

Combination of Rhizobium and Biochar with Iron Fertilizers Boosted Glyphosate-Contaminated Maize Growth

Diego Oswaldo Cumbicos Ortega^{1*}

¹Instituto Superior Tecnológico Los Andes, Loja, Ecuador

KEY WORDS

Nutrient's deficiency,
Mineral fertilizers,
Rhizobium, Biochar,
Yield

ABSTRACT

The broad-spectrum herbicide glyphosate is used globally to kill agricultural weeds. Since the 1970s, Reduced crop productivity due to a lack of nutrients and synthetic fertilisers drove up production costs and prompted researchers to look for new ways to boost crop yields and quality. The purpose of this study was to evaluate the effectiveness of iron supplements with organic fertilisers in maintaining maize (*Zea mays* L.) yields in the face of glyphosate contamination. For this purpose, 24 different pots were selected according to treatment detail and placed them by following completely randomized design (CRD). The chosen pots were treated with 25 g of biochar and 50 ppm of rhizobium before being planted. Iron sulphate was then injected, at a rate of 2.5 tonnes per hectare. The results demonstrated that the agronomic and physiological features of maize were greatly improved by the addition of biochar and rhizobium with iron fertilisers, both individually and in combination with glyphosate. In contrast to the control treatment which was contaminated with glyphosate, the combination of iron fertilizer, rhizobium, and biochar resulted in a significant improvement of 69.8% in root length, 46.9% in shoot length, 65.0% in root fresh weight, 52.2% in shoot fresh weight, and 45.5% in shoot dry weight in maize plants. In glyphosate-contaminated soil, their combined application resulted in increases of 42.8%, 24.5%, 41.6%, 23.9%, and 46.4% in chlorophyll contents, quantum yield, photosynthetically active radiation, fluorescence yield, and electron transport reaction, respectively, compared to the control. The biochemical composition may also be enhanced in contrast to the control with the co-addition of iron fertilisers with rhizobium and biochar. Based on the findings presented here, it appears that iron fertilisers applied in conjunction with rhizobium and biochar can improve soil properties and promote the growth, yield, and quality of maize plants.



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Corresponding Author Email: diegooswalda234@gmail.com

1. Introduction

Globally, maize production consistently outpaces that of other grains (Blomme et al., 2023). It ranks third in Pakistan and is the first significant grain crop globally. Every portion of the maize plant is being put to good use, including human food, poultry feed, and animal fodder. In addition to having a low-fat content (4%), it is a meal that is rich in protein (10%), dietary fiber (72%), and calorie density (365 kcal/100g). Shete et al. (2023) lists its many industrial uses, including in corn oil, sweeteners, sorbitol, starch, liquid glucose, ethanol, and corn gluten. The wet milling method produces a watery by-product known as steep corn liquor. Wood pellets made from its straw are a source of renewable energy.

The increasing use of herbicides in the quest for better and more sustainable agriculture has led to the pollution of agricultural soils by organic and inorganic contaminants. According to Lacroix and Kurrasch (2023), glyphosate is a non-selective herbicide that is applied after a plant has emerged. It kills annual and perennial weeds by

preventing them from making tyrosine and tryptophan, which are important amino acids. The widespread usage of glyphosate has devastating effects on bacterial populations, crops, and cattle. Since glyphosate eliminates several species and increases the variety of phytopathogenic fungus, it changes the diversity of soil microbes. The trifunctional chemical glycolate may serve as both an acid and a base due to its amino, carboxyl, and phosphonate groups. Glyphosate blocks the synthesis of plant growth regulators (Rawat et al., 2023). Products containing glyphosate may cause skin and eye irritation.

