Journal of
Water & Climate Change

IWA PUBLISHING

Journal of Water and Climate Change Vol 15 No 2, 541 doi: 10.2166/wcc.2024.482

The effect of arbuscular mycorrhizal fungi on carbon dioxide (CO_2) emission from turfgrass soil under different irrigation intervals

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ABSTRACT

Increased nutrient and/or water uptake by arbuscular mycorrhizal (AM) symbiosis can affect soil biochemical properties and emission of the greenhouse gas carbon dioxide (CO_2). Therefore, an experiment was designed to investigate the effect of AM fungi (AMF) on CO_2 emissions from turfgrass. Three different AMF species (*Funneliformis mosseae*, *Claroideoglomus etunicatum*, and *Rhizophagus irregularis*) were used in this experiment. Turfgrass plants were cultivated in pots containing both mycorrhizal and non-mycorrhizal soils over a 10-week period. To mimic real-world conditions, the plants underwent irrigation cycles at intervals of 1, 2, and 3 days, replicating common irrigation practices in turfgrass fields. The research aimed to comprehensively understand the effects of AMF and varying irrigation intervals on CO_2 emissions, soil characteristics, plant growth, and AMF parameters. It was observed that the changing irrigation intervals affected the AM symbiosis and this effect increased as the irrigation interval increased. It was determined that this AM symbiosis created with the plant significantly reduced CO_2 emissions. In addition, it was determined that it regulates the soil structure and increases plant growth. In conclusion, it can be said that AMF species reduce CO_2 emissions by reducing the need for water in the turfgrass.

Key words: arbuscular mycorrhizal fungi, carbon dioxide (CO_2) emission, irrigation, turfgrass

HIGHLIGHTS

- Host plants inoculated with arbuscular mycorrhiza (AM) symbiosis are believed to tolerate stressful situations.
- Increased CO_2 emissions in soil may pose a threat to a sustainable ecosystem.
- CO_2 emissions and soil temperature have been reduced with mycorrhiza treatments.
- It has been determined that AM fungi increases plant growth under different irrigation intervals.

INTRODUCTION

Turfgrass production, an essential part of the green ecosystem, directly depends on irrigation. However, while the amount of water used may pose a problem for the water needs of the increasing world population, increasing CO_2 emissions in the soil can pose a danger to a sustainable ecosystem (Allaire *et al.* 2012; Hatfield-Dodds *et al.* 2017; Song *et al.* 2018). To avoid this, the availability of arbuscular mycorrhizal fungi (AMF), which have the potential to increase the water cycle and thus improve environmental quality in various ecosystems, has been reported (Gianinazzi *et al.* 2010; Jackson *et al.* 2012).

It creates arbuscular mycorrhizal (AM) symbiosis by interacting with plant roots of AMF (Smith & Read 2010; Smith & Smith 2011; Boyno & Demir 2022). This symbiosis has been demonstrated by research in which photoassimilate carbon compounds exchange in the uptake of soil nutrients, especially phosphorus (P) (Kiers *et al.* 2011; Fellbaum *et al.* 2014; Boyno *et al.* 2022). It has also been suggested that these fungi may affect CO_2 emissions in the soil through their direct respiration or indirect effects on heterotrophic microorganisms (Johnson & Bridge 2002; Langley & Hungate 2003; Cavagnaro *et al.* 2008).

AM symbiosis can also affect CO_2 emissions in the soil through changes in soil physical properties (Augé 2004; Cavagnaro *et al.* 2012). The amount of water in the soil significantly impacts the microbial communities and changes the processes of

mineralization, gaseous diffusion, oxygen availability, nitrification, and denitrification (Blagodatsky & Smith 2012). A large body of research suggests that, in addition to the direct effects of soil on water retention, AM symbiosis modifies plant–water interactions and makes mycorrhizal plants more resilient to water stress than non-mycorrhizal plants (Augé *et al.* 2001; Burger *et al.* 2005). Mycorrhizal plants often have their stomatal conductivity unchanged for longer than non-mycorrhizal plants when soil moisture is lowered by lengthening the watering interval (Duan *et al.* 1996). Similarly, mycorrhizal plants frequently display better photosynthetic rates in low soil moisture situations, demonstrating a more substantial tolerance to drought and internal water use efficiency (Augé *et al.* 2001; Ruiz-Lozano *et al.* 2012). Mycorrhizal plants typically have more significant plant sizes, allowing hyphae to explore the soil's water and nutrients more deeply, increasing the rate of photosynthetic activity (Augé *et al.* 2001; Birhane *et al.* 2012). However, among plants of comparable size and nutritional content, mycorrhizal and non-mycorrhizal plants vary in their water interactions (Kothari *et al.* 1990). Compared to non-mycorrhizal plants, mycorrhizal plants may absorb more water, which might lead to reduced plant water demand and soil moisture, affecting biogeochemical soil cycles and CO_2 emissions (Augé *et al.* 2001; Augé 2004).

AM symbiosis of plant roots may boost water and nutrient usage effectiveness, enhancing the quality of the environment in many environments (Gianinazzi *et al.* 2010; Jackson *et al.* 2012). The development of sustainable ecological practices depends on understanding how these symbiotic interactions affect plant nutrition and biogeochemical cycles. This study examined how AM symbiosis affects CO_2 emissions from the soil under varying irrigation intervals of turf plants. We hypothesized that mycorrhizal plants would reduce CO_2 emissions by reducing their water requirement. To test this hypothesis, a controlled *in vivo* experiment was designed.

