Advanced Sustainable BIOfuels for Aviation

Deliverable D2.11:
Results on optimal Biochar+Compost agronomic protocol trial at field scale application

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

CO…Coordinator, BEN…Beneficiary

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 789562.

General Information

Call identifier: H2020-LCE-2017-RES-IA
GA Number: 789562
Topic: LCE-20-2016-2017
Start date of project: 01/05/2018
Duration: To 30/06/2023
Work Package: WP2: R&D Long-Term Strategy for HEFA production
Type: Deliverable
Number: D2.11
Title: Results on optimal Biochar+Compost agronomic protocol trial at field scale application
Due Date: 30/06/2023
Submission date: 20/06/2023
Reference Period: 01/05/2018 – 30/06/2023

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Responsible Person: Tommaso Barsali
Dissemination Level: Public

INTERNAL MONITORING & REVISION TABLE					
REV.	**DATE**	**DESCRIPTION**	**PAGES**	**CHECKED**	**APPROVED**
1	**22/05/2023**	**Original**	**28**	**FT, TB**	
2	**19/06/2023**	**Revised**	**31**	**FT, TB, JP, PL**	**All**

Document Type		
PRO	Technical/economic progress report (internal work package reports indicating work status)	
DEL	Technical reports identified as deliverables in the Description of Work	**X**
MoM	Minutes of Meeting	
MAN	Procedures and user manuals	
WOR	Working document, issued as preparatory documents to a Technical report	
INF	Information and Notes	

Dissemination Level		
PU	Public	**X**
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	
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Summary

Biochar is a carbon-rich material obtained through the thermal conversion of organic biomass and presents several opportunities in the context of a circular economy and sustainable agriculture. Its use allows for the recovery and reuse of residues and co-products from agricultural, forestry, and agro-industrial activities, contributing to waste reduction and resource efficiency. The use of biochar as soil amendment can have multiple benefits since biochar supports biological and chemical mechanisms that promote soil fertility, resilience and adaptation to climate change. Accordingly, in the last two decades, a great effort has been made on researches exploring the benefits of biochar land application to soil and ecosystem health, and the majority of findings has been strongly positive (Antonangelo et al., 2021).

Over the past ten years, attention has also been paid to the use of biochar combined with other materials such as compost. The combination of biochar, compost and inorganic fertilizers can provide beneficial effects especially in the context of rain-fed agriculture, where water scarcity is becoming a serious issue. In fact, compost is a material rich in available nutrients, while biochar, due to its porous structure, may have the ability to absorb these nutrients and release them slowly to the plant, avoiding the risk of loss through leaching. The co-application of biochar and compost can enhance soil fertility by increasing soil water retention and at the same time by providing a balanced supply of carbon and nitrogen, thus improving nutrient cycling and overall plant productivity. Responses will likely depend on the type and rate of amendment applied to soil as well as on the initial soil characteristics such as soil EC, pH, CEC and other components of soil fertility. It is expected that different soil fertility treatments in relation to amendment type may differ in their effects on soil biophysical and chemical properties and early crop growth and development (Lehmann et al., 2003a).

Maintaining an appropriate level of soil organic matter and ensuring the efficient biological cycling of nutrients is crucial to the success of soil management and agricultural productivity strategies (Bationo et al., 2006). The application of organic and inorganic fertilizers combined with knowledge of how to adapt these practices to local conditions, are important for maximizing the agronomic use efficiency of the applied nutrients and thus crop productivity (Vanlauwe et al., 2010).

Camelina sativa (L.) Crantz, commonly known as Camelina is an interesting oilseed crop belonging to the Brassicaceae family, considered a valuable oil crop that can be cultivated on marginal lands for Sustainable Aviation Fuel production. Camelina shows valuable agronomic traits due to its minimal input requirements, its high compatibility with reduced tillage systems and its wide adaptability to marginal land with limited fertility and water availability and for this reason Camelina is well suited in the context of low ILUC biofuels. Accordingly, the use of biochar as a soil conditioner accompanied by minimum tillage practices can represent an agronomic practice to develop sustainable cultivation models of Camelina for the establishment of a low ILUC bioenergy chain.

The objective of this trial was to corroborate, to confirm the positive biochar effects observed during the microplot trials in Spain from 2018 to 2023, on a larger scale and in different agronomic conditions. More in detail, the Italian trial was focused on the evaluation of the effect of biochar (alone or mixed with compost) on Camelina yield and biomass and on the chemical and physical fertility of the soil.

In this context, two Camelina varieties (i.e. CCE26 and CCE32) were chosen as a plant model for the cultivation with biochar alone or mixed with compost derived from municipal organic waste processing. The objective of the field trial was to corroborate the effect of biochar-based amendment on soil physical and chemical properties and on Camelina growth and yield. The

experiment was carried out in two different locations of the Tuscany Region to assess for different climate response. Overall, camelina well-responded to the presence of biochar, especially when compost was also added. However, different genotype performances were detected depending on the location.

In one of the two locations, in a year marked by a very low rainfall, Camelina sativa performed very well in the presence of biochar mixed with compost, but also with biochar alone. An improvement of the soil physical characteristics was observed in the biochar-mixed plots. This result confirms that when biochar is incorporated into soil with a high sand content (such as that of Terontola), it causes an improvement in soil structure, which results in greater water retention and greater plant tolerance to prolonged periods of drought.

1. Introduction: aim and approach

In the framework of the H2020 BIO4A project, an agronomic field test was performed to assess the sustainable production of virgin lipids from Camelina (*Camelina sativa L*. Crantz) for aviation fuel production.

The objective of this trial is to corroborate the implementation of biochar addition with conventional machinery as well as to confirm the positive biochar effects observed during the microplot trials in a different location. More in details, the Italian trial was focused on the evaluation of the effect of biochar (alone or mixed with compost) on Camelina yield and biomass and on the chemical and physical fertility of the soil.
Two short cycle varieties of Camelina were selected, (namely CCE26 and CCE32) to account for any genotype effect.
RE-CORD decided to carry out the experimentation in two different locations, in addition to what is mentioned in the Grant Agreement. Both locations were in Tuscany, Terontola (Arezzo) and Montepaldi (Florence), to also evaluate the different pedoclimatic characteristics of the two locations. Both locations showed a poor content in soil organic carbon (<2%), different texture and historical land use: in Terontola, the field had always been subject to intensive arable crops cultivation, while in Montepaldi, the test was conducted on an abandoned field (>10 years).

The experimental design involved the comparison of different amendments and fertilizers:

B) only biochar (3 ton/ha) + NPK (133 kg/ha);
CO) only compost (20 ton/ha) + NPK (133 kg/ha);
BCCO) compost (20 ton/ha) + biochar (3 ton/ha) + NPK (133 kg/ha);
NPK) only NPK fertilizer (133 kg/ha);
CK) control treatment (without fertilizer or amendment).

Soil was sampled for the determination of several physical and chemical properties (pH, electrical conductivity, cation exchange capacity, organic carbon content, total and available elements and water holding capacity). A further sampling at the end of the cultivation was realized to evaluate any significant variation of these properties.
Camelina varieties were both harvested at complete maturity for the determination of plant biomass, seed and oil yield. A qualitative analysis of the oil was performed for the determination of the industrially important parameters (e.g. CHN concentration, element concentrations, LHV and HHV).
The experiments started in March 2022 with the minimum tillage operation aimed at preparing the soil for the Camelina sowing and concluded on July after Camelina seed maturation.

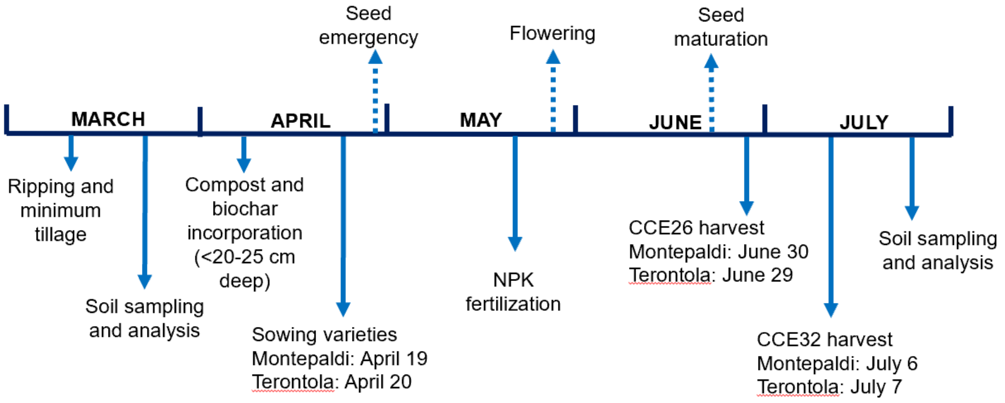


Figure 1. Chronological activities of the agronomic trial with Camelina

2 Materials and methods

2.1 Experimental site and preliminary operations

The experiments were conducted during the spring-summer period (April-July) of the 2022 in two different locations of the Tuscany Region, namely Terontola (Cortona, Arezzo, 3°11'50.7"N 12°01'01.0"E) (**Figure 2A**) and Montepaldi (San Casciano Val di Pesa, Florence, 43°39'42.7"N 11°08'31.2"E) (**Figure 2B**). These fields were selected because of the different pedoclimatic conditions and the agronomic background. Regarding the soil characteristics, both fields showed a low organic matter content (<2%) but a different texture: in Montepaldi, the soil has a sandy clayed texture while Terontola soil showed a higher silt content. Another difference discerning the two localities concerns land use. The Terontola field is annually cultivated for sunflower production, so it has a very loose and light texture. In contrast, the soil in Montepaldi has not been subjected to mechanical tillage and fertilization for more than a decade, so it has a much more compact and firm structure.

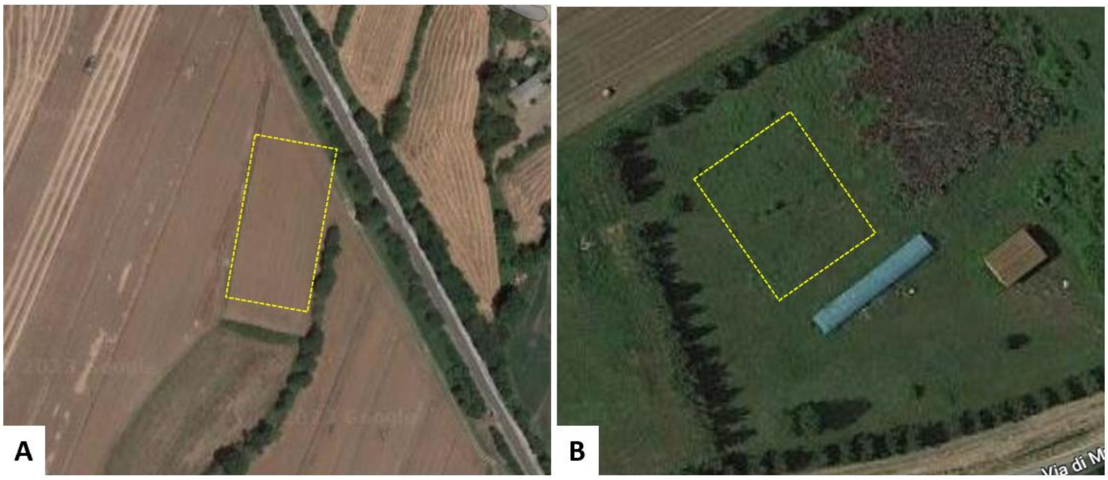


Figure 2. Italian experimental trials located in Terontola (Arezzo) (A) and in Montepaldi (Florence) (B).