With biochar, farmers should expect an improvement in the soil's physical and chemical qualities, which might lead to noticeable effects right away (Kumari et al., 2016). Because of these benefits, agricultural industry sees an instantaneous increase in biomass and grain yield. As an example, when applied to fields, biochar enhanced agricultural output by an average of 10% according to a quantitative assessment by Jeffery et al. (2011). Given that the biochar fraction has a constant, very small quantity of N, this is particularly true. Song and Kim (2023) found that applying 20 and 40 Mg ha⁻¹ of wheat straw biochar to maize

(*Zea mays* L.) plants enhanced grain output by 8.8 and 12.1%, respectively. The sorption properties of biochar were unaffected by soil glyphosate complexation with iron and copper prior to biochar pyrolysis (Bogale et al., 2023). Mayakaduwa et al. (2016) states that biochar may be used to extract glyphosate from soil that has been polluted. Herath et al. (2019) came to a similar conclusion, stating that response surface techniques might be used to statistically optimize the glyphosate adsorption by biochar and activated carbon. Iron is an essential micronutrient for almost all forms of life due to its role in many metabolic processes, including DNA synthesis, respiration, and photosynthesis. Additionally, iron triggers a variety of metabolic processes and is a component of the prosthetic groups of several enzymes. As an added bonus, iron metal may speed up the cleaning procedure. Furthermore, weeds would impede crop development and the many enzyme reactions that may be enhanced by adding iron or zinc, according to the pesticides used in agricultural areas. Being an integral part of several important enzymes—including the electron transport chain's cytochromes—it is required for many biological activities. Ozturk et al. (2008) discovered that glyphosate inhibits ferric reductase activity in roots of sunflowers that are iron deficient. Rhizobium and Azospirillum-treated crop plants showed improved physiology, nutritional status, and growth (Chi et al., 2005). Research conducted by Liu et al. (1991) indicates that the majority of Rhizobiaceae species can break down glyphosate. They may also increase plant growth in unfavorable (Afzal et al., 2020) and water-stressed (Bano et al., 2013) environments, and they can produce and dissolve indole 3-acetic acid (IAA) in the rhizosphere. Rhizobium controls infections and the struggle for nutrients and space, which indirectly helps non-leguminous plants flourish. According to Ansary et al. (2012), plants that were exposed to *Pseudomonas* showed an increase in proline, sugar, and amino acid synthesis, a reduction in leaf water losses, and improvements in root-tissue ratio, water content, aggregate stability, and water potential.

To tackle these problems, scientists need to establish the best way to apply glyphosate to agricultural fields for weed control and figure out how to break it down in soil using both organic (biochar and rhizobium) and inorganic (fertilizer) materials so that plants can't absorb it. Finding out how soil properties and maize growth were affected by iron, rhizobium, and biochar fertilisers was the main objective of the research.

2. Materials and methods

A pot experiment was conducted with maize crops to assess the effects of organic amendments such as biochar and rhizobium with inorganic iron fertilizer (Fe sulfate) on soil quality and growth. This research was at Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad. Soil was extracted from the same testing location. After collection, the dirt was well mixed and let to dry naturally before sieving. A suitable sieve size of 2 mm was used to capture the soil sample (Blaud et al., 2017). Then, up to a final dosage of 900 mg/kg of glyphosate, the soil was spiked with Roudup (Kamdern et al., 2016). After that, 10 kg of earth that had been sieved was added to the pot (Sadiq and Hussain, 1993) and allowed to dry. Corn seeds (DK-6714) bought in Huancavelica, Peru, were used in the experiment. We sourced the rhizobium from the microbiology lab, and the charcoal and iron sulphate from the relevant agronomy farm. We used 25 g of biochar and 50 ppm of rhizobium to treat the selected pots before planting. Afterwards, 2.5 metric tons of iron sulfate per acre was injected. Table S1 in the supplemental materials shows the study's

treatment details. Following the technique, the seeds were planted in the pots after the enriched soil had been added. Every container was seeded with three maize seeds. After germination, the maize was trimmed so that each pot could contain one plant. Prior to planting, the recommended dosages of P (6.53 g), N (2.5 g), and K (0.927 g) for maize crops were introduced using the following sources: single super phosphate, urea, and muriate of potash, in that order. We added K and P as we were planting, and we broke the N application into three parts. By consistently establishing and maintaining the field capacity at 70%, the water level of the optimum crop was maintained. In order to keep the field at 70% capacity, 875 cc of water were used. From that point on, the water level was kept daily at the specified field capacity.

