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Low Concentrations of Biochar Improve Germination and Seedling Development in the Threatened Arable Weed *Centaurea cyanus*

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Abstract: In the context of sustainable agriculture, the search for soil improvers that boost crop growth without harming biodiversity is gaining much attention. Biochar, the solid residue resulting from the pyrolysis of organic material, has recently emerged as a promising bioproduct in enhancing crop yield, but there is a lack of information regarding its effects on arable biodiversity. Thus, in this study, we tested the effect of biochar application on the germination and seedling growth of cornflower (*Centaurea cyanus* L., Asteraceae), a threatened arable weed, under laboratory conditions. We investigated various parameters, including germination percentage (GP%), mean germination time (MGT), germination rate index (GRI), germination energy (GE%), fresh and dry weight (mg) of seedlings, and radicle length (mm) under biochar treatments at different concentrations: 0% (control), 0.1%, 0.2%, 0.5%, 1%, and 2%. Our findings revealed a significant increase in GP, GE, and GRI at biochar concentrations of 0.5% and 1%. MGT slightly increased at 0.1% biochar. Seedling fresh weight was unaffected by biochar application, whereas seedling dry weight exhibited a significant increase at 0.5% biochar. Radicle length showed a substantial increase under 0.1% biochar on day one, and was significantly higher at 0.2% and 1% biochar on day two. However, by day three, no more statistically significant differences in radicle length were observed between biochar-treated diaspores and controls (i.e., biochar had positive effects only in the first stages). These results suggest that the application of biochar at intermediate concentrations (0.5% and 1%) overall provides the most benefit to the germination and seedling growth of *C. cyanus*.

Keywords: arable plant; biodiversity; bio-based product; not-target plant; segetal plant; sustainable agriculture

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1. Introduction

The intensification of agricultural practices that followed the green revolution of the 1950s' caused great changes in the plant diversity of arable lands [1,2]. In Europe, many arable weed species that were once common and widespread began to disappear from agroecosystems, to the point that they are now very rare or locally extinct [3,4]. Intensive fertilization practices and broad herbicide application are among the main reasons for the decline of arable weed diversity. By increasing nutrient availability in the soil, fertilization favored generalist and highly competitive species over more specialized and rare arable weeds in agroecosystems, while herbicides tended to remove any wild species that could compete with the crop [4–8]. These practices resulted in significant shifts in arable plant communities, both in terms of a decrease in species richness and in terms of species turnover [9,10].

Despite their role as crop competitors, the ecological importance of arable weeds in agroecosystems is widely acknowledged, since they provide many ecosystem services [11,12].

There is evidence that species-rich arable weed communities with a high evenness can mitigate crop yield loss, as opposed to arable weed communities dominated by few competitive species [13]. Moreover, they are of high importance for the maintenance of pollinator networks in agricultural landscapes, with benefits for both the environment and agricultural productivity [14]. Based on such evidence, the need for their conservation became more and more relevant to plant scientists. Thus, the search for fertilizers that support crop growth without negatively affecting biodiversity is critical [15]. Biochar has been proposed as a promising, more sustainable fertilizer option [16–19], and it is currently approved as a soil improver in Italian organic agriculture [20].

Biochar is the solid fraction derived from the pyrolysis (i.e., heating in the absence of oxygen) of organic materials, mostly plant-derived, such as wood or crop residues [21]. The production process of biochar involves heating the biomass at relatively low temperatures, typically between 350 and 700 °C [22–25]. Since it is rich in carbon, biochar has a crucial environmental role when added to the soil, being able to store atmospheric carbon (sequestration) and improve physical soil properties like porosity and aeration, as well as chemical soil properties like cation-exchange capacity [26,27]. However, due to its chemical characteristics, and especially to its known Na release and high pH (>8), the recommended biochar dose to be added to agricultural soils should not exceed 15% by weight, or it could lead to undesirable effects on plant growth and development [28]. As regards its use to boost germination, lower concentrations are used, usually lower than 5% [29–31].

