ) molasses + gram floor + *Bacillus* sp. MN54 + animal manure, ( $T_6$ ) molasses + gram floor + rice polish + animal manure, ( $T_7$ ) molasses + gram floor + rice polish + *Bacillus* sp. MN54 + animal manure, ( $T_8$ ) molasses + gram floor + rice polish + mustard oil cake + animal manure and ( $T_9$ ) molasses + gram floor + rice polish + mustard oil cake + *Bacillus* sp. MN54 + animal manure.

The pH was monitored during treatment by inserting a (Hanna portable) pH meter [30]. The pH was reduced in the  $T_9$  treatment from 8.02 to 4.5 within 18 days at  $25^\circ\text{C}$ . For the pot experiment  $T_1$  (non-fermented animal manure) and  $T_9$  (fermented animal manure) were selected and tested with half and full rate of P fertilizer (Table 1). After drying, manure phosphorus content ( $\text{g} \cdot \text{kg}^{-1}$ ) was determined using a spectrophotometer model UV–visible spectrophotometer (T-60) at wavelength 880 nm [31]. Manure nitrogen ( $\text{g} \cdot \text{kg}^{-1}$ ) was determined using the method described by Jackson [32]. Manure K content ( $\text{g} \cdot \text{kg}^{-1}$ ) was determined using a flame photometer (EI 392) at wavelength 767 nm [33]. All reagents and chemicals were analytical grade, provided by Sigma-Aldrich, St. Louis, MO, USA and Merk, Darmstadt, Germany.

### 2.2. Preparation and Analysis of the Experimental Soil

Soil samples were taken randomly from a field area at the Institute of Soil and Environmental Sciences (ISES), UAF Pakistan, from a depth of 0–15 cm, and the selected soil properties were determined (Table 2). Samples were air dried and ground to pass through a  $<2 \text{ mm}$  sieve prior to analysis. Soil EC and pH were determined by preparing a 1:1 ( $w/v$ ) suspension in distilled water [34]. Soil texture was determined by standard methods using a Bouyoucos hydrometer [35]. The saturation percentage of the soil was determined by making a soil saturated paste. Soil phosphorus content ( $\text{mg} \cdot \text{kg}^{-1}$ ) was determined using a spectrophotometer at wavelength 880 nm [31]. Organic matter percentage was estimated following the protocol described by [36]. Soil nitrogen percentage was determined using the method described by Jackson [32]. Soil K content ( $\text{mg} \cdot \text{kg}^{-1}$ ) was determined using a flame photometer at wavelength 767 nm [33]. DTPA extractable micronutrient contents of zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) were determined using atomic absorption spectrophotometer [37].



**Table 1.** The treatment description applied in the pot experiment.

	Treatments	Description
1	Control (no P)	Unamended control
2	P (45 kg·ha <sup>-1</sup> )	DAP applied at the rate of 45 kg·ha <sup>-1</sup>
3	P (90 kg·ha <sup>-1</sup> )	DAP applied at the rate of 90 kg·ha <sup>-1</sup>
4	Non-FAM [pH 7.26]	Non-fermented animal manure of pH 7.26
5	P (45 kg·ha <sup>-1</sup> ) + non-FAM	DAP applied at the rate of 45 kg·ha <sup>-1</sup> combined with non-fermented animal manure
6	P (90 kg·ha <sup>-1</sup> ) + non-FAM	DAP applied at the rate of 90 kg·ha <sup>-1</sup> combined with non-fermented animal manure
7	FAM [pH 4.5]	Fermented animal manure of pH 4.5
8	P (45 kg·ha <sup>-1</sup> ) + FAM	DAP applied at the rate of 45 kg·ha <sup>-1</sup> combined with fermented animal manure
9	P (90 kg·ha <sup>-1</sup> ) + FAM	DAP applied at the rate of 90 kg·ha <sup>-1</sup> combined with fermented animal manure

Note: P applied in the form of DAP; DAP; diammonium phosphate.

**Table 2.** The measured physico-chemical properties of soil with fermented and non-fermented animal manures used in the study.

Properties	Pre-Soil Analysis	Animal Manure Properties	
		Non-Fermented Animal Manure	Fermented Animal Manure
Organic matter/carbon	05 (g kg <sup>-1</sup> )	364 (g kg <sup>-1</sup> )	421 (g kg <sup>-1</sup> )
Texture	Loam	-	-
Saturation percentage	28.0 (%)	75%	23%
EC	1.83 (dS m <sup>-1</sup> )	-	-
pH	8.42	7.26	4.5
N	0.8 (g kg <sup>-1</sup> )	3.40 (g kg <sup>-1</sup> )	7.44 (g kg <sup>-1</sup> )
P	6.7 (mg kg <sup>-1</sup> )	0.67 (g kg <sup>-1</sup> )	3.99 (g kg <sup>-1</sup> )
K	133 (mg kg <sup>-1</sup> )	1.55 (g kg <sup>-1</sup> )	1.92 (g kg <sup>-1</sup> )
Zn	2.24 (mg kg <sup>-1</sup> )	78 (mg kg <sup>-1</sup> )	115 (mg kg <sup>-1</sup> )
Fe	6.25 (mg kg <sup>-1</sup> )	398 (mg kg <sup>-1</sup> )	650 (mg kg <sup>-1</sup> )
Cu	1.15 (mg kg <sup>-1</sup> )	15 (mg kg <sup>-1</sup> )	26 (mg kg <sup>-1</sup> )
Mn	8.40 (mg kg <sup>-1</sup> )	40 (mg kg <sup>-1</sup> )	62 (mg kg <sup>-1</sup> )

### 2.3. Pot Experiment and Cultivation of Wheat Crop

Each pot was filled and settled with 7 kg of soil for study at the wire house of ISES, UAF Pakistan, using both manure types, i.e., non-fermented and fermented with no P (control) or half (45 kg·ha<sup>-1</sup>) or recommended (90 kg·ha<sup>-1</sup>) doses of phosphorus fertilizer. Diammonium phosphate (DAP) was used as a source of phosphorus fertilizer. Non-fermented and fermented animal manures were selected for the pot trial on the basis of high and low pH obtained from the incubation study. Treatments were arranged under factorial complete randomized design with 3 replicates. The treatments included are given in (Table 1). Amendments (fermented and non-fermented manures) were added to all the treatments at rate of 1% of dry soil on w/w basis and mixed thoroughly before sowing. The recommended dose of N (120 kg·ha<sup>-1</sup>), as urea in two splits, and K (60 kg·ha<sup>-1</sup>) as sulphate of potash were applied as the basal dose. In each pot, 7 seeds of winter wheat variety “Akbar 2019” were sown. The seeds of this wheat variety were provided by the Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Three plants were maintained in each pot after germination. The pots were placed into the wire house with



no control of rainfall, temperature, and relative humidity. Water was applied to the pots according to the field capacity of the soil when required.

#### 2.4. Harvesting and Determination of Agronomic Parameters

Wheat plants were harvested at the end of the growth period (120 days). Different agronomic parameters such as plant height, root length and spike length were measured using a meter rod. The number of spikelets per spike, total number of tillers/pot and number of fertile tillers per pot were counted manually. The root fresh and dry weight ( $\text{g} \cdot \text{pot}^{-1}$ ), straw weight ( $\text{g} \cdot \text{pot}^{-1}$ ) and 100-grain weight ( $\text{g} \cdot \text{pot}^{-1}$ ) were determined using a digital balance.