MATERIALS AND METHODS

Plant/fungal materials and study area

Turfgrass seeds (content: 40% *Lolium perenne*, 35% *Festuca rubra rubra*, 15% *Festuca rubra commutata*, and 10% *Poa pratensis*) were obtained from a private company (4D Dominantl Mix, Dr Tohumculuk Co., Turkey). AMF inoculums (*Funneliformis mosseae, Claroideoglomus etunicatum*, and *Rhizophagus irregularis*) were also obtained from the culture collection in Phytopathology Laboratory, at the Department of Plant Protection, Faculty of Agriculture, Van Yuzuncu Yil University, Turkey. The study was carried out in the climate room of the Faculty of Agriculture of Van Yuzuncu Yil University. During the study, the mean temperature and humidity inside the climate room were determined as 22 $\pm$ 3°C and 45 $\pm$ 5% (Figure 1).

Study design

In the research, 16 $\times$ 18 cm plastic pots that can hold 1 l of mixture were used. In the experiment, cultivation soil containing 1:2 sand, which was disinfected by keeping it in an autoclave at 121°C for 1 h, was used. Based on this soil volume, 10% AMF inoculum (*F. mosseae*, *C. etunicatum*, *and R. irregularis*) was added to the cultivation soil and mixed; 10% sterile sand was added to the pots without AMF application. Turfgrass mixture consisting of 40% *L. perenne*, 35% *F. rubra rubra*, 15% *F. rubra commutata*, and 10% *P. pratensis* was planted in this cultivation soil at 50 g m^{-2} seeds per pot (Celebi *et al.* 2009). The sowing rate, amount, and selection of turfgrass varieties were determined considering the purity and the germination rate of the seeds before the study. The seeds were covered with peat, burnt-sieved barn manure, and the soil at a ratio of 1:1:1 and pressurized

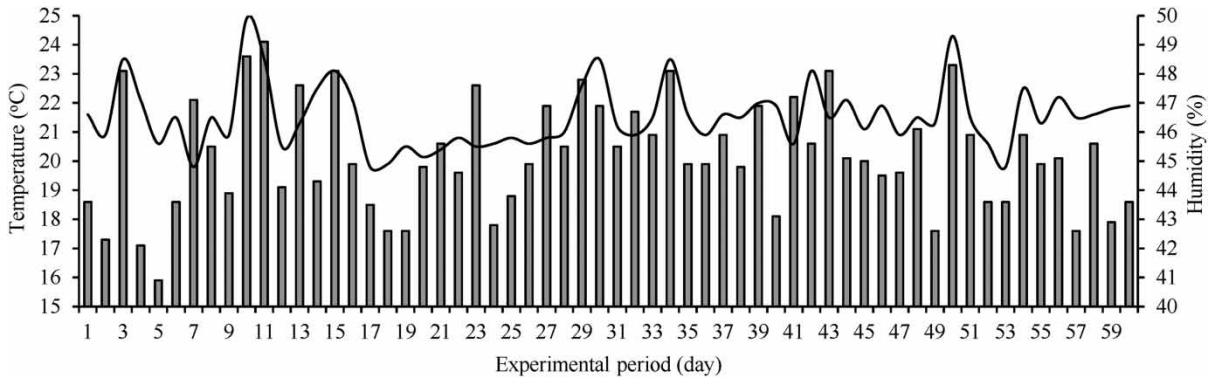

Figure 1 | Daily temperatures [——] and humidity [▬] inside the climate room throughout the experimental period.

by weight. Peat was preferred to protect the soil and seeds from excessive light and other external factors, while barn manure was preferred to increase moisture retention despite the soil's light texture. After the sowing process was completed, 4 g m^{-2} of 26% ammonium nitrate fertilization was applied (Celebi *et al.* 2009). The turfgrass lawn mowing was initially made to be cut from 8 to 6 cm in order not to prevent the development of roots and crops and then to be cut from 5–6 to 3–4 cm approximately every 8–10 days (Morris & Shearman 1998).

The field capacity (pot capacity) and irrigation water amount were determined on a weight basis. The non-mycorrhiza treatment was placed in the water pan and kept in the pan until complete wetting of the soil surface through capillarity. Then it was drained with gravity to remove excess water above the field capacity, and the field capacity was determined as 0.322 m^3 m^{-3}. The irrigation water amount applied in each irrigation was determined with $I = \text{Wfc} - \text{Wi}$ where I is the irrigation water amount (mL), Wfc and Wi are weights of the pot (kg) at field capacity and measurement day, respectively.

Until the turfgrass germinated, the amount of moisture that decreased every day was completed to the field capacity. For this purpose, 18.3 mm of irrigation water was applied in this process. Irrigation treatments (irrigation at intervals of 1, 2, and 3 days) started with the first lawn mowing after the crop height was 8 cm (11th day of the study) and continued. The total amount of irrigation water applied during the study was approximately equal in all treatments (Figure 2).

