Advanced Sustainable BIOfuels for Aviation

Deliverable D2.10:
Results on Lysimeters trials

Consortium:

Acronym	Legal entity	Role
RE-CORD	CONSORZIO PER LA RICERCA E LA DIMOSTRAZIONE SULLE ENERGIE RINNOVABILI	CO
ENI	ENI SPA	BEN
SKYNRG	SKYENERGY BV	BEN
CENER	FUNDACION CENER-CIEMAT	BEN
ETA	ETA – Energia, Trasporti, Agricoltura Srl	BEN
CCE	CAMELINA COMPANY ESPANA S.L.	BEN
JRC	JOINT RESEARCH CENTRE – EUROPEAN COMMISSION	BEN

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Summary

Agricultural production must deal with climate variability, land degradation, and loss of ecosystem functioning as there is an urgent need to develop agricultural methods that balance water and nutrient supply and demand while at the same time improve resilience to climate variability. These challenges coincide with increasing food and energy demand globally driven by growing population. As a result, the agricultural sector's demand for resources – especially water – is increasing and creating competition where resources are limited.

Rainfed agriculture provides the largest share of global food and energy production. Soil water limitations often limit yield and plant growth under non-irrigated agricultural systems, leading to the conversion of agro-ecosystems to soils increasingly close to desertification due to arid and extreme climates.
Thus, there is a need to develop and use agronomic strategies that improve soil water retention as well as water use efficiency and plant available water. To counter the phenomenon of desertification and climate change, attention is given to the use of biochar as an organic soil amendment, which, due to its richly porous structure, significantly enhance soil water retention capacity, and increase the availability of water in the root exploration zone.

Using reduced irrigation techniques such as deficit irrigation along with biochar amendment is considered to have the potential to substantially reduce the amount of irrigation water required, and to enhance crop productivity. To date, there have been relatively few studies that investigated the effects of biochar under water deficit conditions for plant production.

In the frame of the BIO4A project, two trials were realized to corroborate the effect of biochar used as soil amendment on water retention under simulated semi-arid conditions.
The trials were performed in lysimeters under controlled conditions, to simulate the real conditions of the field trials, were devised to produce quantitative results concerning the capacity of biochar to retain water, influence soil humidity, and the N content of water leaving the plant-soil system, compared to the business-as-usual conditions.
Two tests campaigns were realized in a climatic chamber using lysimeters for plant cultivation in order to control and minimize the effect of environmental factors.
In the first experiment, the use of lysimeters was useful to monitor the water loss trough the soil-plant system as well as to collect leachate samples for qualitative analysis, such as the measure of the concentration of soluble elements. In this contest, a further goal of these experiments was to evaluate the ability of biochar to retain nutrients such as nitrates which are very soluble in water and thus susceptible to leakage as occurred under heavy precipitations. These nitrates losses might not only imply a great use of inorganic fertilizer to counter soil fertility and but also pose an environmental adverse effect as eutrophication.
In a second experiment, biochar was used at increasing rates in lysimeters (equivalent to 0, 10, 20 ton/ha), under severe water stress, simulating semi-arid conditions, and provide understanding over the effect on soil humidity and on plant biomass yield.

The two experimental campaigns conducted for the BIO4A project Task 2.2. in controlled conditions with lysimeters, provided some relevant indications regarding the effect of biochar on nitrogen leaching from soil, water stress and plant productivity.
The first indication is that biochar can be used to reduce the total amount of nitrogen forms which exit the soil depth explored by annual plant roots, thus providing an additional tool for the reduction of water pollution and eutrophication phenomena, potentially increasing barley development and productivity in terms of biomass (although no statistical significance was found).
Moreover, when water is not a limiting factor for plant biomass productivity, as in the 1st test campaign, biochar can play a role in conserving soil humidity.
In the framework of water stress conditions, the optimal dose of biochar should be investigated to maximise efficiency, under 20 t/ha d.b. equivalent, as there might be a competition between

water available for plants and stored in biochar under severe conditions (below 15% of field capacity).

1. Introduction

1.1. First test campaign

Aim, approach and duration of the experiment
The aim of the trial was to investigate the effects of the addition of biochar (equivalent to 2 ton/ha) mixed on NPK fertilization on soil water and nitrogen retention and on barley biomass accumulation. The results on soil and plants were compared to the control treatment represented by the presence of only soil and NPK fertilization.
The experiment determined the following parameters:

- Amount of water lost with leaching
- Amount of nitrogen forms lost with leaching
- Barley aboveground and root biomass accumulation

As a scientific approach, it was chosen to simulate a semi-arid Mediterranean environment to better elucidate the biochar performances. Therefore, as a reference, the climatic conditions that occurred during the barley field trial in Spain (Madrid) were replicate in terms of daily and nighty temperatures, precipitations and photoperiod. Moreover, the soil from the same barley field test was used as it was classified as marginal soil.

Due to the limited space of the climatic cell, it was not possible to have three repetitions of the trials simultaneously. Therefore, the experiment was replicated three times in different moments. Each replicate implied a new cycle of barley of about 20 weeks (from seedling to harvest), as well as the soil and biochar removal and lysimeter filling.
The experiment started on September 2020 and ended on April 2022.

1.2. Second test campaign

Aim, approach and duration of the experiment
This trial was conducted in the RE-CORD prototype climatic chamber, which was designed, engineering and developed in the framework of the BIO4A project.
The climatic chamber is a containerized prototype for agronomic experiments in controlled conditions. It is equipped with a PLC, controlled by remote, to regulate and automate daylight, temperature and humidity, simulating different photoperiods and field conditions. It also contains 9 lysimeters and precision scales, ambient sensors for temperature, CO_2 and humidity.

The objective of the prototype was to allow simultaneous treatments, with sufficient replications (3 treatments with 3 replications) for statistical validity. Also, a logic control from remote of critical parameters (daylight duration and photoperiod simulation, temperature, humidity) was possible thanks to the prototype.
The aim of the trial was to investigate the effects of the addition of different biochar doses (equivalent to 0, 10 and 20 ton/ha) mixed with NPK fertilization on soil moisture and biomass yield.
More in details, the experiment determined the following parameters:

- Effect of biochar on the humidity trends in soil
- Influence of increasing rates of biochar (an order of magnitude higher than the field experiments) on biomass production
- Effect of the increasing rates of biochar on water use efficiency

The experiment was operational for pre-trials in June 2022.

2. First test campaign

2.1. Materials and methods

The cultivation trial has been carried out in a climatic chamber under controlled conditions at the University of Florence – Faculty of Agricultural Sciences – DAGRI department, that has a Framework Agreement with RE-CORD.

Lysimeter prototypes

For this experimentation, two lysimeters were specially purchased for barley cultivation, one for cultivation with soil amended with biochar, and one with soil only (used as a control treatment). The dimensions of these lysimeters (67.5 cm in diameter and 60 cm in height and 0.3 m^3 of volume) were suitable for barley growth and for soil moisture monitoring via sensors (**Figure 1A**).
The volume of leachate drained was monitored on two additional lysimeters of reduced size (i.e. mini lysimeter) to facilitate the drainage of leachate and its collection (**Figure 1B**). These mini lysimeters were developed using a plastic container with a hole on the basal part for the drainage of the leachate. The collection of the liquid was done using a plastic bottle placed on a load cell to measure water drained weight variations continuously.
Both types of lysimeters received the same experimental conditions in terms of biochar quantity, NPK fertiliser, irrigated water and barley seed density.

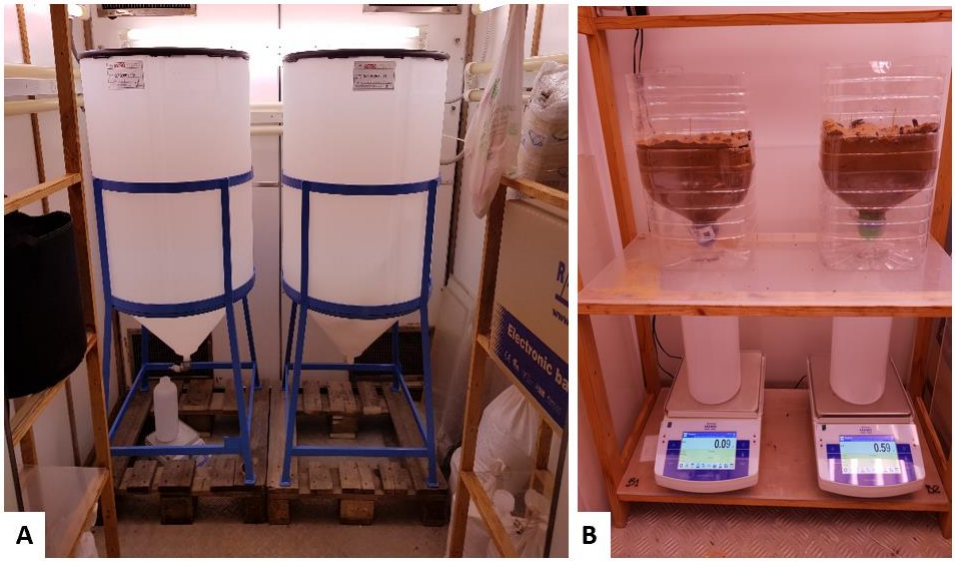

Figure 1 Lysimeters (A) and mini lysimeters (B) used for barley cultivation under controlled environment.