In recent years, the use of biochar in agriculture has been highly investigated, especially in relation to increasing crop yield and quality [32,33]. Moreover, it improves plant resistance to abiotic stress, such as salinity [34,35], drought [36,37], and hydrocarbon-contaminated soils [38,39]. While evidence suggests that biochar enhances growth and yield in cultivated plants [32,33], there is currently a lack of knowledge regarding its potential effects on arable weed diversity within agroecosystems. This topic could be relevant for biodiversity conservation in agroecosystems, since biochar, in addition to crops, may interact also with arable weeds, both in the germination phases and during seedling emergence and early growth stages, which are the main critical phases of a plant's life cycle [40,41]. So far, research on the influence of biochar on seed germination has focused primarily on forest [29,42,43] and crop plant species [30,31,44], with contrasting results, since these studies showed that the effects of biochar application have ranged from positive to negative, depending on the species tested.

Thus, understanding the effects of biochar addition on the plants spontaneously growing in arable fields (i.e., on non-target species) is crucial for a comprehensive exploration of its impact on agroecosystem biodiversity and the development of ecologically friendly crop-management practices. Germination is the initial and fundamental step in the growth of plants, setting the stage for their subsequent development and survival [45–47]. Despite the increasing interest in biochar as a sustainable agricultural amendment, there is a significant gap in the literature regarding its influence on the germination and early growth of non-target plant species in arable fields. To the best of our knowledge, this specific aspect has not been previously studied. This research represents the first effort to address this gap, offering novel insights into the ecological consequences of biochar application from the first stages of plant development, and contributing valuable knowledge to the global discourse on sustainable agriculture.

Cornflower (*Centaurea cyanus* L.; Asteraceae) is one of the most iconic and once widespread arable weeds in Europe [3]. It is very important for pollinators, producing both floral and extrafloral nectar [12]. Due to its high specialization to live among winter cereal crops, and to its vulnerability to intensive agricultural practices, it is an effective indicator of environmental quality in arable ecosystems [48–50]. Moreover, it is widely appreciated as an ornamental plant, and it is one of the most common components of seed mixtures in wildflower strips [51,52]. Despite its wide distribution, both in the wild and in cultivation, as many other strictly arable weeds, it is undergoing a steep decline in the agricultural landscapes of Europe due to modern agricultural practices, with chemical fertilization being one of the main reasons for its disappearance [4].

For the reasons described above, in this study, we used the threatened arable weed *C. cyanus* to assess the effects of biochar (0.1%, 0.2%, 0.5%, 1%, and 2%) application on the germination and seedling development of non-target plants in arable land. We hypothesized that the application of biochar would increase the seed germination and seedling early growth of *C. cyanus*, and that these positive effects would be stronger at the lowest concentrations of biochar.

2. Materials and Methods

2.1. Biochar

The biochar used in our experiment (BioDea®) was produced (by BioEsperia srl, Arezzo, Italy) through pyrolysis at temperatures ranging from 600 to 650 °C, using a mixture of agricultural woody residues, (i.e., *Castanea sativa* Mill., *Robinia pseudoacacia* L., *Fraxinus ornus* L., *Alnus glutinosa* (L.) Gaertn., and *Quercus robur* L.). Subsequently, the biochar was mechanically collected, resulting in a product with minimal ash content and a high concentration of organic carbon. Finally, the biochar was finely ground and put into an aqueous solution to allow for its application by fertigation in crop fields. The chemical characteristics of the used biochar are reported in Table 1.

Table 1. Physicochemical characteristics of the used biochar.

N (%)	<0.4
K (mg kg^{-1})	3020
P (mg kg^{-1})	340
Ca (mg kg^{-1})	9920
Mg (mg kg^{-1})	852
Na (mg kg^{-1})	291
C from carbonate (%)	<0.1
C (%)	68.7
WHC (%)	23.5
EC (mS cm^{-1})	110
pH	9.9
Hash content (%)	4.6
H/C	0.2

N: nitrogen; K: potassium; P: phosphorus; Ca: calcium; Mg: magnesium; Na: sodium; C: carbon; WHC: water holding capacity; EC: electrical conductivity.