2.1. Harvesting of crop

We collected potential parameters throughout plant life and some at the conclusion of the experiment during harvesting of the maize crop, which was harvested at physiological maturity. The plants were carefully selected from the middle rows of each pot and tagged with tape for assessment in order to minimize the impact of border effects. A number of characteristics were evaluated once the crop was harvested 110 days after planting. Then, the right technique and instrument were used to determine the agronomic parameters.

2.2. Physiological parameters

The SPAD meter was used to determine physiological parameters, such as chlorophyll content, in the maize plant. The instrument MINI-PAM-II (WALZ, Germany) was used to note electron transport reaction (ETR), quantum yield (YII), photosynthetic active radiation (PAR), and fluorescence yield (Ft). Simultaneously, the IRGA device captured other physiological variables like as transpirational rate, stomatal conductance, and sub-stomatal conductance.

2.3. Chemical parameters

We acquired the soil-saturated extract by pressing down on the chamber that had soil paste added to it. After filtering, we extracted the extract. The soil extract was kept intact by reducing the amount of salt that precipitated by adding one drop of sodium hexametaphosphate to twenty-five milliliters of extract. The ICARDA handbook outlined a process that was followed to assess the NPK and iron in soil samples.

To find out how much organic stuff is in a sample, scientists utilize the Walkley black protocol (Walkley, 1947). One gram of dirt and ten milliliters of potassium dichromate ($K_2Cr_2O_7$) were combined in a five-milliliter conical flask. The next step was to add 20 ml of sulfuric acid, stir the mixture well, and then set the flask mixture away for 30 minutes. The next step was to chill the mixture before adding 200 ml of tap water and a 10 ml solution of phosphoric acid to the same flask. Using diphenylamine as a color indicator, the solution was titrated against 0.5 M $FeSO_4$ until the color changed from violet-blue to green after cooling. Their ferrous ammonium sulfate use was recorded.

2.4. Calculations

Oxidizable carbon = $(0.38 * \text{Blank sample} * \text{molarity of } FeSO_4) / \text{Soil weight}$ 1

2.5. Total organic carbon

%Total organic carbon = $1.334 * \text{oxidizable carbon}$ 2

%O.M. = $1.723 * \text{TOC}$ 3

2.6. Statistical analysis

The experimental data was analyzed statistically (Statistix 8.1) to compare the various treatments. Here, we used a 5% probability level LSD test for our analysis.

3. Results

Organic fertilizers and microbial treatment considerably raised the germination percentage, according to the results of the present research

(Figure 1).