A homogenous area in terms of exposure and position of about 1500 m^2 (40 x 37.5 m) was identified. Approximately one month before planting in March, each field was subjected to the same operations in terms of tillage. After cutting the weeds, the field underwent typical operations which involved an initial ripping to aerate the first layers of soil and subsequent harrowing operations (**Figure 3**).

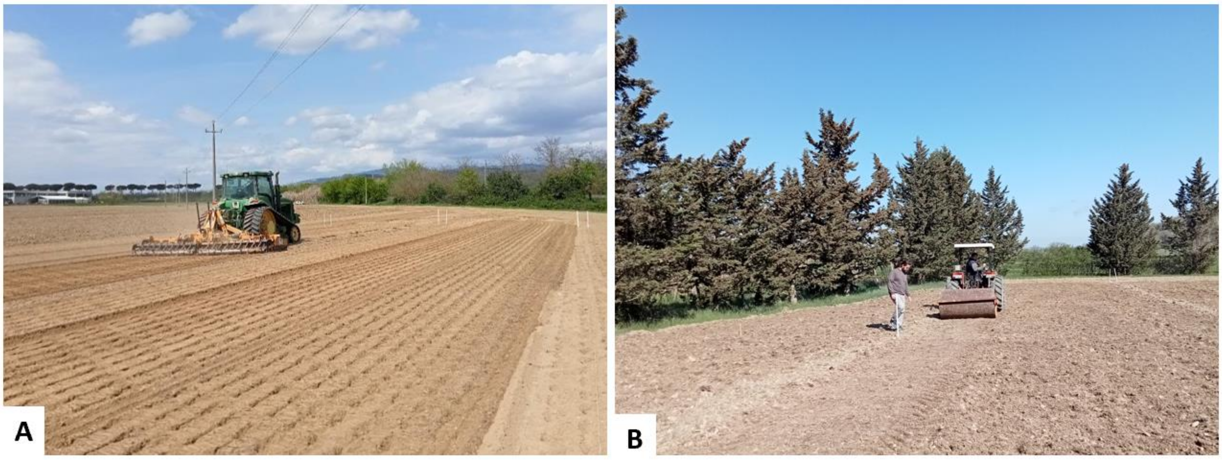


Figure 3. Minimum tillage operations in Terontola (A) and in Montepaldi (B).

After treatment application, barley was manually sown on all four prepared plots at a density of 250 kg/ha.

2.2 Design of the experiment and treatments application

The area was measured and divided into 5 experimental plots, homogeneous for soil type, slope and exposition, of 300 m^2 each. A space of 0.5 m was left between plots to avoid border effects of treatments. The 5 experimental treatments consisted in:

CK) control treatment (without fertilizer or amendment).
NPK) only NPK fertilizer (eq. 133 kg/ha);
CO) only compost (eq. 20 ton/ha) + NPK (eq. 133 kg/ha);
BC) only biochar (eq. 3 ton/ha) + NPK (eq. 133 kg/ha);
BCCO) compost (eq. 20 ton/ha) + biochar (eq. 3 ton/ha) + NPK (eq. 133 kg/ha);

NPK Treatment

All plots were fertilized with an inorganic NPK fertilizer (Poly-Feed,11:44:11+ME containing small traces of Fe, Mo, B, Mn, Md and CU). The fertilizer was applied manually a month after the sowing in May (eq. 133 t/ha) (**Figure 1**), in accordance with the crop requirements.

CO Treatment

For the compost treatment, a mixed composted soil improver (registered with the Ministry of Agriculture registration no. 1386/13) was used, obtained by composting the Organic Fraction of Municipal Solid Waste mixed with green waste and garden pruning. The main physical and chemical properties of the compost are illustrated in (**Table 1**). The application dose was equivalent to 20 t/ha dry basis.

Table 1. Main physical and chemical characteristics and analytical methods of the compost used in this study.

Parameters	Analytical method	Compost
Moisture (%)	UNI EN ISO 13040	28.58
Ash content 550°C (%)	UNI EN 13039: 2012	35.85
Volatiles (%)	UNI EN ISO 18123:2016	53.73
pH	UNI EN ISO 10390: 2022	7.37
Total C (% db)	UNI EN ISO 16948:2015	36.38
Organic carbon (% db)	UNI EN ISO 16948: 2015 + D.M. 13/09/99 Met. V.1	35.74
C/N	Calculated	10.18
Fixed carbon (% db)	UNI EN 1860-2: 2005	10.43
N (% db)	UNI EN ISO 16948:2015	3.51
K (mg/kg db)	UNI EN ISO 16967:2015/UNI EN ISO 16968:2015	24868
P (mg/kg db)	UNI EN ISO 16967:2015/UNI EN ISO 16968:2015	8336

BC Treatment

Biochar was produced through slow oxidative pyrolysis at 500°C of poplar wood chips, originated from agricultural residues at the RE-CORD plant. The biochar used in this study was compliant with the Italian Legislative Decree L.D. 75/10, regulating the properties of biochar as amendment (Annex II, category 16) (

Table 2). Considering contaminants, all metals and polycyclic aromatic hydrocarbons (PAHs) concentrations resulted below the legal threshold and in most cases below the instrumental detection limits, thus, evidencing the complete absence of contaminations risk and the suitability of the biochar for soil incorporation. In addition, screening for other toxic organic micropollutants such as polychlorinated dibenzo(p)dioxins and furans (PCDD/Fs) and polychlorinated biphenyls, (PCBs) which are not regulated by the Legislative Decree, also exhibited clearly negligible values, confirming its safety from an environmental and agronomic point of view.

The application was done manually, with an application dose equivalent to 3 t/ha dry basis.

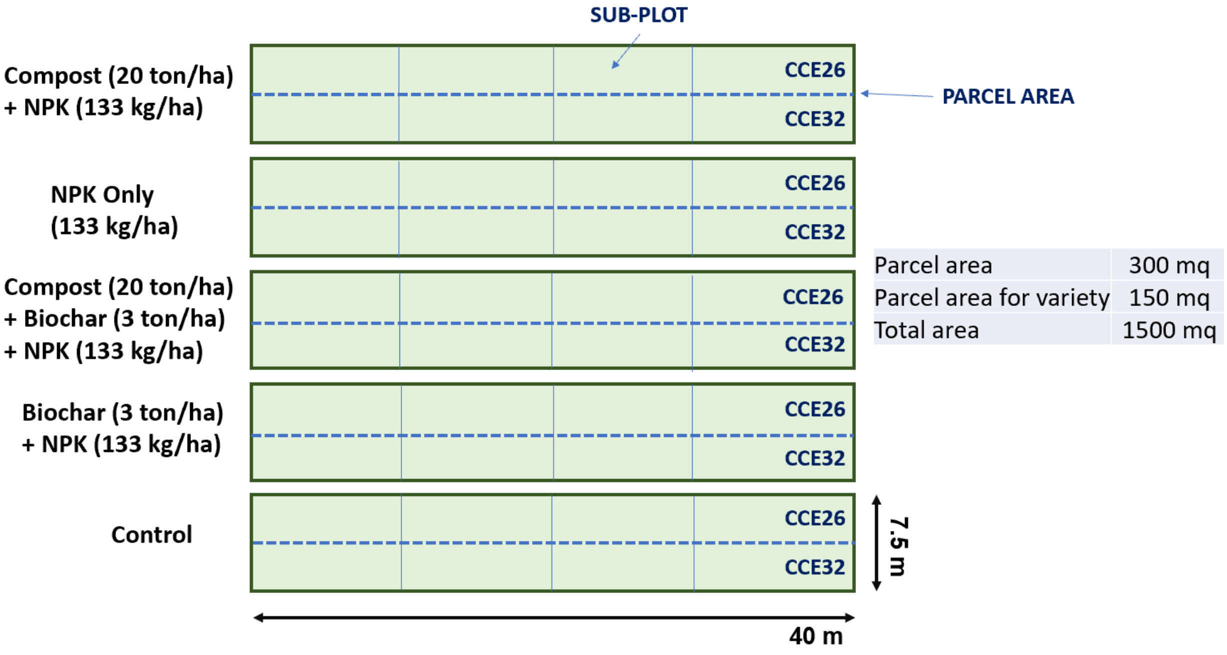
<u>BCCO Treatment</u>

This treatment consisted in the addition of biochar (eq. 3 t/ha) and compost (eq. 20 t/ha) mixed together. The application was done manually.

For all the organic amendments, the incorporation of the material occurred mechanically at the depth of 20-25 cm with conventional machinery.

Two weeks after the incorporation of the organic amendments, the seeding of two varieties of *Camelina sativa* (CCE26 and CCE32) was performed manually on April 19 and 20 for Montepaldi and Terontola respectively, with an approximate seeding density of 12.4 kg/ha After that, a roller was passed over the field to carry the camelina seeds deep into the field to ensure greater germination.

The allocation of treatments and varieties followed the scheme reported in **Figure 4**.



Parcel area	300 mq
Parcel area for variety	150 mq
Total area	1500 mq

Figure 4. Design of the experiment in both locations.

Table 2. Main physico-chemical characteristics, metals and organic contaminants concentrations of the biochar. Limits reported in the Italian Legislative Decrees L.D. 75/2010 (Annex II category 16) and report by the Regalement (EU) n.2019/1009 and n.2021/2088 are shown as a reference.

Parameters	**Analytic method**		**Biochar**	**L.D. 75/2010 (Annex II, category 16)**	**Reg. EU 2019/1009, PFC 3(A) Organic soil improver**	**Reg. EU 2021/2088 CMC 14**
Moisture (%m/m a.s.)	UNI EN ISO 18134-2		2.33	-	-	-
Ashes 550°C (% m/m d.b.)	UNI EN ISO 18122		7.98	≤60.0	-	-
Volatiles (% m/m d.b.)	UNI EN ISO 18123		11.66	-	-	-
Fixed Carbon (% m/m d.b.)	Calculated		80.36	-	-	-
Total C (% m/m d.b.)	UNI EN ISO 16948		86.92	-	-	-
Total H (% m/m d.b.)	UNI EN ISO 16948		2.07	-	-	-
Total N (% m/m d.b.)	UNI EN ISO 16948		0.29	to be declared	-	-
Inorganic C (% m/m d.b.)	Calculated		0.48	-	-	-
Organic C (% m/m d.b.)	Calculated		86.44	≥20	>7.5	-
H/organic C	Calculated		0.28	≤0.7	-	<0.7
Bulk density (kg/m3 ad)	UNI EN ISO 17828		263.00	-	-	-
pH	ISO 10390		8.00	4-12	-	-
EC (mS/m)	ISO 11265		4.35	≤1000	-	-
WHC (% m/m)			143	to be declared	-	-
Granulometry	UNI EN ISO 17827-1				-	-
< 0,5 mm (% m/m)	UNI EN ISO 17827-2		21.37	-	-	-
> 0,5 mm (% m/m)	UNI EN ISO 17827-3		6.19	-	-	-
> 1 mm (% m/m)	UNI EN ISO 17827-4		7.52	-	-	-
> 2 mm (% m/m)	UNI EN ISO 17827-5		16.85	-	-	-
> 5 mm (% m/m)	UNI EN ISO 17827-6		48.07	-	-	-
d50 (% m/m)			1.89	-	-	-
BET (m2/g)			88.00	-	-	-
Elements and metals					-	-
Al (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	667.70	-	-	-
B (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	b.d.l.	-	-	-

