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Biochar and mycorrhiza enhance soil carbon storage and reduce CO_2 emissions in wastewater-irrigated turf

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ABSTRACT

Irrigation with recycled wastewater can reduce freshwater demand and improve soil fertility, but it can also increase CO_2 emissions from soil and contribute to global warming. This study investigated whether biochar and mycorrhiza can reduce CO_2 emissions and enhance soil quality in wastewater-irrigated turf. A factorial experiment was conducted with four levels of biochar (0, 0.5, 1, and 1.5%), two mycorrhiza (with and without), and two types of irrigation water (freshwater and recycled wastewater). Soil CO_2 and H_2O emissions, moisture and temperature, and chemical and physical properties were measured for 3 months. Biochar and mycorrhiza treatments significantly reduced CO_2 emissions by 19.4–45.0% compared to the control treatment. The combination of biochar at a 1.5% level with mycorrhiza had the highest emission-reducing effect. Biochar and mycorrhiza treatments also reduced H_2O emissions by 8.1–14.6%, increased soil organic matter, carbon, and total nitrogen, regulated soil EC and pH, and improved soil porosity and aggregate stability. The results suggest that biochar and mycorrhiza can be effective strategies to mitigate CO_2 emissions and improve soil quality in wastewater irrigation. The combination of biochar with mycorrhiza can have synergistic benefits for soil carbon storage and conservation.

Key words: biochar, CO_2 emission, H_2O emission, mycorrhiza, soil moisture, soil properties

HIGHLIGHTS

- Biochar and mycorrhiza application under wastewater irrigation reduces CO_2 emissions from the turf soil while increasing soil carbon stocks.
- The combination of mycorrhiza with biochar reduces CO_2 emissions from turf soil compared to the use of biochar alone.
- The combination of mycorrhiza with biochar improves soil physical properties.

INTRODUCTION

It is predicted that the world population, which was 7.6 billion in 2017, will reach about 10 billion in 2050 (United Nations 2017). With increasing urbanization and a growing population, wastewater production is also increasing significantly (Hossain *et al.* 2018). This challenge reveals the problems of wastewater discharge and disruptions in wastewater management lead to an increase in wastewater-related diseases (Singh 2021).

Using wastewater for irrigation can both solve the problem of wastewater discharge (Sakib 2022) and reduce pressure on freshwater resources by replacing freshwater resources used for irrigation. Thus, environmental management can be achieved by reducing wastewater discharge to the environment, and freshwater sustainability can be addressed by improving the freshwater supply crisis, which is one of the major threats today. In addition, the presence of wastewater throughout the year despite freshwater shows the continuity of agricultural production and the ability to maintain plant production throughout the year (Zhang & Shen 2019). The high nutrient content of wastewater, which supports plant yields and reduces the use of synthetic fertilizers, can contribute to more economical production practices by increasing plant efficiency and reducing fertilizer use. Yerli *et al.* (2023a) stated that the plant yield increased by 11% in irrigation with wastewater and the need for synthetic fertilizers was completely eliminated in the second year of the study. In addition, wastewater, which is a potential

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source of nutrients with high organic and inorganic contents, improves soil aggregation and porosity and provides better plant growth (Cakmakci & Sahin 2021).

For all the benefits of using wastewater in irrigation, the risks of wastewater should not be ignored. Wastewater with high salt concentrations can increase soil salinity and also lead to nutrient depletion and eutrophication due to its high nutrient content. The heavy metal content of wastewater can affect soil quality and cause yield and quality losses for the plant (Cakmakci & Sahin 2021). Heavy metals, pathogens, and various toxic compounds in wastewater are serious health risk factors (Singh 2021; Asirifi *et al.* 2023). However, all of these negative factors are partially eliminated by treating wastewater, and these negative impacts can be reduced through collaborative management of wastewater impacts on plants, soils, irrigation systems, and human and animal health (Demir & Sahin 2019, 2020; Cakmakci & Sahin 2021; Yerli *et al.* 2023a). Although the possibility of using in irrigation can be increased by reducing the risks of wastewater, soils irrigated with wastewater release significant greenhouse gases into the atmosphere, increasing the magnitude of global warming (Thangarajan *et al.* 2012; Li *et al.* 2020; Yerli *et al.* 2023b). The fact that CO_2 accounts for 82% of greenhouse gases has made it the most important greenhouse gas (Thangarajan *et al.* 2012), and it is considered a problem that requires an urgent action plan according to the Intergovernmental Panel on Climate Change. For this reason, it is very important to control greenhouse gas emissions, especially CO_2, through environmentally friendly practices in irrigation with wastewater to protect freshwater resources.

Biochar, which is defined as a product of thermochemical transformations such as pyrolysis, hydrothermal carbonization, gasification, and roasting, makes the properties of the soil suitable for the plant (Wang *et al.* 2020). Biochar improves the chemical, physical, and biological properties of poor soils (Liu *et al.* 2013; Asirifi *et al.* 2023) and regulates the soil's nutrient and water-holding capacity, pH buffering capacity, and biological indicators (Häring *et al.* 2017). In addition to these, biochar also rehabilitates the global warming conditions of the soil. It is well known that biochar, which has been extensively studied in recent years as an environmentally friendly practice, significantly reduces CO_2 emissions from the soil by regulating soil carbon stocks (Yerli *et al.* 2022). The low decomposition rate of biochar increases its residence time in the soil, protects the existing organic carbon in the soil, and adds organic carbon to the soil. Due to the stable nature of carbon, the residence time of biochar in soil is 10–1,000 times longer than that of another organic soil amendment (Van Zwieten *et al.* 2010). By improving soil aggregation, biochar protects organic carbon in soil aggregates, so biochar not only reduces CO_2 emissions from the soil but also promotes soil productivity (Mukherjee & Lal 2013; Cakmakci *et al.* 2022). Mycorrhiza, living microbial organisms, interact with plant roots and maintain a symbiotic relationship. In this symbiotic relationship, mycorrhiza photoassimilates 2 carbon compounds from plants, while allowing plant roots to take up more nutrients and water more easily and comfortably (Boyno *et al.* 2022). Mycorrhiza helps reduce soil CO_2 emissions by sequestering carbon in the soil during this beneficial exchange (Cavagnaro *et al.* 2008). In addition, mycorrhizal symbiosis can influence soil CO_2 emissions by causing changes in soil chemical and physical forms (Cavagnaro *et al.* 2012).