#### 2.5. Determination of Physiological Parameters

Leaf chlorophyll contents were measured using upper leaves through a chlorophyll content meter (SPAD-502). The relative water content was calculated by taking fresh leaf weight inside 100% humidity following the protocol of [38]. Electrolyte leakage was determined following the protocol described by Lutts [39]. The relative membrane permeability was calculated using the protocol described by Yang [40]. An infrared gas analyzer (IRGA) was used to measure physiological parameters, i.e., photosynthetic rate, stomatal conductance, transpiration rate, evaporation rate and internal  $\text{CO}_2$  concentration.

#### 2.6. Profiling Biochemical Parameters of Wheat Grains

Biochemical parameters, e.g., fat content, was measured using the protocol described by James [41]. The ash and fiber contents were measured using the methodology described by Arlington [42]. Crude protein was calculated by a multiplication factor of 6.25 with nitrogen content [43].

#### 2.7. Determination of the Chemical Parameters

The soil phosphorus content was measured using a spectrophotometer at wavelength 410 nm by taking a 0.5 g soil sample in a 50 mL flask, followed by addition of 5 mL digested liquid and colored reagent [31]), and phosphorus use efficiency (PUE) and P uptake indices were calculated following [12].

$$\text{P uptake} = \text{P content in shoot} \times \text{shoot dry weight per pot} \quad \text{PUE} = \frac{\text{Grain yield (g per pot)}}{\text{P uptake (mg per pot)}}$$

Straw, grain and soil nitrogen contents were estimated using Kjeldahl apparatus [32] (Jackson, 1962), whereas potassium concentration in straw, grain and soil was estimated using a flame photometer [44].

#### 2.8. Statistical Analyses

The collected data were analyzed using analysis of variance (ANOVA) at a  $\leq 5\%$  value of statistical significance [45] for comparison under a factorial complete randomized design (CRD) with the help of statistics 8.1 software. The linear regression and heat map analyses were plotted using the computer-based software R studio.

### 3. Results

#### 3.1. Characteristics of Fermented Manure

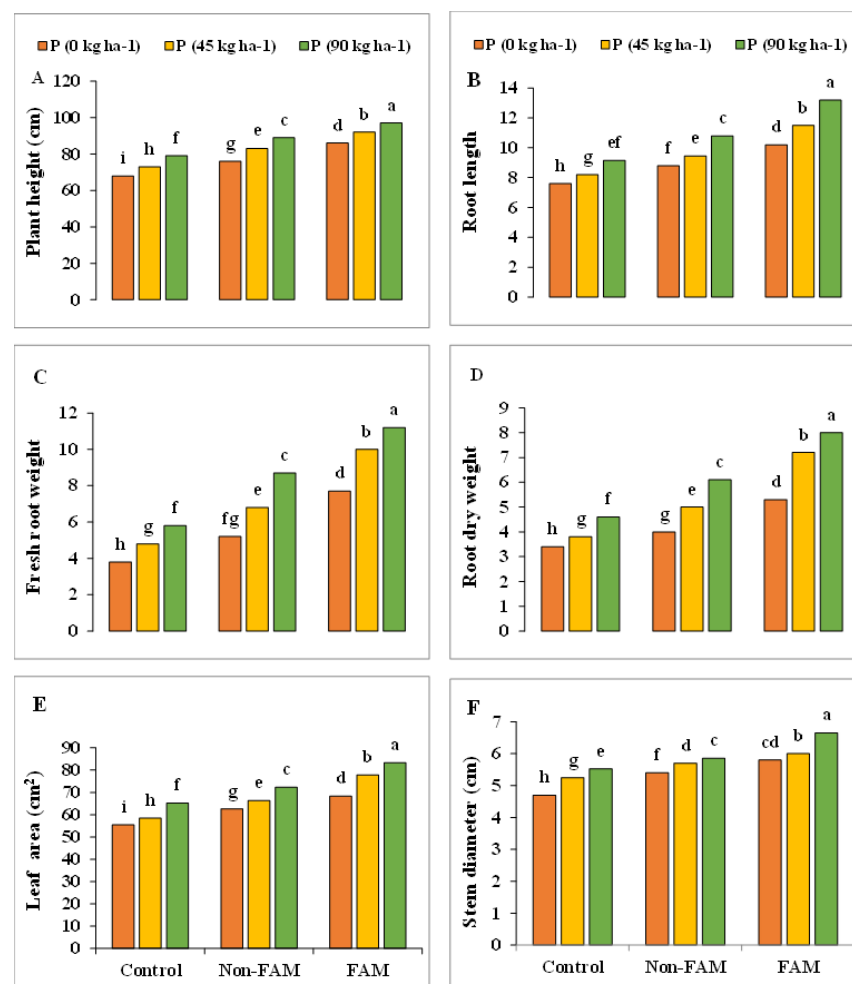
In the incubation study, better performance for fermented animal manure was observed in terms of decreased pH and increases in N, P, K, Zn, Fe, C, and Mn by the addition of different crude sources of carbon (molasses), protein (gram floor), starch (rice polish), fat (mustard oil cake) and cellulase-producing bacteria as compared with the non-fermented manure, as shown in (Table 2). Manure treated with different crude sources of carbon, protein, fat and cellulose-degrading bacteria decreased the pH (4.5) more than non-fermented animal manure (7.26). The study showed that, a mean higher amount of nitrogen under application of fermentation was obtained ( $7.44 \text{ g} \cdot \text{kg}^{-1}$ ) followed by non-fermented animal manure ( $3.40 \text{ g} \cdot \text{kg}^{-1}$ ). In the same way, the mean phosphorus value was also increased



in fermented animal manure ( $3.99 \text{ g} \cdot \text{kg}^{-1}$ ) compared with non-fermented animal manure ( $0.67 \text{ g} \cdot \text{kg}^{-1}$ ). The least pronounced increase in the mean value of K was measured in fermented animal manure ( $1.92 \text{ g} \cdot \text{kg}^{-1}$ ) followed by non-fermented animal manure ( $1.55 \text{ g} \cdot \text{kg}^{-1}$ ). Likewise, the concentrations of trace elements were also increased after the fermentation of animal manure (Table 2).

### 3.2. Crop Performance under Applied Fermented Manure

Pots treated with fermented animal manure and half and recommended rates of P fertilizer enhanced the growth parameters of wheat more than the non-fermented manure and control treatments (Figure 1A–F). The maximum plant height (96.66 cm) was recorded with fermented manure and  $90 \text{ kg} \cdot \text{ha}^{-1}$  P, whereas the non-fermented manure with the same P rate produced 89.00 cm height. Data indicate that the highest increase in root length, 13.23 cm, was achieved by application of fermented manure and  $90 \text{ kg} \cdot \text{ha}^{-1}$  P (Figure 1). In the same way, root fresh weight was decreased in  $90 \text{ kg} \cdot \text{ha}^{-1}$  P compared with its combined effect with animal manure. The least pronounced decrease ( $11.23 \text{ g} \cdot \text{pot}^{-1}$ ) was found in the application of fermented animal manure and recommended amount of P fertilizer. A similar trend was obtained in the case of root dry weight, and it was increased up to  $8.00 \text{ g} \cdot \text{pot}^{-1}$  by application of fermented animal manure with  $90 \text{ kg} \cdot \text{ha}^{-1}$  P fertilizer.