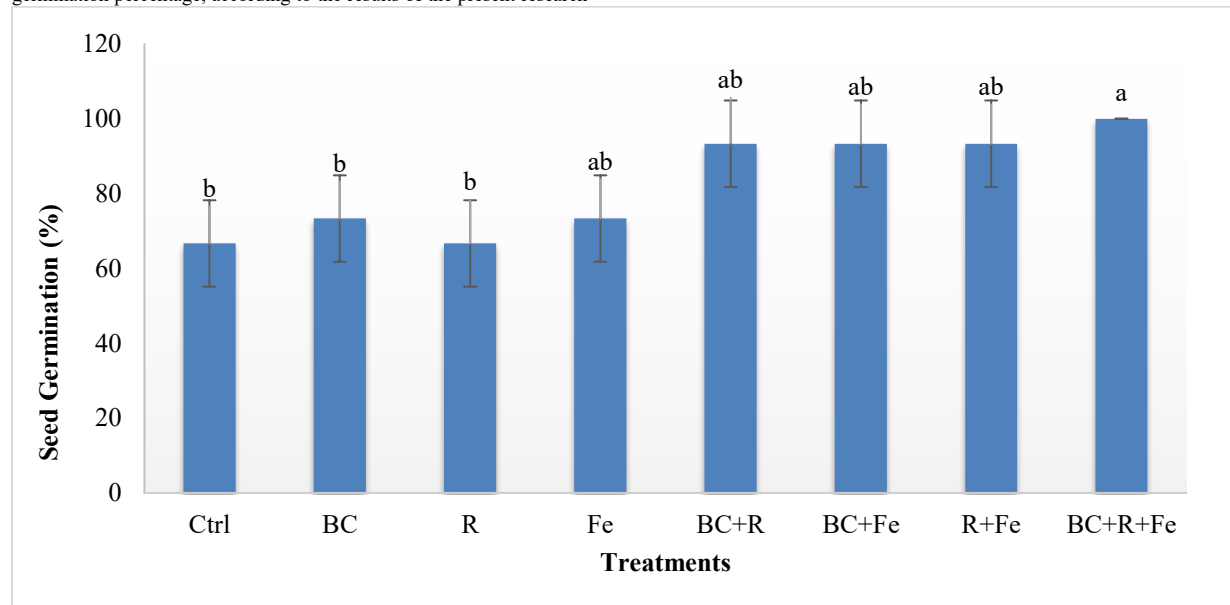


Figure 1 How organic fertilizer and microbial treatment affect the percentage of germinated maize plant seeds. Ctrl denotes control, R stands for rhizobium, Fe denotes iron sulfate, and BC denotes biochar. The LSD test indicates that means with different letters are substantially different at $P \leq 0.05$.

Table 1 shows that other maize plant characteristics were positively affected by the mixed application of biochar rhizobium and iron fertilisers, in addition to increasing plant diameter, leaf number, and fresh and dry weight per plant in glyphosate-contaminated environments. When comparing the control treatment to one that included rhizobium and biochar in addition to iron sulphate under glyphosate contamination, we found that root length, shoot length, stem diameter, and the number of leaves were all improved by 40%, 70%,

32.8%, and 64.0%, respectively. When compared to the control plots contaminated with glyphosate, the treatments with iron sulfate and rhizobium showed the second-best results, increasing the root dry weight by 45.5%, the shoot fresh weight by 65%, and the root fresh per plant by 52.3%. Table 1 shows that compared to the control, the crops whose growth and development were enhanced by the separate applications of compost and cytokinins fared better.

Table 1 Effects of organic fertilizers along with iron sulphate on maize agronomic parameters.

Treatments	Shoot length	Root fresh weight	Root length	Shoot fresh weight	Shoot dry weight	Number of leaves	Root dry weight	Stem diameter
Ctrl	100±19.7c	63.0±10.0c	70.0±6.34c	208±16.1d	103±7.00d	9.33±0.57	44.0±10.0c	6.50±0.10d
BC	138±15.3b	78.0±4.55b	86.7±11.7cd	238±19.6bc	112±6.42abc	12.7±1.01	53.7±3.78bcd	7.21±0.11cd
R	136±16.6b	73.7±11.9bc	83.3±14.4cd	224±12.8cd	107±10.0bcd	11.0±1.15	52.0±3.01cd	6.73±0.11d
Fe	133±10.4b	70.7±8.50bc	80.7±2.91de	218±18.6cd	125±10.1cd	10.7±1.42	50.3±2.08de	6.65±0.39d
BC+R	139±15.2b	80.0±10.0b	90±8.01bcd	243±6.66bc	123±13.5abc	13.0±0.59	58.3±2.08abc	7.43±0.21bcd
BC+Fe	153±12.1ab	83.0±7.21ab	96±9.56abc	257±12.4ab	130±17.1ab	13.0±1.00	59.7±3.21ab	8.07±0.05abc
R+Fe	166±7.13a	85.3±4.51ab	101±6.58ab	257±20.7ab	132±9.16ab	14.7±1.21	61.0±1.03ab	8.40±0.79ab
BC+R+Fe	170±6.62a	96.7±9.50a	110±8.14a	274±13.1a	137±10.7a	15.3±1.02	64.7±3.51a	8.63±1.52a

BC; Biochar, R; Rhizobium, Fe; Iron. Ctrl, control; R, rhizobium; Fe, Iron sulphate; BC, biochar. The LSD test indicates that means with different letters are substantially different at $P \leq 0.05$.