Soil and biochar preparation

Each lysimeter were filled with **soil** derived from the experimental site of the National Institute for Agricultural and Food Research and Technology (INIA) in Madrid, where field trials have been held by Camelina Company España, in the BIO4A framework, on COMBI effects on Camelina sativa yield, soil organic matter content and soil water holding capacity.
Soil material was collected on October 10th, 2019, provided and shipped to Italy by Camelina Company Espana in adequate quantity to fill a volume of 2 m^3. An exploratory excavation at 80 cm has been performed to describe soil profile using FAO guidelines, including number and depth of horizons. Two horizons were described (A, B): at 80 cm, proportions of 25% A and 75%, respectively.
Before the experimentation, soil sample was collected and analysed for the main physical and chemical parameters (**Table 2**). The soil was a silty clay loam with a light texture as denoted by the low value of bulk density. The soil was characterized by a sub-alkaline pH and very low content of organic content and nitrogen concentration (**Table 2**).

Table 1. Soil physical and chemical characteristics at time zero, before barley sowing.

Parameters	Analytic methods	Values
Sand (%)	ISO 11277:2020	52
Silt (%)	ISO 11277:2020	22
Clay (%)	ISO 11277:2020	26
Bulk density (g/cm^3)	UNI EN ISO 17828	1.16
pH	ISO 10390	7.45
Electrical conductivity (dS/m)	ISO 11265	0.76
Cation exchange capacity (cmol(+)/kg)	ISO 11260:2018	16.4
Soil organic matter (g/kg)	D.M. 13/09/99 Met. V.1	9.98
Organic C (% db)	D.M. 13/09/99 Met. V.1	0.85
Total N (% db)	ISO 11261:1995	0.08
Total P (% db)	ISO 11263:1994	532

The **biochar** was prepared using chestnut ligno-cellulosic biomass, pyrolyzed at low temperatures (550°) at RE-CORD plant. The same biochar was also used for the experimentations realized in Spain (in Madrid and Ciudad Real locations).
Table 2 shows the analyses performed on biochar, with the respective analytical methods and the limits imposed by the Italian Legislative Decree D.Lgs 75/2010 regarding the properties of biochar used as a soil conditioner for agronomic purposes.
Since 2015, biochar is included in the list of soil improvers that can be used in soil (Annex 2 of Legislative Decree 75/2010). The definition of biochar adopted at legislative level includes materials obtained from the carbonisation of only virgin products and residues of plant origin from agriculture and forestry, placing stringent limits on heavy metals and organic pollutants (PAHs, PCBs and dioxins). As can be seen from the **Table 2**, all the parameters analysed were within the legal limits, highlighting the suitability of this type of biochar for introduction into the soil as a soil conditioner.

Table 2 Main physical and chemical characteristics of the biochar used in this experimentation. Limits reported in the Italian Legislative Decrees (L.D.) 75/2010 regulating the properties of the biochar used as soil amendment are also shown for a comparison. *b.d.l = below the detection limits; d.b.= dry basis.

Parameters	Analytic method	Unit	Biochar	D.lgs. 75/2010, Annex 2, Category 16, Biochar from pyrolysis and gasification
Moisture	UNI EN ISO 18134-2	% (m/m)	2.9	≥20% for powdery products
Ashes 550°C	UNI EN ISO 18122	% (m/m) d.b.	4.0	≤60 (cl1, cl2, cl3)
Volatiles	UNI EN ISO 18123	% (m/m) d.b.	18.3	
Fixed carbon	Value derived	% (m/m) d.b.	77.7	
C	UNI EN ISO 16948	% (m/m) d.b.	84.7	
H	UNI EN ISO 16948	% (m/m) d.b.	2.3	
N	UNI EN ISO 16948	% (m/m) d.b.	0.6	to be declared
S	ASTM D4239	% (m/m) d.b.	0.03	
H/Corg	Value derived		0.3	≤0,7
Bulk density	UNI EN ISO 17828	kg/mc (d.b.)	166	

pH	ISO 10390		7.2	4-12
Electrical conductivity (1:10)	ISO 11265	mS/m	18	≤1000 mS/m
Sum PAHs16	UNI EN 16181	mg/kg d.b.	0.29	<6
Inorganic C	D.M. 13/09/99 Met. V.1	%(m/m) d.b.	0.21	to be declared
Organic C	Value derived	%(m/m) d.b.	84.5	≥20 (cl1, cl2, cl3)
Elements and metals	UNI EN ISO 16967 UNI EN ISO 16968			
Al		mg/kg d.b.	129	
B		mg/kg d.b.	b.d.l	
Ba		mg/kg d.b.	42	
Ca		mg/kg d.b.	6973	to be declared
Cd		mg/kg d.b.	<0.2	<1.5
Co		mg/kg d.b.	b.d.l	
Cr		mg/kg d.b.	b.d.l	
Cu		mg/kg d.b.	b.d.l	<230
Fe		mg/kg d.b.	147	
K		mg/kg d.b.	2436	to be declared
Li		mg/kg d.b.	b.d.l	
Mg		mg/kg d.b.	660	to be declared
Mn		mg/kg d.b.	64	
Mo		mg/kg d.b.	b.d.l	
Na		mg/kg d.b.	198	to be declared
Ni		mg/kg d.b.	b.d.l	<100
P		mg/kg d.b.	b.d.l	to be declared
Pb		mg/kg d.b.	b.d.l	<140
Si		mg/kg d.b.	153	
Ti		mg/kg d.b.	b.d.l	
V		mg/kg d.b.	b.d.l	
Zn		mg/kg d.b.	b.d.l	<500
Max water retention	DM 01/08/1997 - Met 5	% (m/m)	231	to be declared
Hg	EPA 3051A+EPA 1631E 2002	mg/kg d.b.	<1	<1.5
Cr VI	ANPA 16 Man 3 2001	mg/kg d.b.	<0.2	<0.5
Sum PCDD+PCDF	EPA 31613B 1994	ng TE/kg d.b.	7	<=9 ng TEQ/kg s.s.
PCBs	EPA 3545A 2007+EPA 3620C 2014+ EPA 8270 E 2018	mg/kg d.b.	0	<0.5 mg/kg s.s.
Germination index (diluition at 30%)	UNI 10780:1998 App.K	%	93	to be declared
Granulometry	UNI EN ISO 17827-1			to be declared (mm 0,5-2-5)
< 0,5 mm		% (m/m)	5.7	
> 0,5 mm		% (m/m)	3.3	
> 1 mm		% (m/m)	6.1	
> 2 mm		% (m/m)	14.8	
> 5 mm		% (m/m)	70.1	
d50		mm	2.9	

Barley cultivation

Barley (*Hordeum vulgare*), was selected as a plant model for this experimentation. For lysimeter trials, the *Vinagrosa* variety was chosen since this genotype was also tested in field in Spain during the trials with Camelina and Barley rotation. The **seeds** of variety *Vinagrosa* were provided by Camelina Company in the BIO4A framework and the sowing density was equal to 0.18 ton/ha.

The cultivation period was planned to last between **19-20 weeks** till the phenological stage of the boot, which corresponds to the development of the length of the culms and the subsequent emergence of the leaf sheaths. This phase preludes the reproductive stage and, in the field, occurs in early spring.

Design of the experiment

Due to the limited space in the climate cell, the experiments involve three separate repetitions of barley cultivation replicating the field parameters (**Figure 2**). This enabled the results to be obtained in triplicate to ensure good reproducibility of the experiment and statistical processing. In each repetition, two lysimeters were in operation at once, both filled with pristine soil (**Figure 3**).

The lysimeter 1 was fertilized with 200 kg/ha of NPK (8-24-8) as previously done in field trials in Madrid (Location 1) and it was used as control treatment (**CNT**).

The lysimeter 2 received BIOCHAR + NPK treatment following the same rate (equivalent to 2 ton/ha) and procedure used in field trials in Madrid (**Figure 2**) and it was called biochar treatments (**BC**).

Replication 1		Sowing	Harvest
Ly 1	Control treatment (CNT)	21/09/2020	03/02/2021
Ly 2	Biochar treatment (BC)		
Replication 2		**Sowing**	**Harvest**
Ly 1	Control treatment (CNT)	04/06/2021	19/10/2021
Ly 2	Biochar treatment (BC)		
Replication 3		**Sowing**	**Harvest**
Ly 1	Control treatment (CNT)	01/11/2021	24/03/2022
Ly 2	Biochar treatment (BC)		

Figure 2 Experimental design of the lysimeter experiment and date of sowing and harvest of each replicate.