**Figure 1.** Combined use of fermented/non-fermented animal manure and phosphorus fertilizer on the growth parameters of wheat. (A) plant height, (B) root length, (C,D) root fresh and dry weight, (E) leaf area and (F) stem diameter. Columns with different letters are significantly different from one another at a level of significance of  $p < 5\%$ .



The combined application of fermented animal manure and a full dose of P fertilizer showed a  $83.33 \text{ cm}^2$  leaf area, followed by treatment with fermented manure and a half dose ( $45 \text{ kg} \cdot \text{ha}^{-1}$ ) of P fertilizer. The non-fermented manure also showed a significant increase in leaf area as compared with the untreated control (Figure 1). Likewise, the maximum stem diameter ( $6.65 \text{ mm}^2$ ) was obtained by the combined use of fermented manure and the recommended rate of P fertilizer. Similarly, the total number of tillers (14) and fertile tillers per pot (12) were at their maximums in the treatment where the combination of fermented animal manure and the recommended rate of P fertilizer were applied (Figure 2). Non-fermented manure along with both levels of P fertilizer addition showed a significant response regarding tiller count compared with the control. The mean highest increase in spike length ( $18.33 \text{ cm}$ ) was found with the application of fermented animal manure with  $90 \text{ kg} \cdot \text{ha}^{-1}$  P, whereas the same fertilizer rate with non-fermented manure gave  $16.63 \text{ cm}$  spike length (Figure 2A,B). Application of non-fermented animal manure had a great influence on spike length (Figure 2C).

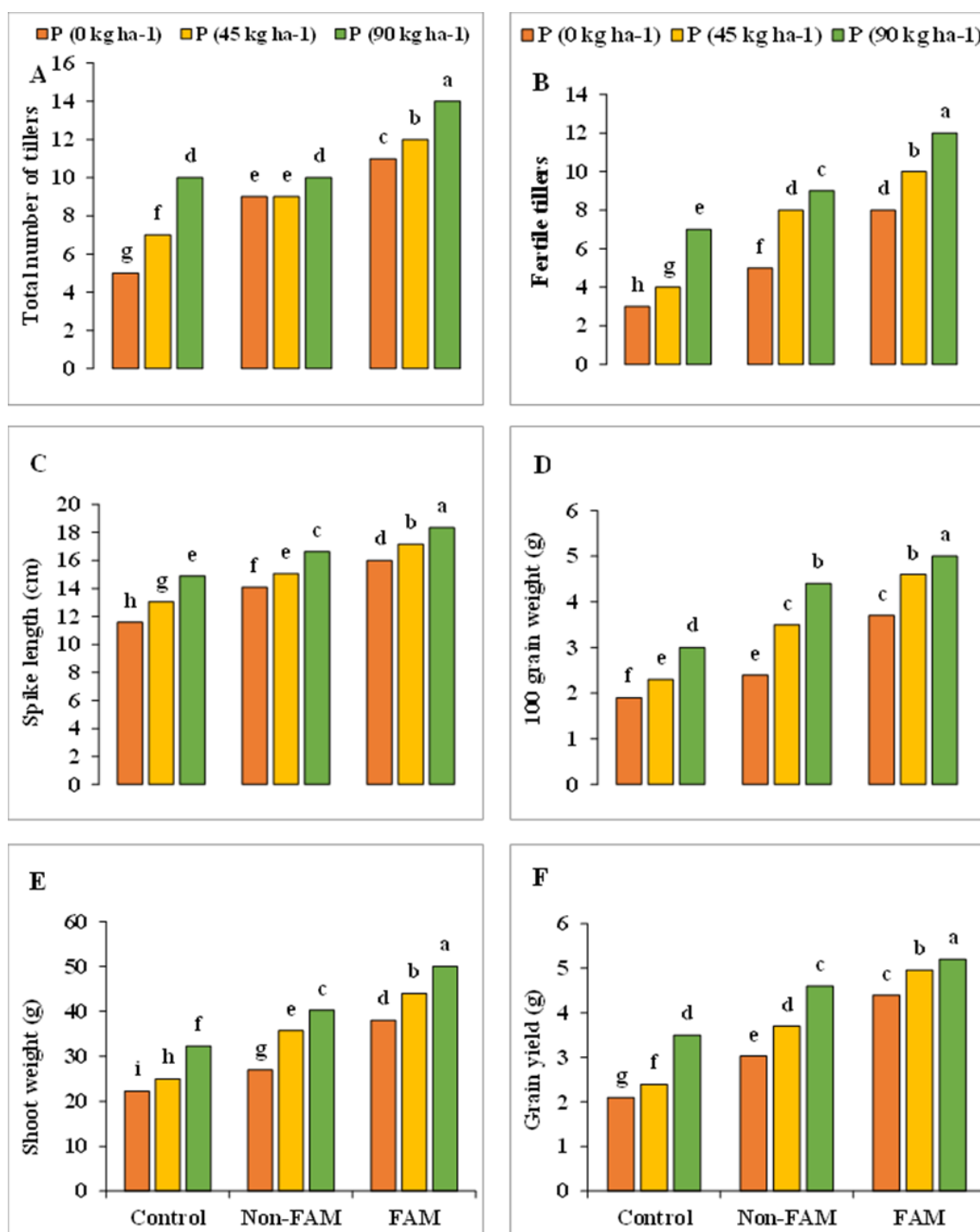
### 3.3. Influence of Fermented Animal Manure and DAP Fertilizer on Yield Parameters

A significant increase in number of total and fertile tillers, 100-grain weight, shoot weight and grain yield was recorded in the application of fermented animal manure with half and recommended levels of P fertilizer as compared with all other treatments, as shown in Figure 2A–F. Further, the maximum increase in the variant was recorded in 100-grain weight ( $5 \text{ g} \cdot \text{pot}^{-1}$ ) through combined application of fermented manure and  $90 \text{ kg} \cdot \text{ha}^{-1}$  P. The results show a similar trend in shoot weight, where  $11.72 \text{ g} \cdot \text{pot}^{-1}$  weight was achieved by same treatment. The highest grain yield ( $5.2 \text{ g} \cdot \text{pot}^{-1}$ ) was observed in the application of fermented manure with  $90 \text{ kg} \cdot \text{ha}^{-1}$  P, whereas non-fermented animal manure with  $90 \text{ kg} \cdot \text{ha}^{-1}$  P showed a  $4.6 \text{ g} \cdot \text{pot}^{-1}$  grain yield. The individual effect of half and recommended levels of P fertilizer showed 2.4 and  $3.50 \text{ g} \cdot \text{pot}^{-1}$  grain yields, respectively. A similar trend in number of total and fertile tillers was observed when compared control.

