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Contributed Paper

Some Physiological Parameters, Biomass Distribution and Carbon Allocation in Roots of Forage Grasses Growing under Different Nitrogen Dosages

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ABSTRACT

The accumulation and distribution of photosynthetically fixed carbon (C) in different types of crops, and especially in roots, is important for determining the input of carbon in ecosystems. The effect of various types of nitrogen (N) fertilization on physiological parameters, biomass allocation between shoots and roots, and carbon accumulation in roots of festulolium and meadow fescue plants was evaluated. The physiological parameters (SPAD index, CO₂ assimilation, transpiration rate, water-use efficiency), yield, mass of roots, harvest index, carbon content and accumulation in roots of grasses were determined. The increase in N dosage improved the SPAD index, CO₂ net assimilation rate, and the harvest index of plants. However, the highest dosage of N reduced the root mass, and also carbon content, and its accumulation in the roots of plant. Regardless of N fertilization, the mean content of C in roots was low when compared with the literature (0.29 g/g). Moreover, plant photosynthetic activity was positively related to the aboveground part of plants. In contrast, it was negatively related to the belowground part. A higher allocation of biomass to the roots was associated with an increase in the C accumulation in grass roots and the reduction of plant CO₂ assimilation and yield.

Keywords: C content in roots, CO₂ assimilation, forage grasses, nitrogen yield

1. INTRODUCTION

Festulolium (*Lolium* × *Festuca*) hybrids and meadow fescue belong to highly productive, nutritious, persistent, seed-producing and well-adapted grasses that are widely used for agricultural purposes as forage grasses [1-2]. Because of their high yield potential when given high dosages of nitrogen, they are used in intensive feed production on arable land and permanent grasslands, as well [3-4].

The productivity of plants depends on photosynthesis intensity, which is a function of the leaf area (interception of radiation) and the leaf photosynthetic activity (CO₂ assimilation rate per unit of leaf area). It also depends on the transport and partitioning of assimilates between roots and shoots since only a portion of the crop will be harvested and the allocation of assimilates for leaf growth will also be beneficial to plant photosynthesis [5]. Carbon dioxide associated in the aboveground biomass of forage grasses quickly returns to circulation together with the yield, while the carbon accumulated in the root mass may be immobilized [6]. Grasslands are considered as a friendly way for agricultural land use and maintaining organic carbon in the soil [7]. The possibility of carbon accumulation by the roots of forage grasses could be an important way of limiting its loss from the soil. The methods proposed for determining the input of carbon in ecosystems and the amount of photosynthetically fixed C that can potentially be sequestered in soil organic matter are specific for particular crops and management practices. There is a need to update them with new experimental results along with measurements of the parameters used to quantify the accumulation and distribution of photosynthetically fixed C in different types of crops [8].

Fertilization is the most important practice in crop production because of its

influence on soil nutrient availability. Nitrogen, as an essential component of protein, is a component of enzymes that catalyze the process in the reduction and assimilation of carbon dioxide. It is also a component of chlorophyll pigments [9]. Nitrogen stimulates growth processes resulting in an increased area of assimilation and better tillering of grasses. A deficiency in nitrogen induces modifications of many physiological processes along with the biomass distribution of plants [10]. However, plants are able to adapt to nutrient deficiency by altering their root system architecture to efficiently explore soil zones containing the limited nutrient [11]. Nitrogen is the nutrient generally applied to soil in large quantity, therefore, it may be subject to degradation through volatilization, immobilization, denitrification and leaching. An adequate application of fertilizers could increase soil nutrients and soil organic carbon [12]. So, the dosage of nitrogen applied to plants should be optimal for both shoot and root development, and for the relations between them as well. There is a need for multi-parametric investigations into the influence of an essential nutrient for supporting the high photosynthesis rate and allocation of plant biomass between root and shoot as an integrated response to environmental factors as related to the application of nitrogen. The aim of this work was to study the effect of nitrogen fertilization on the process of photosynthesis and to evaluate how the latter can modify biomass allocation between shoots and roots, as well as carbon accumulation in the roots of festulolium and meadow fescue plants.