Ba (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	45.27	-	-	-
Ca (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	23141.55	-	-	-
Cd (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	<0.20	<1.5	<2	-
Co (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	b.d.l.	-	-	-
Cr (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	6.46	-	-	-
Cu (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	b.d.l.	<230	<300	-
Fe (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	893.48	-	-	-
K (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	4964.79	to be declared	-	-
Li (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	0.90	-	-	-
Mg (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	2570.89	to be declared	-	-
Mn (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	73.98	-	-	-
Mo (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	b.d.l.	-	-	-
Na (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	321.15	to be declared	-	-
Ni (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	b.d.l.	<100	<50	-
P (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	b.d.l.	to be declared	-	-
Pb (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	b.d.l.	<140	<120	-
Si (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	1129.32	-	-	-
Ti (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	32.62	-	-	-
V (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	b.d.l.	-	-	-
Zn (mg/kg d.b.)	UNI EN ISO 16967	UNI EN ISO 16968	92.23	<500	<800	-
As (mg/kg d.b.)	UNI EN 13657:2004+UNI EN ISO 11885:2009		<2	-	<40 inorganic As	-
Tl (mg/kg d.b.)	UNI EN 13657:2004+UNI EN ISO 11885:2009		<1	-	-	-
Hg (mg/kg d.b.)	UNI EN 13657:2004+UNI EN ISO 11885:2009		<1	<1.5	<1	-
Cr VI (mg/kg d.b.)	DM 08/05/2003		<0.5	<0.5	<2	-
Germination index (%)	UNI 10780:1998 App.K		57	-	-	-
PAHs (mg/kg d.b.)	UNI EN 16181		<4	<4	-	>6 (PAHS 16)
Sum PCDDs+PCDFs (ngTE/kg d.b.)	EPA 31613B 1994		0.65	-	-	<20
PCBs (mg/kg d.b.)	EPA 1668 C 2010		0.0151	-	-	<0.8 (ndl-PCBs)

After one week of planting, seedling emergence occurred. About a month after planting, all plots except the control were fertilized with NPK (April 23 and 24 at Terontola and Montepaldi, respectively) when Camelina reached the stage of five leaves (**Figure 5**).

Figure 5. Camelina plants before NPK fertilization.

Table 3 shows a summary of the amount of nitrogen, phosphorus and potassium elements contained in treatments. NPK and BC treatments only showed the nutrient input provided by NPK fertilisation (equivalent to 133 kg/ha), on the other hand, CO and BCCO treatments contained the nuntrient input provided by both mineral fertilisation and compost. Thus, as can be seen from the table, the plots amended with compost (i.e. CO and BCCO) contained a high amount of macro nutrients. In particular, the most abundant element was N, followed by K and then P.

Table 3. Concentration of total elements in the treatments (% w/w, w.b.), and quantity of elements applied in the 300 m^2 plot (kg w.b.).

Elements	Treatments	Nutrient (kg) applied in the 300 mq
N	NPK	0.44
	CO	21.52
	BC	0.44
	BCCO	21.52
P	NPK	1.76
	CO	6.76
	BC	1.76
	BCCO	6.76
K	NPK	0.44
	CO	15.36
	BC	0.44
	BCCO	15.36

2.3 Environmental parameters

The meteorological data for the period considered was retrieved from the pluvio-thermometric station of Montespertoli and Cortona, part of the Hydrological and Geological Regional

Monitoring System of Tuscany (SIR). These stations are distant approximately 4.6 and 8 km for Montepaldi and Terontola respectively, from each experimental area.

2.4 Soil analysis

Soil samples collection was performed before the incorporation of the amendment (on April) and after Camelina seed harvest (on July) to determine variation for several chemical and physical parameters. More in detail, for each treatment, four sub-samples were collected per sub-plot and grouped together soil samples were collected from 0 to 30 cm depth at each experimental site. The analytical method used for the determination of the soil chemical and physical are illustrated in **Table 4**.

Table 4. Analytical methods used for soil chemical and physical analysis.

Parameters	Analytical method
Moisture	UNI EN ISO 18134-2: 2017
pH	DM 13/9/99 Metodo III.1
Water holding capacity	DM 01/08/1997 Metodo 5
Cation exchange capacity	DM 13/9/99 Metodo XIII.2
CHN	DM 13/9/99 Metodo VII.1
Electrical conductivity	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo IV.1
Organic carbon	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo VII.3
Soil organic matter	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo VII.3
Total P	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo XV.1
Organic P	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo XV.2
Available P	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo XV.3
Exchangeable K	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo XIII.5
Exchangeable Mg	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo XIII.5
Exchangeable Ca	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo XIII.5
Exchangeable Na	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo XIII.5
Total K	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo X.1
Total Mg	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo X.1
Total Ca	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo X.1
Total Na	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo X.1
Cation exchange capacity	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo XIII.2
Total calcare	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo V.1
Nitrate	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo XIV.4 + metodo XIV. 6
Ammonium	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo XIV.4 + metodo XIV. 6
Nitric	Metodi ufficiali analisi del suolo D.M. 13/9/99 - metodo XIV.4 + metodo XIV. 6
Fe solubile in acqiua regia	Metodo UNI EN 13657
Fe assimilabile	Metodi ufficiali analisi del suolo D.M. 11/5/92 - metodo 37
Texture	Metodi ufficiali analisi del suolo D.M. 11/5/92 - metodo 6

2.5 Camelina biomass and grain and oil yield determination and characterization

The Camelina varieties were harvested once the seeds had reached full maturity: the CCE26 variety was harvested one week earlier than the CCE32 variety characterised by a slightly longer cycle. A circular frame was randomly thrown, 12 times per each variety. Biomass samples were collected by cutting all the Camelina plants which stem fell within the frame borders, while weeds were promptly excluded. Determination of epigeal biomass was measured by considering only the stem and leaves. The seed-containing capsules were processed using an electrical thresher to separate capsules from the seeds. Subsequently, the seeds were dried in an oven for the determination of the grain yield.

For each treatment and variety, an aliquot of the seed was collected and prepared for the determination of the oil content. Seeds samples were first dried and manually milled. The oil extraction was performed in hexane at 60°C, about 10 grams of sample were extracted with 150 ml hexane three times, the sample was then filtered, and the oils were obtained by rotary evaporation of the liquid extract. The collected solids were instead dried at 105°C overnight and then weighted in order to calculate the oil yields (%).

After that, oil samples were analysed for assessing of the CHN content and element concentrations according to ISO UNI EN ISO 16948:2015 (for CHN) and to UNI EN ISO 16967:2015 and UNI EN ISO 16968:2015 (for element concentration determination).
Finally, for each oil sample, the HHV and LHV parameters (high heating value and low heating value, respectively) were calculated by multiplying the oil yield expressed in (kg/ha) by the Camelina's HHV and LHV coefficients (equally to 39.6 and 37.0 Mj/kg, respectively), provided by Camelina Company.

2.6 Statistical analysis

Normality and homogeneity of all parameters were tested prior to analysis, and data were normalized by transformation as needed. Data on soil parameters were compared using a 2-sample t-test to determine significant differences within the same treatments before Camelina sowing (March 2021) and after Camelina harvest (July 2022). One-way ANOVA followed by the Fisher multiple range test at the level of significance of 99% ($p < 0.001$) was used to assess the significance of difference in plant yield and biomass and oil parameters. All data elaborations were realized with Minitab®17.1.0, Minitab Inc., State College, PA, USA.

3 Results

3.1 Environmental parameters

Figure 6 illustrates the total monthly rainfall and the average monthly values of the maximum and minimum temperatures occurring in the period between sowing and harvesting of Camelina.

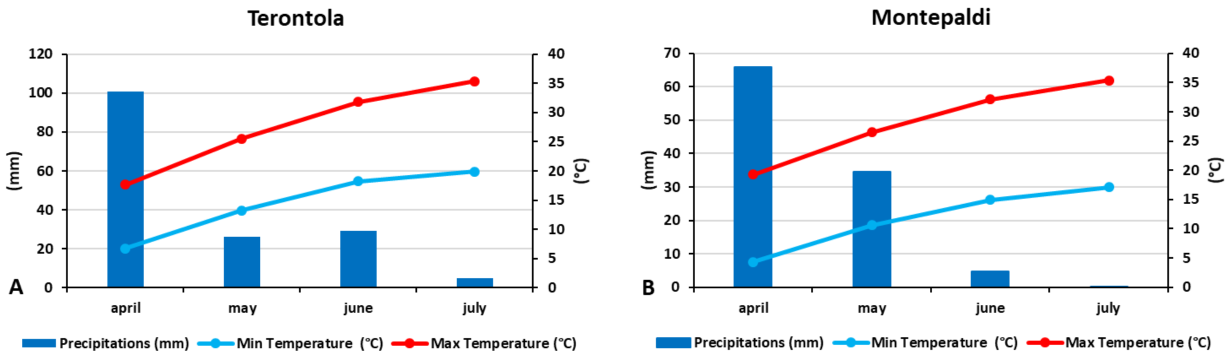


Figure 6. Total monthly rainfall and the average monthly values of the maximum and minimum temperatures occurring in the period between sowing and harvesting of Camelina in Terontola (A) and in Montepaldi (B).

The cited period of cultivation was characterized by a total rainfall of 105.4 and 168 mm in Terontola and Montepaldi, respectively. As in the entire Mediterranean basin, in the 2022, these two locations experienced summer temperatures as early as May, accompanied by a strong lack of rainfall. These climatic conditions inevitably influenced growth and yield of non-irrigated crops such as Camelina.

3.2 Soil chemical and physical characterization

Soils in Terontola and Montepaldi were characterised by a loam texture; however, higher percentage of clay was found in Montepaldi field (**Figure 7**).

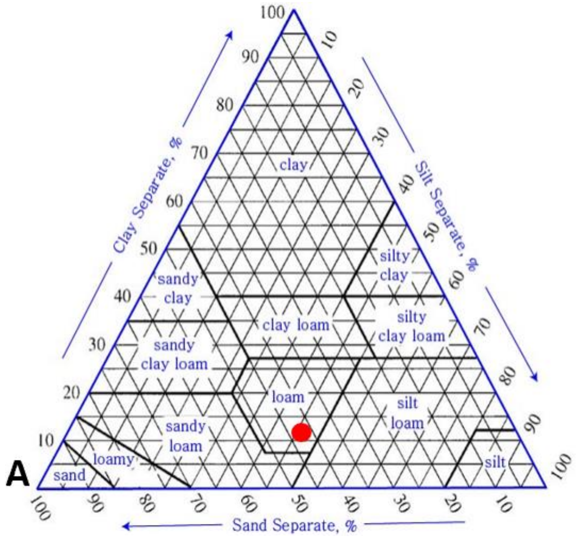


Figure 7. Soil texture characterization in Terontola (A) and Montepaldi (B).

At time zero, the soil pH at Terontola was quite sub-acidic with values at time zero between 5.50 and 5.95 while at Montepaldi, the values were much higher in the range of 6.90 and 7.23, and thus with a neutral, sub-alkaline pH (**Figure 8 andFigure** 9). After harvesting Camelina, a significant increase in pH was observed at both locations for all theses, with the exception of NPK at Terontola. During cultivation, an increase in electrical conductivity (EC) was observed in all theses with the exception of CK at Terontola, and CK, NPK, and CO at Montepaldi (**Figure 8 andFigure** 9). The cation exchange capacity (CEC) was higher in Montepaldi (18-20 meq/100g) than in Terontola (12-14 meq/100g), but it was not affected by any treatment in either location (**Figure 8 andFigure** 9).