### 3.4. Physiological Attributes

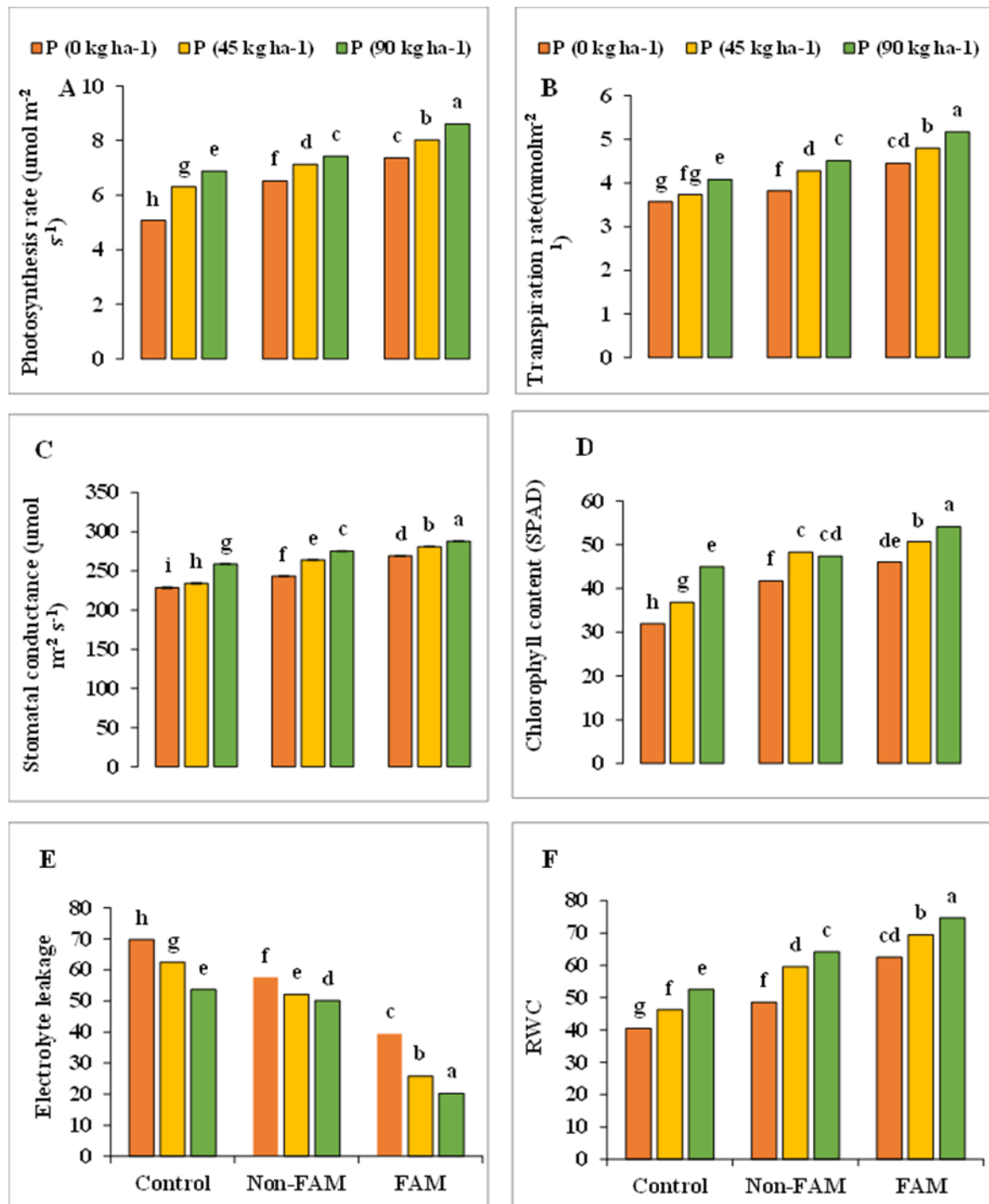
The data for physiological attributes such as RWC (relative water content), chlorophyll content, electrolyte leakage, transpiration rate, photosynthetic rate and stomatal conductance revealed better response in animal manure with 45 and  $90 \text{ kg} \cdot \text{ha}^{-1}$  P compared with the respective controls (Figure 3). The data obtained from consortium application of fermented animal manure and the recommended P amount showed the highest increase in RWC (74.02) compared with other treatments and controls. Compared with controls, non-fermented and fermented manures with 45 and  $90 \text{ kg} \cdot \text{ha}^{-1}$  P, showed higher chlorophyll contents, however, the maximum content (54.13 SPAD) was recorded with fermented manure and  $90 \text{ kg} \cdot \text{ha}^{-1}$  P. The worst condition for electrolyte leakage occurred with control treatment and  $0 \text{ kg} \cdot \text{ha}^{-1}$  P when compared with other treatments. The treatment with the least response was the application of fermented animal manure with  $90 \text{ kg} \cdot \text{ha}^{-1}$  P, which recorded 20.22%. Data regarding the transpiration rate indicated that the maximum increase of  $5.17 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  was observed by combined application of fermented manure and the recommended dose of P fertilizer. A similar trend was observed in the case of photosynthetic rate and stomatal conductance. Combined application of fermented manure and  $90 \text{ kg} \cdot \text{ha}^{-1}$  P showed the maximum photosynthesis and conductance rates ( $8.61 \text{ } \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  and  $287.97 \text{ } \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , respectively) in wheat plants when compared with controls and other treatments.





**Figure 2.** Combined use of fermented/non-fermented animal manure and phosphorus fertilizer on growth and yield parameters of wheat. (A,B) Total and fertile tillers, (C) spike length, (D) 100-grain weight (E) shoot weight, and (F) grain yield. Columns with different letters are significantly different from one another at a level of significance of  $p < 5\%$ .