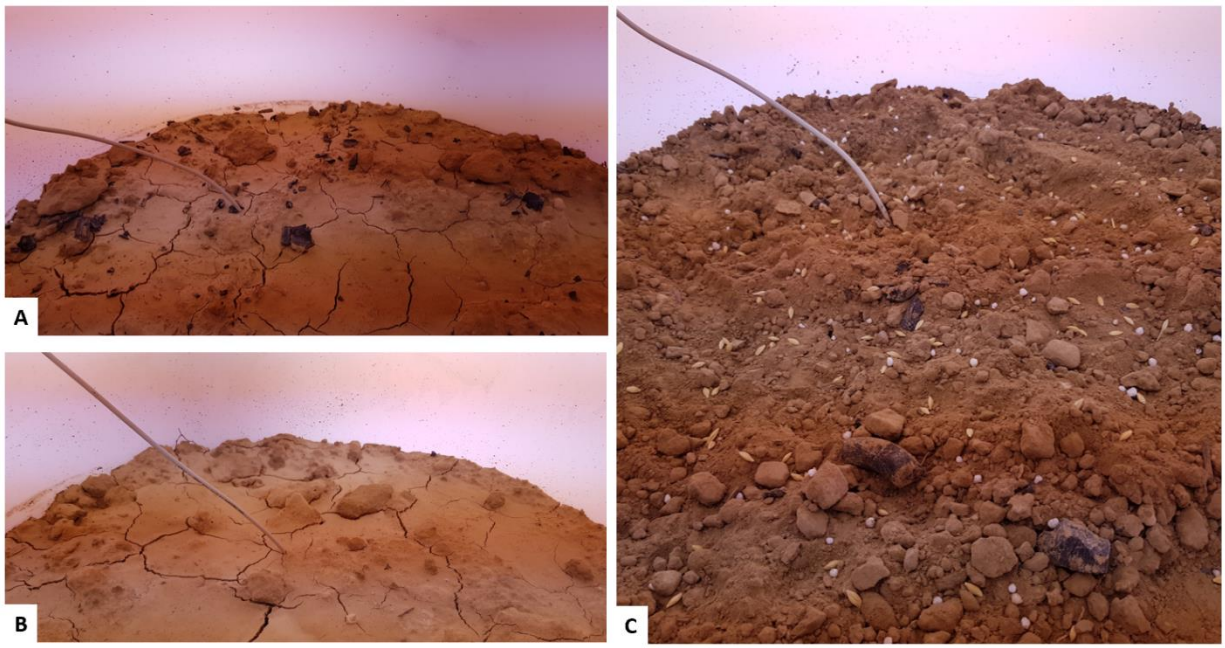

Figure 3 Biochar (A) and control (B) treatment with moisture sensor for humidity monitoring; Biochar treatment with barley seeds and granular NPK fertilization (C).

Environmental conditions

Environmental conditions such as temperature (maximum and minimum), rainfall and photoperiod were replicated following field data obtained during first campaign of barley cultivation at the National Institute for Agricultural and Food Research and Technology (INIA) in Madrid. Meteorological conditions were retrieved online (https://www.esrl.noaa.gov/gmd/grad/solcalc/).

Each week the conditions regarding photoperiod (hours of light and darkness) and temperatures (maximum and minimum) were changed by averaging the daily values. In details, the amount of daylight hours was calculated considering the time of sunrise and the time of sunset**,** while, daytime and night-time temperatures were calculated by averaging the daily average temperature values with the daily maximum and minimum temperature values respectively. Irrigation of the lysimeters took place once a week, providing the cumulative amount of water verified each week. Only for weeks with higher precipitation (> 20 mm for weeks 4 and 7), the watering of the pots took place in two events to avoid water stagnation. Throughout the experiment, the large and small lysimeters received the same volume of water to maintain the same soil moisture and growth conditions for barley.
Temperatures, sunrise and sunset and cumulative precipitations are illustrated in **Figure 4**.
Two moisture probes were installed in the lysimeters to monitor the water content during the cultivation trials.

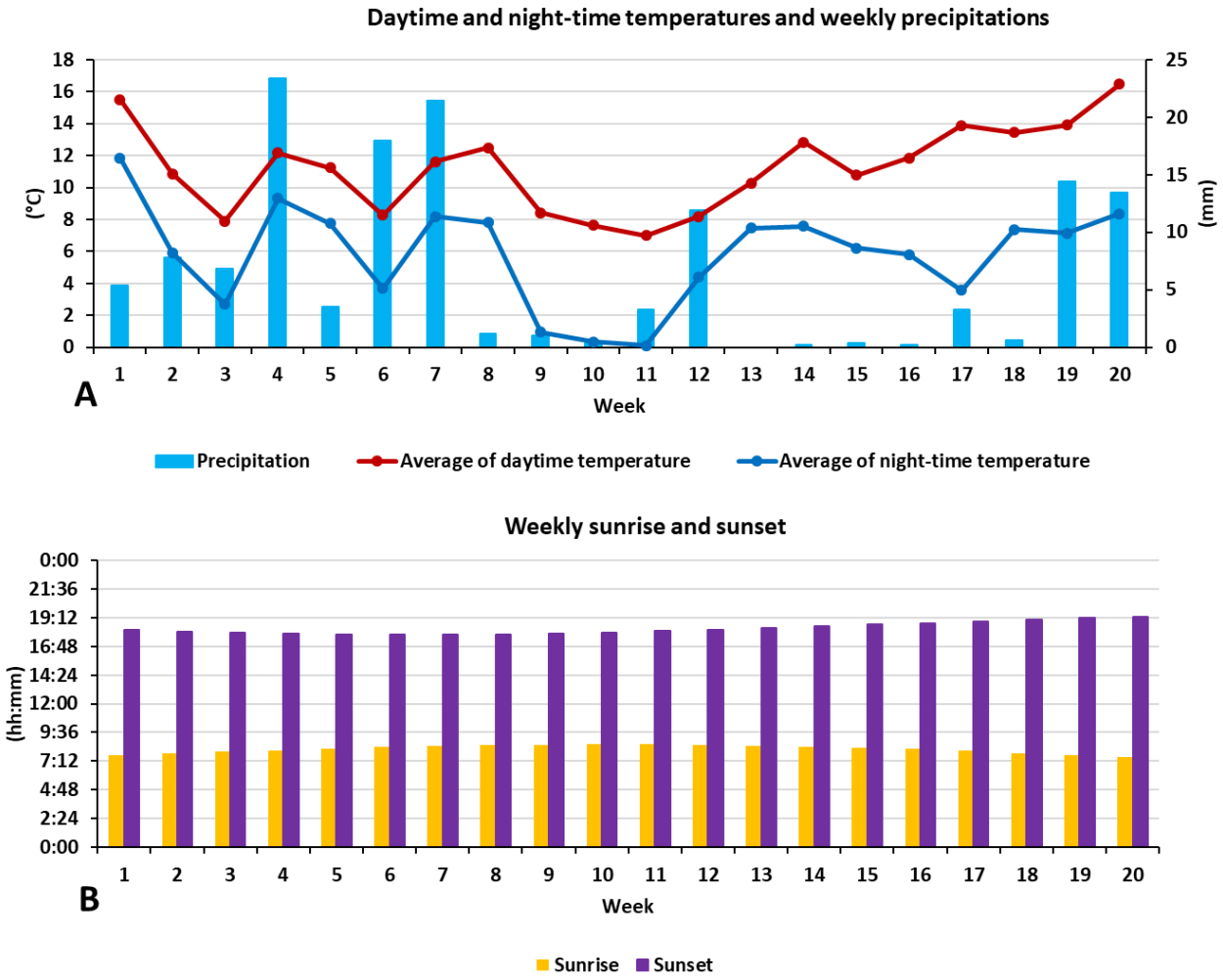

Figure 4 Daytime and night-time temperature values and weekly precipitations (A); Weekly sunrise and sunset values (B) used for setting the climatic cell during the 20 weeks of barley growth.

Drainage collection and leachate analysis

The volume of leachate was continuously monitored using the balance system that allows to estimate the total volume exiting the system from the bottom of lysimeters. Then, the effect of biochar on water saving was determined as followed:

$$WS_{BC}: \frac{(VL_{BC} - VL_{CNT})}{VL_{CNT}} \; x \; 100$$

Where:
WS_{BC} is the effect biochar on water saving (%);
VL_{BC} is the volume leached with biochar (ml);
VL_{CNT} is the volume leached with only soil (control treatment) (ml).

From, it was possible to calculate the percentage of leachate lost as follow:

$$LL: \frac{VL}{VI} \; X \; 100$$

Where:
LL is the leached lost (%) from both lysimeters;
VL is the volume of leached (ml) measured from both lysimeter;
VI is the volume of water supplied as irrigation (ml).

The total amount of leachate lost each week was collected and stored at -20°C for determination of N forms concentrations. In details, the concentration of ammonium ion NH_4^+ and ammonia NH_3 dissolved in the samples was determined by spectrophotometric method using the Ammonium Cell test 14793 Spectroquant ® kit, according to the EPA 350.1 standard, which allowed the measurement of NH_4^+ a 690 nm using the previously calibrated Shimadzu UV-1800 UV-VIS spectrophotometer. This analysis measured the total ammonium and ammonia concentration of the sample considering that generally these two forms are both present in pH dependent equilibrium.
Nitrites (NO_2^-) and nitrates (NO_3^-) concentrations were measured by ion chromatography using a Metrohm 883 Basic IC Plus. Leachate samples were directly injected in the instrument, previously calibrated with a 4-point standard curve for nitrate and nitrites concentration.
Ammonium ion, nitrites and nitrates forms concentrations were used to determine the amount of each form within each leachate sample; then it was possible to estimate the amount of N within each form, and the total amount of N lost with during the leaching.