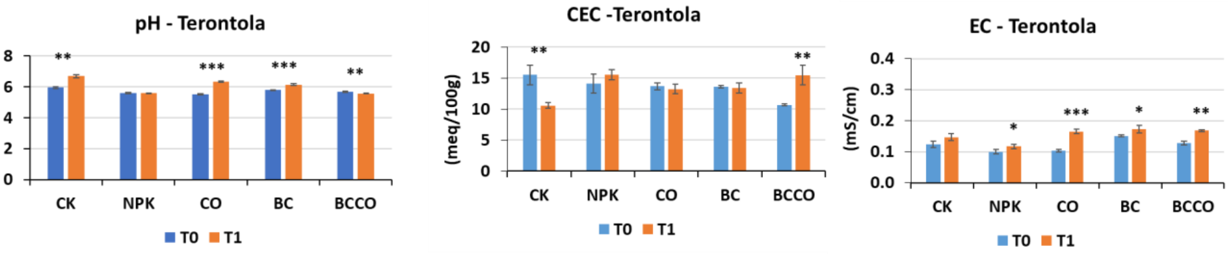


Figure 8. Mean values (n=3) of pH, CEC and EC in soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Terontola. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001

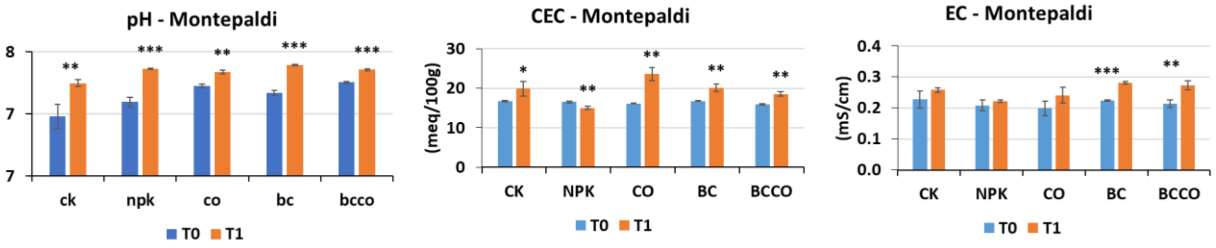


Figure 9. Mean values (n=3) of pH, CEC and EC in soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Montepaldi. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001.

No significant differences were found for organic carbon (C org) and soil organic matter (SOM). However, it is interesting to note that at Terontola, these parameters tended to increase, while at Montepaldi, there was a decrease (**Figure 10 andFigure** 11).

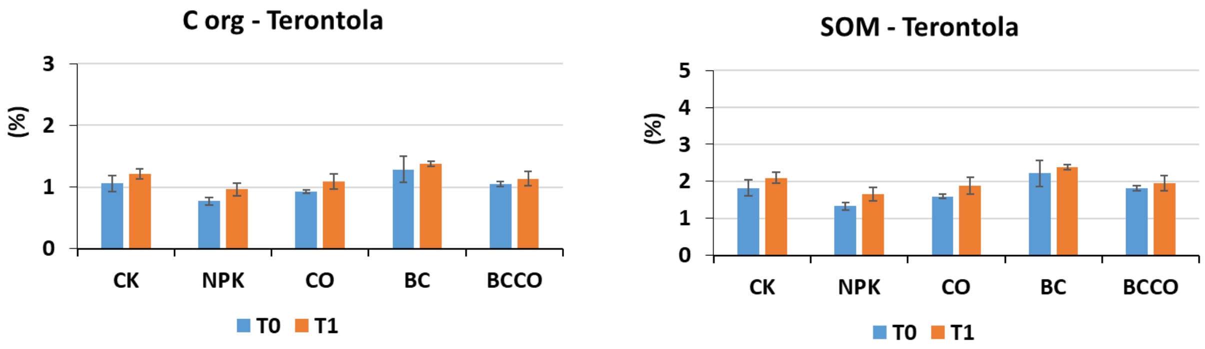


Figure 10. Mean values (n=3) of organic carbon (C org) and soil organic matter (SOM) of soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Terontola. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001.

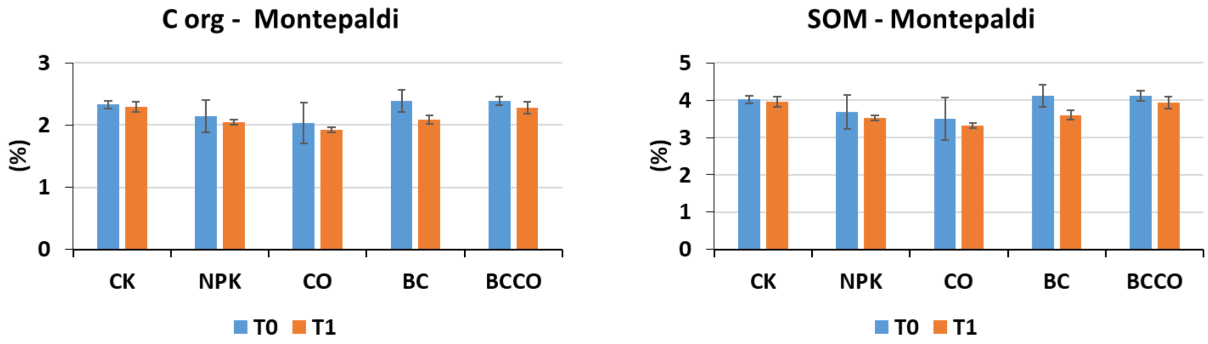


Figure 11. Mean values (n=3) of organic carbon (C org) and soil organic matter (SOM) of soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Montepaldi. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001.

At Terontola, total nitrogen (TN) significantly increased in the NPK, CO and BC theses (**Figure 12**), while at Montepaldi, concentrations decreased for CK and BC and slightly increased for BCCO (**Figure 13**). The C/N ratio significantly decreased for CO and BCCO in Terontola and Montepaldi locations respectively (**Figure 12 andFigure** 13).

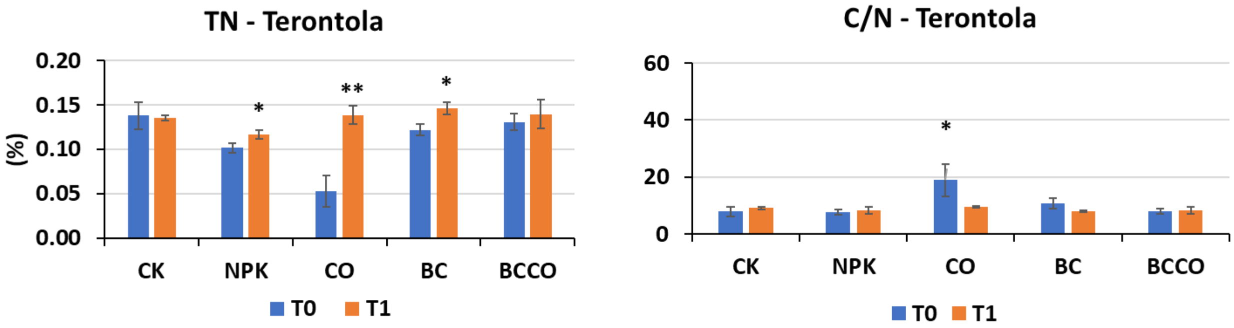


Figure 12. Mean values (n=3) of total nitrogen (TN) and ration C/N of soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Montepaldi. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001.

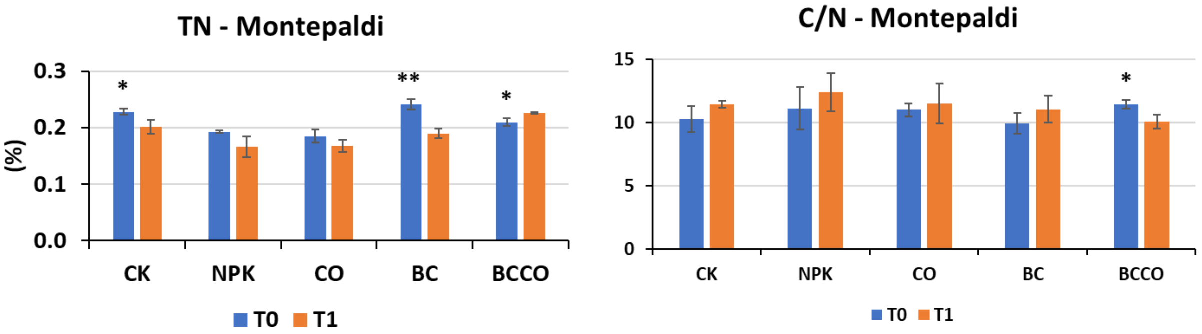


Figure 13. Mean values (n=3) of total nitrogen (TN) and ration C/N of soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Montepaldi. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001.

Nitrogen forms behaved differently depending on the location considered. In detail, in Terontola, a significant decrease in NH_4^+ concentrations were observed for organic-based amendments (**Figure 14**). By contrary, NO_2^- concentrations, strongly decreased after plant harvest in all treatments, reaching values below the limit of determination (<0.3 mg/kg) in all cases, expect for CK treatment. NO_3^- concentrations showed significantly increasing trends for all the investigated cases (**Figure 14**).

In Montepaldi, significantly higher values of NH_4^+ concentrations were observed in all cases, (**Figure 15**). No consistent trends were observed for NO_2^-, which only showed an increase for the control treatment. A reduction of NO_3^- concentrations were detected for NPK, BC and BCCO while CK treatment exhibited a significant increase (**Figure 15**).

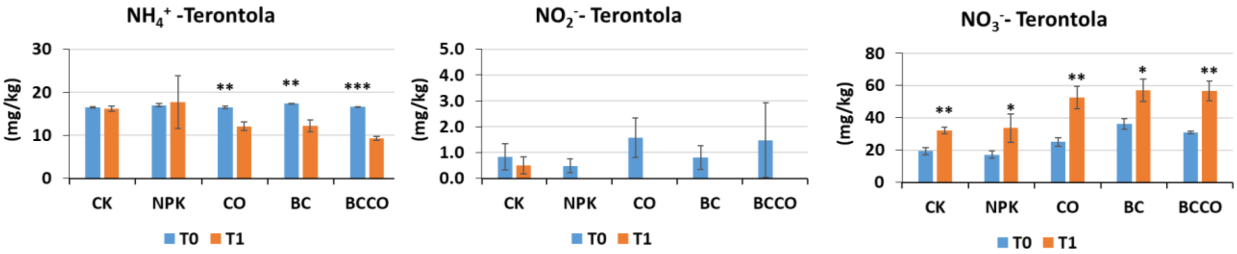


Figure 14. Mean values (n=3) of ammonium (NH_4^+), nitric (NO_2^-) and nitrate (NO_3^-) of the soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Terontola. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001.

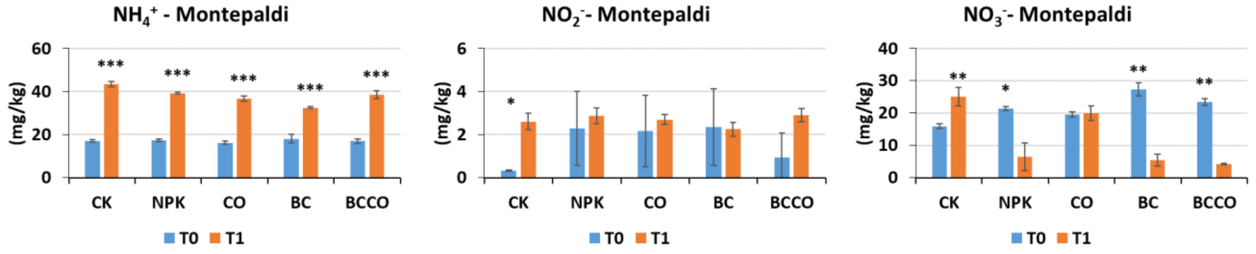


Figure 15. Mean values (n=3) of ammonium (NH$_4^+$), nitric (NO$_2^-$) and nitrate (NO$_3^-$) of the soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Montepaldi. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001.