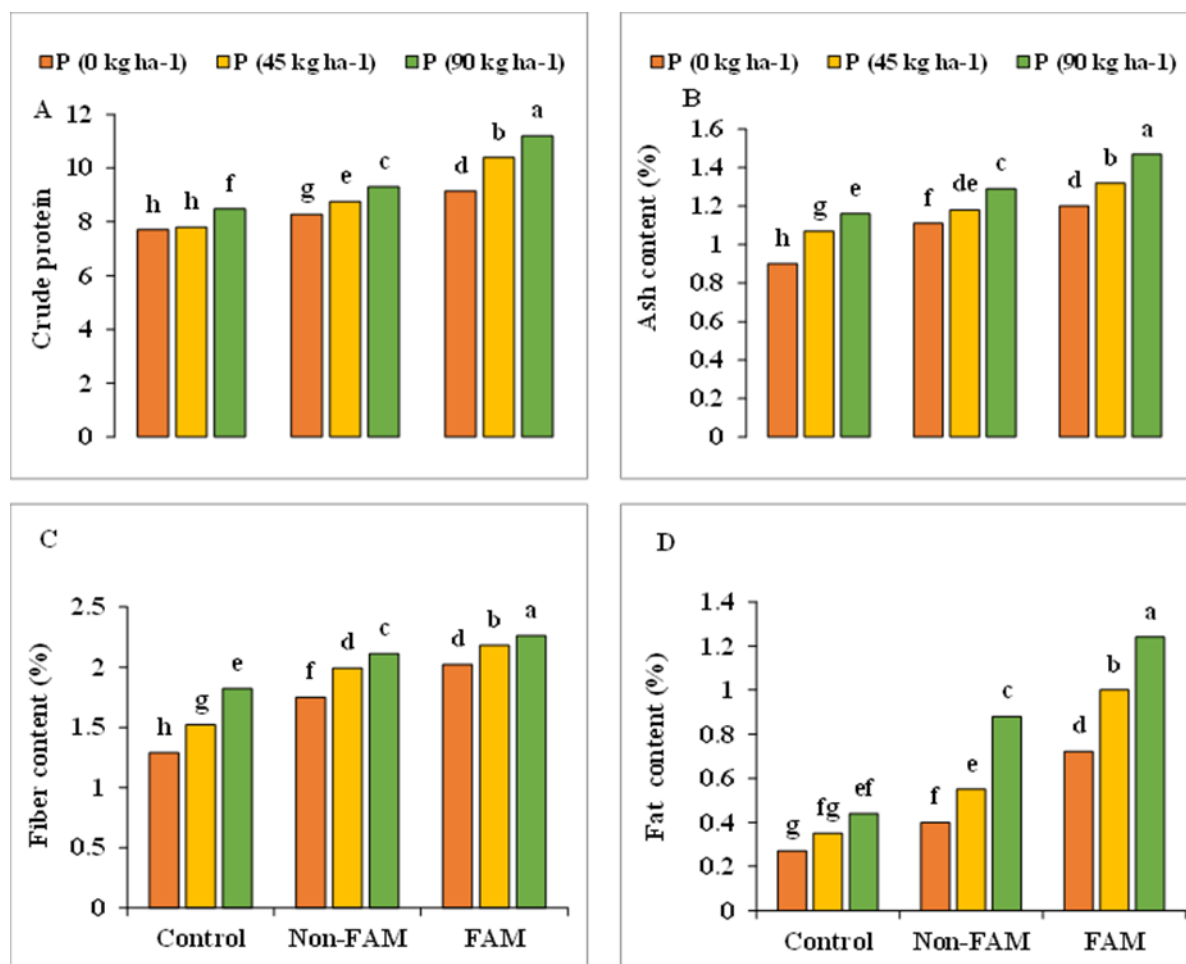
**Figure 3.** Combined use of fermented/non-fermented animal manure and phosphorus fertilizer on physiological parameters of wheat. (A) Photosynthesis, (B) transpiration, (C) stomatal conductance, (D) chlorophyll, (E) electrolyte leakage and (F) relative water content. Columns with different letters are significantly different from one another at a level of significance of  $p < 0.05$ .

### 3.5. Influence of Fermented Animal Manure and DAP Fertilizer on Biochemical Parameters

The data regarding biochemical parameters such as crude protein content, fat content, fiber content and ash content (Figure 4) indicated that the application of fermented animal manure with 90 kg·ha<sup>-1</sup> P to pots significantly increased these parameters over controls as well as non-fermented animal manure. Data regarding crude protein indicated that the maximum content (11.2%) was achieved with fermented manure and the recommended



dose of P fertilizer. In the case of fat content, application of fermented manure with 90 kg·ha<sup>-1</sup> P showed the maximum fat content of 1.24% as compared with other treatments. The same trend was followed for the fiber content and ash content. Fiber and ash contents were higher, i.e., 2.26 and 1.47%, respectively, in the variant with application of fermented animal manure and the recommended dose P fertilizer.



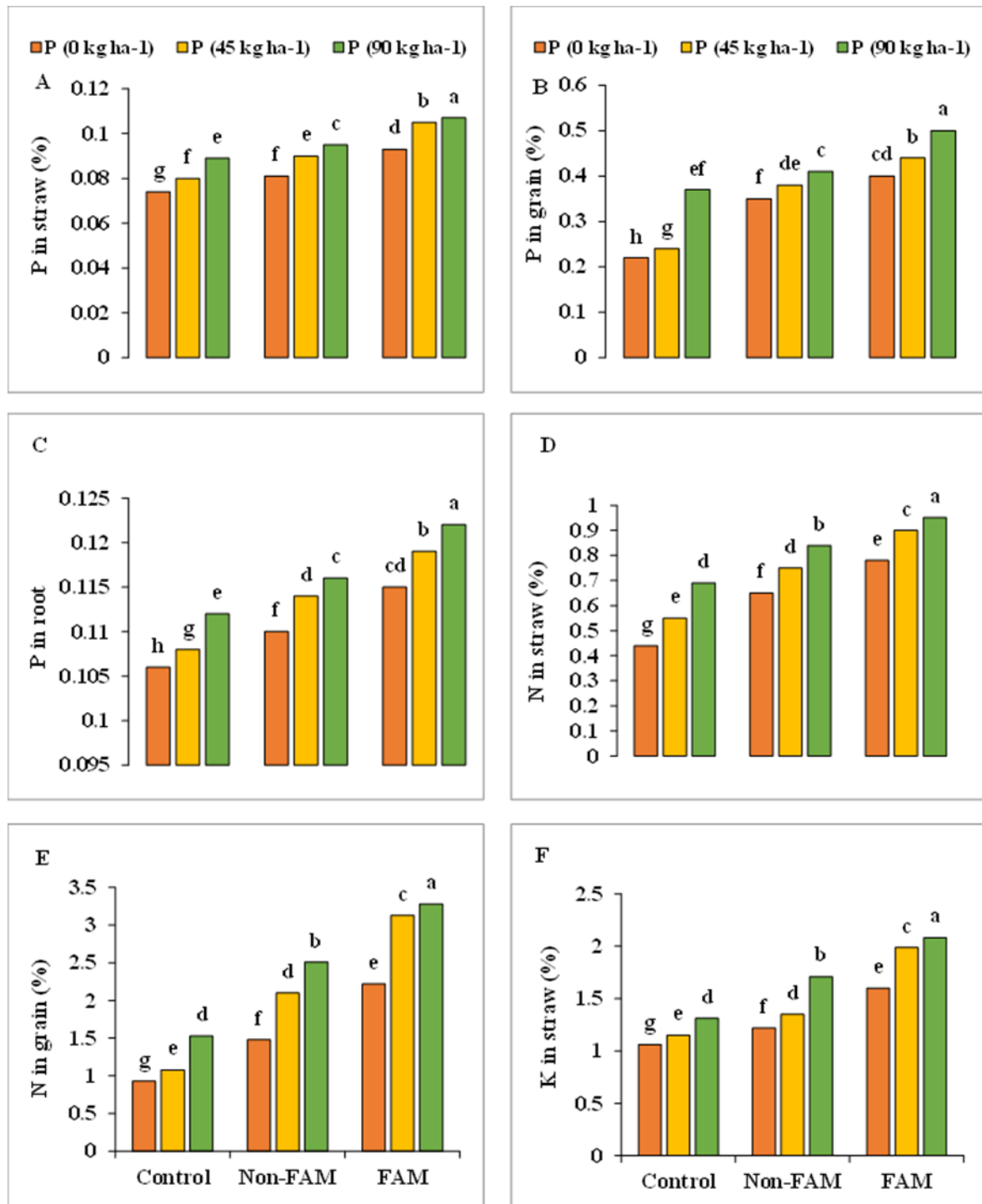
**Figure 4.** Combined use of fermented/non-fermented animal manure and phosphorus fertilizer on the biochemical parameters of wheat. (A) Crude protein content, (B) ash content, (C) fiber content, (D) fat content. Columns with different letters are significantly different from one another at a level of significance of  $p < 5\%$ .

