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Effects of Straw and Biochar Amendments on Grassland Productivity and Root Morphology

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Abstract: The objective of this research was to determine the effect of straw and biochar amendment on the root system morphology and aboveground biomass of a red clover/grass mixture (*Lolium. perenne* L., *Phleum pratense* L., *Festuca pratensis* Huds., *F. arundinacea* Schreb., *L. multiflorum* L., *L. westerwoldicum* Breakw., *Trifolium pratense* L.). A grassland experiment was conducted from 2014 to 2018. Straw was collected from miscanthus (*Miscanthus × giganteus*), winter wheat (*Triticum aestivum* L.), and biochar was produced from the biomass of those species. The following treatments were applied: wheat straw at a rate of 5 t ha^{-1} (WS), miscanthus straw at a rate of 5 t ha^{-1} (MS), wheat biochar at a rate of 5 t ha^{-1} (WBH), wheat biochar at a rate of 2.25 t ha^{-1} (WBL), miscanthus biochar at a rate of 5 t ha^{-1} (MBH), and miscanthus biochar at a rate of 2.25 t ha^{-1} (MBL). A treatment with mineral fertilizer but without organic amendments (MCTR) was used, and a control treatment (CTR) without mineral fertilizer and without any amendments was also tested. The botanical composition and the aboveground yields were determined. The roots were sampled in 2018, and the root morphology parameters were determined using an image analysis system. The applied soil amendments resulted in increased root lengths, surface areas, volumes, and mean root diameters. There were no differences between the treatments with different feedstock types (miscanthus vs. wheat), materials (straw vs. biochar), or amendment rates (5 vs. 2.25 t ha^{-1}). The resulting root system characteristics were reflected in the aboveground biomass productivity. The soil amendments, i.e., the straw and biochar, significantly increased the productivity in comparison to that of the control treatment. However, these differences were noticed only during the first and second cuts. Recommended practice in grassland management is to improve soil with straw. The conversion of straw into biochar does not provide a better effect on grassland productivity.

Keywords: wheat; miscanthus; straw; biochar; grassland; yields; roots; botanical composition

1. Introduction

Organic amendment is a well-known and common practice for providing nutrients to crops and conserving soil and water. The use of straw as an organic amendment is widely recommended in various conservation tillage systems [1]. Straw is usually used as a mulch after crop harvesting because of its beneficial effects on the soil physical, chemical, and biological properties. It reduces the soil bulk density and improves the soil structure and soil pore system [2]. The incorporation of straw into the soil increases the soil organic carbon (SOC) storage and thus mitigates climate change [3]. According to Zhang et al. [4], straw amendment increases the available nitrogen and phosphorus content in the upper soil layers and enhances the urease, phosphatase, and invertase activity levels in the lower soil layers. The incorporation of straw into soil increases crop biomass production [5]. Xu et al. [1] reported that straw return increased the crop yield stability in wheat-maize systems. According to Getahun et al. [6], the incorporation of straw residues into soil had beneficial effects on root development and thus affected aboveground biomass production.

On the other hand, the application of straw can lead to negative effects on the soil environment and crop yields. The decomposition of straw with a high C:N ratio in soil results in microbial N immobilization and a temporary decrease in the crop-available N and thus can reduce crop growth [3,7]. Since this phenomenon appears under typical soil conditions in arable land, straw application should be combined with N fertilizer to increase crop yield and improve soil fertility [8]. Reducing soil N levels also inhibits the establishment of non-native, invasive species with only little effect on the native grasses [9,10].

The solution to the problem of decreasing plant-available N is to convert straw into biochar through pyrolysis. During the past decade, biochar has been recognized as a very promising soil amendment that results in soil improvement and carbon sequestration that may mitigate climate change [11,12]. The application of biochar increases crop production through different mechanisms, i.e., providing a liming effect, increasing the soil water and nutrient retention and reducing N leaching [13–15]. The positive effect of biochar amendment on root growth was confirmed by Rafique et al. [16] in research with maize. This effect can be increased by P fertilizer. However, the effects of biochar application on crop production are sometimes contradictory. The results depend on the feedstock used for biochar production, the parameters of the pyrolysis process, and soil and the climate conditions [17].

Moreover, there is little information on the long-term impacts of straw and biochar on perennial plants in soil under natural environmental conditions. Most studies examining the effects of biochar on plants have been performed on annual crops in agricultural systems. Relatively little is known about how biochar affects plant competition in grasslands. The grasslands in Europe are the main source of forage for ruminant production systems [18]. The botanical composition of grassland is one of the most critical parameters for forage quality and productivity. Grasslands are regarded as diverse and species-rich ecosystems and are usually dominated by perennial ryegrass (*Lolium perenne* L.) in West and Central Europe according to Habel et al. [19].

Mineral or organic fertilizer affects the grassland botanical composition. The tall grasses in grassland communities become more dominant under increased levels of N fertilizer. The results also showed a decrease in the number of species with increasing N fertilizer for forbs and especially legumes [20,21]. The most important nutrient responsible for biomass productivity is N. Even so, the use of N fertilizer to increase dry matter yield inhibits the diversity and results in changes in the botanical composition of meadow sward [22,23]. The relationship between species richness and nutrient supply has been widely investigated [24,25]. Lee and Lee [26] stated that fertilizer with N stimulates grass growth but depresses the growth of legumes. The effects of biochar on the growth of plant species can be explained by changes in the soil physical and chemical properties, such as increased soil pH, increased soil nutrient availability, or altered soil water retention [14,27,28]. These soil features affect the outcomes of competition between plant species and thus plant community composition. Legumes, in particular, have been found to respond strongly to the addition of biochar to soils [14]. Some reports have suggested that biochar can increase the competitive ability of legumes in grassland communities [15]. This effect has been ascribed to N immobilization by the microbial community and the stimulation of biological N fixation [29]. Biochar amendment was shown to significantly increase *L. perenne* yields and shift the plant community from grasses to herbs [30]. The stimulation of these changes was explained by higher SOC contents and increased water retention. It has been recognized that biochar amendment changes plant community composition through two mechanisms: (i) affecting the seed germination process and plant establishment and (ii) affecting the growth and development of the plant species and the functional groups of plants [27]. According to Beltman et al. (2007) [31], the species richness was negatively correlated with aboveground biomass. The interactions between the aboveground and belowground plant biomass present high potential for integrating knowledge of the agriculture practices, soil environment, and plant response [32].