Taking the P forms into consideration, the total P concentration was only significant for the soil amended with compost at Terontola, which suffered a slight reduction (**Figure 16**). The organic P (P org) showed a significant reduction for the soil amended with compost only at Terontola, whereas at Montepaldi the available P showed a significant increasing trend for all treatments (**Figure 17**).

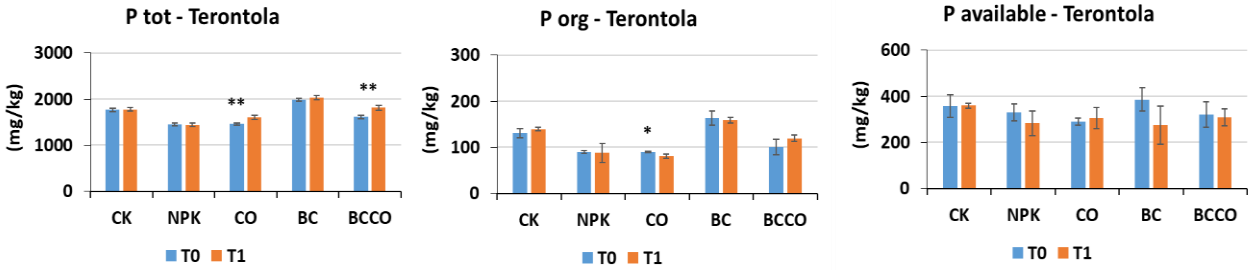


Figure 16. Mean values (n=3) of total P, organic P and available P of the soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Terontola. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001.

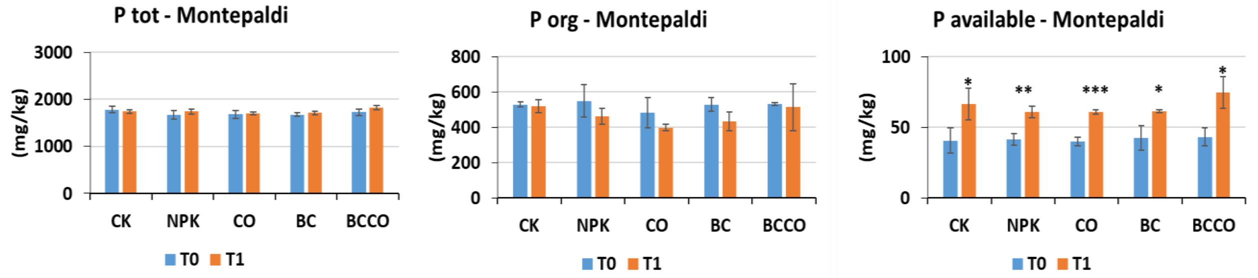


Figure 17. Mean values (n=3) of total P, organic P and available P of the soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Montepaldi. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001.

The exchangeable concentration of K, Ca and Mg found in Terontola and Montepaldi are reported in **Figure 18** and **19.**

In general, K concentration decreased for the CK, CO and BC treatments in Terontola, and only for BC in Montepaldi. Ca showed a significant trend only for CO, BC and BCCO in Terontola. In contrast, the soils sampled in both locations exhibited decreasing trends (**Figure 18 Figure 19**).

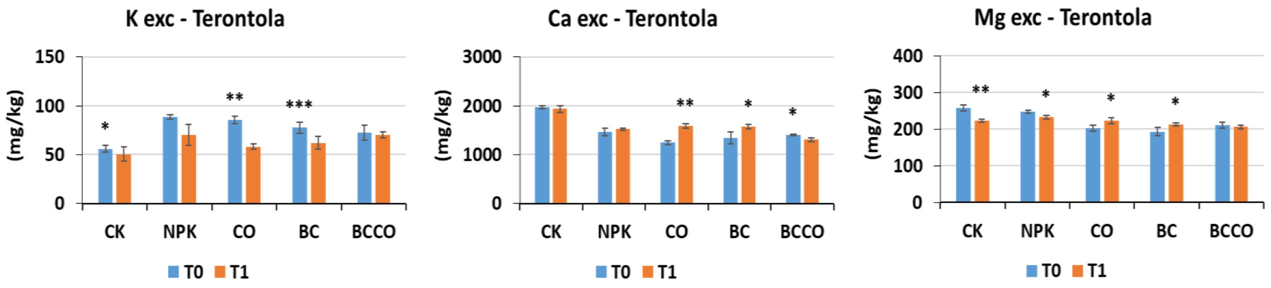


Figure 18. Mean values (n=3) of exchangeable K, Ca and Mg of the soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Terontola. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001.

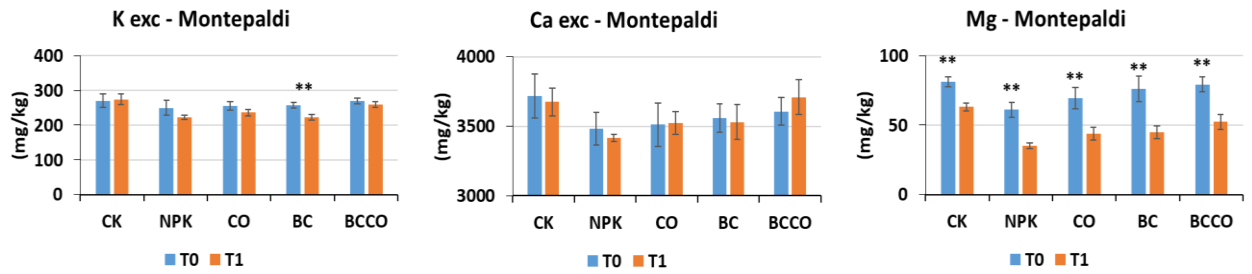


Figure 19. Mean values (n=3) of exchangeable K, Ca and Mg of the soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Montepaldi. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001.

At the end of the test, exchangeable Na decreased significantly for all treatments except CO in Terontola (**Figure 20**). At Montepaldi, exchangeable Na increased in CO and BCCO and decreased in NPK (**Figure 21**). At the end of the test, exchangeable Na decreased significantly for all treatments except CO in Terontola, whereas at Montepaldi, Na concentration increased in CO and BCCO and decreased in NPK. At Terontola, with the exception of BCCO, exchangeable Fe decreased significantly, while at Montepaldi only the BC treatment increased. Only the CK in Montepaldi showed an increased in Fe soluble concentrations (**Figure 20 and Figure** 21).

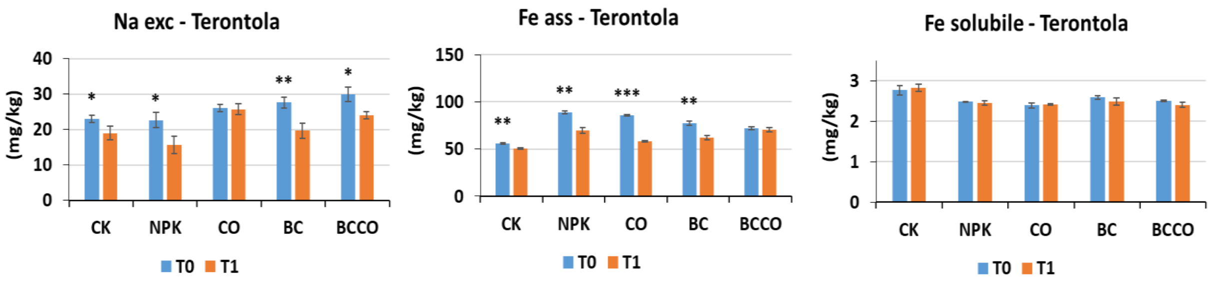


Figure 20. Mean values (n=3) of exchangeable Na, Ca and Mg of the soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Terontola. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001.

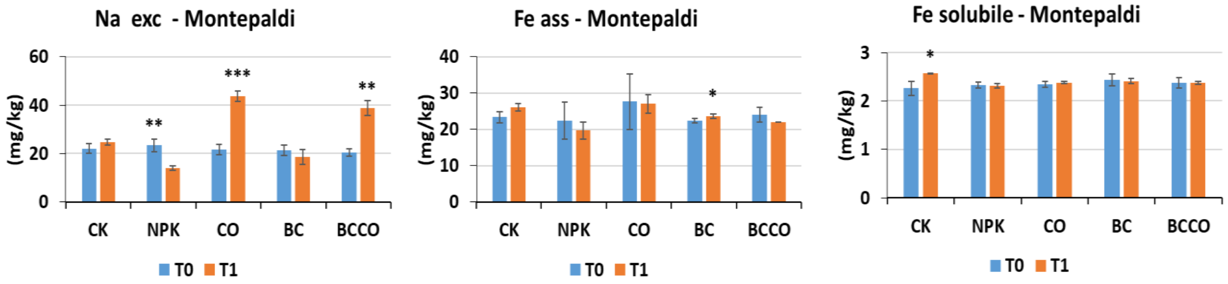


Figure 21. Mean values (n=3) of exchangeable Na, Ca and Mg of the soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Montepaldi. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001.

Taking into account total element concentrations, only in some cases there were significant differences. More in details, CK showed a significant decrease for K and Na elements, and, together with Co treatments an increase in Mg concentrations (**Figure 22**). Ca was only affected by the presence of biochar.

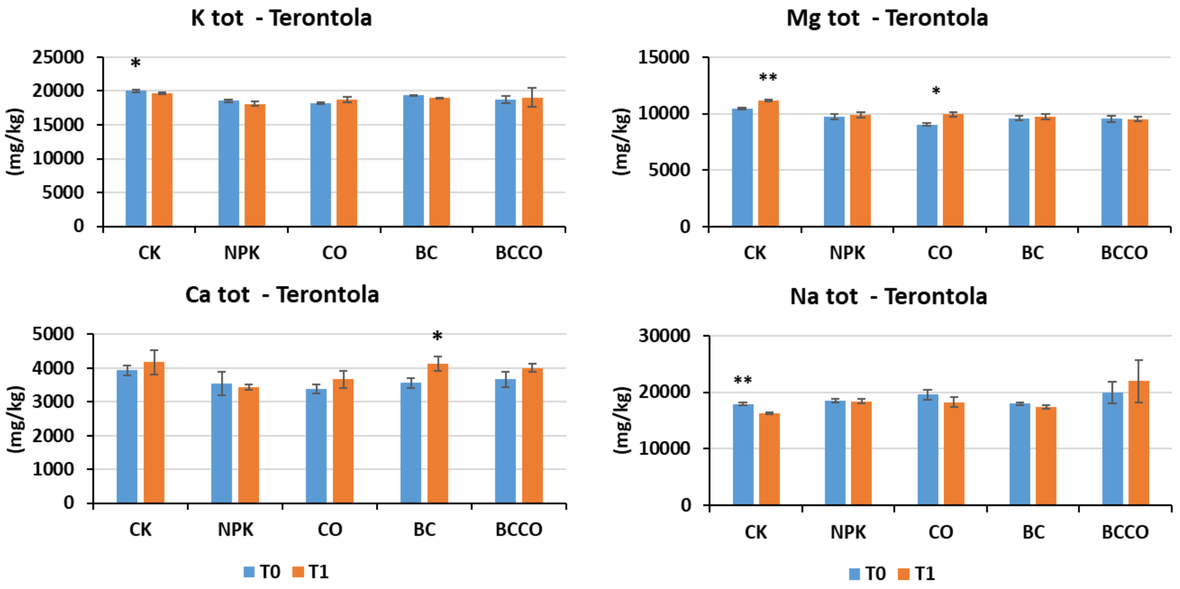


Figure 22. Mean values (n=3) of total K, Mg, Ca, and Na concentration of the soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Montepaldi. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001.

Regarding elements in Montepaldi, almost all the treatments showed no variations among time, except for CK and BCCO which showed decreasing and increasing trend for Mg and Ca concentrations, respectively (**Figure 23**).

