

The cultivation possibility of tall oat grass and red clover mixture on acidic soil in order to develop a sustainable management system

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Abstract

The work aimed to analyze the effects of soil liming on the productivity of the mixture of tall oat grass and red clover during the entire period of exploitation in order to develop sustainable management systems in agriculture. The trial was conducted from 2014 to 2020 on the experimental field in Čačak on an alluvial soil with an acid reaction. The trial was set up as a completely randomized block system with four replicates and a plot size of 5x1 m. Levels of liming included: A1 – control (without CaO); A2 – 3 t ha⁻¹ CaO; A3 – 6 t ha⁻¹ CaO. Crop response to soil liming was determined by the amount of lime applied, the amount of rainfall and the developmental stage of the species during the life cycle. In concrete conditions, the greatest positive effect of soil liming was observed in the second and third years of production. And if it was expected that the proportion of clover and the intensity of nitrogen fixation generally increase with the application of soil liming, this study showed that in some cases the crop responded differently and non-linearly to the application of lime. By growing red clover in a mixture with tall oat grass and applying soil liming on acidic soils, satisfactory yields can be achieved while recovering the soil from intensive exploitation, which can be good practice for developing a sustainable management system in agriculture.

Keywords: forage yield; liming; red clover; sustainable agriculture; tall oat grass

Introduction

The growing demand for food worldwide and pursuit of higher profits over the last century have fundamentally changed agricultural systems. Land use has been intensified, leading to its degradation and pollution (Jaleta *et al.*, 2019; Renard and Tilman, 2019; Beye *et al.*, 2022). The size of farms is increasing, which limits the proportion of grazing in the livestock diet (Kristensen *et al.*, 2022). As a result, agriculture can no longer be managed in the same way as it was two or three decades ago (Ayarza *et al.*, 2022). In recent years, measures for the ecological intensification of agriculture have been increasingly discussed (Reinsch *et al.*, 2021). The conservation and introduction of plant diversity are seen as a key factor for sustainable farming systems

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(Kremen and Merenlender, 2018). On the other hand, the production of sufficient quantities of high-quality animal products depends on the production of high-quality feed, which includes perennial forage legumes (Johansen *et al.*, 2017). Forage production with perennial legumes has the potential to facilitate economically and ecologically profitable dairy production systems.

The inclusion of forage legumes in the grassland composition in the form of grass-clover mixtures reduces dependence on industrial fertilizers and increases protein self-sufficiency, which increases yield stability compared to pure grass crops (Tomić *et al.*, 2019). Increasing the proportion of clover generally improves forage quality (Phelan *et al.*, 2015) and increases milk production due to the higher protein content (Johansen *et al.*, 2017). In contrast to pure legumes, grass-clover mixtures have a low level of nitrogen leaching (Eriksen *et al.*, 2012). Lorenz *et al.* (2020) point out that the harmful effects of intensive milk production on the environment can be reduced by including grasses in mixtures with clovers. In this way, using more diverse pastures can reduce the excess nitrogen in the forage and thus the excretion of excess nitrogen in the urine. Increasing species diversity improves nutrient utilization, stabilizes biomass yield, and controls weeds (Tahir *et al.*, 2022). However, the authors reported that the performance of the low-diversified mixture was better than that of the high-diversified mixture in terms of productivity and nutrient quality. Grass-clover mixtures can improve the biodiversity of a complete ecosystem compared to intensively managed conventional pastures (Beye *et al.*, 2022). Ayarza *et al.* (2022) point out that appropriately managed agricultural systems can be an important fixer of atmospheric CO₂ by increasing the accumulation of organic matter in the soil.

One of the grass species that has proven itself on the European continent for the establishment of lawns is tall oat grass (*Arrhenatherum elatius* L. (P. Beauv.)) (Wrobel and Jankowska-Huflejt, 2006). It is an important forage grass that is native and widespread throughout Europe. The first cultivation of *A. elatius* took place as early as the sixteenth century in southern France, from where the seed was later widely distributed in Europe as “French rye”. The species is often included in seed mixtures for pastures and is used in grassland restoration measures (Michalski *et al.*, 2010). Growing tall oat grass together with red clover (*Trifolium pratense* L.) as a legume component can be very useful in terms of achieving a high yield of high-quality forage with positive effects on soil fertility, which are key elements of sustainable systems in agriculture.