### 3.6. Influence of Fermented Animal Manure and DAP Fertilizer on Chemical Parameters

Application of fermented animal manure with 45 or 90 kg·ha<sup>-1</sup> P increased N, P and K uptake compared with the control, as shown in Figure 5. The highest value for P uptake was recorded at 1.25 g·pot<sup>-1</sup> in the application of fermented animal manure with 90 kg·ha<sup>-1</sup> P. A similar trend was observed in soil P as well as PUE (Figure 6). In the application of fermented animal manure with the recommended P dose, the maximum 16.1 mg P kg<sup>-1</sup> soil was recorded. In the same way, application of manure with both P levels significantly improved PUE, however, maximum PUE (0.856) was achieved in the treatment with the recommended P dose and fermented manure. A similar trend was observed in the case of nitrogen (N) uptake. The application of fermented manure with 90 kg·ha<sup>-1</sup> P showed the highest improvement in N uptake (0.95%) in straw when compared with the other treatments. The maximum concentration of N (3.28%) was recorded when fermented animal manure was added with the recommended dose of P fertilizer. Similarly, fermented manure and the recommended dose of P gave the highest concentration of P in the soil. In the case of

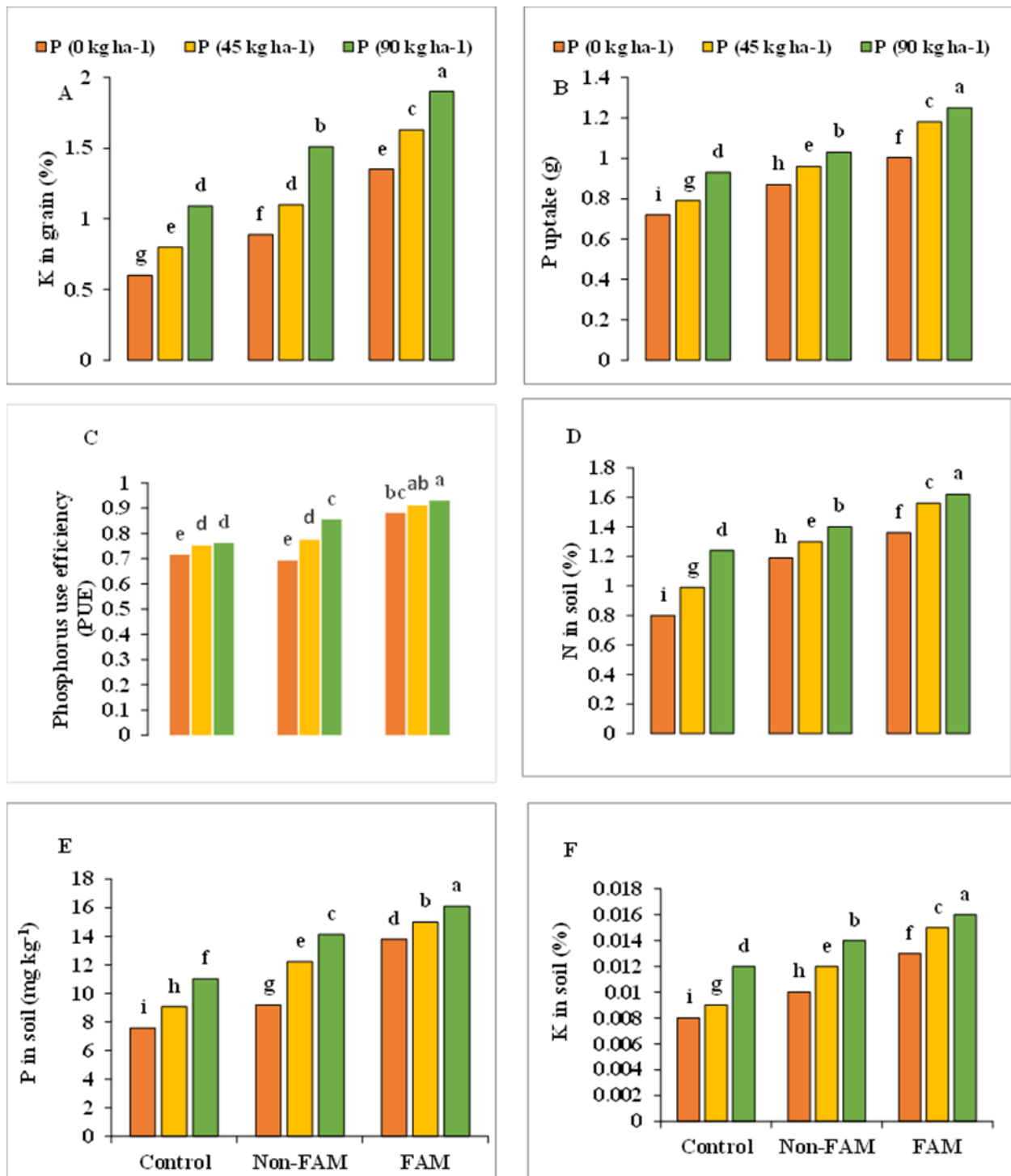


potassium, the application of fermented manure with 90 kg·ha<sup>-1</sup> P markedly improved the K content (2.08%) in straw in comparison with other treatments. The maximum grain K content (1.90%) was observed in the treatment where fermented manure was applied with the recommended dose of P fertilizer. The results show the same trend in soil N (0.016%) by combined application of fermented animal manure and 90 kg·ha<sup>-1</sup> P fertilizer.



**Figure 5.** Combined use of fermented/non-fermented animal manure and phosphorus fertilizer on chemical parameters of wheat. (A–C) P in straw, grain and root, (D,E) N in straw and grain and (F) K in straw. Columns with different letters are significantly different from one another at a level of significance of  $p < 5\%$ .