Barley morphological parameters and biomass determination
At barley boot phenological stage, each lysimeter was flooded to facilitate the extraction of the root system. The following parameters were taken on 20 plants: aboveground vegetation height (from the collar), root length and culm diameter using a calibre. Subsequently, the remaining plants were also extracted and gathered together to determine the biomass weight. More in detail, each single plant was separated into root and apical part, weighed to predict the fresh weight and then oven-dried at 105°C for 24h for measuring dry weight biomass.

Statistical analysis
Data on the volume of leachate lost, nitrogen concentrations and barley growth parameters were compared using a 2-sample t-test to determine significant differences between the averages obtained from treatment with biochar and without biochar, using Minitab®17.1.0, Minitab Inc., State College, PA, US.

2.2. Results and discussions

Leaching volume determination and soil moisture content

However, although there were no statistically significant differences, the average amount of leachate lost was lower for the biochar treatment with an average of 922 ml of leachate and a water saving of approximately 6% compared to the control (**Table 3**). These data indicate that the presence of biochar had a positive effect on soil water retention leading to less water loss from the soil-plant system.

Table 3 Volume leached (ml), Leached loss (%), Water retained (%) and biochar effect on water saving (%) for biochar and control treatments. Values are the means (n=3) and standard deviations (in bracket). Within a same column, different letters mean statistically significant differences (P<0.05).
***The negative sign indicates a reduction of the water leaching.**

Treatments	Volume leached (ml)	Leached loss (% on tot)	Water retained (% on tot)	Biochar effect on water saving (%)
BC	922 (192) a	36 (7) a	64 (7) a	-6 (9)*
CNT	979 (148) a	38 (6) a	62 (6) a	-

Soil moisture data in control and biochar treatments from the 3 replications trials were plotted and illustrated in **Figure 5**. From the graph, an increasing moisture trend can be seen depending on the simulated irrigation and drought periods. In general, it can be observed that the presence of biochar maintained a higher soil moisture of approximately 5-10 %.

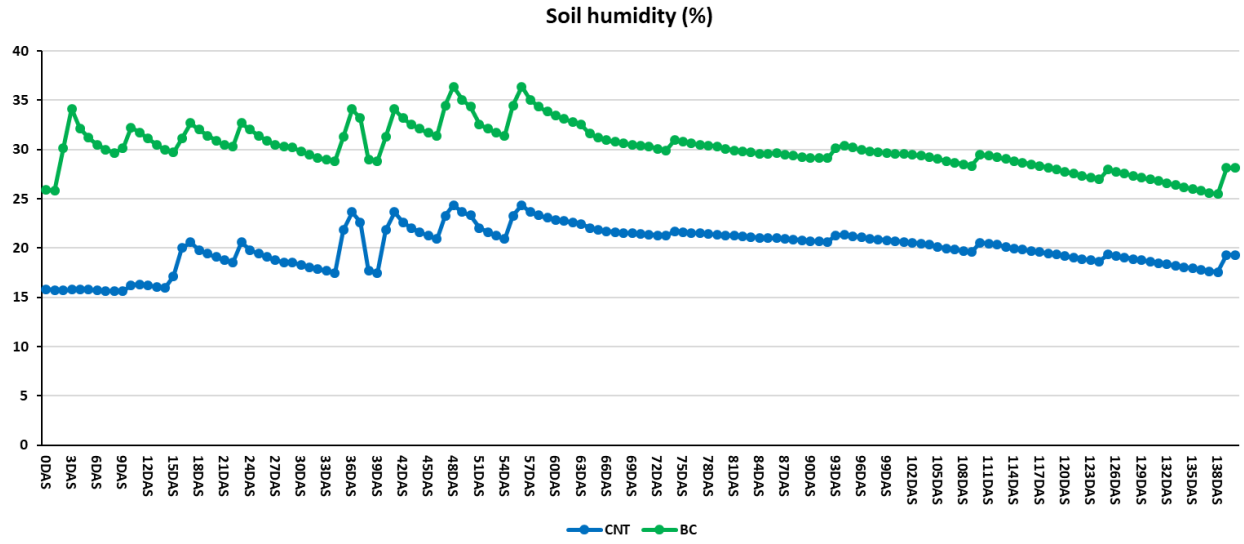

Figure 5 Soil moisture (%) from control and biochar treatments from barley sowing to harvest. DAS=days after sowing

Nitrogen form concentrations within the leachate

The ammonium form (NH_4^+) was below instrumental detection limits (<0.01 mg/l) in all samples analysed. The absence of NH_4^+ in the leachates obtained from the treatments could be due to several mechanisms than can be occurred simultaneously concurring to detect in the circulating solution minute quantities for short period and often in traces:

- NH_4^+ may have been lost through volatilisation after being converted in ammonia (NH_3) which is a volatile compound;
- NH_4^+ may have been totally absorbed by barley roots;
- NH_4^+ may have been nitrified to NO_3^- via NO_2^-;
- NH_4^+ may have been absorbed to the negative charges of the biochar functional groups thanks to its high cation exchange capacity;
- NH_4^+ may have been retained by the absorbing power of the soil, being adsorbed by the residual negatively charged residues on the surface of the phyllosilicates (-OH-) and the organic matter (-COO- and -OH-).

Regarding nitrite and nitrate concentrations, no significant differences were found between the treatments (**Table 4)**, again due to the high variability of the data caused by the technical problem with the climate cell. Despite this, some considerations can be made.

As expected, nitrate represents the most abundant form of nitrogen in both type of leachates, while nitrite represents only a low proportion of the nitrogen lost. In fact, nitrate is among the most abundant forms of inorganic nitrogen, and being easily soluble in water, and having a negative charge, is not retained by the absorbing power of the soil, as the surfaces of clay minerals and organic matter also have electronegative charges.

In general, the average nitrate concentration (although not statistically significant) was higher for the biochar-based thesis with a value of 690.7 mg/l than for the control, which showed an average of 538.1 mg/l (**Table 4**). An inverse trend can be observed for nitrate expressed as a quantity in mass. The amount was in fact calculated by considering the volume of water exiting the system for each leachate sample collected, multiplied by the concentration of nitrate found. Thus, as it can be seen from **Table 4**, the sum of the amounts of nitrate forms (expressed in mg) that came out of the system was lower for the biochar treatment than for the control (325.5 and 365.1 mg for the biochar and control theses, respectively).
This result indicates that the measured average nitrate concentration was higher for biochar than for the control, but, taking into account that less water leaked from the biochar thesis (**Table 3**), less nitrate, and therefore less nitrogen, was lost through leaching.

From this picture, it appears that the presence of biochar resulted in a greater release of nitrate into the circulating solution since the solution was more concentrated, but nevertheless, as less water was lost, there was a saving in nitrate form and nitrogen lost. That means in this case, a reduction of nitrate leaching occurred thanks to the ability of biochar to hold water. In literature this mechanism has been observed by several studies (Ouyang et al., 2013; Zhang et al., 2013, Blanco-Canqui, H., 2017).

Table 4 Nitrites (NO_2^-) and nitrates (NO_3^-) concentrations and quantities, and amount of N within each form and total N loss with the leaching from biochar and control treatments. Values are the means (n=3) and standard deviations (in bracket). Within a same row, different letters mean statistically significant differences (P<0.05).

Parameters	N forms	BC	CNT
Average concentration (ml)	NO_2^-	13.8 (15.6) a	9.3 (6.9) a
	NO_3^-	690.7 (872.2) a	538.1 (497.8) a
Total amount forms (mg)	NO_2^-	8.1 (8.0) a	6.5 (6.3) a
	NO_3^-	325.3 (320.0) a	365.1 (397.8) a
Total amount of N within each form	$N\text{-}NO_2^-$	2.5 (2.4) a	2.0 (1.9) a
	$N\text{-}NO_3^-$	73.5 (72.3) a	82.5 (89.9) a
Total amount of N leached (mg)		76.0 (74.8) a	84.5 (91.8) a

Considering nitrite, the leachates from the biochar treatment showed a greater loss of this form, confirmed both by the higher average concentration (13.8 mg/l) compared to the control (9.3 mg/l), and by the values expressed as quantity (8.1 and 6.5 mg for the biochar and control theses, respectively) (**Table 4**).
This result could suggest that the presence of biochar induced a priming effect, enhancing soil organic matter mineralisation, by promoting ammonification (conversion of ammonium ion to nitrite) and subsequently nitrification (conversion of nitrite to nitrate). This consideration could explain the higher average nitrate (and nitrite) concentration found in samples from biochar treatment.