Soil analysis

In the experimental soil, particle size distribution (Gee & Bauder 1986), bulk density, specific gravity (Blake & Hartge 1986), aggregate stability (Kemper & Rosenau 1986), $CaCO_3$ (Nelson 1982), organic matter (Nelson & Sommers 1982), total nitrogen (Bremner & Mulvaney 1982), available phosphorus (Olsen *et al.* 1982), and available potassium (Knudsen *et al.* 1982) were determined by analysis. Soil reaction (pH) (McLean 1982) and electrical conductivity (EC) (Corwin & Rhoades 1984) were determined using a pH meter and conductometer in the saturation extract, respectively, while the porosity (Danielson & Sutherland 1986) and organic carbon (Avramidis *et al.* 2015) were also calculated. According to the USDA classification, the soil was sandy loam, and the study of soil properties is given in Table 1.

Irrigation water analysis

In the middle of the study, the tap water was used as irrigation water, cations (Ca, Mg, Na, and K) were determined by inductively coupled plasma - optical emission spectrometry (ICP-OES) (Anonymous 2007), CO_3 and HCO_3 were determined by titration with sulfuric acid, CI was determined by titration with silver nitrate using a potassium chromate indicator (Tuzuner 1990), SO_4 was determined by a spectrophotometer (HACH 2010), pH and EC were determined by the pH meter and the conductivimeter by direct reading. The sodium absorption rate (SAR) and percent sodium (%Na) were determined by calculation (Kanber & Unlu 2010). The values regarding irrigation water quality are given in Table 2.

The plant growth parameters

At the end of the study, turfgrass color values were determined by three researchers according to a 1–9 scale (1: yellow, 3: light yellow-green, 5: green, 7: dark green, and 9: highly dark green) and turfgrass quality values were also determined according to the uniformity, density, color, and homogeneous appearance, again with the same method, according to the 1–9 scale (Morris & Shearman 1998). Plant fresh weights were determined by weighing. The plant materials were dried at 70 °C for

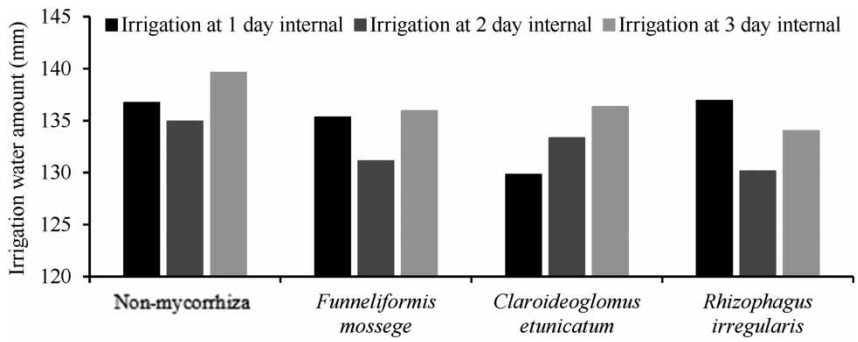

Figure 2 | The total amount of irrigation water applied during the study.

Table 1 | The properties of experimental soil

Property		Property	
Soil texture	Sandy loam	EC (dS m^{-1})	0.615
Sand (%)	66.3	pH	7.17
Silt (%)	15.6	$CaCO_3$ (%)	2.2
Clay (%)	18.1	Organic matter (%)	0.81
Bulk density (g cm^{-3})	1.37	Organic carbon (%)	0.47
Specific gravity	2.69	Total N (%)	0.040
Porosity (%)	49.1	P_2O_5 (kg ha^{-1})	59.0
Aggregate stability (%)	48.1	K_2O (kg ha^{-1})	662

Table 2 | The properties of irrigation water used in the study

Property		Property	
Ca (me L^{-1})	1.96	CO_3 (me L^{-1})	-
Mg (me L^{-1})	3.65	HCO_3 (me L^{-1})	2.91
Na (me L^{-1})	0.42	Cl (me L^{-1})	1.22
K (me L^{-1})	0.22	SO_4 (me L^{-1})	1.97
EC (dS m^{-1})	0.644	SAR	0.25
pH	7.91	%Na	6.72

48 h and measured to estimate the dry weights. The root length (cm) was determined using a digital caliper (Insize-1112–150, Germany).

AMF root colonization, spore density, and mycorrhizal dependency assays

Root cleaning and staining (Phillips & Hayman 1970) and the grid line intersect technique were used to assess the colonization of host plant roots by AMF (Giovannetti & Mosse 1980). Root segments (0.5–1 cm) were treated with 10% KOH (Merck 1.05012.1000, Germany) and 10% HCl (Merck 1.00312.2500, Germany) after being washed in distilled water. The root portions were then dyed with 0.05% Lactophenol blue (Merck 1.13741.0100, Germany), and lactoglycerol was used to clean them 2–3 times. To investigate fungal colonization and structures, microscopic slides were created. These slides were created by cutting 1 cm long segments to represent the root zone. A total of 27 segments were evaluated in this way for each sample. Fungal spores were extracted from soil samples (1 g in three replicates) using the ultrasound centrifugation technique (Boyno *et al.* 2023) and AM fungal spore density in 1 ml was counted using a stereomicroscope (Leica, Germany). Then, the number of AM fungal spores in 1 g of soil was determined by the following formula (Boyno *et al.* 2023).

$$\mathrm{TSN} = [\mathrm{SN} \times W] \div S$$

where TSN is the total AMF spore numbers in 1 g of rhizosphere, SN is the AMF spore numbers in 1 mL of spore suspension, W is the amount of water used (ml), and S is the amount of soil used (g).