Similarly, as compared to the control group, maize crops amended with rhizobium, biochar, and iron fertilizers showed a considerable

improvement in their relative water levels, even when glyphosate contamination was present (Figure 2).

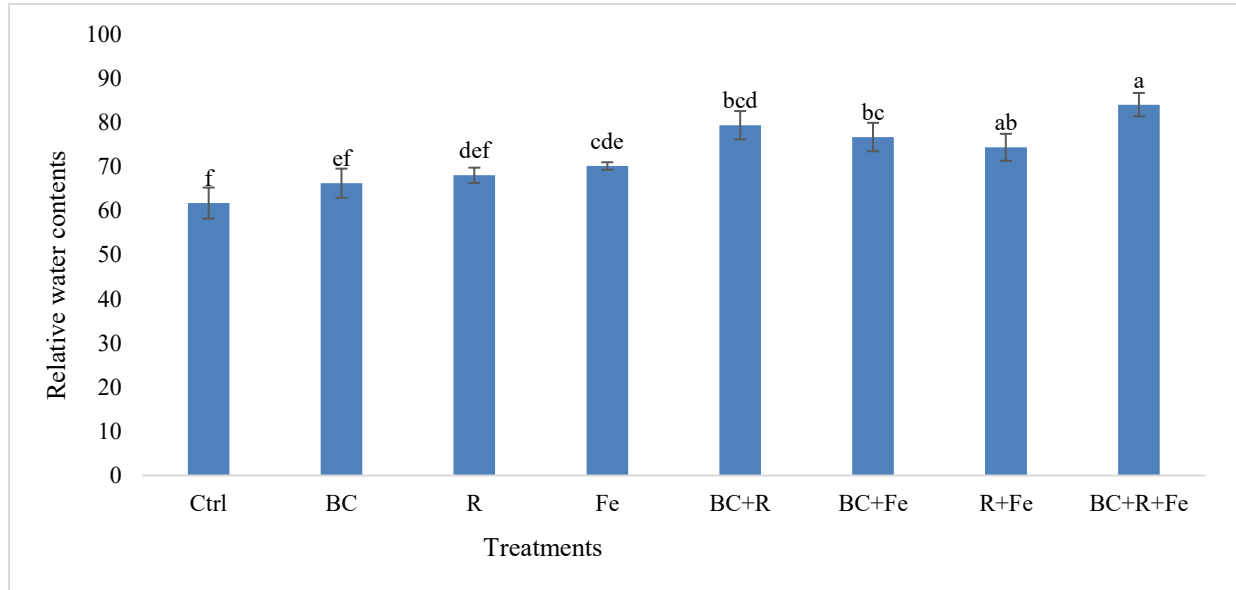


Figure 2 Effects of organic and inorganic iron fertilizers on maize crop relative water contents. Ctrl, control; R, rhizobium; Fe, Iron sulphate; BC, biochar. LSD test indicates that means with different letters are substantially different at $P \leq 0.05$.

The results demonstrated that in glyphosate-contaminated plants, the control treatment exhibited no change in response to several treatments, including rhizobium, iron, and charcoal fertilizers. The physiological traits of the maize crop were greatly enhanced by all of the supplements, both organic and inorganic. When applied to soil that contained glyphosate, organic fertilizer with iron supplements increased the following yields in maize plants: fluorescence yield (23.9%), quantum yield (25.5%), chlorophyll content (42.8%), electron

transport reaction (46.4%), and photosynthetically active radiation (41.7%). Iron supplements with rhizobium came in second. In terms of enhanced physiological metrics compared to the control, microbial, synthetic, and biochar supplementation alone also performed better. Table 3 shows that the physiological features of maize (photosynthesis rate, transpiration rate, stomatal conductance, and sub-stomatal conductance) were significantly enhanced and improved when inorganic and organic fertilizers were mixed.