Mineralization rates in the soil are a function of the C and N pools available to microorganisms. Typically, as C:N ratios decreases mineralization occurs. Adding biochar to the soil adds another dimension to both the C and N pools. In literature, addition of biochar to soils has been shown to decrease net N mineralisation, however, other studies reported an increase of the net N mineralisation. For example, in an incubation study, Mandal et al. (2016) used three fertilizer sources on five soil types with poultry litter biochar and macadamia nut shell biochar (5%) to study ammonification and nitrification rates. Overall, they observed reduction in NH_4^+ concentration (ammonification) and corresponding increase in NO_3^- (nitrification) in early days of incubation across all the treated soils. Similarly, Nelissen et al., 2012 observed accelerated soil N cycling following biochar addition, with increased gross N mineralization (185-221%), nitrification (10-69%) and ammonium consumption rates (333-508%). Moreover, the same authors observed that with the addition of biochar, most of the N was coming from recalcitrant form, whereas mineralization of the control was from a labile N pool, indicating a priming effect of the biochar in stimulation microorganism to mineralize recalcitrant SOM.

Barley morphological parameters and biomass determination

As can be seen from **Table 5**, no significant differences were found for any of the parameters related to fresh, dry biomass and water content. Nevertheless, it can be observed that the plants grown on soil amended with biochar showed higher values of aerial and root biomass than the control thesis, suggesting a favourable condition induced by the presence of the organic soil amendment.

Table 5 Aerial, root and total of the biomass (fresh, dry and water content) of barley grown on biochar (BC) and control (CNT) treatments. Values are the means (n=3) and standard deviations (in bracket). Within a same row, different letters mean statistically significant differences (P<0.05).

Treatments	BC	CNT
Aerial fresh biomass (g)	476 (228) a	383 (262) a
Root fresh biomass (g)	69 (23) a	62 (27) a
Total fresh biomass (g)	545 (250) a	446 (228) a
Aerial dry biomass (g)	99 (65) a	72 (52) a
Root dry biomass (g)	20 (9) a	16 (5) a
Total dry biomass (g)	120 (72) a	89 (56) a
Aerial water content (%)	77 (11) a	81 (7) a
Root water content (%)	72 (5) a	72 (7) a
Total water content (%)	77 (9) a	80 (6) a

With regards to the morphological parameters (**Table 6**), barley plants grown on the control treatment showed a significantly lower average plant height than those soil conditioned with biochar, again confirming the positive effect of the biochar as soil conditioner. Root length and culm diameter were not statistically significantly different, but values were higher for plants cultivated with biochar.

Table 6 Plant height, root length and culm diameter of barley grown on biochar (BC) and control (CNT) treatments. Values are the means (n=3) and standard deviations (in bracket). Within a same row, different letters mean statistically significant differences (P<0.05).

Treatments	Plant height (cm)	Root length (cm)	Culm diameter (mm)
BC	62 (9) a	13.3 (4.4) a	2.5 (0.5) a
CNT	55 (8) b	12.6 (5.7) a	2.3 (0.4) a

3. Second test campaign

3.1. Materials and Methods

The second test campaign took place in Montepaldi (Florence) at the experimental agronomic area of the Consortium RE-CORD. Barley plants were grown under environmental controlled conditions in the climatic chamber prototype, using lysimeters as soil containers (**Figure 6**).

Figure 6 RE-CORD Climatic cell and lysimeters

Lysimeter prototype

For this experimentation, 10 lysimeters were specially developed and purchased with the aim of studying the effect of biochar used as soil amendment on plant parameters and soil chemical and physical fertility (**Figure 7**). Each lysimeter is equipped with wheels for easier logistics. The dimensions of these lysimeters (67.5 cm in diameter and 60 cm in height and 0.3 m^3 of volume) were suitable for barley growth and for soil moisture monitoring via sensors.

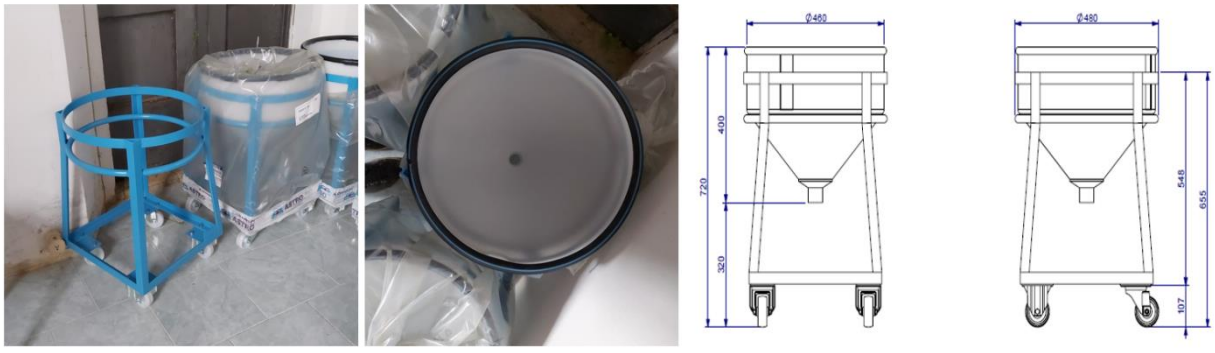

Figure 7. Lysimeter prototype size.

Soil and biochar preparation

The soil used for the tests was agricultural soil, retrieved from the field trial in Terontola, described in deliverable D2.11. At the end of the 2022 summer, in September, about 3 big bags of soil were collected from Terontola field and transported to Montepaldi (**Figure 8**).

Figure 8 Soil collection from Terontola field.

Table 7 Chemical and physical properties of the soil from Terontola field and analytical method. Mean and standard deviation (in brackets) are shown.

Parameters	Analytical methods	Values
Bulk density (kg/m^3)	UNI EN 17828	1118 (50)
Sand (%)	D.M. 11/5/1992 Metodo VI	45
Silt (%)	D.M. 11/5/1992 Metodo VI	43
Clay (%)	D.M. 11/5/1992 Metodo VI	12
pH	D.M. 13/9/1999 Metodo III.1	5.7 (.0.2)
CEC (cmol(+)/kg)	D.M. 13/9/1999 Metodo XIII.2	14 (2)
TN (%)	D.M. 13/9/1999 Metodo VII.1	0.11 (0.03)
EC (mS/cm)	D.M. 13/9/1999 Metodo IV.1	0.12 (0.02)
Organic C (%)	D.M. 13/9/1999 Metodo VII.3	1.02 (0.20)
Soil organic matter (%)	D.M. 13/9/1999 Metodo VII.3	1.75 (0.35)
Total P	D.M. 13/9/1999 Metodo XV.1	1654 (213)
Available P	D.M. 13/9/1999 Metodo XV.3	115 (31)

Before filling each lysimeter, the soil was manually sieved at 2 mm to ensure uniform density and to avoid coarse fragments with the aim to guarantee identical soil particle size within the container (**Figure 9**). Each lysimeter was filled with 45 kg of soil, to ensure a consistent volume of soil.

Figure 9 Soil preparation (i.e. soil sieving) before beginning the test within the climatic chamber.

The **biochar** was prepared using poplar ligno-cellulosic biomass, pyrolyzed at low temperatures (500°) at RE-CORD plant. The same biochar was also used for the field trials realized in Italy (in Montepaldi and Terontola locations) (see Deliverable 2.11 Results on optimal Biochar+Compost agronomic protocol trial at field scale application). As can be seen from **Table 8** all the parameters analysed were within the legal limits, highlighting the suitability of this type of biochar for introduction into the soil as a soil conditioner.

Table 8 Main physical and chemical characteristics of the biochar used in this experimentation. Limits reported in the Italian Legislative Decrees (L.D.) 75/2010 regulating the properties of the biochar used as soil amendment are also shown for a comparison. *b.d.l = below the detection limits; d.b.= dry basis; ar= as arrived.

Parameters	Analytical methods	Unit	Biochar	D.lgs. 75/2010, Annex 2, Category 16, Biochar from pyrolysis and gasification
Moisture	UNI EN ISO 18134-2	% (m/m) ar	2.3	≥20% for powdery products
Ashes 550°C	UNI EN ISO 18122	% (m/m) d.b.	8.0	≤60 (cl1, cl2, cl3)
Volatiles	UNI EN ISO 18123	% (m/m) d.b.	11.7	
Fixed carbon	calculated	% (m/m) d.b.	80.36	
C	UNI EN ISO 16948	% (m/m) d.b.	86.92	
H	UNI EN ISO 16948	% (m/m) d.b.	2.1	
N	UNI EN ISO 16948	% (m/m) d.b.	0.3	to be declared
Inorganic C		%(m/m) d.b.	0.48	to be declared
Organic C		%(m/m) d.b.	86.44	≥20 (cl1, cl2, cl3)