We hypothesized that straw amendment improves aboveground and belowground productivity of the red clover/grass mixture, but biochar as a soil amendment results in a greater effect. The objectives of this study were to (i) determine the effect of straw and biochar amendment on the yields of a red clover/grass mixture and on the root morphology and (ii) determine the effects of the carbon amendments on the botanical composition of the grassland plant communities.

2. Materials and Methods

2.1. Organic Amendments

Organic amendments were added to the soil in 2014. The biochar was produced from the biomass of the following two species: miscanthus (*Miscanthus* × *giganteus*) and winter wheat (*Triticum aestivum* L.). The miscanthus and winter wheat straw were air-dried at 70 °C, ground into fine particles (<4 mm), and mixed to ensure homogeneity. The plant material was pyrolyzed in an electrical laboratory furnace equipped with a temperature controller at a temperature of 300 °C for 15 min; with limited air access to reduce C losses [33,34]. The furnace heating occurred at a rate of 10 °C min^{-1}. The time and temperature were set according to the research of Lu et al. [35], Mendez et al. [36], Gondek et al. [37] and Domene et al. [38]. The basic chemical characteristics of the feedstocks are presented in Table 1.

Table 1. Chemical properties of organic amendments used in field experiments with red clover/grass mixture (means ± standard deviation).

Parameter	Unit	*Triticum Aestivum* L.		*Miscanthus* × *Giganteus*	
		Straw	**Biochar**	**Straw**	**Biochar**
pH (H_2O)		5.84 ± 0.15	6.52 ± 0.60	6.18 ± 0.43	6.28 ± 0.42
EC *	mS·cm^{-1}	4.48 ± 0.21	378 ± 21	3.23 ± 0.45	345 ± 18
DM	g·kg^{-1}	952 ± 0.2	966 ± 2	947 ± 0.3	977 ± 1
Ash	g·kg^{-1} DM	59 ± 2	134 ± 5	54 ± 1	87 ± 3
$C_{org.}$	g·kg^{-1} DM	441 ± 2	628 ± 2	456 ± 2	651 ± 6
N_{total}	g·kg^{-1} DM	7.16 ± 0.32	12.4 ± 0.36	3.97 ± 0.29	7.31 ± 0.09
K	g·kg^{-1} DM	4.95 ± 0.66	11.9 ± 0.29	1.33 ± 0.06	2.81 ± 0.17
P	g·kg^{-1} DM	1.04 ± 0.05	1.17 ± 0.04	0.73 ± 0.04	0.94 ± 0.06

* EC-electrical conductivity; DM-dry matter.

2.2. Experimental Design and Treatments

A field experiment was conducted at the experimental station of the Department of Agricultural and Environmental Chemistry, University of Agriculture in Krakow (50°04′ N, 19°51′ E, 280 m a.s.l.) in the period of 2014–2018. The climate of the experimental site, which is situated in southern Poland, is considered temperate. The average annual temperature during the study period was 9.7 °C, and the average annual precipitation was 639 mm (Figure 1). The soil in the experimental field was a Eutric Cambisol (loamy sand) [39]. Table 2 lists the soil characteristics based on the samples collected in 2014 before the start of the trial.

The straw and biochar were applied on March 2014. The soil amendments were mixed with the soil using a rotary harrow at a depth of 10 cm. After the amendment application, the seeds of the red clover/grass mixture were sown at a seeding rate of 60 kg ha^{-1}; the mixture included perennial ryegrass (*L. perenne* L.) cv. Victorian (with a 20% rate in mixture) and cv. Solen (20%), timothy (*Phleum pratense* L.) cv. Erecta (15%), meadow fescue (*Festuca pratensis* Huds.) cv. Ardeinia (10%), tall fescue (*F. arundinacea* Schreb.) cv. Alix (10%), Italian ryegrass (*L. multiflorum* L.) cv. Gaza (10%), Westerwolds ryegrass (*L. westerwoldicum* Breakw.) cv. Mowester (10%), and red clover (*Trifolium pratense* L.) cv. Dajana (5%).

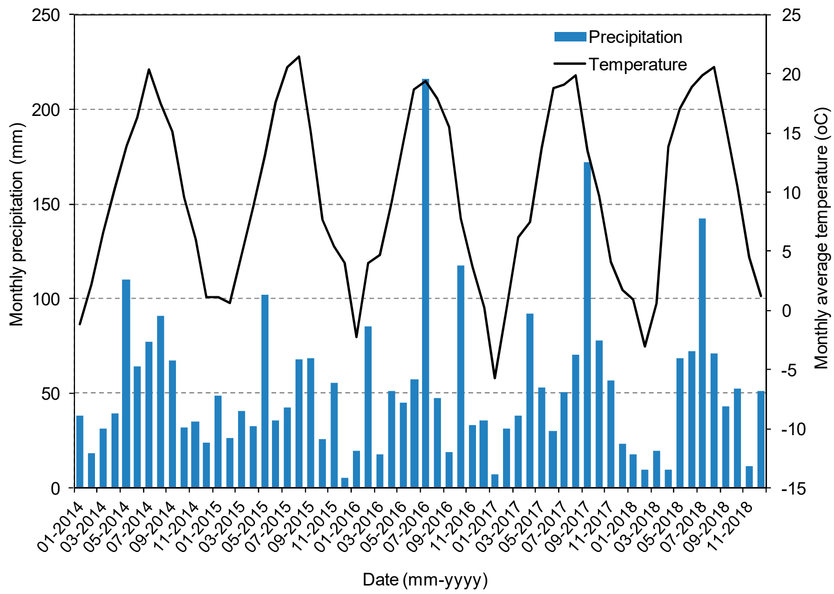

Figure 1. Temporal distribution of monthly precipitation and temperature of the trial location, Mydlniki near Krakow, Poland (50°04′ N, 19°51′ E, 280 m a.s.l) from 2014 to 2018.