2.2. Experimental Design

Achenes of *C. cyanus* harvested in spring 2023 were provided by the Agro-Botanical Garden of the University of Cluj-Napoca, Romania. The achenes were subjected to surface sterilization by immersing them in a 3% sodium hypochlorite (NaClO) solution for two minutes, followed by a thorough washing with deionized H_2O, as suggested by Maresca et al. [53]. Subsequently, a Whatman N1 filter (Whatman International, Maidstone, UK) was placed inside each Petri dish and then saturated with the treatment solutions. The experimental scheme of the study is described in Figure 1. Biochar was applied at five concentrations: 0% (control), 0.1%, 0.2%, 0.5%, 1%, and 2%. Five Petri dishes (statistical replicates) were prepared for each treatment, with 20 achenes placed in each dish, following a design usually adopted for germination tests under laboratory conditions [54–56]. The Petri dishes were placed in complete darkness inside a growth chamber at a constant temperature (18 ± 2 °C) and relative humidity (70%).

From the time of sowing until the end of the experiment (9 days after sowing), digital images of each Petri dish were acquired every 24 h with a digital scanner (Bookeye 5 V2, Image Access GmbH, Wuppertal, Germany), without altering the growing conditions of seedlings. Each digital image was used to record the following daily: (i) the number of germinated seeds, and (ii) their radicle length. To retrieve this information, each digital image was uploaded into the Fiji/ImageJ software (v. 1.54 h), following the method reported by Fedeli et al. [57,58]. We summed up radicle lengths in each Petri dish to obtain a daily cumulative radicle length. On the last day of the experiment (9 days after sowing), the fresh weight of each seedling was determined using a precision balance, after which the seedlings were placed in an oven for 48 h at 80 °C [59]. At the end of the indicated period, the dry weight of each seedling was determined through the same precision balance.

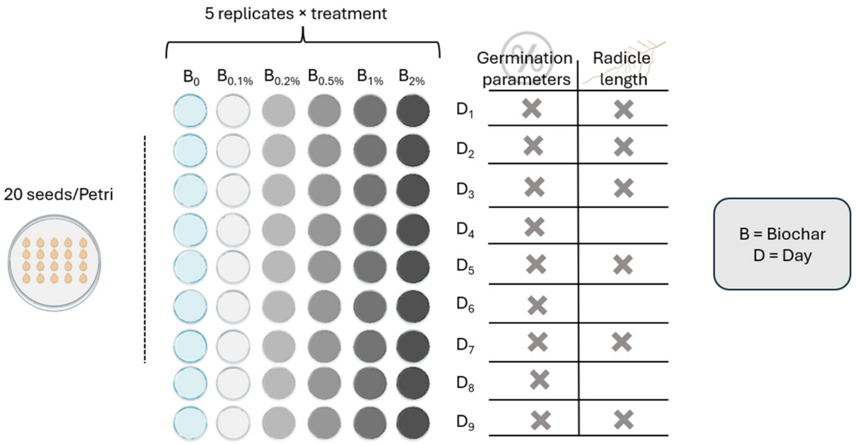


Figure 1. Experimental design (created with BioRender).

2.3. Germination Parameters

Based on the retrieved data, we calculated the following parameters related to germination performance:

1. Germination percentage (*GP*), calculated according to Czabator [60] (Equation (1)):

$$GP(\%) = \frac{Total\ germinated\ seeds}{Total\ number\ of\ tested\ seeds} \times 100 \tag{1}$$

2. Mean germination time (*MGT*), calculated according to Ellis and Roberts [61] (Equation (2)):

$$MGT = \frac{\sum_{i=1}^{j} n_i \times d_i}{N} \tag{2}$$

where n_i is the number of seeds germinated on the ith day, d_i is the ith number of days from the beginning of the test, N is the total number of seeds germinated at the end of the experiment, and j is the total number of days of the experiment.

3. Germination rate index (*GRI*), calculated according to Fowler [62] (Equation (3)):

$$GRI = \sum \frac{G_n}{D_n} \tag{3}$$

where G_n is the number of germinated seeds, and D_n is the number of days since the beginning of observations.

4. Germination energy (*GE*), calculated according to Czabator [60] (Equation (4)):

$$GE(\%) = \frac{Number\ of\ germinated\ seeds\ at\ 4\ DAS}{Total\ number\ of\ tested\ seeds} \times 100 \tag{4}$$

where *DAS* is the number of days after sowing.