H/organic C	calculated		0.28	≤0,7
Bulk density	UNI EN ISO 17828	kg/mc	263	
pH	ISO 10390		8.0	45264.0
Electrical conductivity (1:10)	ISO 11265	mS/m	4.3	≤1000
sum PAHs16	UNI EN 16181	mg/kg d.b.	<4	<6
BET		mq/g	88.0	
Elements	UNI EN ISO 16967 UNI EN ISO 16968			
Al		mg/kg d.b.	668	
B		mg/kg d.b.	b.d.l.	
Ba		mg/kg d.b.	45	
Ca		mg/kg d.b.	23142	to be declared
Cd		mg/kg d.b.	<0.20	<1.5
Co		mg/kg d.b.	b.d.l.	
Cr		mg/kg d.b.	6	
Cu		mg/kg d.b.	b.d.l.	<230
Fe		mg/kg d.b.	893	
K		mg/kg d.b.	4965	to be declared
Li		mg/kg d.b.	1	
Mg		mg/kg d.b.	2571	to be declared
Mn		mg/kg d.b.	74	
Mo		mg/kg d.b.	b.d.l.	
Na		mg/kg d.b.	321	to be declared
Ni		mg/kg d.b.	b.d.l.	<100
P		mg/kg d.b.	b.d.l.	to be declared
Pb		mg/kg d.b.	b.d.l.	<140
Si		mg/kg d.b.	1129	
Ti		mg/kg d.b.	33	
V		mg/kg d.b.	b.d.l.	
Zn		mg/kg d.b.	92	<500
Granulometry	UNI EN ISO 17827-1			to be declared (mm 0,5-2-5)
< 0,5 mm		% (m/m)	21.4	
> 0,5 mm		% (m/m)	6.2	
> 1 mm		% (m/m)	7.5	
> 2 mm		% (m/m)	16.8	
> 5 mm		% (m/m)	48.1	
d50		% (m/m)	1.89	
Max water retention		% (m/m)	143.00	to be declared
As	UNI EN 13657:2004+UNI EN ISO 11885:2009	mg/kg d.b.	<2	
Ta	UNI EN 13657:2004+UNI EN ISO 11885:2009	mg/kg d.b.	<1	
Hg	UNI EN 13657:2004+UNI EN ISO 11885:2009	mg/kg d.b.	<1	<1.5
Cr VI	DM 08/05/2003	mg/kg d.b.	<0.5	<0.5
Germination index	UNI 10780:1998	%	57	

sumPCDD+PCDF	EPA 31613B 1994	ng TE/kg d.b.	0.65	<=9 ng TEQ/kg d.b.
PCBs	EPA 1668 C 2010	mg/kg d.b.	0.0151	<0.5 mg/kg d.b.

Preliminary wetting test

Pre-trials consisted in the saturation of the soil in lysimeters, with only distilled water, to determine the maximum amount of water which could be absorbed by bare soil, as a proxy for the maximum theoretical amount of irrigated volume (**Figure 10**). More in details, two lysimeters were filled with 45 kg of sieved soil, and a certain amount of water was added until complete soil saturation.

Soil moisture was measured with soil moisture sensors. Once the lysimeter stopped draining water (approximately 24-48 h from saturation) the soil moisture inside the lysimeter was considered the amount of moisture at field capacity.

Figure 10 Preliminary wetting test using soil from Terontola field and distilled water.

Barley cultivation

For the second lysimeter campaign, the Barley *Sereno* variety was chosen since this genotype was also tested in field in Spain during the trials with Camelina and Barley rotation. Barley sowing occurred on April 17, 2023 at the density of 25 g/m^2.

The cultivation period was planned to last about **10-12 weeks**.

Design of the experiment

The experimental treatments selected were:

- BC0: 0% of biochar (only soil);
- BC10: dose equivalent to 10 ton/ha of biochar;
- BC20: dose equivalent to 20 ton/ha of biochar.

The experimental design involved setting up the trial with 3 replicates (3 lysimeters) for each treatment (**Figure 11**). In addition, an additional lysimeter was added with only soil (without plants) that was irrigated with the same amount of water to monitor soil moisture in the absence of plants.

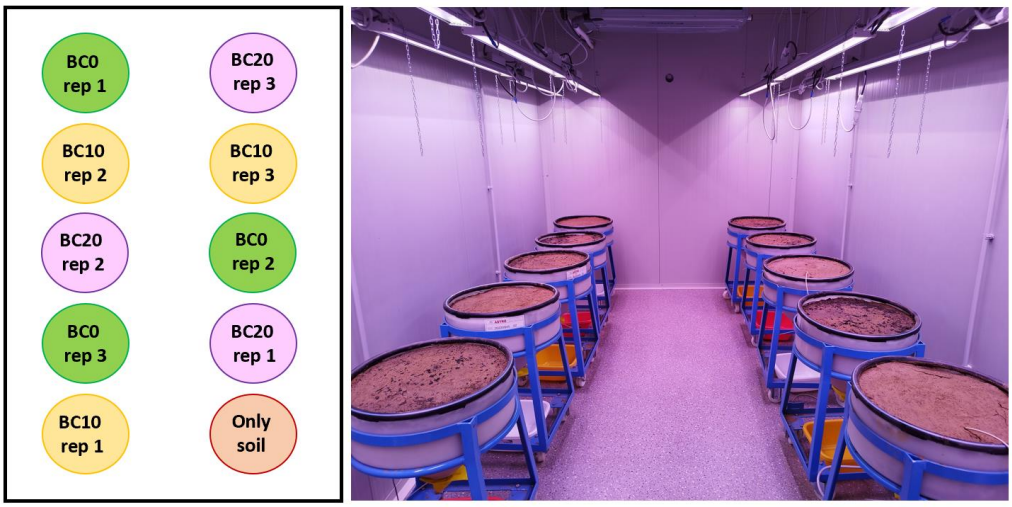

Figure 11 Design of the experiment of the second campaign.

On May 17th, meaning after 5 weeks all pots (expect the lysimeter with only soil) were fertilized with NPK with the equivalent of 200 kg/ha of NPK (8-24-8).

Environmental conditions

For the photoperiod, actual sunrise and sunset values of an indicative period from December to March were considered, representing the barley growth cycle from sowing (winter) to rising (late spring). The climate chamber software allowed the values to be set on a daily basis.

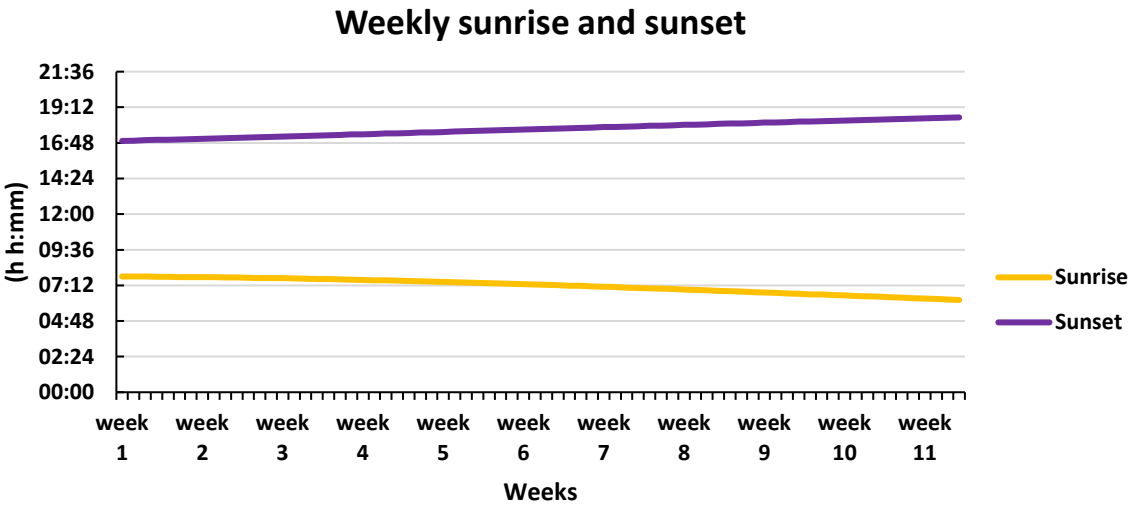

Figure 12 Weekly sunrise and sunset values used for setting the climatic cell during the 12 weeks of barley growth.

Temperatures were set below 20°C for the first 4 weeks, then progressively increased.

Irrigation schedule

The amount of rainfall falling in the Montepaldi area during the period between December 2021 and March 2022 was taken into account for irrigation as it represents the sowing period of an autumn-winter cereal and its development.
This growing period had approximately 102 mm of rain in 2 months as it was one of the driest winters in the last 10 years.
Irrigation was carried out weekly, with double irrigation the first two weeks. Each shift provided an amount of water equal to 1.55 liters, equivalent to 15% of the field capacity for water in soil. In total, the amount of water supplied was 17.05 liters, equivalent to 103 mm of rainfall.

This water quantity corresponds to a severe rainfall deficit for barley.

Soil humidity determination
Soil humidity within the lysimeter was measured with a portable sensor (EB instruments). In details, every week and from each pot, soil moisture was monitored (in 4 replicates) in different area of the pot to have sufficient data for statistical elaboration. The moisture of the lysimeter without the plants (only soil) was monitored as well.

Biomass determination
Barley biomass was determined by cutting at the plant collar the stem. All the biomass was weighted to obtain the fresh weight and then oven-dried at 105°C for 24h for measuring dry weight biomass.