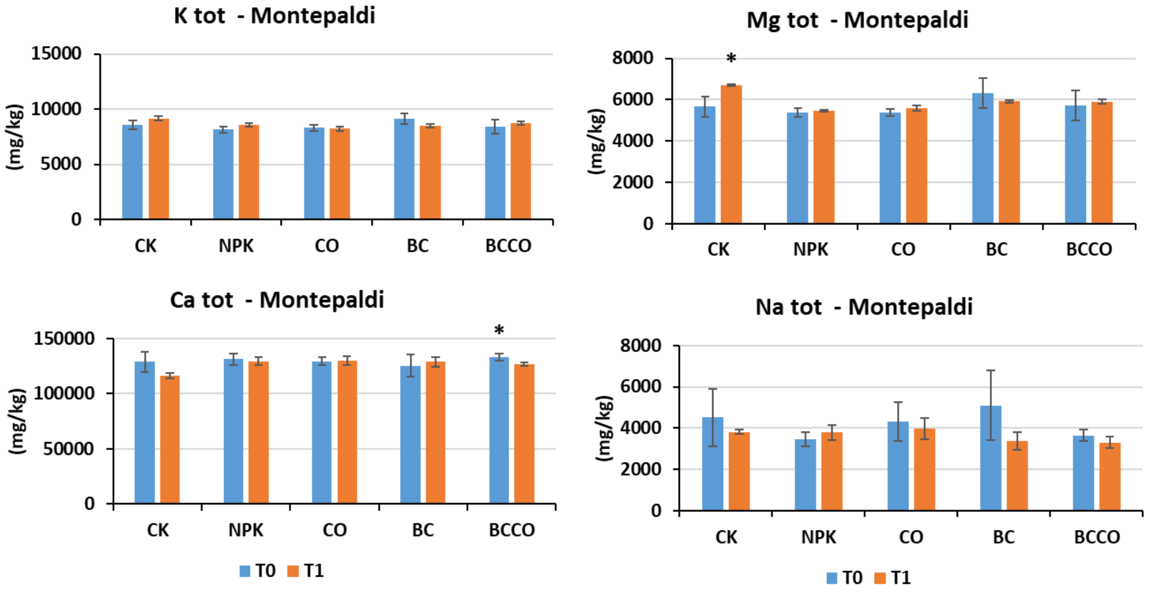


Figure 23. Mean values (n=3) of total K, Mg, Ca, and Na concentration of the soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Montepaldi. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001.

Significant changes in bulk density (BD) were found in Terontola with increased values for the CK, NPK, BC and BCCO treatments, while in Montepaldi the BD did not show significant differences among treatments (**Figure 24 and Figure** 25). At Terontola, porosity increased for the biochar-based treatments, while in Montepaldi the porosity increased for CO and NPK and decreased for BC (**Figure 25**). Taking into account the field capacity, in Montepaldi, NPK, CO and BC exhibited decreasing values from time zero to time 1, while in Terontola, only the BCCO was significantly increased.

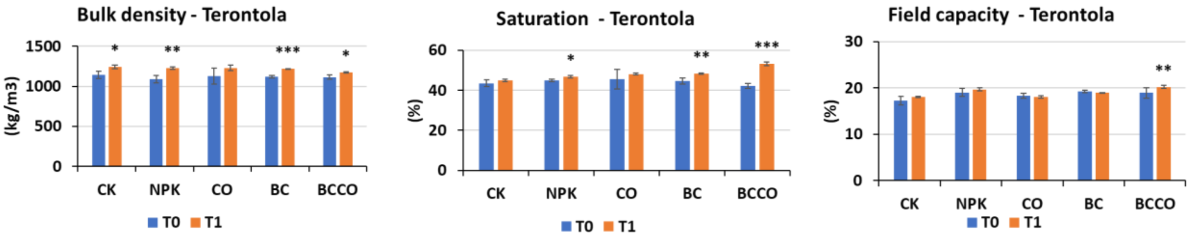


Figure 24. Mean values (n=3) of bulk density, saturation and field capacity of the soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Terontola. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001.

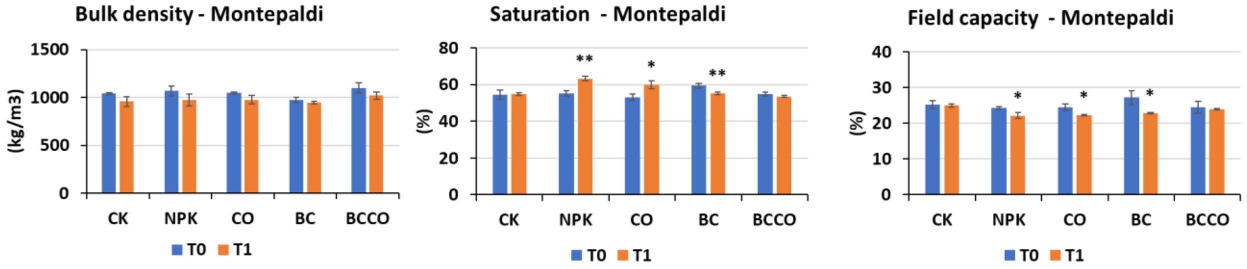


Figure 25. Mean values (n=3) of bulk density, saturation and field capacity of the soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Montepaldi. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001.

Regarding air capacity, BCCO in Terontola and NPK and BC in Montepaldi exhibited increasing trend while CO in Terontola showed a slight decrease (**Figure 26 Figure 27**).
Regarding available water, BC and BCCO in Terontola and CO in Montepaldi showed significant increase, while BC in Montepaldi showed a significant reduction (**Figure 26 Figure 27**).

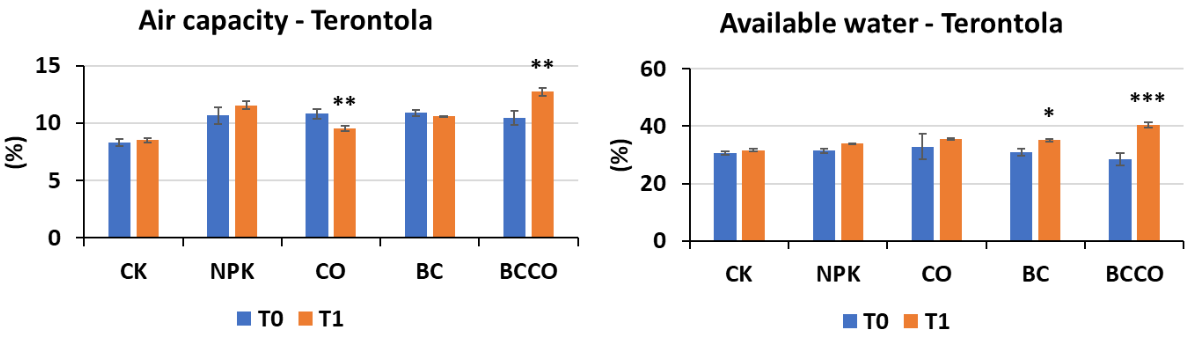


Figure 26. Mean values (n=3) of air capacity and available water of the soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Terontola. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001.

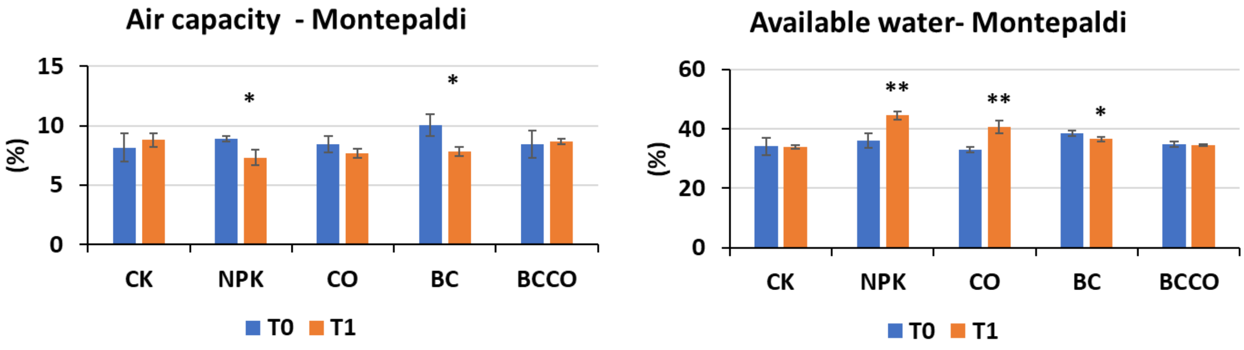


Figure 27. Mean values (n=3) of air capacity and available water of the soil at time 0 (before treatments incorporation) and T1 (after Camelina seed maturation) in Montepaldi. Error bars represents standard deviations. * = P<0.05; ** = P<0.01; *** = P<0.001

3.3 Camelina biomass, seed and oil yield determination

Aerial dry biomass showed significant differences only for Camelina grown in Terontola (**Figure 28A-B**). Regarding CCEE26, the highest biomass was observed for plants grown under BC amendment, followed by BCCO, CK, while NPK and CO showed the lowest values. The trend found for CCE32 was: BCCO> BC and NPK > CO and CK (**Figure 28A**).

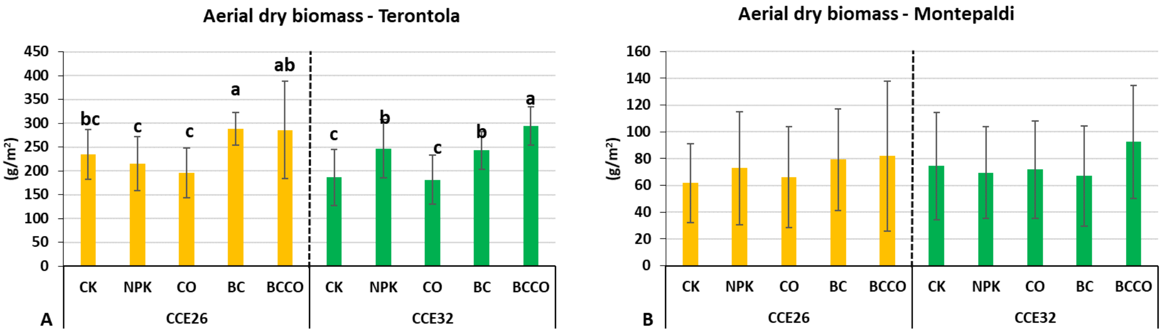


Figure 28. Mean values (n=12) of Camelina aerial dry biomass (g/m²) grown in Terontola (A) and Montepaldi (B). Error bars represents standard deviations. Different letters within the same variety indicates significant differences for P<0.001.

Regarding Terontola, the highest seed yield for Camelina CCE26 was observed in BCCO and BC which gained similar values, while the other treatments exhibited the lowest yield (**Figure 29A**). The highest productivity for CCE32 was detected in BCCO, while the lowest in CK and CO treatments. CCE26 variety was not affected by treatments, while for CCE32, the highest yield was observed for BCCO, whereas the lowest values were detected for CK and NPK treatments (**Figure 29B**).