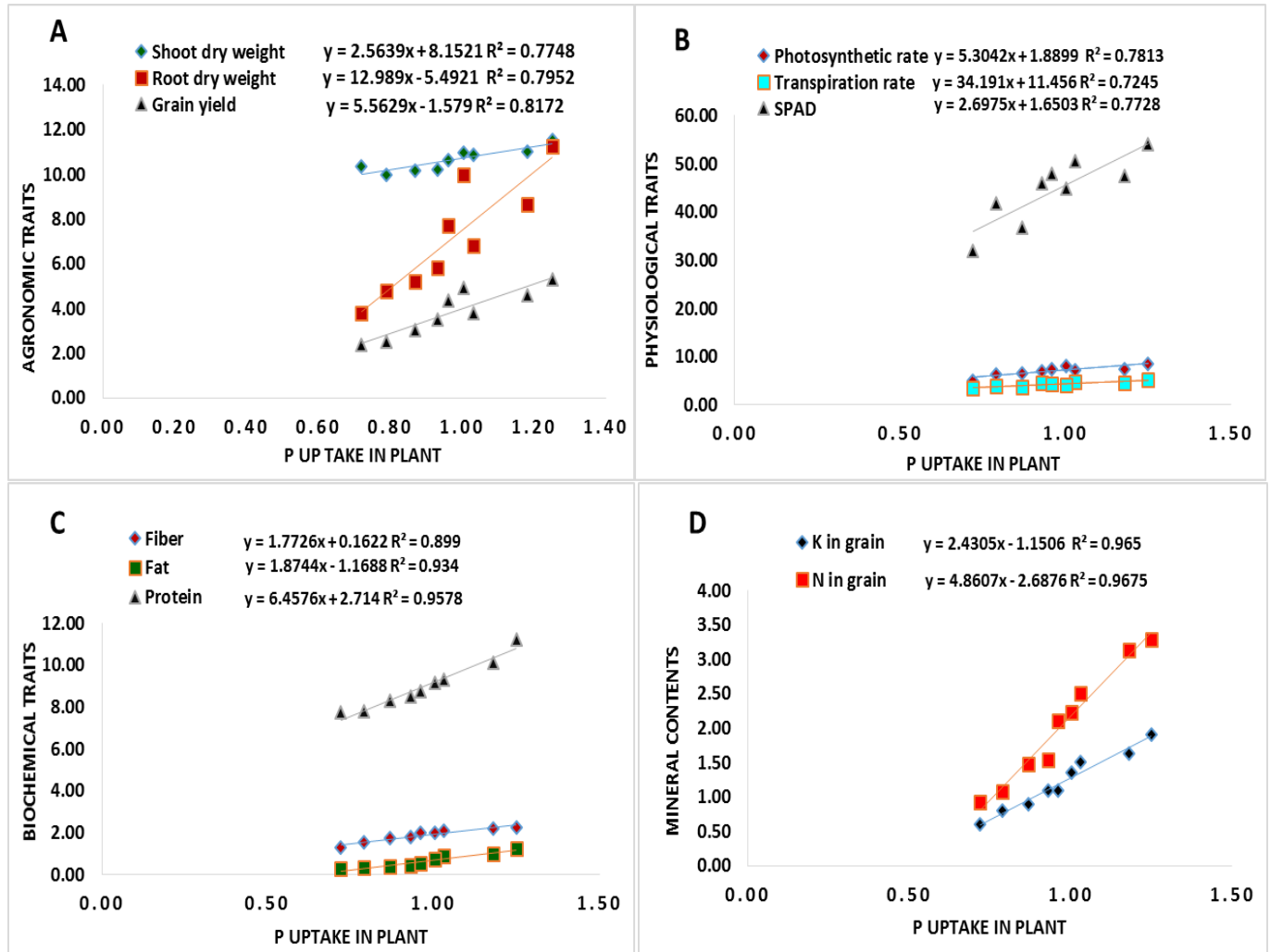
**Figure 6.** Combined use of fermented/non-fermented animal manure and phosphorus fertilizer on biochemical parameters of wheat. (A) K in grain, (B) P uptake, (C) P use efficiency, (D–F) NPK in soil. Columns with different letters are significantly different from one another at a level of significance of  $p < 5\%$ .

### 3.7. Regression and Heat Map Analyses

The plant P uptake was highly significantly correlated with wheat agronomic parameters, with  $R^2$  values ranging from 0.7748–0.8172 (Figure 7A). Moreover, the observed physiological parameters were also positively associated with P uptake, with  $R^2$  values ranging from 0.7245–0.7813 (Figure 7B). The biochemical attributes related to fiber and



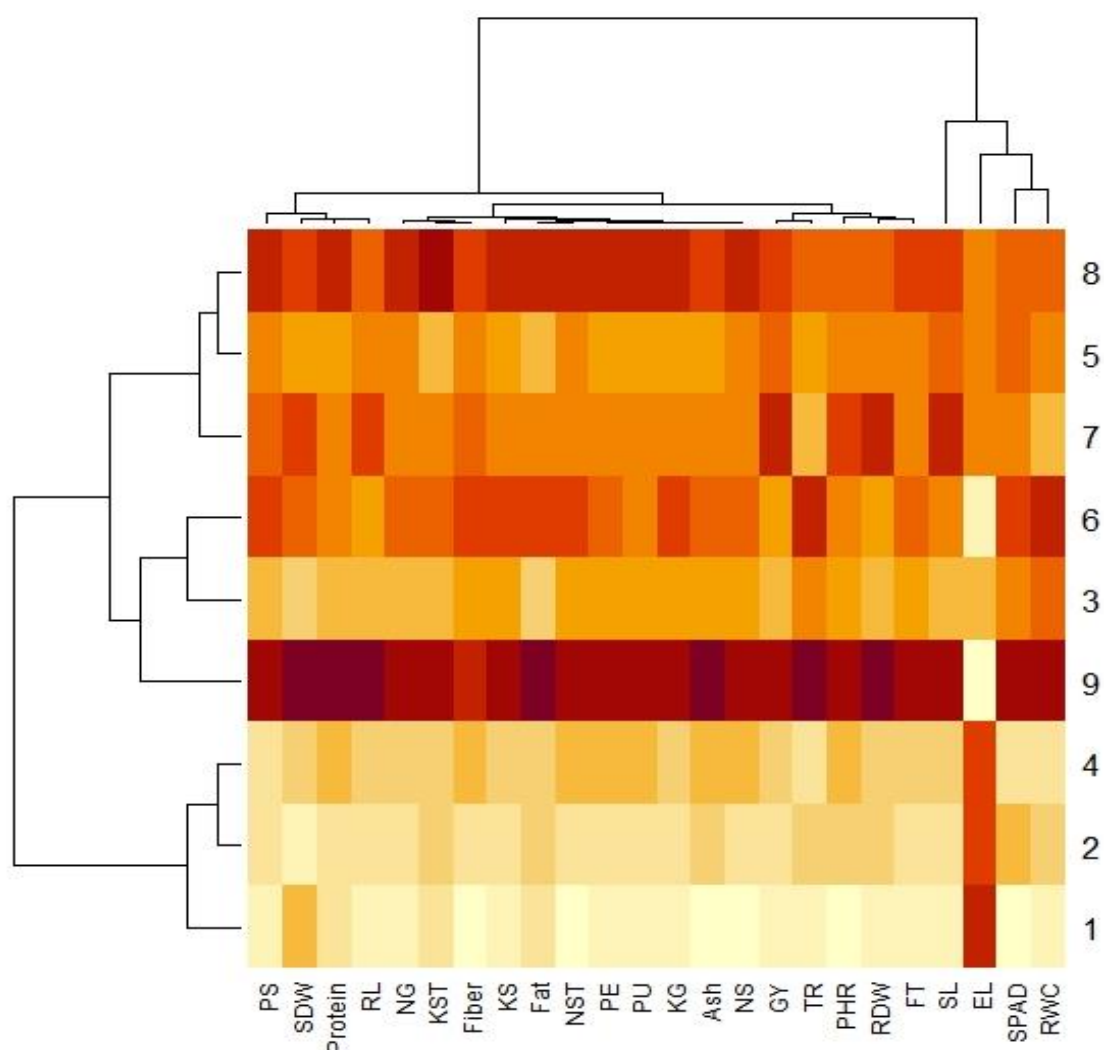
fat and protein contents were highly significantly and positively correlated with P uptake (Figure 7C). The mineral content (K and N in grains of wheat) showed the most significant and positive correlations with P uptake, with  $R^2$  ranging from 0.965–0.9675 (Figure 7D).



**Figure 7.** Relationships between plant P uptake and, (A) agronomic attributes, (B) physiological attributes, (C) biochemical attributes, and (D) mineral contents of wheat.

Moreover, a two-dimensional visual relationship between the applied treatments and the observed parameters was built as a hierarchical dendrogram (Figure 8). The dendrogram shows the relationship between treatments (rows) and observed plant growth, physiological, biochemical and mineral content attributes. The row hierarchical dendrogram clearly differentiated treatment 9 (fermented manure with  $90 \text{ kg} \cdot \text{ha}^{-1} \text{ P}$ ). This suggests that this treatment was most efficient for improving the observed plant and soil parameters.