Aerial biomass was used for determining the water use efficiency (WUE) as follow:

$$WUE = \frac{B_T - B_C}{WA}$$

Where:
WUE is the water use efficiency expressed as g of biomass (dry weight) for mm of water applied;
B_T is the dry biomass from each treatment (i.e. BC10 and BC20) (g/m^2);
B_C is the seed yield from the control treatment (i.e. BC0) (g/m^2);
WA is the total water applied (mm/m^2);

Statistical elaborations
Data on the soil humidity and barley growth parameters were compared trough a one-way ANOVA to detect significant differences between biochar amendments. Data on water use efficiency were compared using a 2-sample t-test to determine significant differences among biochar-based treatments (i.e. BC10 and BC20). All the elaborations were performed using Minitab®17.1.0, Minitab Inc., State College, PA, US.

3.2. Results and discussions

Barley started germination soon after seed sowing (April 17th) and in two weeks barley plantlets were developing properly in all lysimeters independently form the treatment (**Figure 13**), and few weeks later, barley plants were actively growing (**Figure 14**).

Figure 13 Lysimeters after 2 weeks from sowing (picture taken on April 28th).

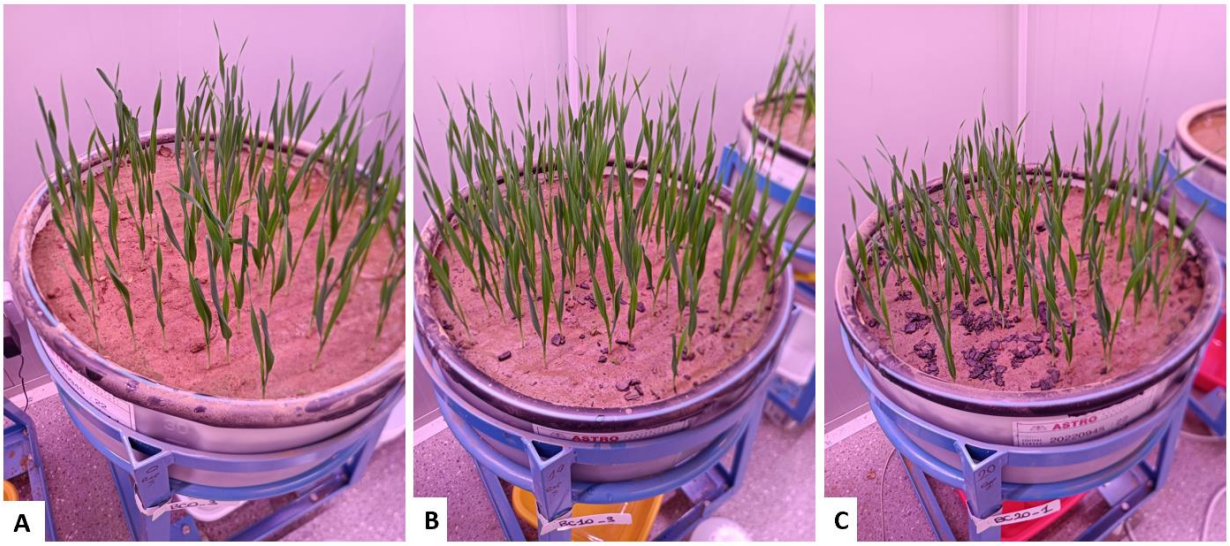

Figure 14 BCO (A), BC10 (B) and BC20 (C) lysimeters after three weeks from sowing (picture taken on May 4th).

Soil moisture monitoring started when germination was complete, on May 10th (**Figure 15 and Figure 16**), to avoid disturbing seeds germinations by using the instrument's sensor (**Figure 16C**).
On May 10th and on May 17th, soil humidity values among the treatments were similar, showing values within the same range (i.e. 15-17.3%) (**Figure 15**, **Table 9**). Also, the lysimeter with only soil showed a very similar values indicating that plants' influence on soil moisture for the first weeks of growth was minimal.

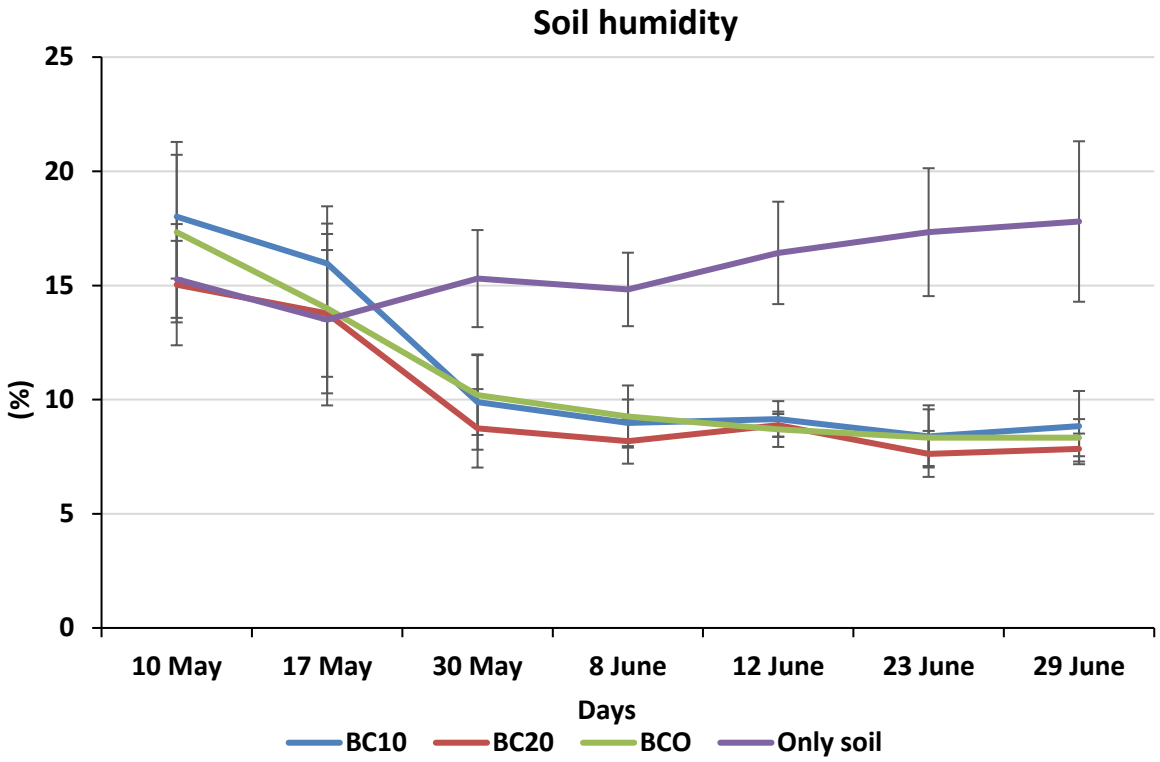

Figure 15 Mean soil humidity in lysimeter amended with BC0, BC10 and BC20. Soil humidity of the lysimeter with no plants (only soil) is also illustrated. Bars represent standard deviation (n=4)

On 30th May, it was possible to observe a clear difference between lysimeters with plants (i.e. BCO, BC10 and BC20) and the lysimeter without plants. For the latter, the moisture curve was higher compared to the treatment's curves (**Figure 15**). In fact, the lysimeters with only soil and no biochar showed a higher water content of about the 36% compared to the mean soil moisture of the treated pots (**Table 9**). At this stage, the plants that saw a very fast tillering, were in an upward phase, with a very strong elongation of the internodes and accumulation of biomass (**Figure 17**).

Table 9 Soil humidity (%) of the lysimeters amended with BC0, BC10 and BC20. Also, the values found for the lysimeter with only soil (no plants) is illustrated. Means (n=4) and standard deviations (in brackets) are shown. Different letters within the same column indicate significant differences among the treatments.

Treatments	10 May	17 May	30 May	8 June	12 June	23 June	29 June
BCO	17.3 (4)	14.0 (3.7)	10.2 (1.7)	9.3 (1.4)	8.7 (0.8)	8.3 (1.2)	8.3 (0.8) ab
BC10	18.0 (2.7)	16.0 (2.5)	9.9 (2.1)	9.0 (1.0)	9.2 (0.8)	8.4 (1.4)	8.8 (1.5) a
BC20	15.0 (2.7)	13.8 (2.8)	8.7 (1.7)	8.2 (1.0)	8.9 (0.5)	7.6 (1.0)	7.8 (0.7) b
No plants	15.3 (1.7)	14.0 (3.8)	15.3 (2.1)	14.8 (1.6)	16.4 (2.2)	17.3 (2.8)	17.8 (3.5)