A major problem in the world today is large areas of acidic soils (about 40%), where the productivity of agricultural crops is reduced (Yan *et al.*, 2021). In acidic soils, nutrients are often present in forms that are inaccessible to plant uptake. High soil acidity is associated with a lack of available forms of Ca, K, Mg, P and Mo and an excess of soluble Al, Mn and other heavy metal ions (Baquy *et al.*, 2017). Soil acidity and Al toxicity limit the activities of soil enzymes, leading to a decrease in the intensity of nutrient cycling (Kunito *et al.*, 2016). In general, soil acidification can be considered as a sum of natural and anthropogenic influences (Rahman *et al.*, 2018). Some of the natural causes are the leaching of bases under the influence of carbonic acid, the accumulation of acidic parent material, the decomposition of organic material and the deposition of atmospheric gases such as SO₂, NH₃, HNO₃ and HCl. Anthropogenic activities that lead to soil acidification, as they are the continuous application of mineral fertilizers and intensive tillage leading to a reduction of organic matter content in the soil and the accumulation of carbonic acid (Behera and Shukla, 2015; Terzić *et al.*, 2019). A humid climate creates a greater potential for acidic soils (Brady and Weil, 2016). The basic agrotechnical measure to reduce soil acidity is soil liming, which must be carried out professionally and carefully (Bolan *et al.*, 2023). In many countries and regions, acid soil management is becoming one of the most important points in the strategy to achieve food security. It is a major challenge that requires investment, analysis, and knowledge to develop and apply sustainable practices (Agegnehu *et al.*, 2021).

The study aimed to carry out a long-term analysis of the effects of soil liming on the productivity of the grass-clover mixture of tall oats and red clover throughout its exploitation period to gain an insight into the possibility of using this measure to develop sustainable management systems in agriculture.

Materials and Methods

Location and soil properties

The trial was conducted from 2014 to 2020 (7 years) on the experimental field of the Veterinary Extension Service in Čačak, Serbia (43°54'39.06" N, 20°19'10.21" E, 246 m a.s.l.). The experiment was conducted on an alluvial soil (pH(H₂O) 4.8; pH(KCl) 3.6) which contained 3.18% organic matter, 0% CaCO₃, 22.1 mg P 100 g⁻¹ soil, 30.0 mg K 100 g⁻¹ soil, 12 mg kg⁻¹ Al, 1.1 mg kg⁻¹ Mn, 82 mg kg⁻¹ Zn, 38.9 mg kg⁻¹ Fe, 25 mg kg⁻¹ Cu, 27.2 mg kg⁻¹ Co, 278 mg kg⁻¹ Na, 141 mg kg⁻¹ Cd, 113 mg kg⁻¹ Ni, 30.4 mg kg⁻¹ Pb, 29.2 mg kg⁻¹ Hg, 79.6 mg kg⁻¹ Cr, 17.2 mg kg⁻¹ As. The previous crop was a natural meadow. In autumn, before sowing and before the beginning of each growing season, 45 kg ha⁻¹ N, 45 kg ha⁻¹ P₂O₅, and 45 kg ha⁻¹ K₂O were used for soil fertilization.

Weather conditions

During the period under study, the mean annual air temperature for the specified area was 12.5 °C, with monthly averages ranging from 2.0 °C to 22.6 °C for January and August, respectively (Table 1). The average annual precipitation was 676 mm. The largest amounts of precipitation during the year were in the period May–June. The annual precipitation totals were highest in 2014 (over 900 mm) and lowest in 2020 (less than 500 mm).

Table 1. Average monthly temperature (T) and monthly precipitation distribution (P) for the experimental period 2014-2020

Year and Index		Month												Mean and sum
		1	2	3	4	5	6	7	8	9	10	11	12	
2014	T (°C)	4.0	6.6	10.2	12.5	16.1	21.1	22.7	22.1	17.0	13.5	8.9	3.1	13.1
	P (mm)	21.5	6.0	52.5	104.5	125.0	103.5	163.0	56.0	101.0	60.0	19.0	90.0	902
2015	T (°C)	0.9	2.7	9.1	13.4	16.5	22.2	23.0	22.4	18.0	16.5	9.2	3.8	13.1
	P (mm)	30.0	63.0	74.0	16.0	53.0	87.0	4.0	25.0	42.0	68.0	41.0	44.0	547
2016	T (°C)	-0.6	2.4	9.5	12.8	16.8	20.8	22.2	22.1	17.6	15.2	8.8	1.2	12.4
	P (mm)	49.0	20.0	182.0	41.5	123.5	74.0	12.5	76.0	44.0	70.0	20.0	55.0	767
2017	T (°C)	-3.5	3.0	10.1	12.5	16.8	23.0	25.3	25.1	19.7	16.2	9.1	3.1	13.4
	P (mm)	27.0	38.0	36.0	82.0	91.0	38.0	16.0	31.0	37.0	38.0	28.0	50.0	502
2018	T (°C)	2.3	1.0	5.6	16.5	18.9	20.2	21.1	22.9	17.3	13.2	4.4	1.1	12.1
	P (mm)	62.0	81.0	165.0	34.4	51.2	97.6	150.0	53.6	28.6	14.4	42.4	43.8	824
2019	T (°C)	1.3	3.8	8.6	12.2	13.7	21.8	21.5	22.7	17.0	12.2	9.6	2.5	12.2
	P (mm)	31.0	27.6	22.4	86.4	153.2	146.2	57.2	27.8	22.4	28.2	54.6	55.4	712.4
2020	T (°C)	-0.3	5.12	6.7	10.8	15.0	19.0	20.9	21.1	17.5	11.3	5.8	4.1	11.4
	P (mm)	21.6	54.4	56.6	46.4	87.2	24.8	29.8	36.0	45.2	53.0	7.8	15.2	478