Table 2. Basic soil physical and chemical properties of Eutric Cambisol from the trial location, 0–20 cm soil layer (means ± standard deviation).

Parameter	Unit	Value
pH (H_2O)		6.46 ± 0.02
pH (KCl)		5.59 ± 0.01
$C_{org.}$	g kg^{-1}	8.87 ± 0.07
N_{total}	g kg^{-1}	0.98 ± 0.01
C:N		9.1
P	g kg^{-1}	2.09 ± 0.02
K	g kg^{-1}	1.66 ± 0.01
Ca	g kg^{-1}	1.38 ± 0.01
Mg	g kg^{-1}	0.81 ± 0.01
EC	mS cm^{-1}	0.02 ± 0.00
Solid particle density	g cm^{-3}	2.65 ± 0.06
Sand	g kg^{-1}	730
Silt	g kg^{-1}	150
Clay	g kg^{-1}	120

The field experiment consisted of 24 plots areas of 1 m^2. Technical strips, 1 m wide, between plots were arranged to avoid cross-contamination between treatments. The experiment was established as a randomized block design with three replications. The following treatments were applied: wheat straw at a rate of 5 t ha^{-1} (WS), miscanthus straw at a rate of 5 t ha^{-1} (MS), wheat biochar at a rate of 5 t ha^{-1} (WBH), wheat biochar at a rate of 2.25 t ha^{-1} (WBL), miscanthus biochar at a rate of 5 t ha^{-1} (MBH), and miscanthus biochar at a rate of 2.25 t ha^{-1} (MBL). A treatment with mineral fertilizer but without organic amendments (MCTR) was used, and a control treatment (CTR) without mineral fertilizer and without any amendments was also tested. The mineral fertilizer was applied every year at rates of 100 kg N ha^{-1}, 40 kg P ha^{-1} and 120 kg K ha^{-1}.

2.3. Yield and Plant Species Composition

The botanical composition was determined in 2018 with the use of a quadrate frame that was subdivided into smaller areas [40]. The frame, which had a size of 0.25 m^2 (0.5 × 0.5 m) and a grid of 0.1 × 0.1 m, was randomly positioned within the plot. In each quadrate, the species were recorded, and the results were expressed as the percentage frequency.

The aboveground biomass was harvested three times per year in May, June, and August in the period from 2014 to 2018. The dry matter (DM) of the yield was determined by harvesting the plants from the whole surface of the plot and drying them at 70 °C to a constant weight.

2.4. Root Measurements

The roots were sampled in 2018 using the soil core method [41]. Four samples per plot were collected using a 50 mm diameter core. The samples were collected from four soil layers: 0–5, 5–10, 10–15, and 15–20 cm. The roots were washed using a hydropneumatic elutriation system [42] to remove mineral particles from the samples. Organic contamination was removed manually, and digital images were obtained with an Epson Perfection 4870 photo scanner (Seico Epson Corp., Tokyo, Japan) at a resolution of 1200 dpi. The images were analyzed using Aphelion Dev image analysis software (ADCIS S.A. and Amerinex Applied Imaging, Saint-Contest, France), and the root characteristics were then determined. The procedure for the image analysis was described by Bauhus and Messier [43] and comprised four main steps: filtering, segmentation, preparation of root skeletons, and morphometric measurements. The obtained root lengths were divided into eight diameter classes (<0.02, 0.02–0.05, 0.05–0.1, 0.1–0.2, 0.2–0.5, 0.5–1.0, 1.0–2.0, and >2.0 mm), and the root morphometric parameters were calculated. The root length density (RLD) was calculated by dividing the total root length by the volume of the soil sample. The mean root diameter (MRD) was calculated as the weighted mean of the root length with the particular diameter classes as the weights. The root surface area (RSA) and root volume density (RVD) were calculated as the product of the root segment lengths and their diameters. After scanning, the roots were dried at a temperature of 70 °C to determine the root dry matter density (RDMD). Then, the specific root length (SRL) was calculated as a ratio of the RLD to the RDMD. Based on the RDMD and the DM of the aboveground yields, the root:shoot ratio (RSR) was determined.

2.5. Statistics

An ANOVA was performed on the randomized block design using the statistical software package Statistica v. 13.3 (StatSoft Inc., Tulsa, OK, USA) to evaluate the significance of the different organic amendments and the soil depth of the root morphometric parameters and plant yields (Table 3). The data were checked for the normality of the distribution using the Shapiro-Wilk test. The homogeneity of the variance was checked using Levene's test. Multiple comparisons were performed using Tukey's HSD test at the $p < 0.05$ level of significance. Pearson's correlation analysis was used to analyze the correlation between the root morphological parameters, and the aboveground biomass production. A regression analysis was carried out to describe the relationship between the root morphological parameters and aboveground biomass.

Table 3. Analysis of variance (ANOVA) for root morphometric parameters.

Factor	df	RLD		RDMD		RVD		MRD		SRL		RSA		RSR	
		F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Amendment	1	2.346	**0.026**	1.005	0.429	1.657	0.123	3.00	**0.005**	1.898	**0.073**	2.428	**0.021**	1.452	0.215
Depth	1	80.510	**<0.001**	45.665	**<0.001**	28.221	**<0.001**	1.86	0.138	2.720	**0.046**	73.593	**<0.001**	75.658	<0.001
A × D	1	1.058	0.398	0.644	0.879	0.825	0.685	0.34	0.996	0.360	0.995	1.073	0.382	0.654	0.958

RLD—root length density; RDMD—root dry matter density; RVD—root volume density; MRD—mean root diameter; SRL—specific root length; RSA—root surface area; RSR—root shoot ratio. *p* values in bold indicate statistical significance.

3. Results

3.1. Red Clover/Grass Mixture Yields

The highest aboveground productivity of the red clover/ryegrass was obtained for the first cut (4.58 t DM ha^{-1} on average in the period from 2014 to 2018); in comparison, the second and the third cuts yielded 2.98 and 1.49 t DM ha^{-1}, respectively (Table 4).