Previous studies have demonstrated the effectiveness of biochar in reducing soil CO_2 emissions. In addition, there are few studies in the literature on the emission-reducing effect of mycorrhiza. However, no study was found in the literature that combined biochar and mycorrhiza to reduce CO_2 emissions from soils irrigated with recycled wastewater. The main hypothesis of this study was that adding a combination of biochar and mycorrhiza to soils irrigated with recycled wastewater would increase soil carbon stocks by helping to conserve organic carbon and significantly reduce CO_2 emissions from turf soils. Therefore, this study aimed to determine the changes in soil CO_2 and H_2O emissions, moisture and temperature, and chemical and physical properties resulting from the incorporation of biochar in varying amounts and the addition of mycorrhiza to turfs irrigated with recycled wastewater.

MATERIALS AND METHODS

Study site

The study was conducted in 1.5-liter pots (diameter: 13 cm, height: 11 cm) in a climate room of the Faculty of Agriculture of Van Yuzuncu Yil University that provided controlled conditions to minimize the influence of external during the 3-month period. According to the temperature and humidity values measured by the automatic climate station (HOBO, Campbell Scientific, USA) during the study period, the average daily temperature and humidity were 23.7 ± 3.6 °C and 46.8 ± 4.6%, respectively.

Study design

The experiment was conducted with three replicates using a completely randomized factorial experimental design considering four different dosages of biochar with and without mycorrhiza in the soil under irrigation conditions with two types of water, namely freshwater and recycled wastewater. The biochar was mixed into the soil at dosages of 0, 0.5, 1.0, and 1.5% on a weight basis (S, B0.5, B1.0, and B1.5), and the mycorrhizal soils contained mycorrhiza in addition to the same biochar dosages (M, B0.5 + M, B1.0 + M, and B1.5 + M). Therefore, 48 pots of turfs were arranged in the experimental plot, considering 2 irrigation water types × 4 biochar and 4 biochar + mycorrhiza treatments × 3 replicates.

Straw biochar was chosen for this study as the biochar raw material that reduces CO_2 emissions the most between different biochar (Yerli *et al.* 2022). Similarly, experimental studies were conducted to determine the mycorrhiza that reduces CO_2 emissions the most between the various mycorrhiza that are well compatible with turfs (*Funneliformis mosseae*, *Claroideoglomus etunicatum*, and *Rhizophagus irregularis*). It was decided to use *Claroideoglomus etunicatum* with the lowest emission.

Properties of soil and biochar

Particle size distribution analysis of the experimental soil (Gee & Bauder 1986) from 0 to 20 cm depth revealed a loamy texture (sand: 37%, silt: 27%, clay: 36%) according to the USDA classification. The dried, sieved, and homogenized biochar material was subjected to a pyrolysis temperature of 400 °C (Yerli *et al.* 2022). Using the analytical methods described in the soil analyses section, soil and biochar analyses were performed prior to the study and are listed in Table 1.

Irrigation waters

Recycled wastewater was taken from the Van Iskele wastewater treatment plant prior to each irrigation with plastic water tanks, brought to the study area, and used for irrigation. Freshwater was obtained directly from the domestic network of Van Yuzuncu Yil University.

To determine the quality characteristics of the water used in the study, samples of the freshwater and recycled wastewater were collected and analyzed in the middle of each month (Table 2). The electrical conductivity (EC) and pH were determined by direct measurement using the EC meter and pH meter, respectively. While cations (Ca, Mg, Na, K) were determined in an inductively coupled plasma optical emission spectrometer (Anonymous 2007), anions (Cl, SO_4, HCO_3, CO_3) were determined by titration with silver nitrate using the potassium chromate indicator (Tuzuner 1990), with the Hach Lange Dr 5000 UV/VIS spectrometer with the SulfaVer 4 ready kit no. HACH 8051 (HACH 2010) and titration with sulfuric acid using the phenolphthalein and bromocrocel green indicators (Tuzuner 1990), respectively. While total nitrogen was determined by the Kjeldahl method (APHA-AWWA-WEF 1989), biological oxygen demand (BOD_5) and chemical oxygen demand (COD) were determined using pre-made kits in the Hach Lange Dr 5000 UV/VIS spectrometer instrument (HACH 2005, 2010). Sodium content (Na%), sodium adsorption ratio (SAR), and residual sodium carbonate (RSC) were determined by calculating the concentrations of anions and cations (Kanber & Unlu 2010).

Table 1 | The properties of study soil and biochar

Property	Soil	Biochar
Particle density	2.67	–
Bulk density (Mg m^{-3})	1.29	–
Porosity (%)	51.69	–
Aggregate stability (%)	46.79	–
EC▫ (dS m^{-1})	0.521	1.853
pH▫	8.25	8.56
Organic matter (%)	1.42	58.21
Organic carbon (%)	0.82	33.76
Total nitrogen (%)	0.092	0.223

▫: in 1:2.5 saturation extract.

Table 2 | The properties of freshwater and recycled wastewater

Property	Freshwater	Recycled wastewater
EC (dS m^{-1})	0.441 ± 0.017	0.832 ± 0.059
pH	8.18 ± 0.15	7.58 ± 0.19
Ca (me l^{-1})	1.24 ± 0.09	1.69 ± 0.15
Mg (me l^{-1})	2.49 ± 0.21	2.71 ± 0.21
Na (me l^{-1})	0.54 ± 0.09	3.12 ± 0.11
K (me l^{-1})	0.39 ± 0.08	1.01 ± 0.04
Cl (me l^{-1})	1.39 ± 0.17	1.94 ± 0.05
SO_4 (me l^{-1})	1.33 ± 0.03	1.91 ± 0.22
HCO_3 (me l^{-1})	2.05 ± 0.05	4.51 ± 0.05
CO_3 (me l^{-1})	–	–
Total nitrogen (mg l^{-1})	–	9.33 ± 0.45
COD (mg l^{-1})	–	38.91 ± 2.17
BOD_5 (mg l^{-1})	–	25.33 ± 3.81
Na%	11.58 ± 0.51	36.57 ± 0.93
SAR	0.40 ± 0.06	2.10 ± 0.15
RSC (me l^{-1})	− 1.68 ± 0.12	0.11 ± 0.24

COD, chemical oxygen demand; BOD_5, biological oxygen demand; Na%, sodium percentage; SAR, sodium adsorption ratio; RSC, residual sodium carbonate; –, not detected; ±, standard error.