Experimental design and analyses

The experiment was designed as a completely randomized block system with four replicates. The plot size was 5 x 1 m and the distance between plots was 0.5 m. The liming levels included: A1 - control (without CaO), A2 - 3 t ha⁻¹ CaO and A3 - 6 t ha⁻¹ CaO. The lime material was applied once in the year of crop establishment by applying the entire amount of lime before the start of the growing season (in March). Unhydrated lime (CaO) was used for liming. After 1 year, the pH (H₂O) of the soil in treatments A2 and A3 was 6.1 and 6.3 respectively.

Sowing took place in the spring of 2014 with 20 cm spacing between the rows. Tall oat grass ('K-12' cultivar) was sown in the same rows as red clover ('Kolubara' cultivar), with a seed rate of 12 kg ha⁻¹ red clover and 12 kg ha⁻¹ tall oat grass. Mechanical weed control was carried out twice. The crop was grown without irrigation. Mowing was carried out during the budding phase of the red clover.

The grassland was mown three times in 2014 and 2018 and twice in the other years in which there was no third cut due to the drought. The forage yield (t ha^{-1}) was determined by measuring the total biomass of the plot immediately after mowing. After the measurement, samples (1000 g) were taken to analyze the forage moisture. The plants were categorized into three functional groups: tall oat grass, red clover, and weeds (other plant species). The material was dried at 65°C to determine the dry matter yield (t ha^{-1}). The proportion of the individual plant groups in the dry sample was then calculated.

The results obtained were subjected to a one-factor analysis of the variance model (soil liming as fixed effects) employing STATISTICA 8 (StatSoft Inc., 2007). Differences between means were tested through the LSD test.

Results and Discussion

Both levels of soil liming had a significant effect on the increase in forage yield ($P \leq 0.05$) already in the first cut in the year of establishment (Table 2), which confirms the studies of many authors about its importance.

Table 2. The influence of soil liming on crop productivity in the period 2014-2017

Year	Cut	Liming	Forage yield (t ha^{-1})	Dry matter yield (t ha^{-1})	Grass proportion (%)	Clover proportion (%)	Weed proportion (%)
2014	1	A ₁	19.92 b	7.42 a	82.0 a	6.6 b	11.41 b
		A ₂	24.62 a	9.12 a	80.8 a	14.7 a	4.49 c
		A ₃	25.62 a	8.49 a	55.6 b	10.2 a	34.16 a
	2	A ₁	20.56 a	7.08 a	60.19 a	29.4 b	10.45 a
		A ₂	23.41 a	6.26 a	43.59 c	46.6 a	9.82 ab
		A ₃	24.52 a	7.21 a	52.64 b	40.8 a	6.59 b
	3	A ₁	10.37 a	2.51 b	88.2 a	10.9 b	0.96 a
		A ₂	12.27 a	3.05 a	76.2 a	21.1 ab	2.65 a
		A ₃	12.22 a	2.65 b	64.6 a	35.4 a	0.00 a
2015	1	A ₁	35.59 b	9.29 c	95.0 a	5.11 a	-
		A ₂	40.81 a	14.19 a	93.1 a	6.89 a	-
		A ₃	37.92 ab	11.84 b	95.2 a	4.79 a	-
	2	A ₁	8.74 b	2.65 c	74.0 c	24.4 a	1.6 a
		A ₂	11.64 ab	3.52 b	89.2 a	10.7 c	0.2 b
		A ₃	15.02 a	4.8 a	84.3 b	15.6 b	0.1 b
2016	1	A ₁	52.7 b	11.74 c	90.6 a	2.49 a	6.9 a
		A ₂	63.8 a	14.43 a	94.4 a	0.64 b	4.9 a
		A ₃	52.7 b	12.89 b	97.5 a	0.34 b	2.2 a
	2	A ₁	13.41 a	4.84 b	71.9 b	24.50 a	3.68 a
		A ₂	15.77 a	7.11 a	99.3 a	0.1 b	0.62 a
		A ₃	13.63 a	5.08 b	87.2 ab	10.8 b	1.96 a
2017	1	A ₁	32.04 a	10.47 a	60.9 b	6.36 a	32.7 a
		A ₂	29.37 a	9.51 b	97.8 a	0.37 b	1.8 b
		A ₃	21.02 b	7.60 c	94.1 a	4.67 a	1.2 b
	2	A ₁	6.71 a	3.28 a	31.7 c	51.8 a	16.5 a
		A ₂	6.95 a	3.40 a	68.7 a	25.9 b	5.5 b
		A ₃	8.30 a	3.28 a	47.1 b	49.0 a	3.9 b