Table 4. Effect of different organic amendments treatments on red clover/mixture aboveground biomass production (t DM ha^{-1}) and root:shoot ratio. Means ± standard deviation of 2014–2018 period.

Treatment	1st cut	2nd cut	3rd cut	Total
CTR	3.69 ± 0.86 b	2.28 ± 0.65 c	1.45 ± 0.45 a	7.42 ± 1.21 c
MCTR	4.20 ± 0.90 ab	2.66 ± 0.52 bc	1.39 ± 0.43 a	8.25 ± 1.26 bc
MS	4.85 ± 0.95 a	3.38 ± 0.65 ab	1.53 ± 0.48 a	9.77 ± 1.12 ab
WS	4.91 ± 0.89 a	3.48 ± 0.64 a	1.46 ± 0.46 a	9.85 ± 1.32 a
MBH	5.18 ± 0.98 a	3.24 ± 0.068 ab	1.62 ± 0.30 a	10.03 ± 1.31 a
MBL	4.50 ± 0.87 a	2.95 ± 0.071 ab	1.43 ± 0.38 a	8.89 ± 1.22 ab
WBH	4.81 ± 0.81 a	3.19 ± 0.069 ab	1.57 ± 0.42 a	9.57 ± 1.23 ab
WBL	4.53 ± 0.92 a	2.62 ± 0.63 bc	1.48 ± 0.39 a	8.64 ± 1.09 abc

For each column, mean values with different letters are significantly different ($p < 0.05$). CTR—control; MCTR—control with mineral fertilization; MS—miscanthus straw; WS—wheat straw; MBH—miscanthus biochar, high rate; MBL—miscanthus biochar, low rate; WBH—wheat biochar, high rate; WBL—wheat biochar, low rate.

The soil amendments, i.e., straw and biochar, significantly increased the productivity in comparison to that of the CTR treatment. Even so, these differences were noticed only during the first and second cut. For the first cut, a significant difference was observed only between the CTR and any other treatment, i.e., the MCTR and the soil with straw and biochar amendments. The plant yield of the CTR was 3.69 t DM ha^{-1}, whereas for other treatments, the yields were 4.71 t DM ha^{-1} on average. During the second cut, the CTR also resulted in the lowest yield (2.28 t DM ha^{-1}), but yield at the MCTR (2.66 t DM ha^{-1}) was at the same level. When comparing the productivity of the plants from the soils with straw or biochar amendments, the highest yield was observed for the WS treatment (3.48 t DM ha^{-1}). A significantly lower yield was obtained from the WBL treatment (2.62 t DM ha^{-1}) with wheat biochar amendment at the rate of 2.25 t ha^{-1}. During the third cut, there were no significant differences between any treatments, and the yield was 1.56 t DM ha^{-1} on average. The differences in the yields obtained during particular cuts also resulted in significant differences between the treatments for the total annual yields. The low values were characterized for CTR (7.42 t DM ha^{-1}) and MCTR (8.25 t DM ha^{-1}). All treatments amended with straw and biochar produced higher yield, at 9.37 t DM ha^{-1} on average.

3.2. Root System Morphology Characteristics

Most of the root morphometric parameters were strongly determined by the soil depth (Figure 2). The highest root biomass was characterized for the upper soil layer (0 5 cm), representing 54% of RDMD for the whole investigated soil layer.

The RDMD percentages for the deeper soil layers, 5 10, 10–15, and 15–20 cm, were 19, 14, and 13%, respectively. Similar proportions were calculated for the RLD parameters, at 57, 19, 12, and 12%, respectively. The most numerous RLD diameter fractions were 0.002–0.005 mm, with 43% of the total RLD value (Figure 3). The root diameter fraction in the range of 0.1–0.2 mm was also characterized by a high share (20.1%) of the root biomass. The effect of the different soil amendment applications on the RLD values was observed for all diameter fractions in equal proportions.

The MRD was not significantly affected by the soil depth. The MRD in the particular soil layers varied from 0.0770 to 0.0837 mm. The treatments used changed some of the root morphometric parameters, i.e., the RLD, MRD, RSA, and RSR (Table 5).