2.4. Data Analysis

To test for significant effects of biochar application, observation day, and their interaction on the GP and radicle length of *C. cyanus* seedlings, we carried out a Permutational Univariate Analysis of Variance based on Euclidean distance matrices. The percentage data were log(x + 1) transformed, and the other values were square-root transformed to improve normality. Using the same analysis, we tested the effect of biochar on MGT, GRI, GE, and fresh and dry weight. The following settings were used for all tests: 999 unrestricted permutations of raw data; $\alpha = 0.05$. Significant terms were then investigated using post hoc pairwise comparisons, with the PERMANOVA t-statistic, and 999 permutations to test for significant differences between the treatments. We also made comparisons between days within biochar concentration. All the analyses were performed using the PERMANOVA routine in the program PRIMER v.6, including the add-on package PERMANOVA+. PERMANOVA accurately computes a suitable pseudo-F statistic for each term in the model, applicable to both multivariate and univariate datasets. Additionally, the permutation method avoids many of the assumptions inherent in parametric statistics [63,64].

3. Results and Discussion

Different biochar concentrations had significant, but contrasting, effects on some of the germination parameters, on dry weight, and radicle length. In particular, we highlighted the significant effects of biochar on GP, MGT, GRI, GE, dry weight, and radicle length, while fresh weight was not affected. We also highlighted a significant interaction between the day and biochar on GP (Table 2).

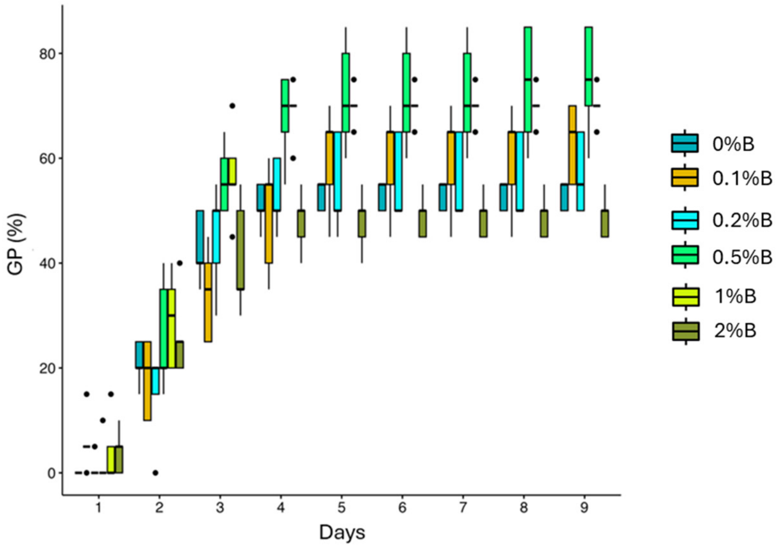
Table 2. PERMANOVA results showing the effect of biochar, day of observation, and their interaction on germination percentage (GP) and radicle length and the effect of biochar on mean germination time (MGT), germination rate index (GRI), germination energy (GE), fresh weight, and dry weight.

Source of variation		**GP**					**Radicle Length**	
	df	MS	F			*df*	MS	F
Day	8	6.96	224.94 **			5	0.01	317.17 ***
Biochar	5	0.19	6.23 ***			5	15,867	7.70 ***
Day × Biochar	40	0.05	1.79″			2.5	3206.3	1.56
Residual	216	0.03				144	2059.8	
Total	269					179		
Source of variation		**MGT**		**GRI**			**GE**	
	df	MS	F	MS	F		MS	F
Biochar	5	0.047	4.22 **	0.01	3.16 *		0.02	7.22 ***
Residual	24	0.01		0.01			0.01	
Total	29							
Source of variation		**Fresh weight**		**Dry weight**				
	df	MS	F	MS	F			
Biochar	5	0.01	0.34	0.01	3.41 *			
Residual	24	0.03		0.01				
Total	29							