The values denoted with different small letters within columns for every cut are significantly different ($p \leq 0.05$) in accordance with the LSD test

According to Yan *et al.* (2021), soil acidity is one of the factors that hinder the cultivation of many crops, both legumes and grasses. Low soil pH affects all stages of plant development, disease resistance, resistance to low temperatures, plant longevity, yield, and forage quality (Jan *et al.*, 2023). Acidic soils contain a high concentration of mobile Al, Mn, and Fe. At a pH below 5.0, Al is soluble in water and becomes the dominant ion in the soil solution. An excess of soluble Al damages the root tip and inhibits its elongation (Marschner, 2011). Poor root growth leads to reduced uptake of water and nutrients, so plants grown on acidic soils are less drought tolerant, leading to a further reduction in growth and yield (Chakraborty *et al.*, 2024). In general, there is active and variable soil acidity (Agegnehu *et al.*, 2021). Active acidity is caused by a high concentration of H⁺ ions in the soil solution. Exchangeable acidity refers to H⁺ and Al³⁺ ions adsorbed on soil colloids. In soil, there is a balance between adsorbed ions and ions in the soil solution, with a dynamic movement from one form to the other. Such a state of balance is very practical because it forms the basis for the buffering capacity of the soil. Lime, which is added to the soil through soil liming, first affects the active acidity in the soil solution. It then binds calcium to the adsorptive complex of the soil, which affects the exchangeable acidity and has a positive effect on reducing the total acidity of the soil. In this way, the harmful effects of Al, Mn, and other metals are reduced. In addition, the application of calcium leads to an increase in microbiological activity in the soil and greater nitrogen mineralisation (Meena and Prakasha, 2021). By reducing the acidity of the soil, favourable conditions are created for the development of symbiotic nitrogen fixers. Burns *et al.* (2020) find that the abundance and microbiological activity of symbiotic nitrogen fixers in perennial legumes is limited by low pH. Under such conditions, the survival of nodule bacteria is difficult and reproduction is slowed, which has an impact on the reduction of legume yields (Alves *et al.*, 2021). Soil liming helps to reduce the amount of highly mobile and toxic forms of Al, Fe, and Mn and to increase the amount of readily available P in the soil (Chakraborty *et al.*, 2024). The application of soil liming influenced a significant increase in forage and hay yield in the grass-clover mixture of red clover and Italian ryegrass (Tomić *et al.*, 2019). By intensifying the process of nitrogen fixation, it is possible to utilise up to 300 kg ha⁻¹ N (Kristensen *et al.*, 2022). Al toxicity and acidity suppress microbial activity and nutrient cycling (Kunito *et al.*, 2016).

In the second and third cuts in the first year of production, no significant influence of soil liming on forage yield was observed in this trial. Soil liming had a significant influence on the increase in forage yield in both cuts in 2015, in the first cut in 2016, and in the second cut in 2020 (Table 3). The results show that the influence of soil liming on forage and hay yield was more pronounced when the plants had a good water supply, while the influence of soil liming on forage yield was lower in cuts with a lack of precipitation. Precipitation mediates species responses to changes in plant mineral nutrition in semi-arid grasslands (Yadav *et al.*, 2020). Changes in climate and soil conditions can modulate the effects of altered soil pH on grassland ecosystems (Ma *et al.*, 2017). Precipitation and soil water balance are crucial factors regulating plant growth and soil pH in grassland ecosystems (Slessarev *et al.*, 2016). Ecosystem responses to changes in grassland soil pH are largely determined by precipitation (Liu *et al.*, 2022).