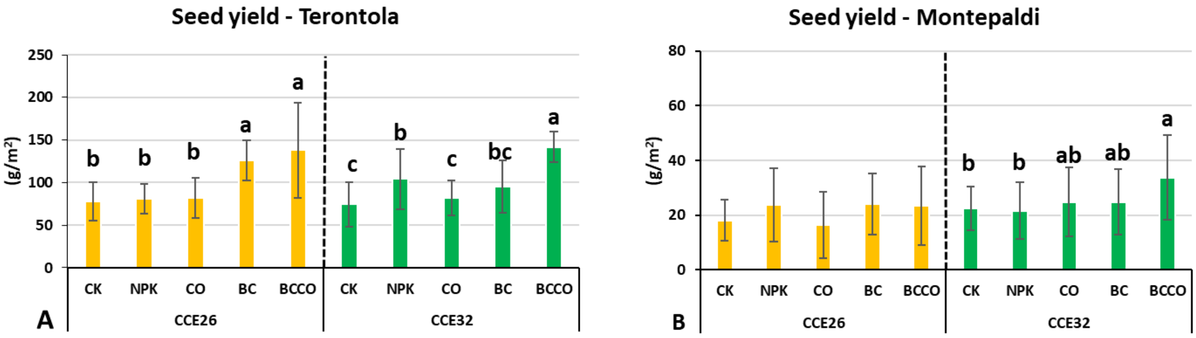


Figure 29. Mean values (n=12) of Camelina seed yield (g/m²) grown in Terontola (A) and Montepaldi (B). Error bars represents standard deviations. Different letters within the same variety indicates significant differences for P<0.001.

Oil yield followed the similar statistical trend observed for seed yield parameter showing no influence of treatments on oil content of seeds (**Figure 30A-B**).

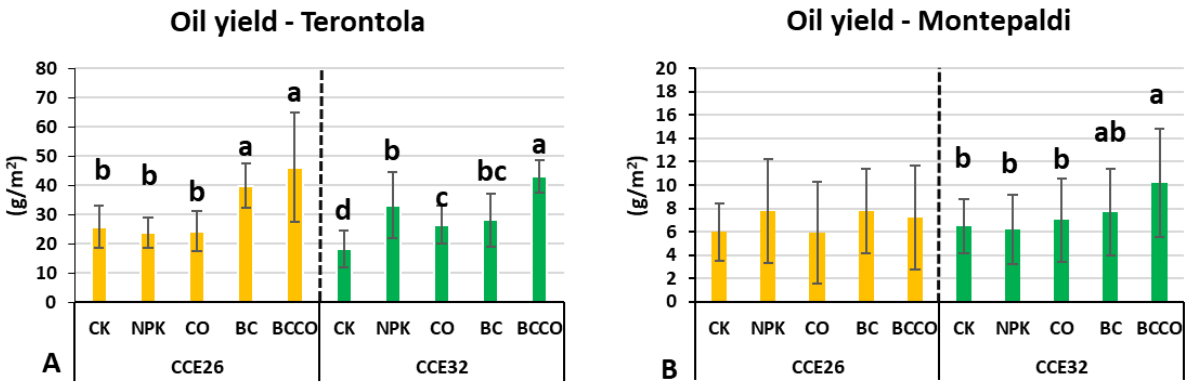


Figure 30. Mean values (n=12) of Camelina oil yield (g/m^2) grown in Terontola (A) and Montepaldi (B). Error bars represents standard deviations. Different letters within the same variety indicates significant differences for P<0.001.

Figures 31 and 32 illustrate the oil HHV and LHV determined in the oil samples. These two parameters reflect the oil quality from the energetic point of view. The statistical trend found in the figures is similar to oil yield values.

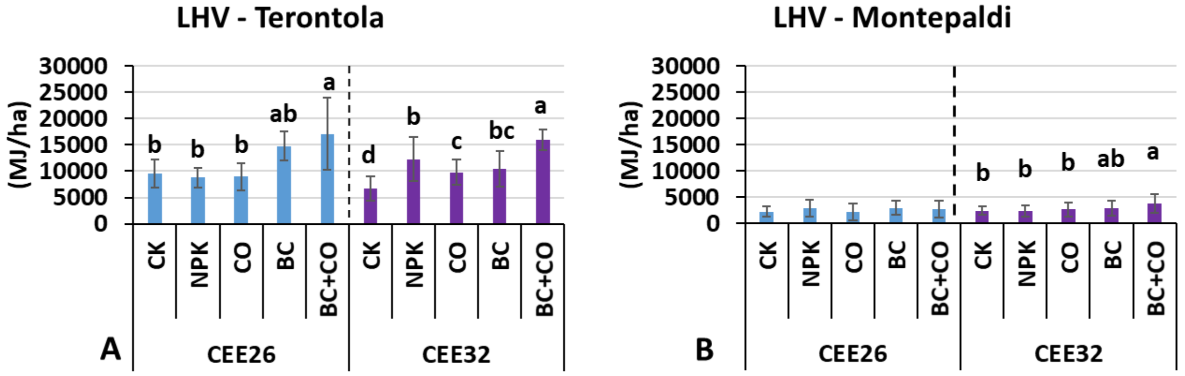


Figure 31. Mean values (n=12) of Camelina oil LHV (MJ/ha) grown in Terontola (A) and Montepaldi (B). Error bars represents standard deviations. Different letters within the same variety indicates significant differences for P<0.001.

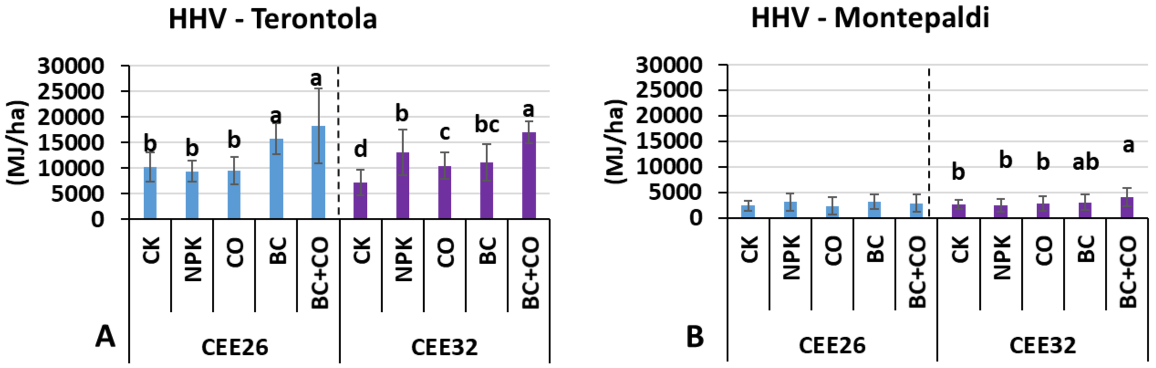


Figure 32. Mean values (n=12) of Camelina oil HHV (MJ/ha) grown in Terontola (A) and Montepaldi (B). Error bars represents standard deviations. Different letters within the same variety indicates significant differences for P<0.001.

The H content in the oil showed very similar values between 10 and 12% considering treatments, variety and location (**Figures 33 and 34**). The average N content observed at Montepaldi in Terontola was 0.14 and 0.12%, respectively. Regardless of variety and location, the oil obtained from the various treatments did not show high variations, except for the CCE32 variety cultivated under BC, which showed the lowest content of 0.06% (**Figure 34**).

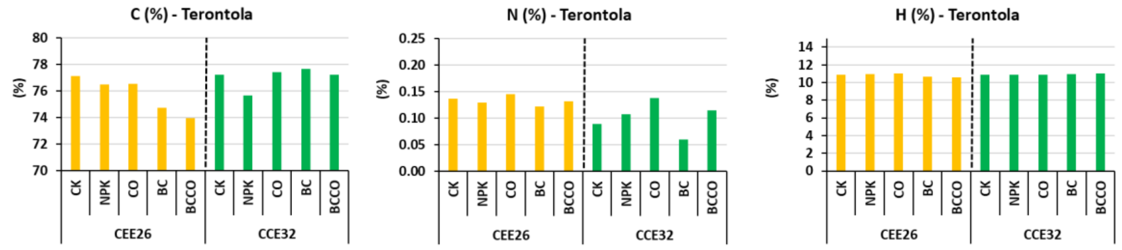


Figure 33. Concentration of C, N and H (%) found in oil samples for plants cultivated in Terontola.

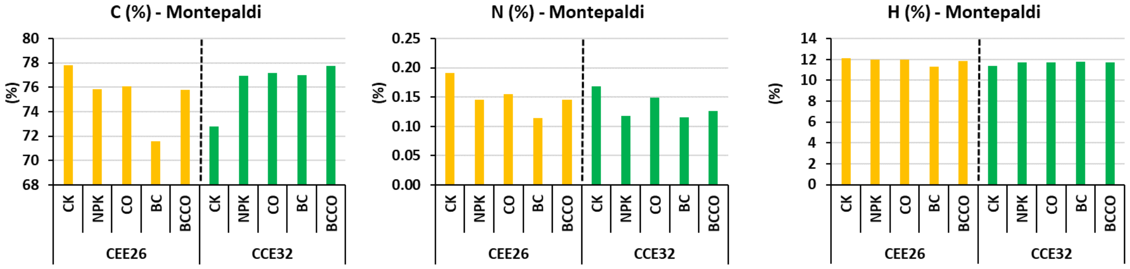


Figure 34. Concentration of C, N and H (%) found in oil samples for plants cultivated in Montepaldi.

Tables 5 and 6 show the element concentrations determined in the various oil samples. Most of the elements were below the limits of determination. In general, oil from plants grown in Terontola showed the presence of Ca, Ba, Si, Ti, and Mg. For these plants, the element with the highest concentration was Si, which in many cases showed concentrations of more than 100 mg/kg. At Montepaldi, the only elements found in the oil samples were Si and Ti, in lower concentrations than those observed at Terontola.

Table 5. Element concentration of the oil for CCE26 and CCE32 varieties cultivated in Terontola.

	Variety CCE26		Variety CCE32	
Treatment	Element	Concentration (mg/kg db)	Element	Concentration (mg/kg db)
CK	Ca	26.1	-	-
	Si	212.0	Si	50.2
	Ti	0.4	Ti	0.3
NPK	Ba	0.1	Ca	1.2
	Si	517.0	Si	37.8
	Ti	0.3	Ti	0.6
CO	Ca	2.6	Ca	26.7
	Si	169.1	Si	35.4
	Ti	0.6	-	-
BC	Ba	0.1	-	-
	Ca	21.2	-	-

	Mg	6.8	-	-
	Si	78.2	Si	97.9
	Ti	0.2	-	-
BCCO	Ba	1.4	-	-
	Ca	17.1	Ca	37.2
	Si	162.4	Si	148.4
	Ti	0.2	-	-

Table 6. Element concentration of the oil for CCE26 and CCE32 varieties cultivated in Montepaldi.

	Variety CCE26		Variety CCE32	
Treatment	**Element**	**Concentration (mg/kg db)**	**Element**	**Concentration (mg/kg db)**
CK	Si	66.4	Si	83.9
	-	-	Ti	0.1
NPK	Si	213.0	Si	76.8
	-	-	Ti	0.3
CO	Si	98.2	Si	52.0
	-	-	Ti	0.5
BC	Si	99.5	Si	27.4
BC	Ti	0.1	Ti	0.6
BCCO	Si	69.1	Si	32.9
	Ti	0.1	Ti	0.5

4 Discussion

Comparison of Camelina behaviour in the two locations

Despite the same agronomic conditions in terms of type of treatment and soil tillage, Camelina plants behaved differently depending on the location. The best performance was observed in Terontola, since Camelina plants produced 3 and 4 fold more biomass production, grain and oil yield than those observed in Montepaldi, regardless varieties and treatments (**Figure 28Figure** 30).
The remarkable differences observed in the behavior of the two varieties can be attributed to several factors and among them, precipitation intensity and frequency play a crucial role for plant development and health.