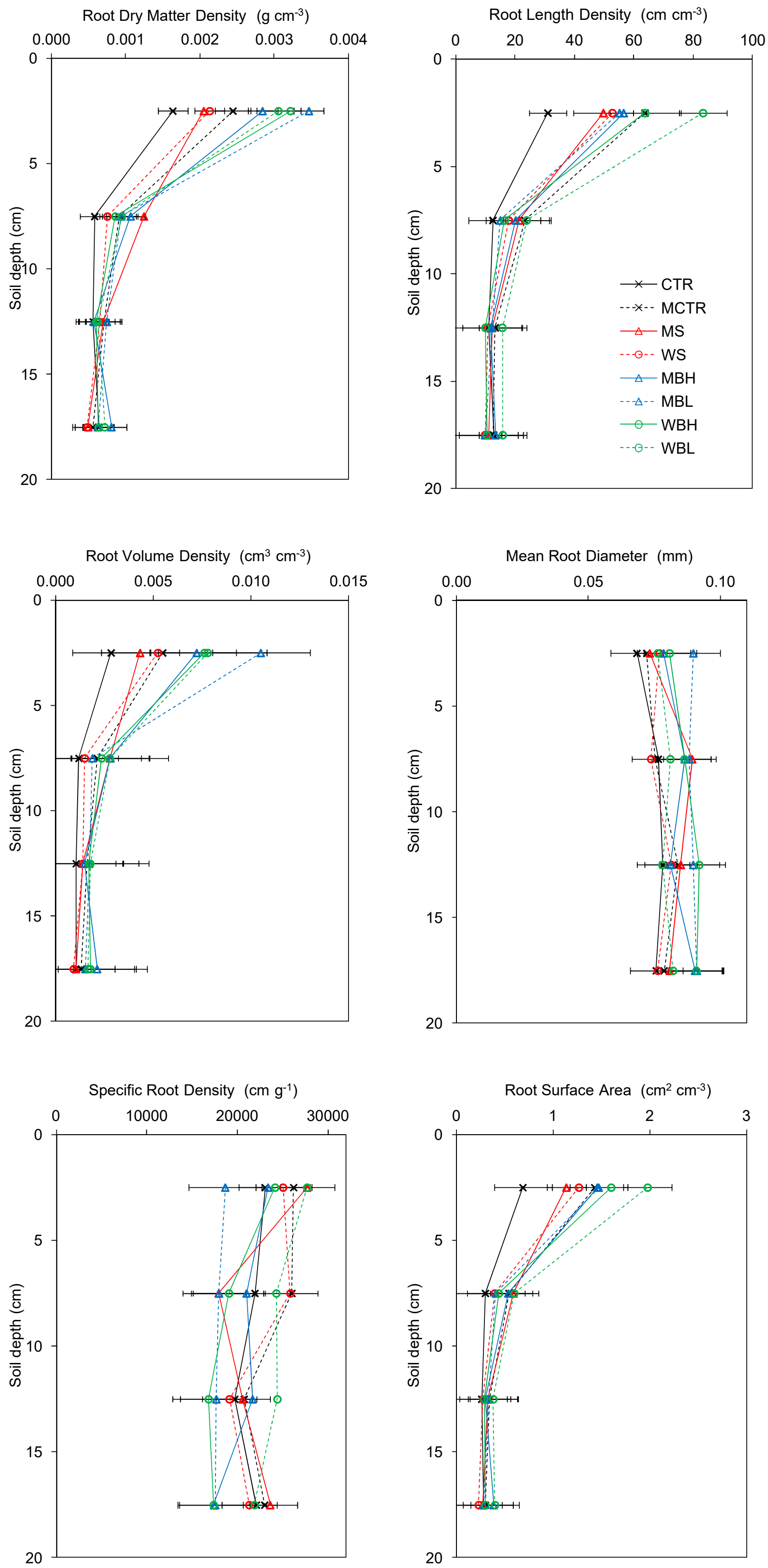

Figure 2. Root morphological parameters for red clover/grass mixture on soil with straw and biochar amendments. CTR: control; MCTR: control with mineral fertilization; MS: miscanthus straw; WS: wheat straw; MBH: miscanthus biochar, high rate; MBL: miscanthus biochar, low rate; WBH: wheat biochar, high rate; WBL: wheat biochar, low rate.

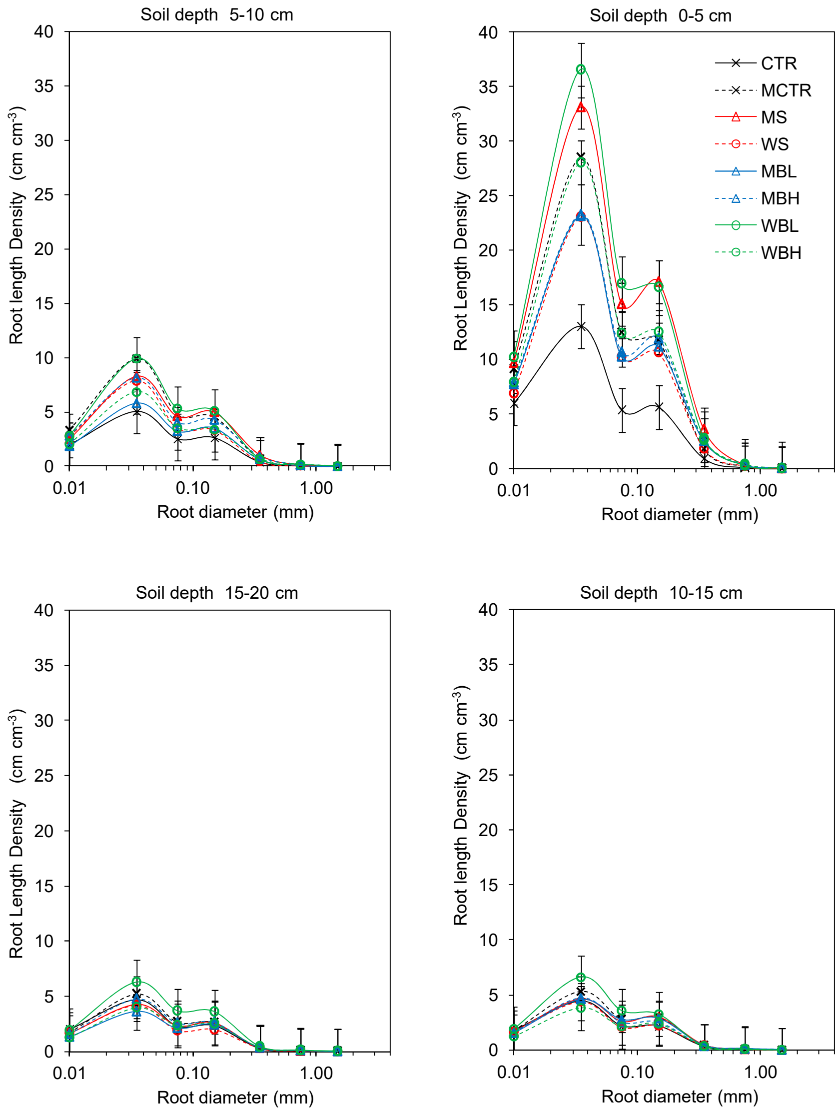

Figure 3. Root length density (RLD) distribution at the different root diameter values for red clover/grass mixture on soil with straw and biochar amendments. CTR: control; MCTR: control with mineral fertilization; MS: miscanthus straw; WS: wheat straw; MBH: miscanthus biochar, high rate; MBL: miscanthus biochar, low rate; WBH: wheat biochar, high rate; WBL: wheat biochar, low rate.

Table 5. Root morphometric characteristics. Mean ± standard deviation values for 0–20 cm soil layer.