Treatments and cultivation

In the biochar treatments (S, B0.5, B1.0, and B1.5), sieved, air-dried soil was mixed with biochar in the indicated amounts (0, 0.5, 1.0, and 1.5%) on a weight basis and placed in pots (Supplementary 1). The biochar was combined with mycorrhizal treatments (M, B0.5 + M, B1.0 + M, and B1.5 + M), 2.5 g of *Claroideoglomus etunicatum* inoculum containing 150 spores g^{-1} was added to the soil as mycorrhiza after mixing the biochar with the soil according to Boyno *et al.* (2022).

A turf mixture consisting of 40% *Lolium perenne*, 30% *Festuca rubra rubra*, 10% *Poa pratensis*, and 10% *Festuca rubra commutata* was sown at 50 g m^{-2} seed in each pot (Celebi Zorer *et al.* 2009). To protect the sown seeds from external factors, seeds were covered with a thin layer of peat, sieved manure, and soil in a 1:1:1 ratio. After sowing, fertilization with 4 g m^{-2} 26% ammonium nitrate was applied with the first irrigation as basic turf fertilization (Celebi Zorer *et al.* 2009). The turf was mowed approximately every 10 days, reducing plant height from 5–6 cm to 3–4 cm (Morris & Shearman 1998).

Irrigation applications

Irritation was done daily until the plants reached a height of 5–6 cm, with all treatments irrigated equally with freshwater. The amount of irrigation water at each irrigation was determined based on the weight loss of the pots considering the pot weight at field capacity (pot) in the control treatment (S) (Supplementary 1). Then, considering that about 30% of moisture in pot capacity decreases in 3 days, irrigation with freshwater and recycled wastewater was continued every 3 days. During this period, the irrigation water amounts were determined according to the field capacity every 3 days in the control pots (S), taking into account the decreasing moisture (current moisture) content. During the study period, a total of 121 mm of irrigation water was applied in each treatment. The pot capacity in the control treatment was determined to be 0.327 m^3 m^{-3} by using the bulk density of the soil after the weight of the pot was determined when the drainage was completely stopped as a result of saturating the pot by cover to prevent evaporation.

Soil analyses

After completion of the 3-month study period, soil samples were collected from each treatment with replicates, and analyses were performed. EC and pH of the samples were determined in 1:2.5 saturation extract. The organic matter was determined using the Walkley-Black method (Nelson & Sommers 1982), and organic carbon was calculated from organic matter. Total nitrogen in the soil samples was determined using the Kjeldahl method (Bremner & Mulvaney 1982). A pycnometer and a soil roller were used to determine particle and bulk densities, respectively. Then porosity was obtained by calculating these values (Danielson & Sutherland 1986). Wet aggregate stability was determined by wet sieving (Kemper & Rosenau 1986).

Soil CO_2 and H_2O emissions, moisture, and temperature measurements

During the 3-month study, soil CO_2 emissions were measured by taking three measurements in each pot every day. Simultaneously with these measurements, H_2O emissions from the soil, soil moisture, and temperature were also monitored at the time of CO_2 emission measurements. An EGM-5 (PPSystems, Stotfold, UK) infrared gas analyzer operating on the dynamic closed-space principle (Yerli *et al.* 2022, 2023b) was used to measure CO_2 and H_2O emissions and soil temperature. While the SRC-1 non-steady state through the flow chamber of this instrument was used to measure CO_2 and H_2O emissions, soil temperature was measured with an STP-1 soil temperature probe connected to the same instrument at a soil depth of 5 cm from the surface in the central region of the pot (Yerli *et al.* 2023b). Pots were weighed for each measurement to determine soil moisture (Yerli *et al.* 2022).

Statistical analysis of the data

SPSS software (Version 23.0) was used for the statistical analysis of the data. After analyzing the obtained data with the general linear model, the means that were found to be significant were separated with the Duncan multiple range test at a probability level of 5%. In addition, a correlation plot was created in the software RStudio with a scatter plot, a correlation coefficient, and the distribution of the variables to examine the relationships between parameters.

RESULTS AND DISCUSSION

Soil chemical properties

The effect of mycorrhizal biochar treatments and irrigation water types on organic matter, carbon, and total nitrogen was significant ($p < 0.01$) (Table 3 and Supplementary 2). The increase in organic matter, carbon, and total nitrogen in the biochar treatments can be related to the high organic matter and carbon and nitrogen content of biochar (Table 1). The rich organic matter and carbon and nitrogen content of biochar are resistant to degradation due to biochar's aromatic structure and retain biochar's effectiveness in the soil over a long period time. Therefore, biochar application increases soil organic matter, carbon, and total nitrogen content (Alaboz & Isildar 2018). More biochar in the soil is an incentive to further improve soil organic matter and carbon and total nitrogen stocks (Mukherjee & Lal 2013). It is thought that when biochar was combined with mycorrhizal treatments, organic matter and carbon and total nitrogen gain are provided in the soil as a result of mycorrhiza's inhibition of mineralization. By aggregating the soil, mycorrhiza supports organic matter and carbon conservation by reducing organic matter and carbon oxidation (Daynes *et al.* 2013). In addition, the fact that the plant root supplies photosynthetically fixed carbon to the mycorrhiza may have supported the increase in soil organic carbon stocks (Leigh *et al.* 2009). The mycorrhiza converts nitrogen in the soil into a form that can be taken up by the plant (nitrate or ammonium) thanks to their hyphae. As a complex structure, mycorrhiza store more nitrogen than the plant needs (Reynolds *et al.* 2005). Thus, mycorrhiza can prevent further mineralization of nitrogen. The high content of organic matter, carbon, and total nitrogen in recycled wastewater irrigation can be explained by the chemical composition of the wastewater (Table 2). The COD and BOD_5 and total nitrogen content of recycled wastewater have the characteristics of liquid fertilizer (Yerli *et al.* 2023a) and enrich organic matter and carbon and total nitrogen content in the soil (Demir & Sahin 2020; Cakmakci & Sahin 2021).