In the first cut in 2017 and 2018, a significantly lower forage yield was recorded in the A3 treatment compared to the control treatment. This is a consequence of the increased weed proportion in the control treatment due to the reduction and lack of yield of red clover, which disappeared from the trial at this time. In the treatments with soil liming, there was significantly less mass of weeds and the forage yield was slightly lower. The reason for this phenomenon could be the higher tolerance of the plants in the treatments with soil liming compared to the control to a long and cold period with temperatures below -10 °C, which occurred during the previous winter and influenced crop thinning of the plants. According to Tomić *et al.* (2019), weeds occur massively in rare crops, consume nutrients and water, and affect the reduction of the yield of the cultivated crops, while crops with a denser structure suppress weeds and prevent their more massive occurrence. Tahir *et al.* (2022) confirmed that the composition of the mixture has a significant effect on the biomass yield of mixtures of legumes and grasses. The productivity of the mixture in this experiment was related to the

dominant species in the mixtures. According to Liu *et al.* (2022), a change in soil pH had a negative effect on the species richness of grassland. Fluctuations in soil pH can change the turnover of species and thus the composition of functional groups. This can change the growth ratio of the different plant species and the productivity of the grassland (Kamble *et al.*, 2014).

The dry matter yield in the year of crop establishment was significantly higher in the third cut in the A2 treatment compared to the control treatment. In both cuts in 2015 and 2016 and the second cut in 2019 and 2020, higher dry matter yields were recorded in the treatments with soil liming compared to the control treatment. A significant negative influence of soil liming on dry matter yield, as well as on the forage yield was observed in the first cut of 2017. The reason for this is the same as for forage yield. The influence of soil liming on dry matter yield was also more pronounced under more favourable growing conditions (better water supply to the plants).

Table 3. The influence of soil liming on crop productivity in the period 2018-2020

Year	Cut	Liming	Forage yield (t ha ⁻¹)	Dry matter yield (t ha ⁻¹)	Grass proportion (%)	Weed proportion (%)
2018	1	A ₁	25.21 a	7.93 b	77.0 b	23.0 a
		A ₂	24.84 a	8.76 a	83.0 ab	17.0 ab
		A ₃	22.03 b	8.00 b	89.7 a	10.3 b
	2	A ₁	9.20 a	3.06 a	81.9 b	18.1 a
		A ₂	10.19 a	3.47 a	91.0 a	9.0 b
		A ₃	9.49 a	2.75 a	89.0 a	11.0 b
	3	A ₁	4.47 a	2.54 a	84.2 ab	15.8 ab
		A ₂	4.31 a	2.48 a	80.5 b	19.5a
		A ₃	4.58 a	2.74 a	95.7 a	4.3 b
2019	1	A ₁	32.9 a	10.1 a	98.4 a	1.6 b
		A ₂	34.6 a	10.6 a	98.4 a	1.6 b
		A ₃	27.3 a	9.2 a	94.7 b	5.3 a
	2	A ₁	8.04 a	2.73 b	78.3 b	20.7 a
		A ₂	8.84 a	3.61 a	93.5 a	6.5 b
		A ₃	7.57 a	2.58 b	79.8 b	20.2 a
2020	1	A ₁	15.6 a	7.12 a	98.4 a	1.6 a
		A ₂	17.9 a	7.12 a	98.9 a	1.1 a
		A ₃	23.6 a	11.18 a	99.1 a	0.9 a
	2	A ₁	5.63 b	3.37 ab	83.7 a	16.3 b
		A ₂	8.07 a	3.06 b	86.6 a	13.4 b
		A ₃	8.56 a	3.68 a	69.2 b	30.8 a

The values denoted with different small letters within columns for every cut are significantly different ($p \leq 0.05$) in accordance with the LSD test

Soil liming had the greatest influence on forage and dry matter yield in the second and third years of utilization (2015 and 2016). In the fourth and fifth years of exploitation when the red clover disappeared, the positive influence of soil liming on forage and dry matter yield was absent, and in the sixth and seventh years, it was minimal (mostly positive in the second cut). This confirms the fact that the residual effect of soil liming usually lasts for five to seven years. Agegnehu *et al.* (2021), for example, point out that the highest yield in barley is obtained in the third year after liming, which means that the effectiveness of lime is greater in the following years than in the first and second years after application. Although the proportion of clover and the intensity of nitrogen fixation generally increase with the application of lime fertilizer, in some cases they responded differently and by no means linearly (Kristensen *et al.*, 2022), which was also confirmed in this trial. Over time,

precipitation leaches soluble nutrients such as Ca, Mg, and K, which reduce acidity, and these are replaced by Al^{3+} ions (Yadav *et al.*, 2020).