2. MATERIALS AND METHODS

2.1 Plant Material and Growth Conditions

A field experiment was carried out

during 2012-2013 at the experimental station of the Warsaw University of Life Science in central Poland (52°92' N, 21°32' E). The study was conducted using a randomized split-plot design with four replications for each treatment in Luvisol soil [13] with a loamy sand texture. The seeds of two forage grass species were used, i.e., festulolium (*Festulolium braunii* K. Richt. A. Camus - cv. Felopa and Sulino) and meadow fescue (*Festuca pratensis* Huds. - cv. Pasja and Wanda) and were sown in August 2011 in pure sand on arable land in rows spaced by 10 cm every 4.0 and 3.6 g/m² (respectively). The plants were harvested three times during the vegetation period. The total organic carbon content of the soil (C_{org}) was 11.63 g/kg, and its chemical properties were as follows: pH (in 1 mol/L KCl) - 5.1, total nitrogen content (N_{tot}) - 0.95 g/kg, available phosphorus (P) - 90.5 mg/kg and potassium (K) - 80.0 mg/kg. Three levels of nitrogen (N) fertilization were used: 60, 120 and 180 kg/ha. Nitrogen was added in the form of ammonium nitrate in three equal amounts in the early spring and at each regrowth. Phosphorus at a single rate (P - 35 kg/ha) was applied as a triple superphosphate in the spring, while potassium (K - 100 kg/ha) in the form of potassium chloride was applied in two equal amounts, in the spring and after the first harvest.

Weather conditions were recorded during the period under study (Figure 1). The temperature distribution was favourable for the plants during the growing seasons, with the temperature in spring 2012 being especially moderate (March 4.5°C, April 9.2°C). The highest air temperature occurred in July (21°C in 2012 and 20°C in 2013). The total precipitation varied during the years and amounted to 382 mm in 2012 and 477 mm in 2013. In the first year of study the dry months were August

(35 mm) and September (31 mm), while in the subsequent year in July (20 mm). The highest precipitation was recorded in May 2013 (120 mm), however, there was an intensive one-day rainfall.

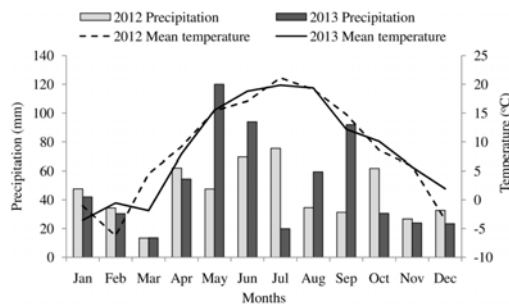


Figure 1. Weather conditions during growing seasons during the years 2012 and 2013: monthly sum of precipitation (mm) and monthly mean of temperature (°C).

2.2 Methods and Measurement

The leaf greenness index was measured with a SPAD optical chlorophyll meter (Minolta, Japan). An LCpro+ Photosynthesis System (ADC BioScientific Ltd., Hoddesdon, England) was used to measure the net photosynthetic rate (A , $\mu\text{mol CO}_2/\text{m}^2/\text{s}$) and the transpiration rate (E , $\text{mmol}/\text{H}_2\text{O}/\text{m}^2/\text{s}$). Measurements were performed at an ambient CO_2 ($\mu\text{mol}/\text{s}/\text{m}^2$) concentration and a saturating irradiance ($1000 \mu\text{mol}/\text{m}^2/\text{s}$). The gas exchange parameters and SPAD readings were monitored during the vegetation seasons on the youngest fully developed leaves, of which four were randomly selected plants from each plot and three from each regrowth. The plant water-use efficiency (WUE) was determined as the ratio A/E .

Yield of dry mass (YDM, kg/m^2) of grasses (above 5 cm) was determined at each harvest for each plot. The dry mass of stubble to 5 cm aboveground (SDM, kg/m^2) and the root dry mass (RDM, kg/m^2) from two soil surfaces below: 0-10 and 10-20 cm

was evaluated by a soil core technique [14]. Samples of grass stubble and roots were collected from each plot, two times during each growing season (in May and in October). Roots were cut from the aboveground mass at the tillering nodes. In order to separate the roots of plants from the soil the samples were sieved and washed out of the soil in a gentle stream of water through a sieve with a mesh size of 2.0 - 0.2 mm. To obtain above- and belowground biomass plant samples were dried at 70°C until a constant weight was reached, and then weighed. The harvest index (HI) values was estimated from these primary data:

$$HI = YDM / (YDM + SDM + RDM)$$

Carbon content in the plants roots was measured by the direct method using the spectrophotometer - analyzer C MAT 55 00. The accumulation of carbon in the roots of plants was evaluated, based on the carbon content and root dry mass.