Treatments	RLD (cm cm^{-3})	RDMD (mg cm^{-3})	RVD (cm^3 cm^{-3})	RSA (cm^2 cm^{-3})	MRD (mm)	SRL (cm g^{-1})	RSR
CTR	16.9 ± 2.12 c	0.00086 ± 0.00015 a	0.00154 ± 0.00020 a	0.382 ± 0.056 c	0.0747 ± 0.0084 c	21688 ± 3531 a	0.691 ± 0.089 c
MCTR	28.3 ± 2.32 ab	0.00116 ± 0.00010 a	0.00264 ± 0.00021 a	0.653 ± 0.056 ab	0.0775 ± 0.0098 c	23985 ± 3689 a	0.842 ± 0.096 ab
MS	23.7 ± 2.06 bc	0.00113 ± 0.00008 a	0.00239 ± 0.00023 a	0.579 ± 0.059 bc	0.0821 ± 0.0087 abc	22440 ± 3265 a	0.691 ± 0.078 cb
WS	22.9 ± 2.21 bc	0.00101 ± 0.00009 a	0.00227 ± 0.00029 a	0.542 ± 0.061 bc	0.0772 ± 0.0085 c	22819 ± 2298 a	0.612 ± 0.081 c
MBH	25.5 ± 2.16 abc	0.00132 ± 0.00011 a	0.00341 ± 0.00032 a	0.668 ± 0.071 ab	0.0828 ± 0.0094 abc	20861 ± 2873 a	0.792 ± 0.086 ab
MBL	23.1 ± 2.35 bc	0.00145 ± 0.00014 a	0.00391 ± 0.00035 a	0.622 ± 0.0.059 abc	0.0904 ± 0.010 a	17955 ± 2951 a	0.976 ± 0.098 a
WBH	25.3 ± 2.41 bc	0.00134 ± 0.00012 a	0.00351 ± 0.00033 a	0.656 ± 0.064 ab	0.0883 ± 0.0099 ab	24566 ± 3941 a	0.835 ± 0.084 ab
WBL	34.7 ± 2.98 a	0.00133 ± 0.00011 a	0.00338 ± 0.00028 a	0.838 ± 0.059 a	0.0800 ± 0.0079 bc	19352 ± 3245 a	0.930 ± 0.086 a

For each column, mean values with different letters are significantly different ($p < 0.05$). RLD: root length density; RDMD: root dry matter density; RVD: root volume density; MRD: mean root diameter; SRL: specific root length; RSA: root surface area; RSR: root shoot ratio.

The RDMD, RVD, and SRL were not significantly different for any treatments used in this trial. The CTR was characterized by the lowest RLD value (16.9 cm cm^{-3}). Straw and biochar application resulted in higher RLDs, with the highest value for WBL (34.7 cm cm^{-3}). A similar relationship was observed for the RSA. The lowest value was calculated for the CTR (0.382 cm^2 cm^{-3}) and the highest for WBL (0.838 cm^2 cm^{-3}). Organic amendment resulted in higher MRDs. The CTR and MCTR were characterized by low MRDs below 0.08 mm, but MRD was at the same level as WS. The MRD increased when straw or biochar was applied, with the highest root diameter (0.0904 mm) with MBL. Whether there was a relationship between the root morphometric parameters and the aboveground shoot biomass was assessed. Only the MRD was correlated with plant yield (Table 6). However, Pearson's correlation coefficient was significant only for the 1st cut (Figure 4).

Table 6. Correlation coefficients from Pearson's correlation analysis between root morphological parameters and aboveground biomass production of red clover/grass mixture.

	RLD	RDMD	MRD	RVD	RSA	SRL
1st cut	−0.3109	0.1762	0.5238 *	0.1715	−0.1349	−0.0473
2nd cut	−0.1308	−0.1480	0.1694	0.0254	−0.0918	0.0193
3rd cut	−0.1879	−0.2201	0.0420	−0.0827	−0.1815	0.0332

* Significance at $p < 0.05$. RLD: root length density; RDMD: root dry matter density; MRD: mean root diameter; RVD: root volume density; RSA: root surface area; SRL: specific root length.

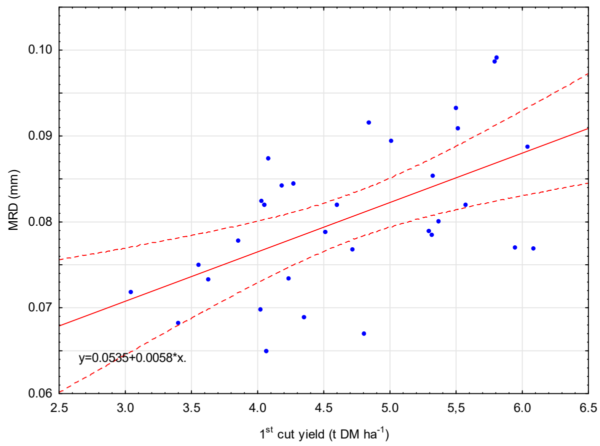

Figure 4. Relationship between mean root diameter (MRD) and biomass productivity of red clover/grass mixture during 1st cut. Solid line is the fitted linear regression, dotted lines represent 0.95 confidence interval.

The RSR was found to be a more sensitive root morphometric parameter. The RSR was affected not only by the soil depth but also by the treatments used in this trial. Low values of the RSR, which were below 0.7, were observed for the CTR, MS, and WS treatments. When biochar was applied, the RSR increased to values above 0.7. The RSR was at a similar level with the MCTR treatment (0.842). A higher RSR was obtained for the treatments with biochar applied at a higher rate (5 t ha^{-1}) than for treatments with biochar applied at a lower rate (2.25 t ha^{-1}).

The differences for most of the root morphometric parameters were noticed mainly between the CTR, without any amendments, and other treatments where straw or biochar produced from miscanthus or wheat were applied. However, there were no differences between the feedstock type (miscanthus vs. wheat), materials (straw vs. biochar) or amendment rates (5 vs. 2.25 t ha^{-1}).

3.3. Botanical Composition

The results of the botanical composition of the investigated grassland community are presented in Table 7. The percentage of empty space was similar among all treatments and ranged from 4.3 to 8.9%. The botanical composition was totally changed during the five years of the experiment.