Mycorrhizal dependency (MD) was also determined after 10 weeks of the host plant's growth (Declerck *et al.* 1995), as follows:

$$\mathrm{MD}\ (\%) = [A - B] \times 100 \div A$$

where A is the AMF (+) total dry weight of the plant and B is the AMF (–) total dry weight of the plant.

Carbon dioxide (CO_2) measurements

Carbon dioxide emission from soil was measured with a gas analyzer instrument (EGM-5, PP Systems, Stotfold, UK), which is a portable dynamic closed chamber infrared gas analyzer system, by taking three measurements from each treatment at the same time every day and continued until the end of the study. The non-steady state through the flow chamber (SRC-1, PP Systems, Stotfold, UK; volume 1,334 cm^3 and area 78.5 cm^2) had only one opening to the soil. In addition, these values were recorded at the time of carbon dioxide measurement to determine the relationship of carbon dioxide with soil temperature and soil moisture. Soil temperature was measured at a depth of 5 cm with an STP-1 soil temperature probe connected to the EGM-5 (Buragiene *et al.* 2019; Yerli & Sahin 2021), while the soil moisture was determined according to weight.

Statistical analysis

Statistical analyses were performed by using the SPSS software (V23.0). ANOVA was applied to the data, and Duncan's multiple range test was used to compare significant means. In addition, linear regression analysis was used to evaluate the relationship of CO_2 emission with soil temperature and soil moisture.

RESULTS

Soil properties

The effects of AMF species (*F. mosseae*, *C. etunicatum*, *and R. irregularis*) on soil properties at different irrigation intervals are presented in Table 3. It was determined that mycorrhizal treatments had no effect on EC and pH values in the soil compared to non-mycorrhizal treatment at varying irrigation intervals. However, it was determined that mycorrhizal treatments increased other parameters (organic matter, organic carbon, total nitrogen (N), P_2O_5, and K_2O). In particular, the highest values for organic matter, organic carbon, and total N ratios were determined in *C. etunicatum* and *R. irregularis* treatments, while the highest values in P_2O_5 and K_2O ratios were determined in *C. etunicatum* treatment. When the mean day (1, 2, and 3 days) intermittent irrigation treatments were examined, statistical differences were not observed in all parameters (Table 3).

The plant growth parameters and AMF analyzes

The effects of AMF species on the growth parameters of plants at different irrigation intervals are presented in Table 4. While these parameters were at the highest values in 1-day interval irrigation conditions, gradual decreases were determined in 2- and 3-day interval irrigations. However, it was determined that the plants forming AM symbiosis had the highest mean growth parameters in general compared to the plants non-mycorrhiza. In the leaf color and turfgrass quality mean values, it was determined that the growth parameters of plants forming symbiosis with *C. etunicatum* and *R. irregularis* were higher, while the highest value in other parameters occurred in plants forming symbiosis with *C. etunicatum* (Table 4).

Considering the AMF parameters, it is seen that *C. etunicatum* treatment has the highest percentage of AMF root colonization with an mean of 63.2%, while *R. irregularis* treatment has the highest soil spore density with an mean of 157 spores g $soil^{-1}$ (Table 4). One-day interval irrigations in plants forming symbiosis with *F. mosseae*; 1 and 2 days interval irrigations in plants forming symbiosis with *C. etunicatum*; 2-day interval irrigations in plants forming symbiosis with *R. irregularis*, the highest AMF colonization rate, and soil spore density occurred. While it was determined that mycorrhizal dependence occurred in all treatments, a parallel increase was observed according to the irrigation intervals in general. Especially *C. etunicatum* has the highest values in mycorrhizal dependence (Table 4).

Carbon dioxide (CO_2) emission

The effects of AMF species on the CO_2 emissions of plants at varying irrigation intervals are presented in Figure 3. In general, CO_2 emissions were at the highest values in 1-day interval irrigation conditions, while parallel reductions were determined in 2- and 3-day interval irrigations. However, in the non-mycorrhiza treatment, the mean CO_2 emission from the soil was 3.5, 5.6, and 5.2% higher, respectively, compared to the *F. mosseae*, *C. etunicatum*, and *R. irregularis* mycorrhiza treatments (Figure 3). Similarly, in all treatments, the soil moisture values decreased as the irrigation intervals increased (Figure 4). The soil temperature parameter increases as the irrigation interval increases (Figure 5). The linear regression analysis results showed that the relationship of CO_2 with soil moisture and soil temperature was quite significant ($P < 0.01$) (Figure 6).

Table 3 | Chemical soil properties in different mycorrhiza species and irrigation intervals