The effect of mycorrhizal biochar treatments and irrigation water types on EC was significant at the $p < 0.01$ level (Table 3 and Supplementary 2). Higher EC values for biochar treatment and irrigation with recycled wastewater could be related to the salinity of the biochar and wastewater (Tables 1 and 2). While the EC content of the different biochar materials applied to the soil's direct effect on soil salinity (Yerli *et al.* 2022), soil salinity increases during irrigation with recycled wastewater depending on the natural form of the wastewater, and soil salinity needs to be monitored during long-term irrigation with wastewater (Demir & Sahin 2020; Cakmakci & Sahin 2021; Yerli *et al.* 2023a). In this study, the statistically similar EC under the conditions with mycorrhiza and without mycorrhiza (Table 3) showed that mycorrhiza did not affect EC.

The effects of mycorrhizal biochar treatments and irrigation water types on pH were significant at the level of $p < 0.01$ (Table 3 and Supplementary 2). Depending on the treatment, the decrease in soil pH may be related to increased nitrogen input to the soil (Table 3), nitrification and the release of protons, and increased soil acidity due to mineralization of organic matter. The further decrease in soil pH under mycorrhizal conditions may be explained by the greater nitrogen conservation

Table 3 | The changes in soil chemical properties in biochar and mycorrhiza treatments under irrigation conditions with freshwater and recycled wastewater

Treatment		**Organic matter (%)**	**Organic carbon (%)**	**Total nitrogen (%)**	**EC (dS m^{-1})**	**pH**
Irrigation with freshwater	S	1.29 ± 0.02	0.75 ± 0.01	0.079 ± 0.001	0.497 ± 0.012	8.21 ± 0.02
	M	1.38 ± 0.02	0.80 ± 0.01	0.082 ± 0.001	0.506 ± 0.009	8.14 ± 0.01
	B0.5	1.45 ± 0.01	0.84 ± 0.01	0.098 ± 0.002	0.540 ± 0.005	8.05 ± 0.06
	B1.0	1.51 ± 0.04	0.88 ± 0.02	0.115 ± 0.004	0.576 ± 0.020	7.90 ± 0.07
	B1.5	1.59 ± 0.02	0.92 ± 0.01	0.128 ± 0.002	0.633 ± 0.010	7.81 ± 0.04
	B0.5 + M	1.51 ± 0.04	0.88 ± 0.02	0.101 ± 0.003	0.542 ± 0.016	7.98 ± 0.03
	B1.0 + M	1.62 ± 0.03	0.94 ± 0.02	0.118 ± 0.002	0.571 ± 0.011	7.86 ± 0.06
	B1.5 + M	1.70 ± 0.04	0.99 ± 0.02	0.134 ± 0.002	0.639 ± 0.006	7.73 ± 0.04
	Mean	1.51 ± 0.03 B	0.87 ± 0.02 B	0.107 ± 0.004 B	0.563 ± 0.011 B	7.96 ± 0.04 A**
Irrigation with wastewater	S	1.41 ± 0.04	0.82 ± 0.02	0.092 ± 0.007	0.835 ± 0.040	8.06 ± 0.03
	M	1.51 ± 0.07	0.88 ± 0.04	0.102 ± 0.004	0.827 ± 0.058	7.94 ± 0.02
	B0.5	1.61 ± 0.05	0.94 ± 0.03	0.115 ± 0.002	0.880 ± 0.018	7.92 ± 0.02
	B1.0	1.70 ± 0.09	0.98 ± 0.05	0.130 ± 0.002	0.940 ± 0.030	7.74 ± 0.02
	B1.5	1.79 ± 0.07	1.04 ± 0.04	0.145 ± 0.002	0.987 ± 0.007	7.63 ± 0.01
	B0.5 + M	1.65 ± 0.03	0.96 ± 0.02	0.119 ± 0.001	0.879 ± 0.038	7.90 ± 0.01
	B1.0 + M	1.78 ± 0.08	0.97 ± 0.01	0.136 ± 0.003	0.936 ± 0.016	7.70 ± 0.01
	B1.5 + M	1.83 ± 0.01	1.03 ± 0.02	0.152 ± 0.001	0.990 ± 0.001	7.59 ± 0.01
	Mean	1.66 ± 0.04 A**	0.96 ± 0.02 A**	0.124 ± 0.004 A**	0.909 ± 0.015 A**	7.81 ± 0.04 B
Mean	S	1.35 ± 0.03 E	0.78 ± 0.02 E	0.086 ± 0.004 F	0.666 ± 0.078 D	8.14 ± 0.04 A**
	M	1.45 ± 0.04 D	0.84 ± 0.02 D	0.092 ± 0.005 E	0.667 ± 0.076 D	8.04 ± 0.04 AB
	B0.5	1.53 ± 0.04 CD	0.89 ± 0.03 CD	0.107 ± 0.004 D	0.710 ± 0.076 BC	7.98 ± 0.05 BC
	B1.0	1.61 ± 0.06 BC	0.93 ± 0.03 BC	0.123 ± 0.004 C	0.758 ± 0.083 B	7.82 ± 0.04 D
	B1.5	1.69 ± 0.05 AB	0.98 ± 0.03 AB	0.137 ± 0.004 B	0.810 ± 0.079 A	7.72 ± 0.03 DE
	B0.5 + M	1.58 ± 0.04 C	0.92 ± 0.02 C	0.110 ± 0.004 D	0.710 ± 0.078 BC	7.94 ± 0.05 C
	B1.0 + M	1.70 ± 0.04 A	0.99 ± 0.02 A	0.127 ± 0.004 C	0.754 ± 0.082 B	7.78 ± 0.04 D
	B1.5 + M	1.77 ± 0.02 A**	1.02 ± 0.02 A**	0.143 ± 0.004 A**	0.815 ± 0.078 A**	7.66 ± 0.04 E

S, soil; M, mycorrhiza; B0.5, 0.5% biochar; B1.0, 1.0% biochar; B1.5, 1.5% biochar; B0.5 + M, 0.5% biochar + mycorrhiza; B1.0 + M, 1.0% biochar + mycorrhiza; B1.5 + M, 1.5% biochar + mycorrhiza; ±, standard error.
**$p < 0.01$.

under mycorrhizal conditions (Table 3). The significant ($p < 0.001$) negative correlation of pH with total nitrogen also supports this situation (Figure 1). Similarly, Aula *et al.* (2016) also pointed out that soil pH decreases with nitrogen input to the soil and that pH and total nitrogen have a negative correlation. Liu & Zhang (2012) and Cakmakci *et al.* (2022) found that biochar treatment to soil and irrigation with wastewater lower soil pH, respectively. Another explanation for the decrease in soil pH could be that the increased organic matter and carbon in the soil (Table 2) release more organic acid. This is also supported by the significant ($p < 0.001$) negative correlation of pH with organic matter and carbon (Figure 1). In addition to the soil, acidity can also increase due to the increase of acids from soil organic content.