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

3.1. Germination Parameters

Our results showed how the response of germination parameters varies depending on the dose of biochar applied. On the first day, there was a higher GP (mean = 6%) at 0.1% biochar. Starting as early as the third day after achene placement in Petri dishes, we observed an increase in the GP of seeds under biochar concentrations of 0.5% and 1%. At the end of the experiment, we highlighted an increase in GP of 56% at 0.5 biochar, and 57% at 1% biochar, compared to the control (Figure 2). Interestingly, at the highest biochar concentration (2%), the GP decreased back to the same levels of the control (mean = 42%), suggesting a hormetic effect of biochar, i.e., that, although beneficial at low doses, higher doses can have a toxic effect (Figure 2). In addition, biochar can contain and release contaminants that could have detrimental effects on seed germination and plant development [49]. The outcomes obtained for GP were consistent with those found for GE and GRI, for which we also observed a statistically significant increase at biochar concentrations of 0.5% (+33% and +39%, respectively) and 1% (+35% and 44%, respectively) (Figure 3). Concerning MGT, we only witnessed a statistically significant increase (+25%) in seeds grown under biochar treatment at 0.1%. These results, again, suggest a dose-dependent effect of biochar on the germination parameters of *C. cyanus*, highlighting that the 0.5% biochar has the most positive effects (Figure 3). This agrees with Solaiman et al. [65], who reported higher germination (GP) of wheat species (*Triticum* spp.) at a lower biochar concentration (0.125% vs. 0.25%). The effects of biochar on germination depend on dosage, species, and biochar type. Pyrolysis impacts particle size, porosity, and water retention, influencing seedling water and oxygen availability [66]. Biochar's chemical properties, like pH and nutrient concentration, are also crucial. For example, biochar pH can change soil pH, and nutrient levels affect plant growth [67,68].



	Day								
Biochar (%)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
0	a	a	a	a	a	a	a	a	a
0.1	b	a	a	a	ac	ab	ab	a	ab
0.2	ab	a	ab	a	ac	a	a	ac	ac
0.5	ab	a	b	a	bc	b	b	bc	b
1	ab	a	b	a	bc	b	b	bc	b
2	ab	a	ab	a	a	a	a	a	c

Figure 2. Germination percentage for each day (GP) of *C. cyanus* seeds. B = biochar. Different letters indicate statistically significant differences ($p < 0.05$) between the different biochar concentrations.

3.2. Fresh and Dry Weight

Our results did not show any statistically significant difference in seedling fresh weight (Figure 4). However, the dry weight of seedlings exhibited a statistically significant increase (+19%) for seeds grown under 0.5% biochar (Figure 4). This is consistent with previous evidence that biochar can directly influence plant growth in various ways, owing to its physico-chemical properties [21]. It slowly releases essential nutrients for plants, such as nitrogen, phosphorus, and potassium, which can be absorbed directly by roots [69]. Even in the absence of a growth substrate, biochar could help retain moisture around roots, creating a more favorable environment for growth. Additionally, biochar could influence root respiration, improving the efficiency of energy usage, thereby facilitating growth [70].

Although the different effects of biochar on fresh and dry weight may seem contradictory, fresh weight is a measure of the total amount of water contained in the roots, in addition to their biomass [71], so that fresh weight may not be related to dry weight. Conversely, dry weight represents the mass of seedlings after all water has been removed, providing a more accurate measure of the present amount of plant biomass [72,73].