Mycorrhiza	Irrigation intervals	EC (ds m^{-1})	pH	Organic matter (%)	Organic carbon (%)	Total N (%)	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})
Non-mycorrhiza	1 day	0.652 ± 0.002ns	7.49 ± 0.17 ns	0.88 ± 0.02 ns	0.51 ± 0.01 ns	0.042 ± 0.001 ns	60.3 ± 0.2 ns	666 ± 9 ns
	2 days	0.644 ± 0.013	7.34 ± 0.10	0.87 ± 0.01	0.50 ± 0.01	0.045 ± 0.002	60.4 ± 0.5	667 ± 5
	3 days	0.640 ± 0.011	7.39 ± 0.07	0.89 ± 0.02	0.51 ± 0.01	0.042 ± 0.001	61.4 ± 0.2	668 ± 8
	Mean	0.642 ± 0.005ns	7.41 ± 0.07ns	0.88 ± 0.01 C**	0.51 ± 0.01C**	0.043 ± 0.001 B**	60.7 ± 0.2 C**	667 ± 4 C**
F. mosseae	1 day	0.624 ± 0.008 ns	7.47 ± 0.10 ns	0.93 ± 0.02 ns	0.54 ± 0.01 ns	0.047 ± 0.003 ns	68.4 ± 1.4 ns	678 ± 4 ns
	2 days	0.638 ± 0.013	7.39 ± 0.02	0.94 ± 0.03	0.55 ± 0.02	0.050 ± 0.003	67.5 ± 2.3	675 ± 5
	3 days	0.620 ± 0.004	7.32 ± 0.17	0.97 ± 0.03	0.56 ± 0.02	0.045 ± 0.003	67.4 ± 2.4	672 ± 8
	Mean	0.627 ± 0.005	7.39 ± 0.06	0.95 ± 0.01 B	0.55 ± 0.01 B	0.047 ± 0.002 B	67.8 ± 1.0 B	675 ± 3 BC
C. etunicatum	1 day	0.646 ± 0.012 ns	7.39 ± 0.05 ns	0.99 ± 0.02 ns	0.57 ± 0.01 ns	0.055 ± 0.006 ns	70.7 ± 0.5 ns	684 ± 8 ns
	2 days	0.618 ± 0.010	7.44 ± 0.14	1.08 ± 0.05	0.63 ± 0.03	0.051 ± 0.004	70.9 ± 0.4	691 ± 6
	3 days	0.634 ± 0.003	7.52 ± 0.07	1.00 ± 0.01	0.58 ± 0.01	0.059 ± 0.009	70.9 ± 0.5	691 ± 9
	Mean	0.633 ± 0.006	7.45 ± 0.05	1.02 ± 0.02 A	0.59 ± 0.01 A	0.055 ± 0.003 A	70.8 ± 0.2 A	689 ± 4 A
R. irregularis	1 day	0.635 ± 0.014 ns	7.29 ± 0.06 ns	1.00 ± 0.07 ns	0.58 ± 0.04 ns	0.053 ± 0.003 ns	69.1 ± 1.1 ns	687 ± 2 ns
	2 days	0.617 ± 0.003	7.48 ± 0.06	0.98 ± 0.01	0.57 ± 0.01	0.054 ± 0.001	69.5 ± 0.4	680 ± 3
	3 days	0.625 ± 0.010	7.59 ± 0.05	0.99 ± 0.05	0.63 ± 0.03	0.061 ± 0.001	69.6 ± 0.5	372 ± 4
	Mean	0.626 ± 0.006	7.45 ± 0.05	0.99 ± 0.03 A	0.59 ± 0.02 A	0.056 ± 0.002 A	69.4 ± 0.4 AB	680 ± 2 AB
Mean	1 day	0.635 ± 0.005 ns	7.41 ± 0.05 ns	0,95 ± 0.02 ns	0.55 ± 0.01 ns	0.049 ± 0.002 ns	67.1 ± 1.3 ns	678 ± 4 ns
	2 days	0.629 ± 0.006	7.41 ± 0.04	0,97 ± 0.03	0.56 ± 0.02	0.050 ± 0.002	67.1 ± 1.3	678 ± 3
	3 days	0.630 ± 0.004	7.45 ± 0.05	0.96 ± 0.02	0.57 ± 0.01	0.052 ± 0.003	67.3 ± 1.2	676 ± 4

Values with the lowercase letters (a,b,c) in each treatment are not significantly different when followed by Duncan's multiple range test at $*P < 0.05$ and $**P < 0.01$, ns, non-significant.
Values with the capital letters (A,B,C) in each treatment's mean are not significantly different when followed by Duncan's multiple range test at $*P < 0.05$ and $**P < 0.01$, ns, non-significant.
Data in the table are indicated as mean ± SE.

Table 4 | Turfgrass growth and mycorrhiza analyzes in different mycorrhiza species and irrigation intervals