Soil physical properties

The effect of mycorrhizal biochar treatments and irrigation water types on particle density was not significant, but the effect on bulk density was significant at the $p < 0.01$ level (Table 4 and Supplementary 2). The reduction in bulk density in the treatments can be attributed to the organic matter added to the soil with biochar and recycled wastewater (Table 3). The lower bulk density of organic matter and better aggregation with organic matter may reduce soil compaction. The significant ($p < 0.001$) negative correlations of bulk density with organic matter, bulk density, and wet aggregate stability also support this (Figure 1). Similarly, Celik *et al.* (2010) indicated that the addition of organic matter to the soil reduces soil compaction by improving its physical properties. Verheijen *et al.* (2019) and Demir & Sahin (2019) also reported that the addition of organic matter to soil by adding biochar and irrigating with wastewater reduces soil bulk density, respectively.

The effects of mycorrhizal biochar treatments and irrigation water types on porosity and wet aggregate stability were significant ($p < 0.01$) (Table 4 and Supplementary 2). Treatment-dependent trends in porosity and wet aggregate stability can be associated with organic matter added to the soil by biochar and the combination of biochar with mycorrhizal treatments and

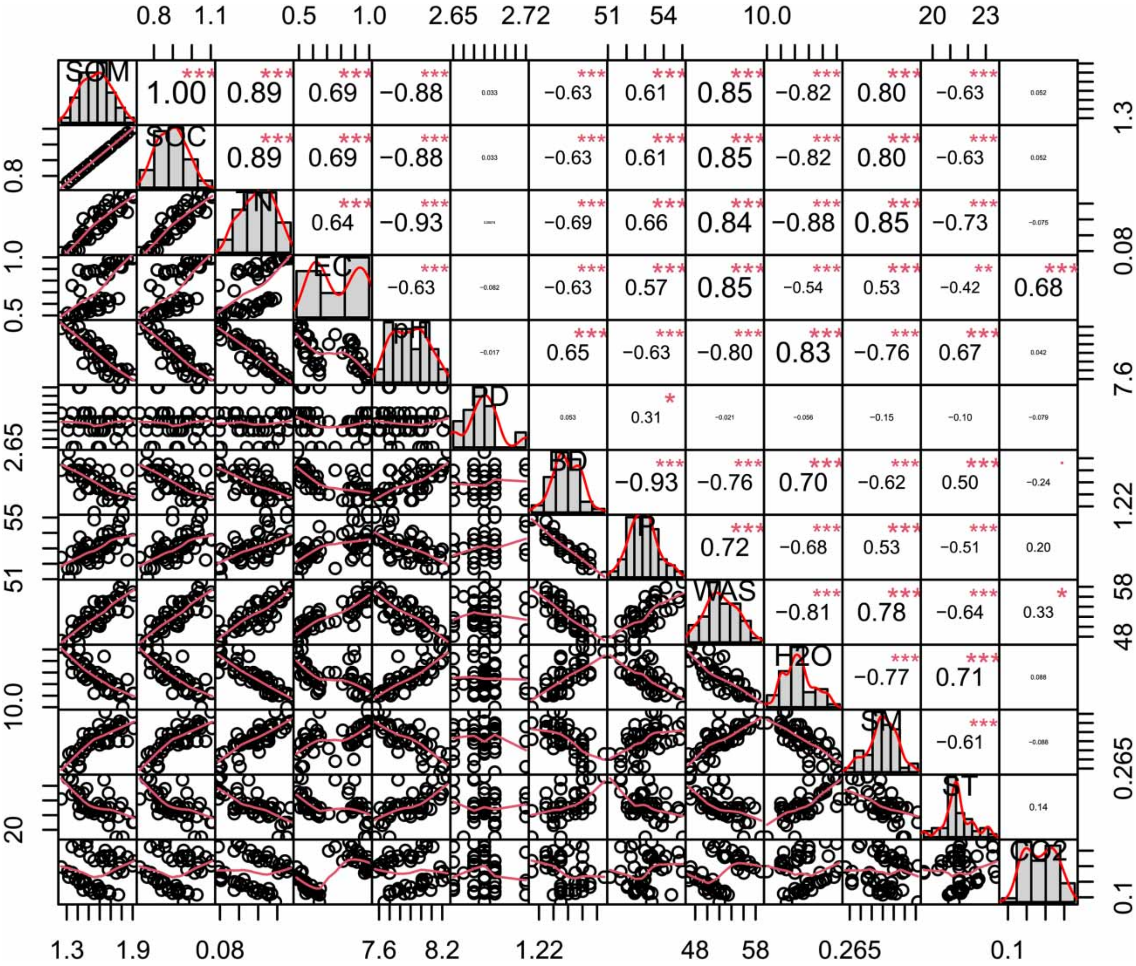

Figure 1 | Correlation matrix for data. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. SOM, soil organic matter; SOC, soil organic carbon; TN, total nitrogen; EC, electrical conductivity; pH, soil reaction; PD, particle denstiy; BD, bulk density; P, porosity; WAS, wet aggregate stability; H_2O, H_2O emission from soil; SM, soil moisture; ST, soil temperature; CO_2, CO_2 emission from soil.

irrigation with recycled wastewater (Table 3). Further increase in porosity and stability of wet aggregates under mycorrhizal conditions could be explained by higher retention of organic matter under mycorrhizal conditions (Table 3). The development seen in soil organic matter is very effective in improving the physical and hydraulic properties of the soil, especially its porosity and wet aggregate stability (Demir & Sahin 2019). The significant ($p < 0.001$) positive correlations of porosity and wet aggregate stability with organic matter also support this (Figure 1). In addition, lower bulk density resulted in increased porosity, as there was a significant ($p < 0.001$) negative linear correlation between porosity and bulk density (Figure 1). Organic matter increases soil porosity by reducing soil compaction and creating more voids (Chaudhari *et al.* 2013). Organic matter improves soil aggregate development by supporting the formation of large clay-humic complexes and water-repellent coatings in the soil (Heikkinen *et al.* 2019). In addition, Kul *et al.* (2021) explained the increase in soil porosity and wet aggregate stability by the characteristic properties of biochar, while Cakmakci & Sahin (2021) explained the increase in porosity and wet aggregate stability when irrigated with recycled wastewater directly by the contribution of organic matter from wastewater.