Table 7. Botanical composition (% ± standard deviation) of the red clover/grass mixture five years after straw and biochar amendment.

Treatment	CTR	MCTR	WS	MS	WBH	MBH	WBL	MBL
Empty space	6.4 ± 1.2	5.9 ± 1.6	8.9 ± 1.9	6.0 ± 1.4	4.3 ± 1.8	5.8 ± 1.6	7.3 ± 1.9	4.8 ± 1.9
Grasses								
Festuca arundinacea Schreb.	53.0 ± 1.5	46.1 ± 1.6	50.0 ± 1.2	66.1 ± 1.5	65.6 ± 1.5	58.6 ± 1.8	58.4 ± 1.9	61.0 ± 1.6
Dactylis glomerata L.	0.0 ± 0.0	6.3 ± 1.4	14.0 ± 1.6	3.3 ± 1.8	2.3 ± 1.4	1.8 ± 1.4	9.5 ± 1.6	2.2 ± 1.4
Lolium multiflorum Lam.	0.0 ± 0.0	17.1 ± 1.6	9.1 ± 1.1	8.4 ± 1.6	1.5 ± 1.2	0.0 ± 0.0	1.9 ± 1.4	1.8 ± 0.8
Holcus lanatus L.	1.3 ± 1.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Legumes								
Trifolium pratense L.	8.4 ± 1.3	0.0 ± 0.0	0.0 ± 0.0	1.6 ± 1.3	1.9 ± 1.4	0.0 ± 0.0	3.1 ± 1.7	0.0 ± 0.0
Forbs								
Stellaria graminea L.	29.21.4	18.4 ± 1.8	15.5 ± 0.8	12.1 ± 1.1	22.8 ± 1.6	30.8 ± 1.4	17.4 ± 1.5	23.4 ± 1.1
Rumex acetosa L.	1.2 ± 1.8	3.2 ± 1.7	0.0 ± 0.0	1.0 ± 0.2	0.0 ± 0.0	1.0 ± 0.8	0.0 ± 0.0	1.3 ± 0.8
Capsella bursa-pastoris (L.) Medik.	0.6 ± 1.1	0.0 ± 0.0	0.6 ± 1.1	0.0 ± 0.0	0.4 ± 1.1	1.0 ± 0.9	0.0 ± 0.0	1.0 ± 0.8
Geranium pusillum L.	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 1.2	0.0 ± 0.0	1.3 ± 1.3	0.4 ± 1.1	0.0 ± 0.0	2.6 ± 0.9
Melandrium pratense Röhl.	0.0 ± 0.0	1.3 ± 1.4	0.0 ± 0.0	1.6 ± 1.4	0.0 ± 0.0	0.4 ± 1.1	2.4 ± 1.5	0.0 ± 0.0
Ranunculus acris L.	0.0 ± 0.0	1.7 ± 1.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Senecio vulgaris L.	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 1.1	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 1.1	0.0 ± 0.0	1.8 ± 1.4

CTR: control; MCTR: control with mineral fertilization; MS: miscanthus straw; WS: wheat straw; MBH: miscanthus biochar, high rate; MBL: miscanthus biochar, low rate; WBH: wheat biochar, high rate; WBL: wheat biochar, low rate.

L. multiflorum Lam. showed the highest participation (17%) in the sward on the MCTR plot and lower on the WS and MS plots, at 9.1 and 8.4%, respectively. The application of biochar reduced the prevalence of *L. multiflorum* Lam. to below 2%. The soil amendments decreased red clover percentage. The highest red clover content was observed on the CTR (8.4%). Any amendments applied resulted in definitely lower red clover participation and did not exceed 3.1%. Some new grass species appeared, i.e., *Dactylis glomerata* L. and *Holcus lanatus* L. *D. glomerata* L. was observed on all treated plots except the CTR. However, *H. lanatus* L. was noticed only on the CTR. During the five-year period, some forb species appeared in the investigated grassland community, i.e., *Capsella bursa-pastoris* (L.) Medik., *Geranium pusillum* L., *Melandrium pratense* Röhl., *Ranunculus acris* L., *Rumex acetosa* L., *Senecio vulgaris* L. and *Stellaria graminea* L. Higher percentages of these species groups were noticed with the WS and MS treatment.

4. Discussion

4.1. Straw and Biochar Effects on Aboveground Biomass

This research showed that straw application can lead to negative effects on the soil environment and crop yields. The decomposition of straw with a high C:N ratio in soil results in microbial N immobilization and a temporary decrease in the crop-available N and thus can reduce crop growth [3,7]. However, this study did not confirm this thesis. The analysis of the data from the experimental trial with the red clover/grass mixture amended with straw and biochar produced from wheat and miscanthus revealed a positive effect on the root system and the aboveground productivity. As expected, the first cut had a significantly higher biomass than the second and third cuts. This effect is usually ascribed to the weather conditions, soil moisture, and the dynamics of humic substance mineralization [23,44]. The beneficial effects of straw amendment on crop productivity were consistent with Zhao et al. [5] and Xu et al. [1], who reported that straw return increased the crop yield of annual crops. According to Getahun et al. [6], straw residues incorporated into soil have beneficial effects on root development and thus affect aboveground biomass production. This aligns with current studies on root morphology. The positive effects of straw application were observed for most root morphometric parameters. Interestingly, the same effect was observed with straw, even after the pyrolysis process was applied.

Some reports have shown that biochar is a source of nutrients for plants and thus improves crop yields [16,45,46]. In this study, an increase in the yield was obtained after the application of organic materials before and after thermal conversion. According to Alburquerque et al. [47], crop yields are influenced less by the dose of biochar than by the type of feedstock used in the production of biochar. They studied 5 types of biochar (olive stone, almond shell, wheat straw, pine woodchips, and olive tree pruning) introduced in doses from 15 to 225 t ha^{-1}. The authors stated that the effects of biochar addition on plant dry biomass were greatly dependent upon the biochar application rate and the biochar type. This effect was associated with the biochar nutrient content. In the present study, the results in the red clover and grass mixture did not confirm the effects of the type of feedstock and its rate. The straw and biochar rates were likely too low to show this effect. However, the rates used here were more likely to be representative of the actual field use. According to Reed et al. [48], the greatest positive yields arise from biochar applications of above 30 t ha^{-1}, which is a much greater rate than that applied in this study. Although such high rates of biochar are theoretically possible, from a practical point of view, the application of high quantities of biochar for large field areas on commercial farms is not reasonable because of the economic costs involved in production, processing, and transport.