Mycorrhiza	Irrigation intervals	Leaf color (1–9)	Turfgrass quality (1–9)	Fresh weight ($g\ m^{-2}$)	Dry weight ($g\ m^{-2}$)	Root length (cm)	AMF colonization (%)	AMF spore density (spore $g\ soil^{-1}$)	Mycorrhizal dependency
Non-mycorrhiza	1 day	5.6 ± 0.6 ns	5.4 ± 0.1 a**	283 ± 24a*	68 ± 7 a**	2.5 ± 0.3 b**	–	–	–
	2 days	4.8 ± 0.1	5.1 ± 0.5 a	222 ± 26a	50 ± 1 b	3.8 ± 0.2 a	–	–	–
	3 days	5.1 ± 0.1	3.6 ± 0.1 b	155 ± 27b	17 ± 2 c	2.6 ± 0.3 b	–	–	–
	Mean	5.2 ± 0.2 C**	4.7 ± 0.3 C**	220 ± 23 C**	45 ± 8 D**	3.0 ± 0.3 B**	–	–	–
F. mosseae	1 day	7.0 ± 0.4 ns	6.9 ± 0.3 a*	349 ± 25a**	82 ± 18a**	3.6 ± 0.3 a**	66.5 ± 3.7 a**	153 ± 3 a*	+17.1
	2 days	7.0 ± 0.2	6.6 ± 0.4 ab	284 ± 14b	63 ± 4b	3.7 ± 0.3 a	58.2 ± 1.8 b	130 ± 6 b	+26.0
	3 days	6.7 ± 0.2	5.6 ± 0.1 b	165 ± 12c	32 ± 3c	2.1 ± 0.3 b	32.3 ± 6.5 c	137 ± 3 b	+46.9
	Mean	6.9 ± 0.1 A	6.3 ± 0.2 A	266 ± 28 BC	59 ± 9 C	3.1 ± 0.4 B	52.3 ± 5.6 B**	140 ± 4 B**	+30.0
C. etunicatum	1 day	7.8 ± 0.6 ns	7.6 ± 0.4 a*	403 ± 29a**	128 ± 12a**	4.8 ± 0.3 a*	64.5 ± 2.9 ab*	153 ± 9 a*	+46.9
	2 days	6.8 ± 0.1	6.8 ± 0.4 ab	379 ± 64b	110 ± 7a	3.6 ± 0.3 b	68.8 ± 2.7 a	147 ± 3 ab	+54.5
	3 days	7.1 ± 0.1	5.6 ± 0.1 b	270 ± 80c	67 ± 4b	3.9 ± 0.3 b	56.4 ± 4.0 b	133 ± 7 b	+74.6
	Mean	7.2 ± 0.2 A	6.6 ± 0.3 A	351 ± 29 A	102 ± 10 A	4.1 ± 0.4 A	63.2 ± 2.4 A	144 ± 4 B	+58.7
R. irregularis	1 day	6.6 ± 0.6 ns	6.4 ± 0.4 a*	350 ± 12a**	119 ± 3a**	4.5 ± 0.4 a*	49.7 ± 2.9 b*	143 ± 3 b*	+42.9
	2 days	5.8 ± 0.1	5.8 ± 0.5 a	289 ± 45b	78 ± 8b	3.5 ± 0.3 b	54.5 ± 4.5 a	167 ± 9 a	+35.9
	3 days	6.1 ± 0.1	4.6 ± 0.1 b	228 ± 13c	47 ± 7c	3.8 ± 0.3 b	48.1 ± 3.2 b	160 ± 6 ab	+63.8
	Mean	6.2 ± 0.2 B	5.6 ± 0.3 B	289 ± 23 B	81 ± 11 B	3.9 ± 0.2 A	50.8 ± 2.1 B	157 ± 5 A	+47.5
Mean	1 days	6.7 ± 0.4 A**	6.6 ± 0.2 A**	346 ± 16 A**	99 ± 9 A**	3.9 ± 0.3 A**	45.2 ± 8.2 A**	112 ± 20 ns	+26.7
	2 days	6.1 ± 0.2 B	6.1 ± 0.3 B	293 ± 25 B	75 ± 7 B	3.7 ± 0.1 A	45.4 ± 8.1 A	111 ± 20	+29.1
	3 day	6.3 ± 0.2 AB	4.8 ± 0.3 C	204 ± 16 C	41 ± 6 C	3.0 ± 0.3 B	34.2 ± 6.7 B	108 ± 19	+46.3

Values with the lowercase letters (a,b,c) in each treatment are not significantly different when followed by Duncan's multiple range test at $*P < 0.05$ and $**P < 0.01$, ns, non-significant.
Values with the capital letters (A,B,C) in each treatment's mean are not significantly different when followed by Duncan's multiple range test at $*P < 0.05$ and $**P < 0.01$, ns, non-significant.
Data in the table are indicated as mean ± SE.

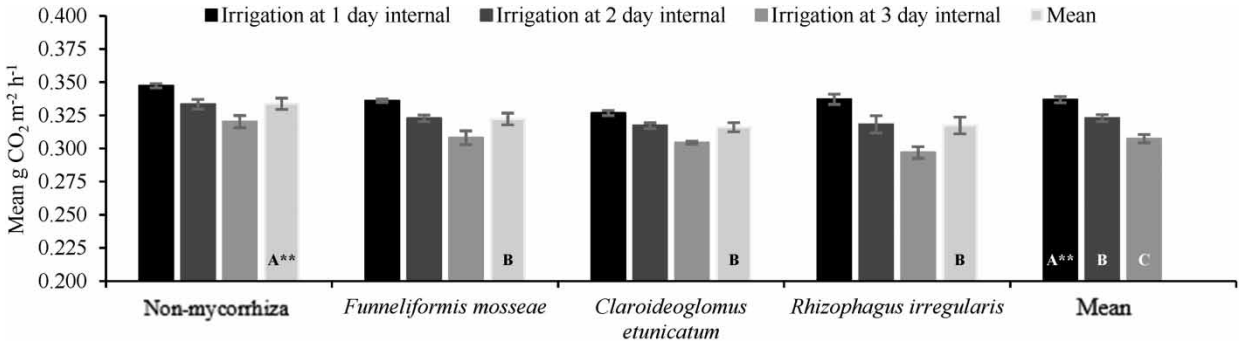

Figure 3 | Mean carbon dioxide emission in different mycorrhiza types and irrigation intervals.