Observed by year, contrary to expectations, a high dry matter yield was recorded in 2014 for all treatments, spread into the three cuts (Figure 1). The reason for this was the rainy season during the vegetation period with a large amount of regularly distributed rainfall so that there were no dry periods. Under these conditions, the newly planted crop developed quickly began to grow, and maintained this pace until the end of the growing season. The result is that in the second year of production (2015), when there were dry spells, the total dry matter yield in the treatments with soil liming was at the level of the first year (otherwise it should be higher), and in the control treatment it was significantly lower. In the third year of production, when rainfall was also moderate, dry matter yields in treatments A1 and A3 also remained at a similar level to the two previous years, and a higher yield was recorded in treatment A2. Then comes 2017 (the fourth year of production), which was extremely dry, with a lack of rainfall, especially in the second part of the growing season. For this reason, there was no third cut and dry matter yields were at their lowest at this time. Yields also fluctuated in the further course of the trial depending on the weather conditions and the amount of precipitation but remained significantly lower compared to the first three years of cultivation. In the last year of the trial, a slightly higher dry matter yield was recorded for treatment A3 compared to treatments A1 and A2, which can be seen as a lasting effect of a higher lime dose in combination with the weather conditions. The first symptoms that appear on the plants when grown on acidic soil are root injury due to Al toxicity (Chakraborty *et al.*, 2024). Damage to root growth of sensitive plant species occurs when the Al content in the soil solution exceeds 1 mg kg^{-1} and in this case, 60% or more of the exchangeable capacity of the soil is occupied by Al (Agegnehu *et al.*, 2021). Damage can also be caused by Mn, which becomes highly soluble at a pH of less than 5.5 (Lu *et al.*, 2023). In these cases, the root system becomes stunted and thorny. With stunted roots, the plant's ability to extract water and nutrients, especially low mobile nutrients such as P, is significantly reduced (Chakraborty *et al.*, 2024). Consequently, plants become sensitive to drought and susceptible to nutrient deficiency. Wrobel and Jankowska-Huflejt (2006) point out that the persistence of nine grass species from the grass and legume family is largely related to weather conditions, especially low rainfall.

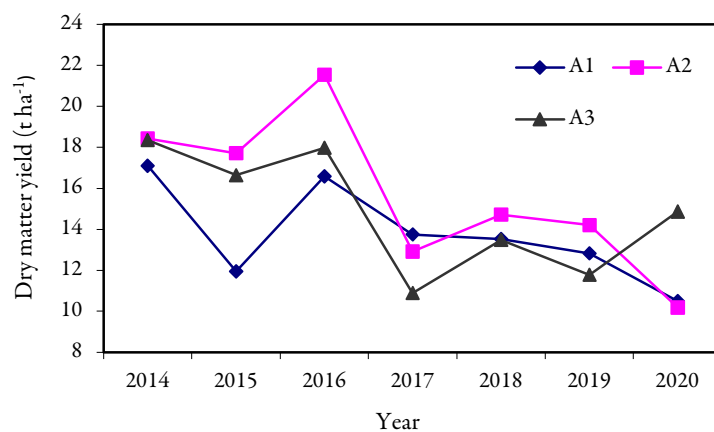


Figure 1. Dry matter yield of the grass-clover mixture for all cuts in the period 2014-2020

In the first year, in the first cut at the A3 treatment, a significant decrease in the proportion of tall oat grass in the dry matter of the forage was recorded compared to the control treatment (Table 2). At the same time, the proportion of red clover increased in both soil liming treatments and weeds in the A3 treatment. In the second cut, a decrease in the proportion of tall oat grass with an increase in the proportion of red clover was observed in both treatments with soil liming and a decrease in the proportion of weeds only in treatment A3. In the third cut, soil liming did not affect the proportion of grasses and weeds, but the proportion of red clover

was significantly higher in treatment A3. Grasses tend to tolerate acidic soils better than legumes so a pH value of 5.5 can be tolerated without reducing yields. However, legumes require more Ca and perform best at a pH between 6.5 and 7.5 (Agegnehu *et al.*, 2021). However, according to Yang *et al.* (2021), red clover is characterized by a slightly higher tolerance to acidic soils compared to most other legumes. The results of Tomić *et al.* (2019) show that in the variant with a pure red clover crop, the application of soil liming did not significantly influence the change in the red clover proportion in any cut, while in the mixture with Italian ryegrass, soil liming had a positive effect on its proportion. Quantitatively, N₂ fixation in a grass-clover mixture is a product of two factors: the proportion of clover and the intensity of symbiotic activity. Both are strongly influenced by the competition between grass and clover, as the clover content is determined by the competition for light above the soil surface and the symbiotic activity by the competition for inorganic N in the soil (Kristensen *et al.*, 2022).