Table 2 Changes in maize plant physiology brought about by organic and inorganic fertilizers

Treatments	Fluorescence yield	PAR	SC	YII	TR	Chlorophyll contents	ETR
Ctrl	589±22.0d	72.0±2.65e	312±2.52c	0.804±0.007g	0.30±0.05c	58.6±3.82d	84.0±12.0d
BC	640±15.2bc	84.0±6.01d	327±12.6abc	0.840±0.015d	0.41±0.08abc	71.6±3.69abc	102.8±9.46abcd
R	613±16.1cd	80.0±5.03d	323±10.4abc	0.835±0.013e	0.38±0.03abc	69.7±2.81c	95.7±5.50bcd
Fe	603±10.1cd	78.3±6.02cd	319±12.4bc	0.824±0.009f	0.33±0.06bc	68.4±2.07cd	89±5.56cd
BC+R	681±12.6ab	90.0±2.64c	332±8.18abc	0.856±0.022d	0.40±0.05abc	72.7±2.68ab	110±6.24abc
BC+Fe	689±35.8ab	94.7±3.05bc	338±16.1abc	0.886±0.016c	0.43±0.03abc	75.1±2.57ab	113±10.7abc
R+Fe	700±20.0a	98.7±3.52b	346±14.0ab	0.949±0.069b	0.44±0.02ab	79.1±3.85a	119±10.0ab
BC+R+Fe	730±26.5a	102±3.76a	355±8.54a	1.001±0.119a	0.49±0.04a	83.7±8.25a	123±12.0a

Ctrl, control; R, rhizobium; Fe, Iron sulphate; BC, biochar. The LSD test indicates that means with different letters are substantially different at $P \leq 0.05$.

According to the results, using biochar and iron sulphate rhizobium had a significant impact on the soil's chemical properties (Table 2). After harvest, the various versions had a noticeable impact on soil organic carbon, organic matter, N, K, and P levels, even when exposed to glyphosate (Table 3). Soil organic matter levels ranged from 0.86% to 1.47% after applying rhizobium, biochar, and iron sulphate, with the

experimental treatment achieving the highest rate of growth. In a similar vein, all other quantifiable chemical metrics did as well. All of these soil properties showed a significant connection at various treatment doses ($P < 0.05$). Soil organic carbon, soil nitrogen, and organic matter were all significantly increased by the organic additions, which also improved soil properties (Table 3).

Table 3 Effects of organic and inorganic supplements on soil characteristics of maize plants

Treatments	Soil N	Soil P	Organic carbon	pH	Soil K	Organic matter (%)
Ctrl	6.34±0.05f	6.5±0.10e	0.57±0.04e	8.43 ± 0.047a	108±1.0d	0.86±12.03
BC	6.73±0.03de	6.88±0.02d	0.72±0.05bcde	8.08 ± 0.063d	118±2.1c	1.23±0.04bcde
R	6.58±0.05ef	6.77±0.03d	0.68±0.05cde	8.14 ± 0.032bc	113±2.6cd	1.16±0.08cde
Fe	6.45±0.04ef	6.61±0.01e	0.67±0.03de	8.21 ± 0.012b	111±2.7d	1.15±0.05de

BC+R	6.94±0.05cd	7.07±0.03c	0.78±0.04abcd	8.01 ± 0.154d	134±2.9b	1.34±0.02abc
BC+Fe	7.13±0.09c	7.21±0.06bc	0.79±0.02abc	7.99± 0.070de	137±3.2ab	1.35±0.04abc
R+Fe	7.49±0.22b	7.31±0.04b	0.81±0.03ab	7.86±0.002e	138±2.5ab	1.39±0.03ab
BC+R+Fe	7.86±0.13a	7.46±0.14a	0.86±0.05a	7.84±0.023e	143±3.0a	1.47±0.01a

Ctrl, control; R, rhizobium; Fe, Iron sulphate; BC, biochar. The LSD test indicates that means with different letters are substantially different at $P \leq 0.05$.