Although camelina is suited to grow in a semi-arid environment, plant development may not be optimal under unequally distributed rainfalls throughout the cultivation period. If most of the rainfall occurs in the pre-flowering period, a reduction of the soil water availability can occur, leading to decrease nutrient uptake and to limit growth rate and biomass accumulation (Sintim et al., 2015). In Italy, the 2022 field campaign was characterized by very low rainfall in both locations (161 and 105 mm for Terontola and Montepaldi, respectively), thus providing a representative example of a dry spell (**Figure 6**). In Montepaldi, the poorer precipitation experienced, and the excessively high temperatures recorded in May certainly resulted in further inhibition of Camelina growth and productivity.

Soil type was certainly an important factor in influencing the performance of Camelina plants. The soil structure of Montepaldi field resulted heavier due to the high coarse fraction and clay percentage, which reduced air capacity under non-optimum value (<10%) compared to the field in Terontola (**Figure 27**) which had a lighter structure thanks to the high percentage of sand. Furthermore, at Terontola, the soil was also looser due to the frequent tilling that periodically worked the first 30 cm deep. Looking at the chemical fertility, the soil in Montepaldi was characterized by total higher N and P concentrations, but, compared to Terontola, the availability of nitrates and phosphates were lower (**Figure 14Figure** 17), probably reduced by the sub-alkaline pH of Montepaldi field (i.e. range 6.9-7-4, **Figure 9**), contributing to limit plant development.

All these physical and chemical characteristics of the soil in combination with a very dry spring-summer season possibly disadvantaged camelina, creating an unsuitable condition for a proper development in Montepaldi.

Comparison between the performances of the varieties in the two locations

The two varieties responded differently depending on the fertilization or amendment application, within the same location.

In Terontola, Camelina CCE26 showed consistent trends for vegetative and productive parameters, with the best results when cultivated under biochar-based amendment (i.e. BC and BCCO) (**Figure 28 and Figure** 30). By contrast, there wasn't a positive effect under NPK and CO treatments as CCE26 plants exhibited similar results of the control treatments (**Figure 28A and Figure** 30**A**). Since all the treatments - expect CK - were equally fertilized, this finding indicated that this variety responded well to the presence of biochar, demonstrating that a dose of 3 t/ha of biochar plus fertilizer (133 kg/ha) can gain high seed and oil yield.

Differently, Camelina CCE32 was positively affected by BCCO amendment. The use of BCCO in Camelina CCE32 provided the highest biomass and productivity, while the application of biochar alone (BC) did not lead to significant improvements (**Figure 28A and Figure** 30**A**). A similar trend was also observed with CCE32 in Montepaldi. This behaviour could suggest that a higher demand for nutrients and can be associated to the CCE32 genotype. In fact, CCE32 is a short cycle variety as CCE26, but it needs some days more to reach the full maturity. This behaviour could suggest that the CCE32, probably may show higher nutrients requirement for a proper growth compared to CCE26. However, the stress induced by high temperatures have shortened the cycle of this variety, anticipating seeds maturity three weeks earlier than expected.

In Montepaldi, the abiotic stresses (low rainfall, high temperature) influenced Camelina CCE26 plants that the supplied of fertiliser/amendment did not cause any beneficial effect on vegetative and productive organs.
An interesting aspect was observed with the CCE32 variety. Although biomass was not affected by the treatments, an increase in seed yield of 44% was detected with the presence of BCCO compared to the other treatments (**Figure 29Figure 30B**), indicating that the productivity of this genotype was positively influenced by the co-presence of biochar, compost and nitrogen fertilisation.

Effect of the treatments on soil in Terontola

In Terontola, the best plant performances were observed under BC and BCCO suggesting that the presence of biochar was relevant for the improvement of plant growth and productivity.

Considering soil analysis, at the end of Camelina cycle, the main physical variations were observed under biochar-based amendments. Bulk density, porosity and available water were significantly increased with both BC and BCCO towards more optimal values (**Figure 24 Figure 26**). The amelioration of the physical properties, surely have a positive effect on the biomass of the biochar-amended plants which benefit from the improved water retention conditions, resulting in a major translocation of water and nutrients from soil to plants, thus promoting plant growth and yield. Previous studies have observed similar results, noting an increase in the biomass of plants amended with biochar alone or mixed with compost accompanied by an improvement of the physical properties (Suliman et al., 2017).

Regarding nutrients, as expected, total elements (i.e. K, Ca, Mg and Na) showed in most cases constant values between time 0 and 1 likely due the short period among the two sampling campaigns (**Figure 22**). On the contrary, the available nutrients exhibited in most cases a significant reduction in their concentration compared to the beginning of the test, mainly due to the plant uptake (**Figure 18Figure 20**). However, it should be noted that BC alone was the only treatment that led to a significant increase in the available forms of Ca and Mg (**Figure 18**) within the nutrient soil solution. The increase in their concentrations could be attributable to the retention effect of positive ions (i.e. Mg^{2+} and Ca^{2+}) exerted by the negatively charged biochar surface and to the consequently release in the soil water solution. Several authors observed biochar has a great ability to retain nutrients in an exchangeable form and slowly released into the environment in a plant-available forms, leading to avoid nutrient leaching (Agegnehu et al., 2015). Unexpectedly, the CEC remained unchanged for most of the theses except for CK and BCCO. In CK, the reduction in CEC can be attributed to the absence of fertiliser treatment and thus to the removal of nutrients present at a colloidal level in the soil at time zero (**Figure 8**). On the other hand, the increase in CEC of the soil fertilised with BCCO indicates that the amount of nutrients available to the plant is increased and that there is less risk of leaching and loss of elements to the environment.

Unexpectedly, P total, organic and available concentrations did not show consistent variation among time (**Figure 16**), although all the plots (except the CK) were fertilized with a product with a high title of P (i.e. 133 kg/ha of Polyfeed 11:44:11). Only total P concentration increased in the composted plots (i.e. CO and BCCO). Undoubtedly this increase can be attributed to the high amount of P supplied with the compost in the plots (**Table 3**), however, the lack of increase in available P in these two plots indicates that much of the removed P has probably been immobilised.

Regarding nitrogen, a significant reduction in the NH_4^+ and NO_2^- ions followed by an increase in NO_3^- concentrations was observed in all treatments at the end of the cycle (**Figure 14**). These data indicated the N cycle was shifted towards ammonification and nitrification processes given the low NH_4^+ and high NO_3^- values found especially in those plots amended with biochar and compost (i.e. BC, CO, BCCO). Thus, these treatments stimulated the availability of nitrates, which are the most readily available form of nitrogen for plants.
Compost provided the highest amount of Nitrogen (**Table 3**). By contrast, the contribution of N supplied with the BC was definitely lower as it was supplied only with the NPK fertilization. Nonetheless, with BC treatment, nitrate availability was significantly increased, and this finding could be the result of a synergistic effect between BC and fertilization. Since biochar significantly increased water availability (**Figure 26**), it is possible that BC treatment also increased nitrate retention by making them available for root uptake, resulting in higher plant biomass and yield. On the other hand, despite the large nitrogen supply with compost, plants grown with only CO, despite fertilization, did not benefit from this.

Effect of the treatments in Montepaldi

As mentioned in the paragraph above, in the case of Camelina plants in Montepaldi, despite the use of soil improvers and fertilizers, there was no significant amelioration in plant biomass and yield. Compared to Terontola, Camelina plants were very small in size and looked stressed. In fact, at soil level, no positive change was observed from the physical and chemical point of view.

Nitrogen was added as NPK fertilizers in May, when plants were at the stage of rosette, since nitrogen must be available before plant bolting, which is the maximum requirement period. Nevertheless, nitrogen fertilization did not appear to have a beneficial effect on biomass production, even on those plants grown on the compost-based amendments (i.e. BCCO and CO) which were supplied with 15 kg/ha of NPK and about 700 kg/ha of N with compost (**Table 3**). Probably, the overall N cycle in the Montepaldi field was inhibited by other factors (e.g. water scarcity, soil initial characteristics). Accordingly, in Montepaldi, a notable rise in ammonium levels was observed in soil for all treatments, regardless of whether they were fertilized or not. However, this increase did not correspond to a rise in nitrate levels (**Figure 15**). It is possible that at the microbial level, the microorganisms responsible for converting ammonium into nitrate were inactive, resulting in a consistent accumulation of unused ammonium. Accordingly, Camelina plants were probably experiencing nitrogen deficiency, which resulted in poor growth and development of small yellow leaves. Previous authors observed that when Camelina is under nitrogen deficient, developed small leaves, greenish yellow in colour are developed, and the crop tend to mature earlier, producing less silicles and small seeds (Solis et al., 2013).
Nitrogen is a crucial nutrient for plant growth and it is generally required in relatively large quantities. However, the N requirement of Camelina plant is less than that needed by other Brassica oilseeds such as canola (*B. napu*s L.) (Solis et al., 2013). Zubr (1997) found that nutrient requirements for Camelina were moderate to low, approximately 100 kg N/ha and recommended to apply fertilizer at the beginning of spring for winter crops.

Different studies have reported varying recommendations for N application rates, ranging from 78 to 100 kg/ha, 12 kg/ha N per 100 kg of expected grain yield, and even up to 100 kg/ha depending on soil fertility, residual nutrient levels, and weather conditions (Jackson, 2008; Wysocki el., 2013). Other authors suggested that the response to N fertilizer application in camelina can be complex, with some studies showing a quadratic response, indicating that the yield increases up to a certain point and then plateaus or decreases with further N application (Johnson and Gesch, 2013). For example, increasing N levels from 60 to 130 kg/ha resulted in a 30% yield increase but decreased oil concentration (Agegenehu and Honermeier, 1997). Therefore, studies found in literature reveal a wide range of responses to N fertilizer application signifying the need to have site specific recommendations and protocol for Camelina production.

Phosphorous is an important macro-nutrient for plant since it promotes flowering and seed formation. However, Camelina does not respond to P if this availability exceeds 13 ppm (Camelina Company personal communications). At Montepaldi, in fact, the increase in available P above 50 ppm (**Figure 17**) did not lead to significant increases in grain and oil yields, indicating that this element (along with nitrogen), was not efficiently utilised by Camelina plants.

Regarding the physical characteristics of the soil, no improvement in water holding capacity properties was observed (**Figure 25Figure 27**). A possible explanation could be related to the high content of clay found in Montepaldi field which have which made the effect on physical properties less noticeable compared to the Terontola field which exhibited a lower amount of clay.

5 Conclusions

At Terontola, Camelina performed very well in the presence of biochar mixed with compost alone, but also with biochar alone. An improvement of the soil physical characteristics was observed in the biochar-mixed plots. This result confirms that when biochar is incorporated into soil with a high sand content (such as that of Terontola), it causes an improvement in soil structure, which results in greater water retention and greater plant tolerance to prolonged periods of drought. In addition, certainly another parameter that increased the performance of Camelina is nitrogen. Despite the lower amount of nitrogen provided by the biochar treatment, the high nitrate content measured in the solution circulating the biochar-fertilised plot leads to the inference that the plants utilized more efficiently the nitrogen they had available. These may indicate that 3 tons/ha of biochar (with mineral fertilisation) are sufficient to ensure an increased yield of seeds and oil compared to the use of 20 tons/ha of compost, in a field characterized by a light and sandy structure.

The results obtained at the Montepaldi site, suggest that rainfall is also a relevant factor for species such as Camelina, which is tolerant of growing in semi-arid environments. Camelina is recognized as a high drought tolerance crop, however, in a four-month cycle, this resilience is reduced, since Camelina needs to develop properly the root system to gain drought tolerance. Despite the suboptimal plant performance, an increase in seed yield was observed in the presence of biochar and compost. Therefore, considering the results collected in Montepaldi, it is important to optimise the protocol for using biochar (alone or in a mixture with compost) by considering the initial characteristics of the soil and adjusting agronomic practices according to the most important environmental parameters, such as rainfall and temperature.

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