Soil H_2O emission and moisture

The effects of mycorrhizal biochar treatments and irrigation water types on H_2O emission from soil and soil moisture were significant ($p < 0.01$) (Table 5 and Supplementary 2). It is thought that organic matter, which increases in the soil depending on the treatment (Table 3), reduces H_2O emission from the soil by increasing moisture retention in the soil. Significant

Table 4 | The changes in soil physical properties in biochar and mycorrhiza treatments under irrigation conditions with freshwater and recycled wastewater

Treatment		Particle density	Bulk density (Mg m^{-3})	Porosity (%)	Wet aggregate stability (%)
Irrigation with freshwater	S	2.68 ± 0.006	1.29 ± 0.009	51.74 ± 0.38	47.08 ± 0.10
	M	2.69 ± 0.020	1.28 ± 0.003	52.48 ± 0.39	48.05 ± 0.64
	B0.5	2.70 ± 0.010	1.28 ± 0.006	52.59 ± 0.11	49.40 ± 0.24
	B1	2.67 ± 0.012	1.27 ± 0.012	52.50 ± 0.23	50.36 ± 0.50
	B1.5	2.66 ± 0.007	1.26 ± 0.007	52.82 ± 0.22	51.19 ± 0.60
	B0.5 + M	2.67 ± 0.003	1.27 ± 0.006	52.55 ± 0.27	50.89 ± 0.06
	B1 + M	2.67 ± 0.012	1.26 ± 0.009	52.74 ± 0.31	51.63 ± 0.77
	B1.5 + M	2.70 ± 0.012	1.25 ± 0.006	53.64 ± 0.39	52.78 ± 0.43
	Mean	2.68 ± 0.004	1.27 ± 0.003 A**	52.63 ± 0.14 B	50.17 ± 0.40 B
Irrigation with wastewater	S	2.67 ± 0.009	1.27 ± 0.009	52.50 ± 0.48	51.09 ± 0.46
	M	2.68 ± 0.003	1.26 ± 0.012	53.05 ± 0.44	52.12 ± 0.67
	B0.5	2.68 ± 0.003	1.25 ± 0.009	53.42 ± 0.34	53.40 ± 0.37
	B1	2.68 ± 0.003	1.25 ± 0.020	53.54 ± 0.75	54.29 ± 0.65
	B1.5	2.67 ± 0.007	1.24 ± 0.003	53.66 ± 0.12	55.52 ± 0.39
	B0.5 + M	2.67 ± 0.003	1.24 ± 0.007	53.55 ± 0.19	55.01 ± 0.07
	B1 + M	2.68 ± 0.022	1.24 ± 0.007	53.79 ± 0.52	56.27 ± 0.34
	B1.5 + M	2.68 ± 0.006	1.23 ± 0.009	54.23 ± 0.43	57.89 ± 0.57
	Mean	2.68 ± 0.003	1.25 ± 0.004 B	53.47 ± 0.17 A**	54.45 ± 0.46 A**
Mean	S	2.67 ± 0.006	1.28 ± 0.008 A**	52.12 ± 0.32 C	49.08 ± 0.92 F
	M	2.68 ± 0.009	1.27 ± 0.007 AB	52.76 ± 0.29 BC	50.09 ± 0.99 E
	B0.5	2.69 ± 0.007	1.26 ± 0.009 AB	53.01 ± 0.25 B	51.40 ± 0.69 D
	B1	2.68 ± 0.006	1.26 ± 0.012 BC	53.02 ± 0.42 B	52.33 ± 0.95 CD
	B1.5	2.67 ± 0.006	1.25 ± 0.004 BC	53.24 ± 0.22 AB	53.36 ± 1.02 B
	B0.5 + M	2.67 ± 0.002	1.26 ± 0.007 BC	53.05 ± 0.27 B	52.95 ± 0.92 BC
	B1 + M	2.68 ± 0.011	1.25 ± 0.008 BC	53.27 ± 0.36 AB	53.95 ± 1.11 B
	B1.5 + M	2.69 ± 0.007	1.24 ± 0.007 C	53.94 ± 0.29 A**	55.33 ± 1.19 A**

S, soil; M, mycorrhiza; B0.5, 0.5% biochar; B1.0, 1.0% biochar; B1.5, 1.5% biochar; B0.5 + M, 0.5% biochar + mycorrhiza; B1.0 + M, 1.0% biochar + mycorrhiza; B1.5 + M, 1.5% biochar + mycorrhiza; ±, standard error.

**$p < 0.01$.

($p < 0.001$) positive and negative correlations of organic matter with soil moisture and H_2O emission from the soil, respectively, could explain this result (Figure 1). An increase in soil organic matter from 1 to 2% results in an increase in soil water retention of 3 liters per approximately 0.03 m^3 of soil (Gould 2015). Increasing organic matter in the soil increases the water-holding capacity of the soil, allowing less H_2O to be released from the soil, and thus reducing the loss of water from the soil (Lal 2020). Organic matter can retain soil water longer due to organic matter's low bulk density and high porosity, aggregate stability, absorption capacity (de Rouw & Rajot 2004), and hydrophilic binding properties (Bronick & Lal 2005). Thus, increased soil moisture retention results in lower H_2O emissions (Yerli *et al.* 2022). The significant ($p < 0.001$) negative correlation of soil moisture with H_2O emission from the soil also supports this result (Figure 1). Moreover, the protective effect of biochar on soil moisture and thus the reduction of H_2O emission can be explained by biochar's ability to retain soil moisture due to its spongy structure (Cakmakci *et al.* 2022), while the effect of mycorrhiza is because mycorrhizal hyphae improve soil porosity and aggregate stability (Table 4), transforming the soil into a physical format that loses less moisture and instead conserves it (Cavagnaro 2016). In addition, similar to the results of this study, Yerli *et al.* (2023b) reported that the increase in soil organic matter during irrigation with recycled wastewater has a protective effect on soil moisture and reduces H_2O emissions from the soil.