In the second year of production (2015), in the first cut, the proportion of tall oat grass and red clover did not differ significantly between treatments, and there were no weeds. In the second cut, both levels of soil liming led to a significant increase in the proportion of tall oat grass and weeds at the expense of a decrease in the proportion of red clover. The reason for this is the better development of tall oat grass in the second year, as it is a perennial grass, and the greater indirect influence of soil liming on tall oat grass in the second year. The cultivation of grasses in a mixture with legumes leads to an increase in the biogenicity of the soil and the fixation of a considerable amount of nitrogen by bacteria of the genus *Rhizobium*, which can be utilized by the plants (Kurhak *et al.*, 2020). The increased biogenicity of the soil also has an effect on increasing the mineralization of organic matter in the soil (Grigorova-Pesheva and Hristov, 2021), which contributes to the more intensive growth of grasses and the achievement of higher yields. In this experiment, it took some time to establish a positive effect of increasing the biogenicity of the soil and the mineralization of organic matter, which had a positive effect on the growth of tall oat grass from the second year of cultivation. Considering that it is a grass of a tall stem, it competed more strongly with red clover, the proportion of which decreased to a lesser extent. This is confirmed by the results of Tomić *et al.* (2019), according to which in the variant with red clover and Italian ryegrass, the proportion of Italian ryegrass in the total hay yield increased significantly in all cuts due to the application of soil liming at the expense of reducing the proportion of red clover, which is a consequence of the greater competitiveness of Italian ryegrass compared to red clover under optimal conditions.

In the third year of production, a similar trend to the second year continued. In the first cut, the only significant reduction in the proportion of red clover was observed in the treatments with soil liming compared to the control treatment. In the second cut, the proportion of tall oat grass increased significantly in treatment A2 compared to the control treatment, the proportion of red clover decreased in both treatments with soil liming, and the proportion of weeds did not differ significantly between treatments. As with the second cut in the second year of production, the decrease in the proportion of red clover is the result of a pronounced reaction of the tall oat grass to soil liming. The growing conditions and the timing of the occurrence of dry and rainy periods in the second and third year of production were also similar. The better response of tall oat grass to soil liming in the second and third year compared to red clover may be related to drier conditions compared to the first year of production, a stronger indirect influence of soil liming due to a higher biogenicity of the soil, and the maximum development of the species considering the fact that it is a perennial grass. Also, as red clover contains more water, it reacted better to soil liming when there was more rainfall.

In the fourth year, the trend from the previous two years was changed. In both cuts, there was a significant increase in the proportion of tall oat grass and a decrease in the proportion of weeds in both treatments with soil liming. This year, a small amount of precipitation was recorded, and a very long and pronounced dry period from the end of May until the end of the growing season, which contributed to the crop being worse and the yield reduced. In such conditions, soil liming had a favorable indirect effect on the development of tall oat grass plants. The proportion of red clover, for which this was the last year of life was significantly lower in treatment A2 compared to treatments A1 and A3.

In the fifth year of production (2018) and beyond, there was still no red clover, which led to an increase in the proportion of weeds. In 2018, the proportion of tall oat grass increased in the first and second cuts in treatments with soil liming compared to the control at the expense of a reduction in the proportion of weeds. In contrast, in the sixth year of production (2019), the proportion of tall oat grass decreased significantly in the first cut and increased in the second cut in the A2 treatment compared to the control. The decrease in the proportion of tall oat grass in the first cut may be related to the very high rainfall recorded during the growth phase of the plants, which increased weed growth, especially in the A3 variant with the largest amount of lime applied. In the seventh year of production (2020), the proportion of tall oat grass in the second cut was significantly reduced in the A3 treatment compared to the control. At this time, there was a depletion and thinning of the tall oat grass, which normally grows 6-7 years old. In addition, a more severe dry period followed in the second cut, which further contributed to the weaker development of tall oat grass, weeds favoured by soil liming developed, and their proportion in the A3 treatment was over 30%. In the eighth year of life, tall oat grass plants were rare, and weediness was high, indicating that the exploitation life of the crop was over, so no further measurements were taken.

Observed by year, the proportion of tall oat grass was generally lowest in the first cut in the first year of production (Figure 2).