3.1. Correlation

Correlation analysis showed that there was a significant ($P < 0.01$) association among the growth, physiological, and biochemical

parameters of maize plants under glyphosate contamination. Figure 3 revealed a correlation matrix graphically by corplot.

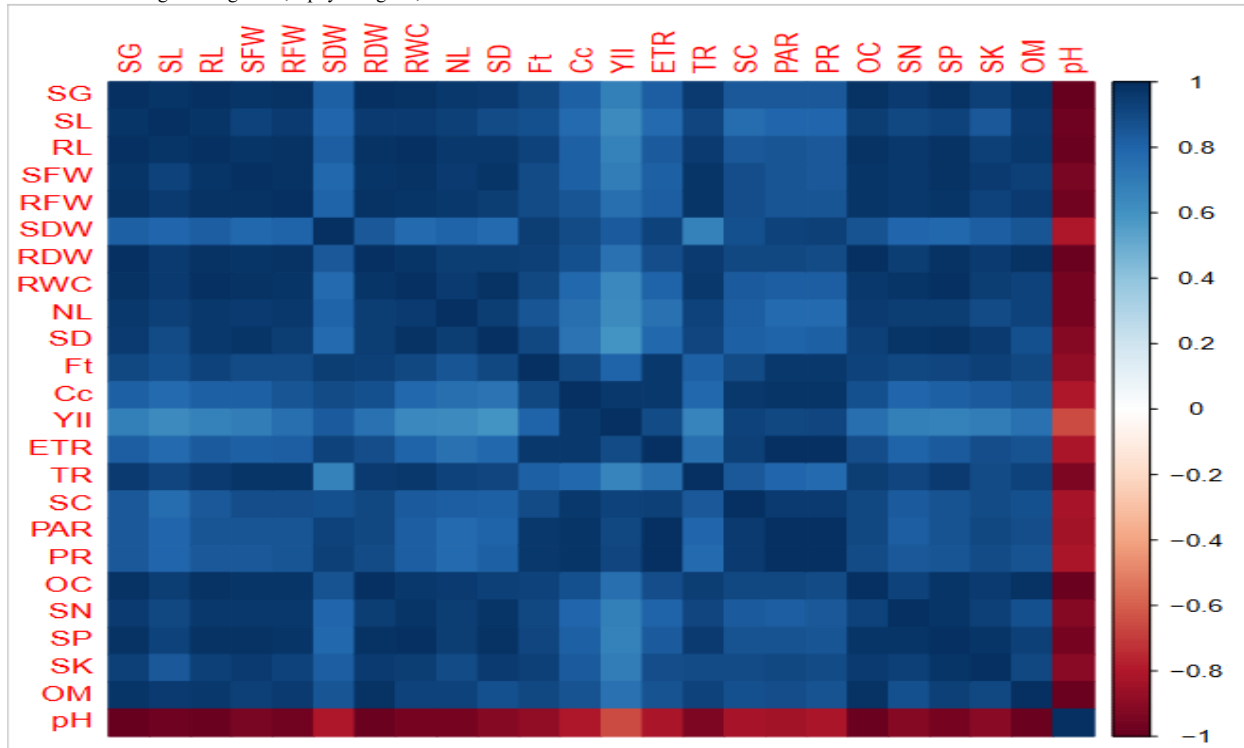


Figure 3 The correlation matrix among the various maize traits and treatments is shown by a corplot, also called a correlation plot. Light blue and sky blue indicate less link among the measured parameters, but dark blue indicates a substantial positive correlation. The correlation coefficient and its accompanying colors are shown in the color caption on the right-hand side of the corplot. The abbreviations are as Fresh weight of fruit per plant (FWFPP), Root length (RL), shoot length (SL), root fresh weight (RFW), shoot fresh weight (SFW), root dry weight (RDW), shoot dry weight (SDW), Diameter (DM), Chlorophyll contents (CC), Fluorescence yield (FT), Photosynthetically active radiation (PSR), Electron transport reaction (ETR), Quantum yield (YII), Photosynthetic rate (PR), Transpiration rate (TR), Phosphorus in soil (PS), Nitrogen in soil (NS), Potassium in soil (PS), Organic matter in soil (OM), Organic carbon in soil (OC).

4. Discussion

Among the agronomic and physical traits evaluated, the combination of rhizobium, biochar, and iron sulphate had the most impact on increasing the length, equatorial diameter, shoot fresh weight, and root fresh and dry biomasses of the maize plant. The recent findings on the enhanced growth of maize plants by biochar corroborated the findings of Tanure et al. (2019), who demonstrated that rhizobium might enhance plant development by increasing soil microbial activity and nutrient availability. This might be because organic supplements promote the growth of microbes such as bacteria and fungus, which in turn decompose organic matter into humus, a material rich in nutrients (Yarrow, 2014; Quadar et al., 2023). Consistent with Ahmad et al. (2015), we found the same thing. A similar set of findings is reported by Ali et al. (2022). Gibberellins and indole-3-acetic acid are examples of growth hormones that the bacteria may be secreting, which might aid plants in developing a resistance to diseases (Li et al., 2014). Based on