2.3 Statistical Analysis

The data presented are the mean values for the years of the study, and resulted in a similar response of the plants examined to the levels of nitrogen applied during both study periods. The statistical analysis of the data was performed using the Statistica 12.0 software (Statsoft, Inc. Tulsa, USA). The significance of differences between means was determined using the Tukey HSD test at the significance level of 0.05. The relationship between the physiological parameters selected and the features of the structure of plant biomass was assessed by correlation analysis ($P \leq 0.05$, $P \leq 0.01$).

3. RESULTS AND DISCUSSION

Nitrogen (N) deficiency affects the whole metabolism of the plant because it is mostly

used for the synthesis of components of the photosynthetic apparatus. About 75% of leaf N is allocated in the chloroplasts. Nitrogen content is correlated with the content of most common enzyme Rubisco and plays an important role in CO₂ assimilation [15]. Our studies have shown that one dosage of N significantly increased the values of the leaf greenness index (SPAD) and the intensity of CO₂ assimilation (A) (Table 1). A significant interaction between plant cultivars and nitrogen fertilization was noted as well. The values of SPAD and A in plants were increased by a higher dosage of N. These results are consistent with those of Olszewska [10, 16], who also recorded the highest values of SPAD and A in festulolium plants under increased dosages of N. Our data showed that meadow fescue plants were characterized by a greater (by 8.75%) relative chlorophyll content as compared to festulolium, especially to Sulino. Low SPAD values in the Sulino cultivar have also been reported by Olszewska [16] and Mastalerczuk *et al.* [17].

In our study, N fertilization led to a more efficient use of water by plants, with the values of WUE increasing significantly at higher dosages of N (120, 180). An increase in WUE in response to N fertilization of festulolium cultivars was observed previously by Mastalerczuk *et al.* [17]. The values of the transpiration rate (E) of tested plants also increased when N was applied, especially at 180 kg N/ha. Other studies report contradictory conclusions about the effect of N deficiency on E . In the study by Olszewska [10], grass species exhibited the highest rate of E at 60 kg N/ha, while plants fertilized with 120 kg N/ha showed significantly reduced values of this parameter. A limited water evaporation from the leaf area unit under N fertilization was shown by Grygierzec [18]. The species

and cultivars tested differed insignificantly with respect to A , E and WUE. The study also revealed only a slight differentiation of WUE values among individual cultivars under conditions of applied N fertilization.

Felopa cultivar of festulolium was characterized by higher WUE values at a dosage of 120 kg/ha and Pasja of meadow fescue at 180 kgN/ha.

Table 1. Mean leaf greenness index (SPAD), CO₂ assimilation (A), transpiration rate (E) and photosynthetic water-use efficiency (WUE) of festulolium (Felopa, Sulino) and meadow fescue (Pasja, Wanda) cultivars at different N fertilization levels (60, 120, 180 kg/ha) (2012-2013).

Features	N dosage (kg/ha)	Festulolium		Meadow Fescue		Total
		Felopa	Sulino	Pasja	Wanda	
Leaf greenness index - SPAD (SPAD unit)	60	31.03 ^{ab}	29.79 ^a	33.39 ^{bc}	31.98 ^{ab}	31.55 ^A
	120	33.78 ^{bc}	33.19 ^{bc}	36.96 ^{cd}	34.33 ^{bc}	34.57 ^B
	180	37.41 ^{cd}	37.10 ^{cd}	39.32 ^d	37.95 ^d	37.94 ^C
		34.07 ^{AB}	33.36 ^A	36.56 ^C	34.75 ^B	
Total		33.7 ^A		35.7 ^B		
CO ₂ assimilation - A ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)	60	6.57 ^{ab}	6.03 ^a	6.28 ^a	5.96 ^a	6.21 ^A
	120	8.21 ^{bc}	7.65 ^b	7.59 ^b	8.02 ^{bc}	7.87 ^B
	180	8.93 ^c	8.93 ^c	9.13 ^c	8.89 ^c	8.97 ^C
		7.90 ^A	7.54 ^A	7.67 ^A	7.62 ^A	
Total		7.72 ^A		7.65 ^A		
Transpiration rate - E ($\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$)	60	2.59 ^a	2.42 ^a	2.51 ^a	2.54 ^a	2.51 ^A
	120	2.74 ^a	2.96 ^a	2.75 ^a	2.98 ^a	2.86 ^{AB}
	180	3.12 ^a	2.92 ^a	3.02 ^a	3.06 ^a	3.03 ^B
		2.82 ^A	2.77 ^A	2.76 ^A	2.86 ^A	
Total		2.79 ^A		2.81 ^A		
Water-use efficiency - WUE ($\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$)	60	2.64 ^a	2.71 ^a	2.68 ^a	2.44 ^a	2.62 ^A
	120	3.40 ^b	2.76 ^a	3.00 ^a	2.82 ^a	2.99 ^B
	180	3.24 ^a	3.35 ^a	3.42 ^b	3.12 ^a	3.28 ^B
		3.09 ^A	2.94 ^A	3.03 ^A	2.79 ^A	
Total		3.02 ^A		2.91 ^A		