**Figure 8.** Heat map analysis based on the correlation of the observed variables under applied fermented and non-fermented manures with different levels of phosphorous. Colour intensity reflects level of significance among different treatments.

#### 4. Discussion

It was observed in the incubation experiment that the pH of manure was decreased in the variants where crude sources of carbon, protein, starch, fat and hydrolytic (cellulase)-enzyme-producing bacteria were added (Table 2). In incubation, we believe that methane was produced in all treatments except for T<sub>9</sub>. This may be due to those hydrolytic enzymes breaking down the complex materials to simple organic compounds that were further converted into volatile fatty acids (acetic acid, propionic acid) by acidogenic bacteria [46,47]. It is likely that due to the toxic effects of organic and fatty acids, methane production was abolished in the T<sub>9</sub> treatment. This sudden decrease in pH reduced the efficiency of anaerobic digestion [48]. Moukaize [49] noted that the shortcoming of the decrease in pH induced by the accumulation of volatile fatty acids is expected to be alleviated by the role of ammonia nitrogen as an alkalinity buffer in feedstock, such as biochemical methane potential and semi continuous assay, used in his study.

In the present study, through the application of organic fermented animal manure with inorganic phosphorus fertilizer (DAP), a positive effect on growth parameters was observed (Figures 1 and 2). However, it has been found that the contribution of fermented manure to crop yield tended to be higher in the case of nutrient shortages. Fermented manure application was identified as the key cause of variability in soil fertility and maize yield response to fertilizer application in the smallholder farming systems of western



Kenya [50]. The explanation for this is that when mineral fertilizer was lacking, fermented manure had a relatively more important role in total nutrient input so that crop yield will not be greatly affected. This was further supported by the obtained results, whereby enhanced micronutrients and macronutrients were observed under fermented animal manure treatments (Table 2). This might be the other reason for the enhanced responses in soil fertility and crop growth under applied amendments. This increment in plant nutrients under fermented manure might be due to the activity of bacteria that might have produced organic acids, resulting in enhanced dissolution of nutrients into the final product [51,52].

However, when organic fertilizer was used as a corresponding nutrient source with chemical fertilizer, it would increase the contribution of fertilizers to yield, thus decreasing yield variability of wheat [53]. Our results show that fermented manure application strongly affected crop yield by increasing soil P supply and plant P uptake. The higher P supply could be as a result of less transfer from labile to stable P pools [54,55], and the weaker P fixation due to the higher organic matter content [56]), as fermented manure is the principal source of organic input in the western Kenya region [57,58]. Our results are further supported by the heat map analysis, which also showed the fermented manure combined with the recommended dose of P as the most influential strategy affecting crop growth and yield formation attributes (Figure 8).

Earlier, it was documented that leaf chlorophyll content, relative water content, photosynthesis rate, transpiration rate, stomatal conductance and soil physical attributes were improved with the addition of organic amendments and inorganic amendments. One possible explanation might be due to the role of organic amendments in improving soil biological activities that are directly involved in nutrient uptake and ultimately improve growth and yield parameters [59–62]. The improvement in soil microbial activity enhances soil nutrient mineralization and ultimately improves nutrient availability and their uptake into crop plants [63,64]. Notably, application of fermented manure alone resulted in a relatively low crop yield because the manure came from within the farmland system and its amount was limited. Furthermore, the highest wheat grain yield was obtained with addition of organic manure along with inorganic fertilizers and this might be due to the improvement in the soil organic matter content, which supplied not only the additional quantities of NPK directly, but also secondary and micronutrients that were limited in the soil [65,66].

Biochemical parameters such as protein, crude fat, fiber and ash contents were higher in treatments where organic and inorganic sources were applied together in the form of fermented animal manure + 90 kg·ha<sup>−1</sup> DAP (Figure 4). The improvement in protein content can be attributed to its important role in providing secondary and micronutrients along with primary nutrients and improving the physical chemical and biological properties. Similar to our results are the findings of Liu et al. [67] and Saha and Mondal [68]. Fat content in wheat may be higher due to the balanced supply of nutrition. The calculation is same as that reported by Singh et al. [69] and Patil et al. [70]. Wheat contains higher amounts of bran portions, which also results in higher ash content compared with other treatments. Nitika and Khetarpaul [71] reported that the ash content in wheat varieties grown under organic and inorganic conditions ranged from 1.82 to 2.14%. Likewise, the maximum fiber content was recorded when 100% P was applied with the processed manure, which might be due to the better growth and uptake of nutrients resulting from the balanced use of fertilizer with an organic amendment. Similar to our results, Chauhan et al. [72] reported improved productivity and biochemical attributes of wheat through integrated use of organic and inorganic fertilizers.

Chemical parameters, including EC (electrical conductivity), pH, N, P and K were measured in treatments where organic and inorganic sources were applied together in the form of fermented animal manure and P fertilizer. Our results indicated that combined application of fermented animal manure and chemical P fertilizers increased phosphorus and nitrogen concentrations in plants in higher amounts than sole application of either organic or inorganic P sources as well as controls. This might be attributed to the improved growth of roots by fermented animal manure addition that ensures improved nutrient



acquisition from soil and higher microbial activity. Similar findings were revealed by Dordas et al. [73], where he added cattle manure and observed higher nutrient uptake and yields of maize compared with their sole applications. The increase in N, P and K uptake noted in the present study might be due to the indirect improvement of soil physicochemical properties. On the one hand, manure could improve soil physical structure and water conditions, thereby promoting crop growth and nutrient acquisition [74]. K uptake in the shoot might be due to the stimulatory effect of organic materials on the cation exchange capacity (CEC) of soil. On the other hand, with the mineralization and decomposition of organic matter, partial nutrients including P will be released for crop utilization. Several studies indicated the conversion of non-soluble P to soluble P could be achieved in the case of manure application [75,76]. The use efficiency of P fertilizer usually ranges from 10 to 30% during the growing season of application [77]. Thus, most residual fertilizer P remains in the soil owing to the strong holding capacity of soil and continues to exert its residual effect for crops [78,79]. Higher P uptake and use efficiency were recorded through combined application of fermented manure and DAP fertilizer when compared with other treatments. Regression analysis further suggested that the fermented animal manure combined with DAP had positive influences on plant growth, physiological, mineral and biochemical attributes as revealed by the existence of highly positive and significant relationships (Figure 7A–D). These findings support the premise that use of fermented manure adds to the effect of phosphorus fertilizer by enhancing crop productivity and reducing P fixation in calcareous soil.

## 5. Conclusions

Based on our findings, application of fermented animal manure with the recommended amount of DAP significantly improved the growth, yield and phosphorus use efficiency of wheat crops as compared with the controls and use of non-fermented animal manure, which suggests that the inclusion of animal manure with mineral fertilization could be an attractive approach for sustaining crop yields. Similarly, combined application of organic and inorganic amendments also improved the nutrient status of soil and plants by a temporary change in the pH of the soil and by enhancing the use efficiency of applied fertilizers. Thus, this approach could be an innovative strategy for enhancing wheat productivity; however, multi-site field trials need to be performed to warrant successful performance in the field.

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