Carbon amendments, such as the application of straw, are used in grasslands to inhibit invasion of non-native plants by reducing the soil nitrogen [9,10]. This study confirmed this effect when miscanthus and wheat straw were used. However, biochar did not show such an effect. It was expected that the biochar application would also alter the competitive ability of plant species in grassland communities. In particular, leguminous species have been shown to benefit from biochar amendment [29]. Some mechanisms were recognized to explain the higher competitive ability of legumes with biochar as the soil amendment: (i) reductions in available N, (ii) increases in soil pH, (iii) increases in the content of soil P, K, Mg, and other nutrients, and (iv) stimulation of biological nitrogen fixation [14,15,29]. The most important nutrient responsible for biomass production was nitrogen. However, the N fertilizer used to increase the DM yield inhibited the diversity and resulted in changes in the botanical composition of meadow sward [22]. Oram et al. [15] reported that biochar had a beneficial effect on red clover under N limiting conditions due to an increase in the K availability. According to Mevlut et al. [49], large concentrations of P are associated with low floristic diversity but play a favorable role in the occurrence of legumes. Concentrations of K appear to increase diversity [22]. In this study, the highest red clover content was observed in the control plot, which was not fertilized. In all other treatments with organic amendments, the red clover nearly disappeared. According to previous authors' report [46], there is no effect of straw and biochar at rates 5 and 2.25 t ha^{-1} on the N content in soil four years after organic amendment application.

Four grass species disappeared: perennial ryegrass, timothy, Westerwolds ryegrass, and meadow fescue. These species were replaced by tall fescue. This effect can be explained by the lower precipitation during the summer periods of 2014 and 2015. Tall fescue is characterized by a thick and dense root system, which makes this species more resistant to soil water deficits. Changes in the botanical composition are strictly related to herbage productivity [23]. In this study, this effect can be ascribed to the higher participation of *D. glomerata* L. and the lower participation of forb species in the control treatment.

4.2. Modification of Root Traits

To date, the effects of biochar application on the root traits remains controversial and highly variable. Root biomass may increase, decrease, or remain relatively stable under biochar application [27,50,51]. In the current study, root biomass was increased after biochar amendment application. According to the optimal partitioning theory, plants allocate their biomass to the organ that has the most limited resources [52,53]. The expression of such optimization is the change in allocation between the biomasses of shoots and roots in response to nutrient availability. When the water supply or nutrient availability increases, plants allocate less mass to their roots because less effort is required to acquire these resources [52,54]. However, this study did not confirm this theory.

Root system characteristics were related to changes in root morphology of particular plants treated with examined soil amendment. However, these parameters also depended on changes in botanical composition as indirect effects of straw and biochar application. Higher legumes and forbs percentage in control grassland community can explain lower RLD values. These two plant groups are characterized by a less dense root system than grasses. The straw and biochar amendments increased the RLD and RDMD. There were also other reports with similar relationships. According to a meta-analysis by Xiang et al. [51], biochar application significantly increased the RDMD, RVD, RSA, MRD, RLD, and the number of root tips. This relationship was confirmed for annual crops [55] and perennial plants [56,57]. The RSR was not significantly altered by the biochar application, while the SRL was significantly enhanced. These beneficial effects were higher in the annual plants than in the perennial plants. In the current research, the differences for most root morphometric parameters were noticed mainly between the control, without fertilizer and any amendments, and treatments where the straw or biochar was applied. However, there were no differences between the particular treatments where wheat, miscanthus straw, and biochar were applied.

In the present study, the relationship between the MRD and the plant yields was significant. This confirmed the thesis that the MRD treatment was beneficial for belowground biomass accumulation, whereas the RLD and RDMD are usually assumed to be proportional to the water or nutrient acquisition. Since grasses have a greater RDMD, they are effective competitors for nutrients, potentially decreasing the proportion of legumes in grass–legume mixtures under nutrient-limiting conditions [15]. On the other hand, Kulmatiski et al. [58] suggested that root systems are foraged independently for different resources. The RSR parameter was found to be a very useful parameter for characterizing the plant reactions to the applied soil amendments. Due to the variations in the root and shoot growth due to various soil and climatic conditions among the regions and during different periods of crop growth, the RSR can vary over a wide range; for perennial grasses, it ranges from 0.18–6.25 [59]. In this study, the RSR ranged from 0.61 to 0.98. Lower values of RSR resulted from the predominant percentage of the grass species in the botanical composition.

5. Conclusions

The results of this five-year field experiment undertaken on a meadow red clover/grass mixture on loamy sand soil have demonstrated that straw and biochar amendments significantly affect aboveground parts of plants and their root system morphology. All tested soil amendments resulted in a lower red clover percentage, but only straw application reduced forbs content with a higher grass percentage.

The soil amendments applied resulted in higher root length, surface area, volume, and mean root diameter. However, the differences for most of the root morphometric parameters were noticed mainly between the control, which did not have any amendments, and the other treatments, where straw or biochar produced from miscanthus or wheat were applied. There were no differences between the treatments with different feedstock types (miscanthus vs. wheat), materials (straw vs. biochar), or amendment rates (5 vs. 2.25 t ha^{-1}).

The results of the root system characteristics and botanical composition were reflected in the aboveground biomass productivity. The soil amendments, i.e., straw and biochar, significantly increased the productivity in comparison with that of the control treatment. However, these differences were noticed only during the first and second cuts. It can be concluded that pyrolysis of straw does not improve this material as a soil amendment. A similar fertilization effect was obtained for both soil amendments.

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