Mean values marked by the same lowercase (for interactions: cultivar x dosage of N) and uppercase (in column for N dosage or row for species, cultivars) are not significantly different at $P \leq 0.05$ by the Tukey HSD test.

The results showed a gradual increase in grass yield under an N dosage increased from 60 to 180 kg/ha (by 40%) (Table 2). Festulolium plants were characterized by higher yields of dry matter, and there were no differences between cultivars studied. A layer of stubble made up 14% of total plant biomass, independent from both the

N dosage and species. The study also found no effect from N fertilization on the amount of stubble. Festulolium cultivars, especially Sulino, were characterized by a higher mass of stubble compared to meadow fescue cv. The collected data showed that the share of the total root mass in plant biomass was 46%, irrespective of N dosage

and cultivar. The greater proportion was located in the layer 0-10 cm (approximately 95%). Among the species tested festulolium formed a higher root mass than meadow fescue, regardless of the N applied. According to the literature [19] the size and location of the root mass is dependent not only on the grass species but also on the abiotic conditions. In our study the nitrogen applied varied significantly by the root mass of plants, particularly in the 0-10 cm layer of soil, with the highest dosage of N significantly

decreasing the root mass of plants as compared to other N dosages (60 kg/ha by 7.6 and 120 kg/ha by 13.8%). At a deeper soil level, there was no differentiation between the root mass, depending on the N fertilization of the examined species and the cultivars. These results are in agreement with the findings of Holub *et al.* [20] and Mastalerczuk *et al.* [17], who found that the total belowground biomass of meadow plants decreased at the highest N level (180 kg/ha).

Table 2. Mean dry mass DM of yield, stubble and roots, harvest index (HI), carbon (C) content, and accumulation in roots of festulolium (Felopa, Sulino) and meadow fescue (Pasja, Wanda) cultivars with different N fertilization (60, 120, 180 kg/ha) (2012-2013).

Features	N dosage (kg/ha)	Festulolium		Meadow Fescue		Total
		Felopa	Sulino	Pasja	Wanda	
DM of Yield (kg/m ²)	60	0.85 ^{abc}	0.78 ^{ab}	0.67 ^a	0.67 ^a	0.75 ^A
	120	1.06 ^{abc}	1.04 ^{abc}	1.01 ^{abc}	0.91 ^{abc}	1.00 ^B
	180	1.28 ^c	1.26 ^c	1.25 ^c	1.16 ^{bc}	1.24 ^C
total		1.07 ^A	1.02 ^A	0.98 ^A	0.92 ^A	
DM of Stubble (kg/m ²)	60	0.32 ^{ab}	0.32 ^{ab}	0.32 ^{ab}	0.28 ^a	0.31 ^A
	120	0.41 ^{ab}	0.36 ^{ab}	0.29 ^a	0.36 ^{ab}	0.35 ^A
	180	0.40 ^{ab}	0.47 ^b	0.31 ^{ab}	0.26 ^a	0.36 ^A
total		0.37 ^{AB}	0.38 ^B	0.31 ^A	0.30 ^A	
DM of total Roots 0-20cm (kg/m ²)	60	1.08 ^{ab}	1.24 ^b	1.08 ^{ab}	1.15 ^{ab}	1.14 ^B
	120	1.33 ^b	1.31 ^b	1.08 ^{ab}	1.15 ^{ab}	1.22 ^B
	180	1.10 ^{ab}	1.17 ^{ab}	0.96 ^a	0.98 ^a	1.06 ^A
total		1.17 ^{BC}	1.24 ^C	1.04 ^A	1.10 ^{AB}	
DM of Roots 0-10cm (kg/m ²)	60	1.04 ^{abc}	1.19 ^{cde}	1.02 ^{abc}	1.09 ^{bcd}	1.08 ^B
	120	1.28 ^c	1.24 ^{de}	1.02 ^{abc}	1.09 ^{bcd}	1.16 ^B
	180	1.05 ^{abc}	1.12 ^{cde}	0.90 ^a	0.93 ^{ab}	1.00 ^A
total		1.12 ^B	1.18 ^B	0.98 ^A	1.04 ^A	
DM of Roots 10-20cm (kg/m ²)	60	0.04 ^a	0.06 ^a	0.06 ^a	0.06 ^a	0.06 ^A
	120	0.05 ^a	0.07 ^a	0.06 ^a	0.06 ^a	0.06 ^A
	180	0.05 ^a	0.05 ^a	0.07 ^a	0.05 ^a	0.06 ^A
total		0.05 ^A	0.06 ^A	0.06 ^A	0.06 ^A	
total		0.05 ^A		0.06 ^A		