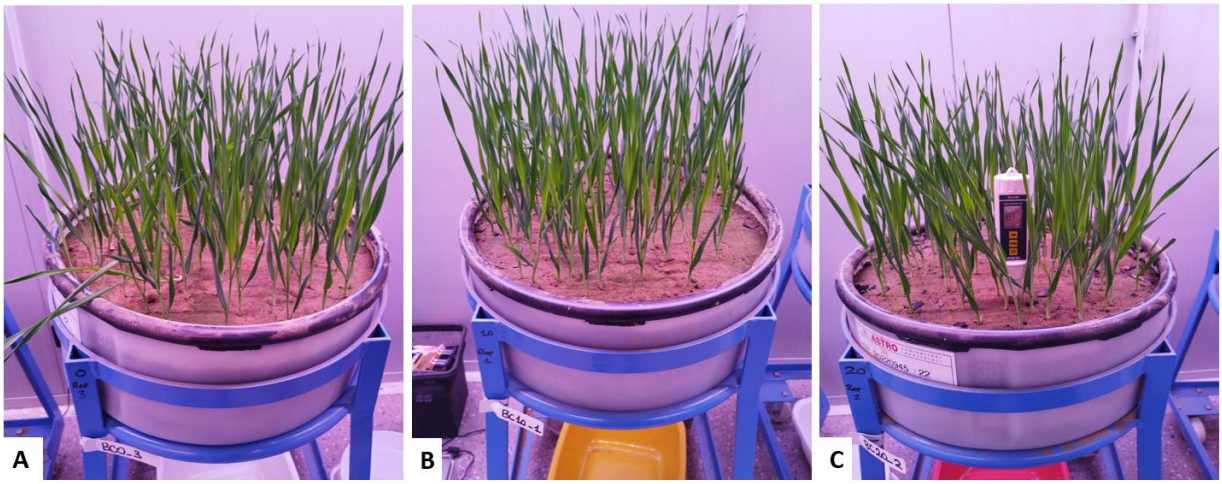

Figure 16 BCO (A), BC10 (B) and BC20 (C) lysimeters after three weeks from sowing (picture taken on May 10th).

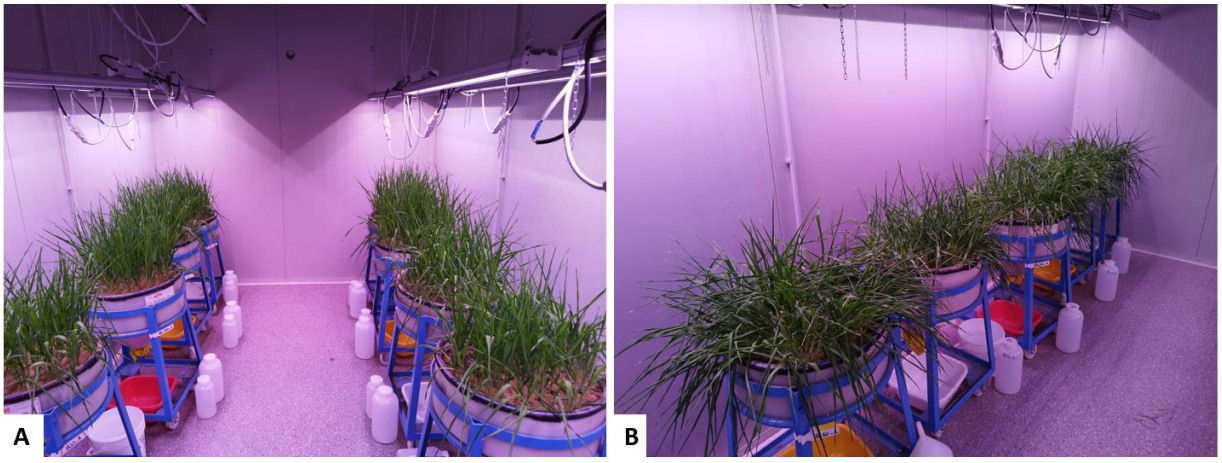

Figure 17 Plants after 4 (A) and 6 (B) weeks from sowing (pictures taken on May 17th and May 30th, respectively).

In the second phase of the trial, in June, the plants started to wither and showed a state of stress certainly due to the limited irrigation (**Figure 18A**). In fact, the soil moisture monitoring carried out in June produced very low moisture values of about 9-8% (**Figure 15**, **Table 9**). On the other hand, the value of soil humidity for the lysimeter with only soil was about 15%, and a loss of leached water from the container was observed immediately after irrigation, indicating that the soil had reached saturation much faster than expected due to the absence of water-absorbing plants. (**Figure 18B**).

On the last day of monitoring (June 29th), significant differences were observed between the three experimental treatments with significantly higher soil moisture values for BC10 (8.8%) compared to the BC20 treatment (7.8%), while the moisture values recorded for BC0 (8.3%) were significantly similar to the other two treatments (**Table 9**).

Data on biomass weight and water content were gathered after the manual harvest of the barley (**Figure 19**). There were no statistically significant differences among treatments for fresh and dry biomass (**Table 10**). Regarding plant water content (%), plants amended with BC0 showed significantly higher water content compared to plants treated with BC10, while barley plants grown on BC20 showed similar values to BCO and BC10 (**Table 10**).

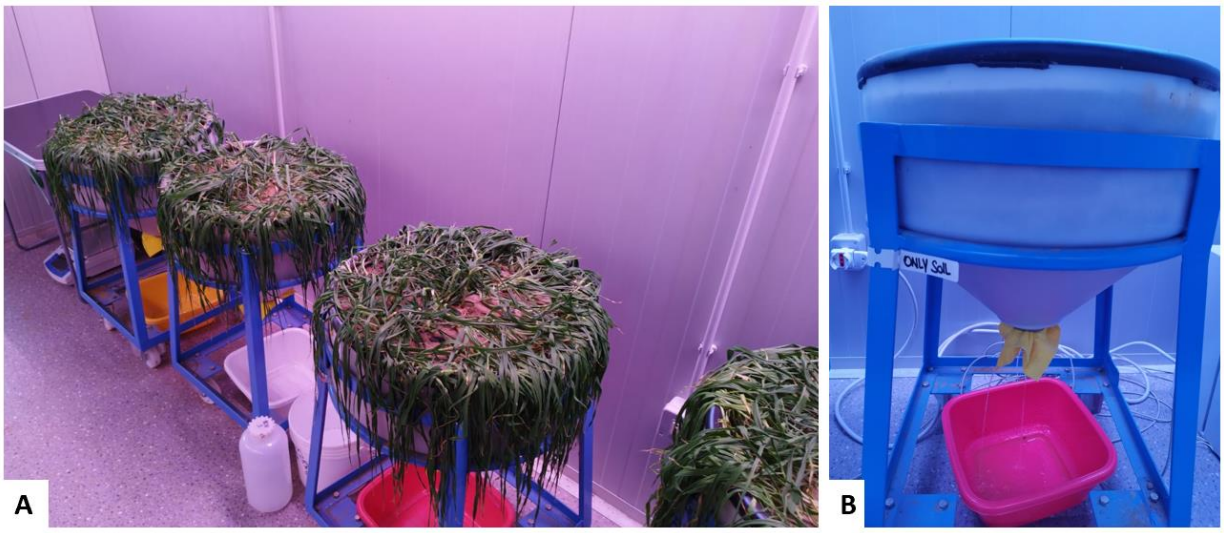

Figure 18 Plants after 7 weeks from sowing (A). Lysimeter with only soil (B) draining water. Both pictures were taken on June 8th.

Figure 19 Barley biomass harvest on June 29th.

Table 10 Barley fresh, dry biomass (g) and water amount (%) at the end of the trial. Means (n=3) and standard deviations (in brackets) are shown. Different letters within the same column indicate significant differences among the treatments. In the last column it is shown the water use efficiency (g/l) calculated only for BC10 and BC20 treatments. For this parameter, means (n=3) and standard deviations (in brackets) are shown.

Treatments	Biomass fresh weight (g)	Biomass dry weight (g)	Water amount (%)	Water use efficiency (g/l)
BC0	167 (17)	53 (3)	68 (1) a	-
BC10	155 (15)	55 (6)	65 (1) b	0.07 (0.35)
BC20	147 (12)	50 (3)	66 (1) ab	-0.02 (0.17)

From these results, it can be stated that regardless of biochar, all plants suffered from water stress as there was no difference between dry and fresh biomass. However, also considering the soil moisture monitoring, the biochar dose equivalent to 10 ton/ha (BC10) was the one that maintained a slightly higher moisture content than the other two treatments. These data could indicate that under severe water stress conditions, a high dose of biochar (such as 20 ton/ha) is not optimal as it may retain water, making it unavailable to the soil-plant system. The water use efficiency calculated (**Table 10)**, appear to confirm this hypothesis, since the lysimeter with BC10 showed an higher percentage compared to BC20 which reached negative values.

4. Conclusions

The two experimental campaigns conducted for the BIO4A project Task 2.2. in controlled conditions with lysimeters, provided some relevant indications regarding the effect of biochar on nitrogen leaching from soil, water stress and plant productivity.
The first indication is that biochar can be used to reduce the total amount of nitrogen forms which exit the soil depth explored by annual plant roots, thus providing an additional tool for the reduction of water pollution and eutrophication phenomena, potentially increasing barley development and productivity in terms of biomass (although no statistical significance was found).
Moreover, when water is not a limiting factor for plant biomass productivity, as in the 1st test campaign, biochar can play a role in conserving soil humidity.
In the framework of water stress conditions, the optimal dose of biochar should be investigated to maximise efficiency, under 20 t/ha d.b. equivalent, as there might be a competition between water available for plants and stored in biochar under severe conditions (below 15% of field capacity).

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