Soil temperature

The effect of mycorrhizal biochar treatments and irrigation water types on soil temperature was significant at $p < 0.01$ and $p < 0.05$ (Table 5 and Supplementary 2). The lower soil temperature in the biochar treatments with and without mycorrhiza and irrigation with wastewater could be explained by the higher soil moisture in these treatments (Table 5) because a significant ($p < 0.001$) negative correlation of soil temperature with soil moisture was found (Figure 1). An increase in soil moisture

Table 5 | The changes in mean soil H_2O and CO_2 emissions and soil moisture and temperature values in biochar and mycorrhiza treatments under irrigation conditions with freshwater and recycled wastewater

Treatment		H_2O emission ($g\ m^{-2}\ h^{-1}$)	Soil moisture ($m^3\ m^{-3}$)	Soil temperature (°C)	CO_2 emission ($g\ m^{-2}\ h^{-1}$)
Irrigation with freshwater	S	12.5 ± 0.06	0.267 ± 0.003	23.1 ± 0.48	0.2634 ± 0.0188
	M	12.0 ± 0.06	0.271 ± 0.003	22.5 ± 0.06	0.2275 ± 0.0156
	B0.5	11.7 ± 0.12	0.275 ± 0.005	22.0 ± 0.29	0.1945 ± 0.0032
	B1	11.4 ± 0.06	0.279 ± 0.003	21.7 ± 0.09	0.1623 ± 0.0086
	B1.5	11.1 ± 0.09	0.283 ± 0.001	21.4 ± 0.15	0.1389 ± 0.0089
	B0.5 + M	11.5 ± 0.06	0.279 ± 0.001	21.6 ± 0.12	0.1786 ± 0.0199
	B1 + M	11.1 ± 0.07	0.282 ± 0.003	21.4 ± 0.15	0.1368 ± 0.0057
	B1.5 + M	10.7 ± 0.06	0.287 ± 0.005	21.0 ± 0.09	0.1024 ± 0.0167
	Mean	11.5 ± 0.11 A**	0.278 ± 0.002 B	21.8 ± 0.15 A*	0.1756 ± 0.0110 B
Irrigation with wastewater	S	12.0 ± 0.29	0.272 ± 0.003	22.6 ± 0.53	0.4421 ± 0.0091
	M	11.6 ± 0.30	0.278 ± 0.001	22.0 ± 0.71	0.3966 ± 0.0175
	B0.5	11.3 ± 0.12	0.280 ± 0.001	21.7 ± 0.39	0.3743 ± 0.0138
	B1	11.0 ± 0.19	0.283 ± 0.003	21.3 ± 0.12	0.3438 ± 0.0079
	B1.5	10.6 ± 0.06	0.287 ± 0.001	20.8 ± 0.37	0.3081 ± 0.0159
	B0.5 + M	11.2 ± 0.12	0.285 ± 0.001	21.4 ± 0.26	0.3528 ± 0.0108
	B1 + M	10.7 ± 0.15	0.288 ± 0.009	20.9 ± 0.72	0.3242 ± 0.0385
	B1.5 + M	10.4 ± 0.19	0.292 ± 0.002	20.5 ± 0.58	0.2857 ± 0.0110
	Mean	11.1 ± 0.12 B	0.283 ± 0.001 A**	21.4 ± 0.20 B	0.3534 ± 0.0112 A**
Mean	S	12.3 ± 0.17 A**	0.270 ± 0.005 E	22.8 ± 0.34 A**	0.3528 ± 0.0410 A**
	M	11.8 ± 0.16 B	0.274 ± 0.006 DE	22.3 ± 0.34 AB	0.3121 ± 0.0392 B
	B0.5	11.5 ± 0.13 C	0.278 ± 0.008 CD	21.9 ± 0.23 BC	0.2844 ± 0.0407 BC
	B1	11.2 ± 0.13 DE	0.281 ± 0.009 BC	21.5 ± 0.10 BCD	0.2530 ± 0.0409 CD
	B1.5	10.9 ± 0.13 F	0.285 ± 0.011 AB	21.1 ± 0.23 CD	0.2235 ± 0.0387 DE
	B0.5 + M	11.3 ± 0.10 CD	0.282 ± 0.006 BC	21.5 ± 0.14 BCD	0.2657 ± 0.0402 C
	B1 + M	10.9 ± 0.12 EF	0.285 ± 0.009 AB	21.2 ± 0.35 CD	0.2305 ± 0.0454 D
	B1.5 + M	10.5 ± 0.11 G	0.290 ± 0.003 A**	20.8 ± 0.29 D	0.1941 ± 0.0419 E

S, soil; M, mycorrhiza; B0.5, 0.5% biochar; B1.0, 1.0% biochar; B1.5, 1.5% biochar; B0.5 + M, 0.5% biochar + mycorrhiza; B1.0 + M, 1.0% biochar + mycorrhiza; B1.5 + M, 1.5% biochar + mycorrhiza; ±, standard error.
$^{**}p < 0.01$, $^{*}p < 0.05$.

has a cooling effect on the soil, which affects soil heat capacity and lowers soil temperature (Yerli *et al.* 2022). Water infiltrating the soil causes a change in the radiant energy delivered to the soil by the sun's rays and alters the energy flux, especially at the soil surface (Chiemeka 2010). Although the heat distribution in the soil is more evident in wet soils, where the pores contain both water and air, than in dry soils, where the soil pores are filled only with air, greater cooling is observed (Onwuka 2016). Zhang *et al.* (2013) pointed out that the increased moisture in the soil causes a decrease in soil temperature at a level greater than 1.5 °C, especially in the surface soil (5 cm). Especially in the irrigation of dry soils, the increased moisture in the soil can significantly decrease the soil temperature through a cooling effect.