Table 2. Continued.

Features	N dosage (kg/ha)	Festulolium		Meadow Fescue		Total
		Felopa	Sulino	Pasja	Wanda	
Harvest index HI	60	0.38 ^{abc}	0.33 ^{ab}	0.33 ^{ab}	0.31 ^a	0.34 ^A
	120	0.37 ^{abc}	0.38 ^{abc}	0.43 ^{abc}	0.36 ^{abc}	0.39 ^A
	180	0.45 ^{bc}	0.43 ^{abc}	0.49 ^c	0.48 ^c	0.47 ^B
		0.40 ^A	0.38 ^A	0.41 ^A	0.40 ^A	
total		0.39 ^A		0.40 ^A		
Carbon content (g/kg DM)	60	286 ^{ab}	293 ^b	298 ^b	292 ^b	292 ^B
	120	294 ^b	293 ^b	284 ^{ab}	294 ^b	291 ^B
	180	274 ^a	291 ^b	288 ^b	289 ^b	285 ^A
		284 ^A	292 ^B	290 ^B	291 ^B	
total		289 ^A		290 ^A		
Carbon accumulation (kg/m ²)	60	37.0 ^{bcd}	41.6 ^d	35.0 ^{bcd}	37.4 ^{bcd}	37.75 ^B
	120	38.5 ^{cd}	35.7 ^{bc}	32.3 ^{bcd}	36.3 ^{bcd}	35.70 ^B
	180	32.7 ^{bc}	33.9 ^{bc}	26.3 ^a	26.7 ^a	29.92 ^A
		36.1 ^C	37.1 ^C	31.2 ^A	33.5 ^B	
total		36.6 ^B		32.3 ^A		

Mean values marked by the same lowercase (for interactions: cultivar x dosage of N) and uppercase (in column for N dosage or row for species, cultivars) are not significantly different at $P \leq 0.05$ by the Tukey *HSD* test.

Harvest index (HI) reflects the partitioning of photosynthesis efficiency into efficient parts. An increase in the value of HI indicates crop improvement [8]. In our study the production yield of grasses, regardless of the species and fertilization, accounted for approximately 40% of the total biomass of plants (Table 2). The values of HI were increased in particular by the highest N dosage. The data presented is in agreement with the results of Gastal *et al.* [21], who stated that with lowering the supply of N, plants allocate proportionally more of their biomass into the roots. According to Kacorzyk *et al.* [22] the share of yield harvested from the total biomass of forage grasses differs depending on the species. Whereas, our results showed no difference in the values of HI between the species studied and the cultivars, regardless of other factors involved.