the findings, it can be concluded that agronomic properties such as relative water contents, germination percentages, plant fresh and dry biomasses, and development aspects of glyphosate-contaminated maize were enhanced by combining organic and inorganic treatments. By improving nutrient absorption, which in turn affected the growth of the plant's lateral roots, stems, and blossoms, iron fertilisers mixed with biochar and rhizobium enhanced yields (Mazhar et al., 2021). The reason behind this is that iron supplements and biochar provide the majority of the nutrient's plants need for growth. These nutrients promote the expansion of plant organs, particularly those responsible for carbohydrate formation and translation, which in turn increases both the quantity and quality of the fruits produced.

The present investigation found that organic nutrients and inorganic iron significantly improved maize plant physiology. According to earlier studies conducted by Paradiković et al. (2011), the presence of biostimulants in organic fertilizers enhances the capacity of plants to

use solar energy for photosynthesis, leading to higher chlorophyll concentrations. Many other physiological parameters have also shown considerable increases, including quantum yield, electron transport reaction, photosynthetically active radiation, and fluorescence yield. Biostimulants and composting enhance intracellular enzymatic activity, leading to an increase in chlorophyll synthesis and a better ability to absorb essential nutrients (Abdul Rahman et al., 2021). Similarly, rhizobium and biochar increased all physiological measures, which may be attributable to changes in soil qualities and cytokinin's nutritional content, but which also suggests that a typical dosage might be very effective in boosting caigua plants. The use of microbial, inorganic, and organic fertilizers together improved chemical parameters such as organic matter, highest organic carbon, N, K, and P. These results were similar to those of Shabir et al. (2023) in maize crops. The presence of organic matter in soil is crucial since it improves the soil's chemical, physical, and biological qualities, leading to higher quality yields.

5. Conclusion

The biochemical composition may also be enhanced in contrast to the control with the co-addition of iron fertilisers with rhizobium and biochar. Based on the findings presented here, it appears that iron fertilisers applied in conjunction with rhizobium and biochar can improve soil properties and promote the growth, yield, and quality of maize plants.

6. Conflict of interest

Authors declared no conflict of interest each other.

7. Acknowledgments

All the authors are highly thankful to Institute of Soil and Environmental Sciences for their moral support.

8. Contributions

Authors contributed equally in the current manuscript.

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