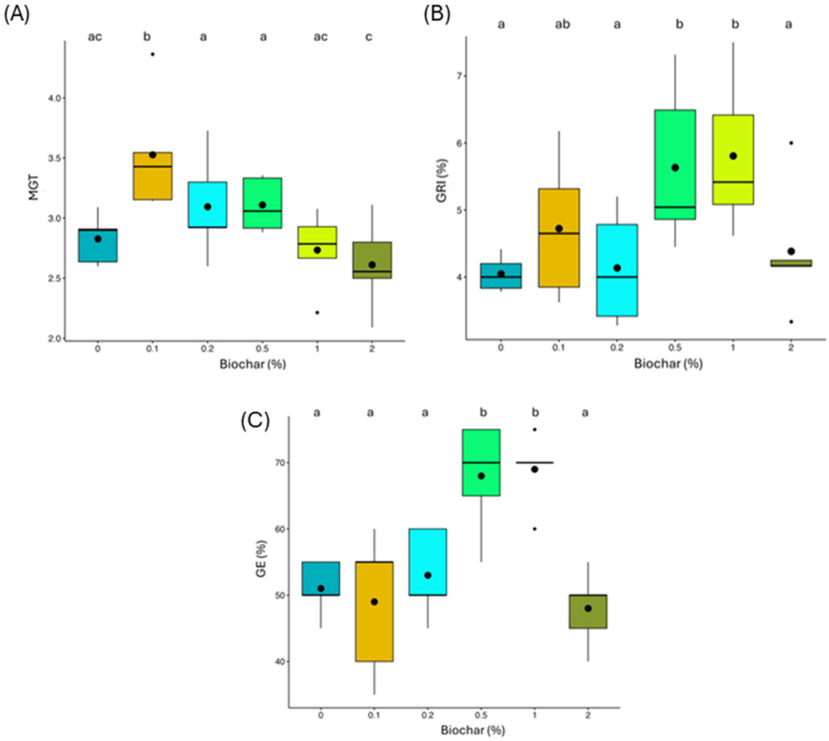


Figure 3. (**A**) Mean germination time (MGT), (**B**) germination rate index (GRI), (**C**) germination energy (GE) of *C. cyanus* seeds. Different letters indicate statistically significant differences ($p < 0.05$) between the different biochar concentrations.

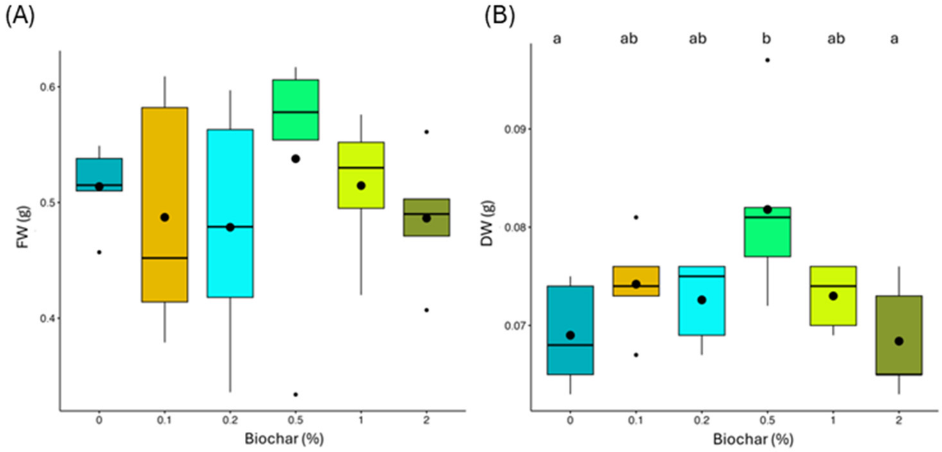
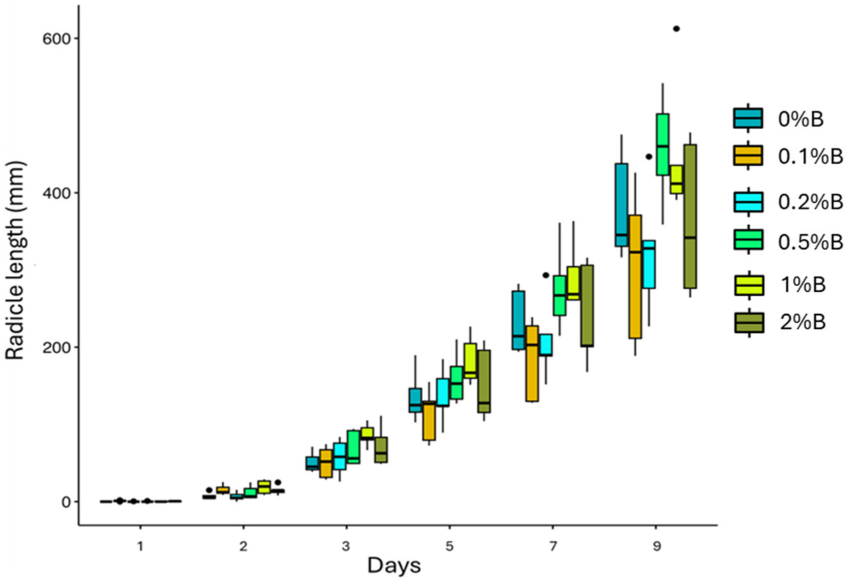


Figure 4. (**A**) Fresh weight (FW), (**B**) dry weight (DW) of *C. cyanus* seeds. Different letters indicate statistically significant differences ($p < 0.05$) between the different biochar concentrations.