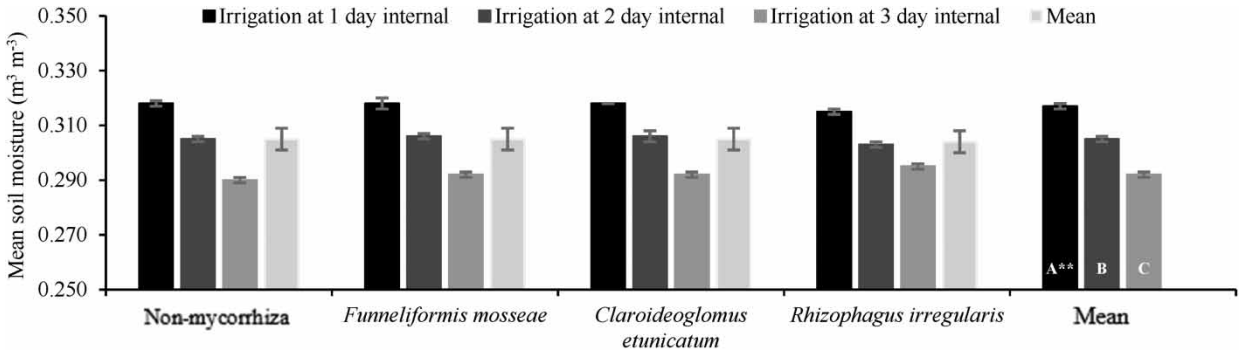

Figure 4 | Mean soil moisture in different mycorrhiza types and irrigation intervals.

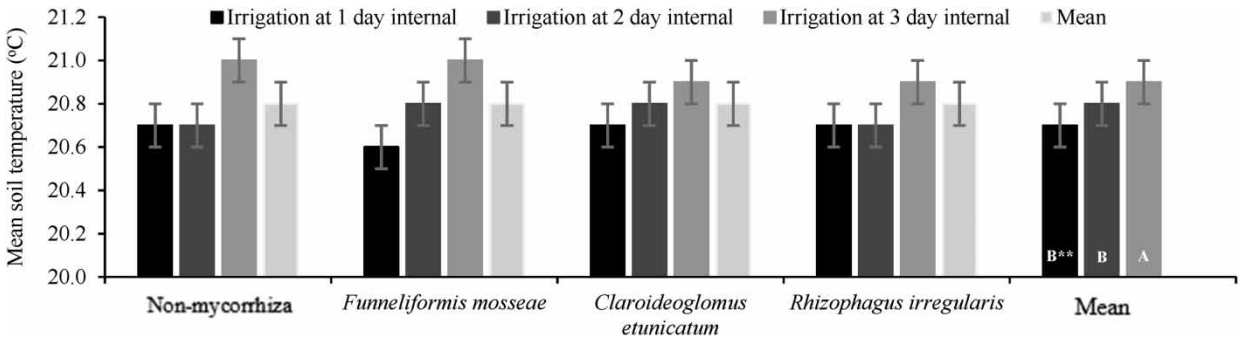

Figure 5 | Mean soil temperature in different mycorrhiza types and irrigation intervals.

DISCUSSION

This research investigated the effects of AM symbiosis on rhizosphere soil, turfgrass growth, and CO_2 emissions from soil under different irrigation intervals (intervals of 1, 2, and 3 days). While there was no change in EC and pH in mycorrhizal rhizosphere soils at all irrigation intervals, an increase was determined in other parameters (organic matter, organic carbon (C), total N, P_2O_5, and K_2O) (Table 3). It is known that plant C allocation to mycorrhizal fungi in the rhizosphere plays a dominant role in the formation and stabilization of organic matter through the production of mycorrhizal biomass, exudates, and necromass (Schmidt *et al.* 2011; Cotrufo *et al.* 2013; Frey 2019). In addition to being an important nutrient store for plants, this organic matter creates more than twice as much carbon (Schlesinger & Andrews 2000) and more

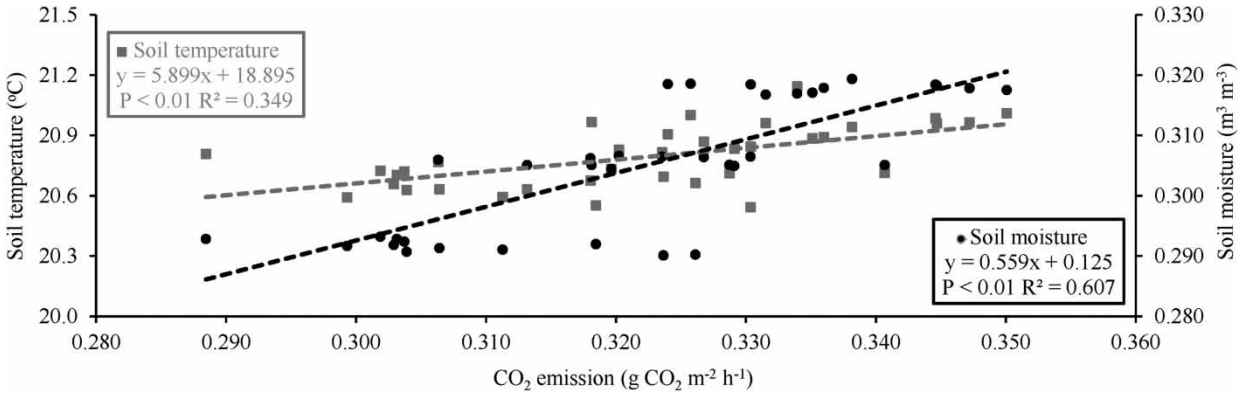

Figure 6 | The linear relationship of carbon dioxide with soil moisture and soil temperature.