Interactions among aboveground carbon allocation are more clearly

characterized than those of belowground processes. Bolinder *et al.* [8] show that the distribution of C in plant parts is usually calculated from agricultural yield. The aboveground part of plants most often measured using HI values or related regression relationships and belowground C inputs are calculated from shoot-to-root (S:R) ratios. However, the plant C allocation coefficients should be updated as new measurements emerge. Based on our results, it should be noted that the C content in grass roots was influenced by the N fertilization. A significant decrease was noted between plants treated with the N dosage of 180 kg/ha and other fertilization levels. We assessed that the C content in roots of festulolium and meadow cultivars ranged from 274 to 298 g/kg DM (0.274-0.298 g/g). According to the literature [8] the C concentration in all crop plant parts was 0.45 g/g. Only a few studies about the amount of photosynthetically fixed C that

can potentially be sequestered in soil organic matter include a variation in C content in individual organs of aboveground plant parts [23].

The accumulation of carbon in plant roots, calculated on the basis of the C content and DM roots, was significantly higher under conditions of under fertilization by 60 and 120 kg N/ha. Species and cultivars tested also differed with this feature between them. The cultivars of meadow fescue, especially Pasja, are characterized by a lower C accumulation in the plant roots. At the same time, the values of this feature increased under the lowest N dosage. Roots of meadow fescue plants differed significantly by a lower C accumulation under conditions of the highest dosage of N. In the study by Stypiński and Mastalerczuk [6] a similar trend in the reduction of C accumulation under intensive management (high N fertilization associated with frequent mowing) in moderately dry and wet sites was observed in plant roots.

Statistical analysis similarly showed a significant correlation among physiological

parameters (SPAD, A , and WUE) in both species (Table 3). Higher values of A were associated with a higher chlorophyll content (SPAD index) and a better use of water by plants. Simultaneously, high values of WUE reduced the transpiration rate. The parameters of biomass distribution (YDM, RDM and HI) of plants under study and tested were also correlated with each other in both species. A dependency between the physiological parameters related to the photosynthetic activity of plants (A and SPAD) and the features of biomass distribution was positive regarding the aboveground part of plants (YDM) and negative regarding the belowground part (RDM). An increased allocation of plant dry mass to their roots in response to a lower N dosage was associated with a reduction in A , WUE, and SPAD. Our results showed that a higher C content and its accumulation in the roots was related to reduced values of A , SPAD, YDM, and HI. At the same time, there was a positive dependence between C content in roots, C accumulation, and also the DM of roots.

Table 3. Correlation coefficients (r) between selected physiological parameters (SPAD-leaf greenness index, CO_2 assimilation- A , transpiration rate- E , photosynthetic water-use efficiency-WUE), features of structure biomass (YDM-dry matter of yield, RDM-dry matter of roots), root carbon content and the accumulation of festulolium and meadow fescue cultivars.

Variable	SPAD	A	E	WUE	YDM	RDM	HI	C _{cont}	C _{accum}
SPAD	1								
CO ₂ assimilation (A)	0.74**	1							
Transpiration rate (E)	0.09	0.07	1						
Water-use efficiency (WUE)	0.35*	0.49**	-0.65**	1					
DM of Yield (YDM)	0.77**	0.82**	0.08	0.32*	1				
DM of Root total 0-20cm (RDM)	-0.67**	-0.52**	-0.20	-0.16	-0.60**	1			
Harvest index (HI)	0.75**	0.77**	0.12	0.23	0.93**	-0.74**	1		
Carbon content (C _{cont})	-0.30*	-0.30*	0.19	-0.22	-0.40**	0.91	-0.38**	1	
Carbon accumulation (C _{accum})	-0.69**	-0.55**	-0.15	-0.19	-0.65**	0.98**	-0.78**	0.38**	1

The coefficient values marked by an asterisk are significant: * $P < 0.05$, ** $P < 0.05$.

4. CONCLUSIONS

The increase in the nitrogen dosage positively enhanced relative chlorophyll content (SPAD), CO₂ net assimilation rate (*A*), and the harvest index (HI) of festulolium and meadow fescue plants. The application of the highest dosage of nitrogen (180 kg/ha) reduced the root mass, as well as the content and accumulation of carbon (C) in plants roots.

The mean content of C in the roots of grasses tested was low (0.29 g/g) when compared to the literature (0.45 g/g), regardless of N fertilization. A higher biomass allocation in the roots was associated with an increase in the C accumulation in the roots of grasses and the reduction of plant CO₂ assimilation and yield. Festulolium cultivars were characterized by higher yields, root dry mass (RDM), and C accumulation in the roots, while meadow fescue exhibited greater SPAD values.

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