3.3. Radicle Length

Radicle length showed statistically significant differences under different biochar concentrations only in the first days of germination. On the 1st day, we found a statistically significant increase (+740%) in radicle length for seeds grown at 0.1% biochar. On the 2nd and 3rd days, radicle length was significantly higher (+63% and +31%, respectively) under 1% biochar (Figure 5). Starting from the 5th day, no statistically significant difference in radicle length was observed anymore between the seeds grown under biochar and those of the controls, although the radicles grown under 0.5% biochar showed a higher, though not statistically significant, average value than those grown under all the other concentrations (Figure 5). Thus, biochar sped up radicle development in the first growth stages, but did not influence the final cumulative radicle length of seedlings. Previous findings from works that investigated the effects of the addition of different types of biochar at different concentrations (0.1% and 0.25%) on the germination and early developmental stages of wheat showed how four of the five tested types of biochar did not affect the final radicle length [67]. Other works highlighted that concentrations of biochar ranging within 0.1–0.3% increased the final radicle length of fodder crops [74]. These contrasting results are due to the different species used and the type of biochar applied. Different species react uniquely to biochar, influenced by their specific biological characteristics. Additionally, the properties of biochar, determined by its feedstock and production methods, significantly impact its effectiveness. Factors such as the particle size, porosity, water retention, pH, and nutrient concentration of biochar play crucial roles in these outcomes [68].



	Day					
Biochar (%)	1	2	3	5	7	9
0	a	ab	a	ab	ab	ab
0.1	b	ac	a	a	a	a
0.2	a	b	ab	ab	a	a
0.5	ab	abc	ab	ab	b	b
1	ab	c	b	b	b	b
2	ab	ac	ab	ab	ab	ab

Figure 5. Radicle length of *C. cyanus* seeds. B = biochar. Different letters indicate statistically significant differences ($p < 0.05$) between the different biochar concentrations.

4. Conclusions

In this work, we showed for the first time that biochar might have beneficial effects on the germination performance and seedling development of the threatened arable weed *C. cyanus*. In particular, we showed that all the tested concentrations of biochar, apart from the highest concentration (2%), have no toxic effects, and that the 0.5% concentration increases some germination parameters (i.e., the final GP, the GRI, and the GE) and the dry weight of the seedlings. Overall, by looking simultaneously at the GP and the radicle-length values of seedlings, higher values of GP were observed in the presence of 0.5% biochar, whereas radicle length did not differ from controls. Since biochar increased dry weight without affecting final radicle length, we might speculate that biochar could positively influence above-ground more than below-ground development in the early growth stages. From an ecological perspective, the use of biochar at low concentrations might be a useful practice to improve the biological performance of *C. cyanus*, both in the context of agro-environmental schemes applied to agroecosystems and in the cultivation of the species for ornamental purposes. Further studies are, however, necessary to evaluate the effects of biochar on *C. cyanus* germination in open-field conditions, where the concentrations of biochar used to promote crop growth are higher (2.5–5%) due to its dispersion in the soil and consequent attenuated effect. In this context, it will be relevant to assess the effects of biochar, considering also crop–weed and weed–weed competition and root/shoot development, and those of different types of biochar on different arable weed species.

Author Contributions: Conceptualization, R.F. and E.F.; methodology, R.F., L.d.S. and E.F.; investigation, R.F., E.F., T.F. and L.d.S.; resources, S.L., S.M. and C.A.; data curation, E.F., R.F. and S.M.; writing—original draft preparation, R.F. and E.F.; writing—review and editing, R.F., T.F., L.d.S., S.L., C.A., S.M. and E.F.; supervision, S.L., C.A. and S.M. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data that support the findings of this study are available from the authors on request.

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Conflicts of Interest: The authors declare no conflicts of interest.

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