than 80% of soil nitrogen (N) than the atmosphere and terrestrial vegetation combined (Simpson *et al.* 2007). Yang *et al.* (2011) also revealed that AMF treatments at different irrigation intervals can be a critical factor in the fixation of organic carbon and total N in the soil. In particular, the organic carbon attached to the soil increases the mineralization of organic phosphorus (P) (Zhang *et al.* 2016, 2018). As a result, AMF can increase plant growth by regulating the soil structure and helping the plant to take up water and nutrients that it cannot reach (Wu *et al.* 2008; Celebi *et al.* 2010; Erman *et al.* 2011; Lazcano *et al.* 2014). In addition, some researchers have shown that the effect of AMF on plant growth is highest even in soils with limited irrigation (Birhane *et al.* 2012; Ruzicka *et al.* 2012; Veresoglou *et al.* 2012). In our study, the increase in the growth parameters of trufgrass plants forming AM symbiosis supports this result (Table 4). This effect is especially prominent in *C. etunicatum* and *R. irregularis* species. As a matter of fact, when AMF parameters are examined, it is seen that *C. etunicatum* treatments have the highest AMF colonization percentage, while *R. irregularis* treatments have the highest soil spore density (Table 4). It has been observed that plants form a more effective symbiosis with mycorrhizal fungi under water stress (Manoharan *et al.* 2010; Demir *et al.* 2022). In particular, the fact that *C. etunicatum* has the highest values in both root colonization and mycorrhizal dependence shows that it forms a high degree of symbiosis with grass (Table 4). Suharno *et al.* (2017) reported that *C. etunicatum* forms an effective symbiosis with plants belonging to the Poaceae family.

The mean carbon dioxide (CO_2) emission from soil was 3.5, 5.6 and 5.2% lower in *F. mosseae*, *C. etunicatum* and *R. irregularis* treated soils, respectively (Figure 3). This case was evaluated as mycorrhiza provides organic carbon retention in the soil. As a result of the study, the high organic carbon content in mycorrhizal soils also supports this case (Table 3). Mean CO_2 emissions were 4.3 and 9.6% higher in 1-day intermittent irrigation compared to 2- and 3-day intermittent irrigations (Figure 3). Cheng *et al.* (2012) and Averill *et al.* (2014) reported that mycorrhiza treatments reduce carbon emissions from the soil. Paterson *et al.* (2016) also stated that mycorrhiza reduces organic matter mineralization in soil. As a matter of fact, nitrogen in the soil can be oxidized and cause CO_2 emissions (Yu *et al.* 2014). The ratio of organic C to total N in the soil can affect CO_2 emissions by changing the oxidation process and the amount of organic matter (Yerli & Şahin 2021). This case can be evaluated in relation to the fact that irrigation frequently changes the organic matter dynamics in the soil and increases oxidation due to increased microorganism activity. Jabro *et al.* (2008) reported that increased microbial activity with increasing soil moisture causes decomposition of organic matter and thus increases CO_2 emissions. Similarly, Sinaie *et al.* (2019) stated that with the decrease in soil moisture, microorganism activities slowed down and CO_2 emissions decreased accordingly. In our study, higher mean soil moisture (Figure 4) and lower mean soil temperature occurred in soils with frequent irrigation (Figure 5). This case can be evaluated in relation to the cooling effect of water ingress into the soil. Similarly, it is stated that the continuous application of water to the soil increases soil moisture and decreases soil temperature (Yerli & Şahin 2021). Mancinelli *et al.* (2015) reported that water ingress into the soil can create a cooling effect by increasing soil moisture. This suggests that it activates the soil biology and increases the release of CO_2 from the soil as a result of mineralization. Soil moisture balance and processes can increase emissions by affecting the oxidation scale of organic C and N (Morugan-Coronado *et al.* 2011). Senyigit & Akbolat (2010) stated that by reducing soil moisture, CO_2 emissions decreased. Yerli *et al.* (2019) noted that scarce irrigation practices reduce emissions. Du *et al.* (2019) and Zhao *et al.*

(2020) revealed that soil temperature, which affects mineralization, is positively related to CO_2 emissions. Jabro *et al.* (2008) reported that soil temperature increased CO_2 emissions by 59%. In fact, the CO_2 emission reduces when the water need of the plants forming the AM symbiosis lowers (Cheng *et al.* 2012; Averill *et al.* 2014), and the results of our research are consistent with this.

CONCLUSION

The study findings showed that AM symbiosis increases with increasing irrigation interval. In particular, CO_2 emissions in soil decreased in colonized plants of AMF. It can be said that the reason for this decrease is that AMF reduces the water requirement in the plant. However, this symbiosis has also shown that turf plants improve growth and regulate soil structure under varying irrigation intervals. Soil management that enhances the colonization of plant roots by arbuscular mycorrhizae can contribute to more efficient use of water in changing environmental conditions, as well as reduce CO_2 emissions and, therefore, the environmental impact of agricultural practices.

RESEARCH INVOLVING HUMAN PARTICIPANTS AND/OR ANIMALS

The research involved no human participants or animals.

INFORMED CONSENT

The research involved no human participants and animals, so a statement on the welfare of animals is not required.

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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First received 21 August 2023; accepted in revised form 25 December 2023. Available online 19 January 2024