Soil CO_2 emission

The effects of mycorrhizal biochar treatments and irrigation water types on soil CO_2 emissions were significant at the $p < 0.01$ level (Table 5 and Supplementary 2). CO_2 emissions from the soil in biochar treatments (B0.5, B1, and B1.5) decreased by 19.4, 28.3, and 36.6%, respectively, while the emissions in mycorrhiza and the combination of biochar with mycorrhiza treatments (M, B0.5 + M, B1 + M, and B1.5 + M) decreased by 11.5, 24.7, 34.7, and 45.0%, respectively, compared with the control treatment (S). In addition, irrigation with recycled wastewater resulted in approximately two times higher soil CO_2 emissions than freshwater.

The effect of biochar on reducing CO_2 emissions from soil can be evaluated in terms of soil organic carbon stabilization and soil organic carbon storage due to the spongy structure of biochar. The stable polycyclic aromatic carbon of biochar is capable of maintaining soil carbon stocks for years (Bass *et al.* 2016). This endemic feature of biochar increases the carbon stock of the soil and stabilizes the organic carbon. Thus, CO_2 emissions from the soil are prevented by biochar, with the reduction of excessive mineralization (Yang *et al.* 2017). Kuzyakov *et al.* (2014) reported that only 6% of biochar applied to soil produced

CO_2 emissions through mineralization in the first 8.5 years. The biochar with a spongy structure retains moisture and carbon in the soil and reduces CO_2 emissions from the soil by stabilizing carbon in the soil (Cakmakci *et al.* 2022; Yerli *et al.* 2022). Biochar absorbs carbon in soil (Kan *et al.* 2016) and reduces soil CO_2 emissions by preventing carbon oxidation by creating resistance to microbial degradation (Sheng & Zhu 2018). Biochar limits the use by soil microorganisms of carbon that is present and has been made available to the soil, resulting in fewer oxidation and fewer emissions (Mukherjee & Lal 2013).

The effect of mycorrhiza on reducing CO_2 emissions from the soil can be explained by the fact that mycorrhizal hyphae store carbon in the soil and prevent mineralization in the symbiosis of mycorrhiza with the plant. It contributes to the exchange of soil organic carbon in the network formed by plant roots and mycorrhizal hyphae without oxidation (Zhou *et al.* 2020). Since organic carbon is used more effectively by mycorrhiza and plants under mycorrhizal conditions, the risk of CO_2 emissions from the soil may be reduced (Cavagnaro *et al.* 2008). In addition, promoting the development of soil aggregates by mycorrhiza (Table 4) may have reduced the conversion of organic carbon to CO_2 by increasing the conservation of organic carbon in aggregates. Promoting the proliferation of aggregates under mycorrhizal conditions (Wilson *et al.* 2009) reduces mineralization by increasing the stock of organic matter in soil aggregates (Panneerselvam *et al.* 2020). Mycorrhiza help stabilize soil organic carbon by transferring carbon from the area of high respiration in the soil to the soil matrix, including soil aggregates (Zhu & Miller 2003). This process occurs when mycorrhiza forms extramatric hyphae outside the plant root (Leake *et al.* 2004). In addition, the photoassimilation of two carbon compounds from the plant by mycorrhiza (Boyno *et al.* 2022) can be considered another way to reduce emissions.

Further reduction of CO_2 emissions from the soil when biochar is combined with mycorrhizal treatments can be attributed to the fact that more carbon stocks can be provided in the soil aggregate due to better development of soil aggregates in these treatments (Tables 3 and 4). In addition, the possibility of better mycorrhizal development in these treatments may have reduced soil CO_2 emissions by helping to maintain more soil organic carbon. Boyno *et al.* (2022) found that plant root colonization and mycorrhizal spore numbers were better under mycorrhizal conditions, while Warnock *et al.* (2007) reported that plant root colonization and mycorrhizal spore numbers were much better under biochar conditions. Mickan *et al.* (2016) found that this growth can expand the soil carbon pool by improving organic carbon conservation. In addition, the better and wider network of mycorrhizal hyphae with plant roots on the biochar surface provides both organic matter storage and comfortable conditions for plant uptake of nutrients (Hammer *et al.* 2014). Thus, organic carbon stored or sequestered in this network shows carbon stability in the soil (Warnock *et al.* 2007), which not only reduces CO_2 emissions from the soil but also improves soil fertility.

The effect of irrigation with recycled wastewater on increasing soil CO_2 can be explained by the contribution of dissolved organic matter from wastewater to soil (Table 3), which is due to the chemical and BOD_5 of wastewater (Table 2). When organic carbon, which is a derivative of organic matter, cannot be retained in the soil for various reasons, it combines with oxygen and is released from the soil to the atmosphere in the form of CO_2 (Yerli *et al.* 2022, 2023b). Thangarajan *et al.* (2012) found that the increase in soil CO_2 emissions in irrigation with wastewater was due to the organic carbon that the wastewater added to the soil, while Fernández-Luqueño *et al.* (2010) reported that emissions from field soils irrigated with recycled wastewater were nearly 2.5 times higher than those from freshwater. Similarly, Biswas & Mojid (2018) indicated that 20% more organic carbon in soils irrigated with recycled wastewater than in soils irrigated with freshwater can cause a significant increase in CO_2 emissions.

CONCLUSION

In this study, it was found that increasing the dose of biochar was effective in reducing CO_2 emissions in turf soils irrigated with recycled wastewater in the range of 19.4–36.6%, and the emission–reduction effect of combining biochar with mycorrhiza was even more effective in the range of 11.5–45.0%. Increasing biochar and combining biochar with mycorrhiza reduced H_2O emissions by 4.0–6.4% compared to the control treatment, and soil temperature by 2.6–9.1% by conserving soil moisture, while combining biochar with mycorrhiza further reduced H_2O emissions and soil temperature by supporting soil moisture retention. The combination of biochar and mycorrhiza promoted greater organic matter and carbon sequestration in the turf soil, increased the total nitrogen contribution of the soil, thereby regulating the EC and pH of the soil, and improving the physical properties of the soil such as bulk density, porosity, and wet aggregate stability. It could be recommended to apply the combination of biochar at a rate of 1.5% with mycorrhiza to turf soils, as it reduces CO_2

emissions caused by global warming, which is the greatest risk under current conditions, and increases moisture retention in the soil by reducing H_2O emissions. However, as a result of this study, it was also considered advisable to conduct more detailed field studies under different climatic conditions.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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