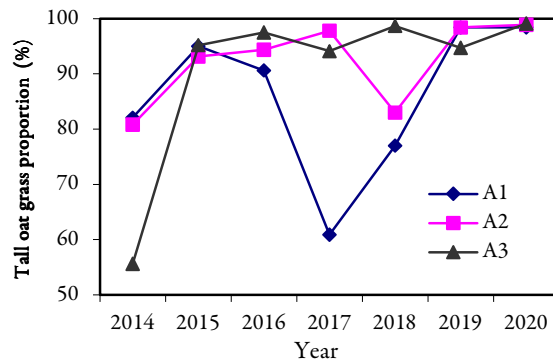


Figure 2. Tall oat grass proportion in the first cut in the period 2014-2020

Then it is the lowest proportion of tall oat grass was recorded in treatment A3, which is a consequence of the increased proportion of weeds in this treatment (Figure 6). In the second and third production years, the proportion of tall oat grass was high and generally did not vary more depending on soil liming, to further in the fourth year of production, which was very dry, it also remained at a similar level in treatments A1 and A2, while in the control treatment, its proportion was significantly lower (Figure 2). In the fifth year of production, a lower proportion of tall oat grass was found in the A2 treatment as well as in the control treatment. Under these conditions, with very high rainfall during plant development and taking into account the absence of red clover, the proportion of tall oat grass was highest in the treatment with the highest lime application. In the sixth and seventh years of the first cut, the proportion of tall oat grass did not vary significantly depending on the application of soil liming and generally remained at a high level. In the second cut, in general, the proportion of tall oat grass was lower compared to the first cut, at the expense of a higher proportion of red clover (Figure 3).

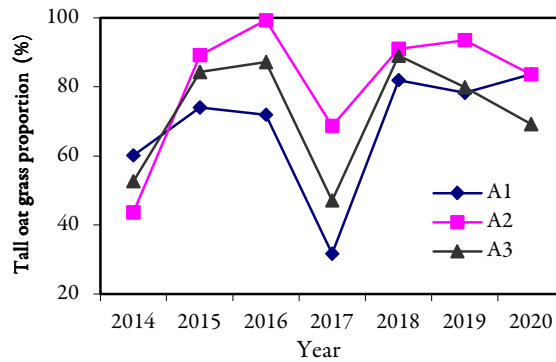


Figure 3. Tall oat grass proportion in the second cut in the period 2014-2020

As in the first cut, the proportion of tall oat grass was also higher in the second and third years compared to the first year. In the fourth year of production (2017), when there was a particularly severe drought during the growth of the second cut, a decrease in the proportion of tall oat grass was then observed in all treatments at the expense of a higher proportion of red clover and more drought-tolerant weeds. In 2018, 2019, and 2020, the proportion of tall oat grass increased again in the second cut in all treatments, as red clover was no longer present.

The proportion of red clover in the first cut was generally highest in the year the crop was established and decreased in later years (Figure 4). The reason for this is that tall oat grass grows more slowly than red clover in the first year and develops predominantly vegetatively, while the generative stems, which have the greatest influence on yield, only develop in the second year. This is a consequence of the increase in yield of tall oat grass, whose competitive ability is slightly more pronounced compared to clover (Gselman *et al.*, 2022). In the last year of the existence of red clover (2017), at the time of growth of the first cut, there was rainy weather with a higher amount of precipitation, which is why its proportion was partially increased. In the second cut, a similar trend to the first cut was recorded (Figure 5).

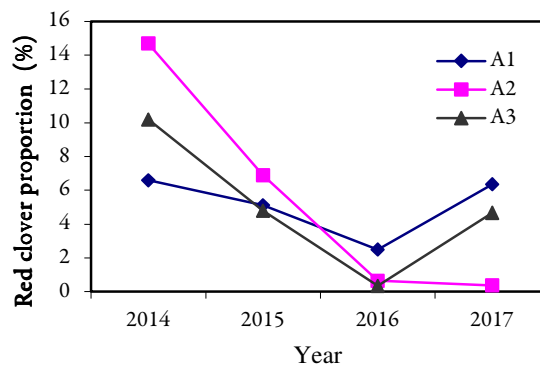


Figure 4. Clover proportion in the first cut in the period 2014-2017

The proportion of red clover was highest in the year the crop was established, then decreased in the following two years, and in the last year of the red clover's existence, an increase in its proportion was recorded again, which was due to a decrease in the proportion of tall oat grass. In all years, the red clover had a larger proportion in the second cut than in the first. The reason for this is its greater tolerance to dry conditions that occurred during the second growth. Similar results were provided by Tomić *et al.* (2019), according to which a significantly higher forage yield was recorded in the treatment with a pure culture of red clover compared to the grass-clover mixture in pronounced drought conditions. According to the authors, this is a consequence of the significantly higher tolerance of red clover to drought compared to grass and indicates that red clover is the

carrier of the yield under dry conditions. Also, tall oat grass in the second cut mainly develops only vegetative stems, which contributes that the proportion of red clover in the second cut would be higher than in the first cut.

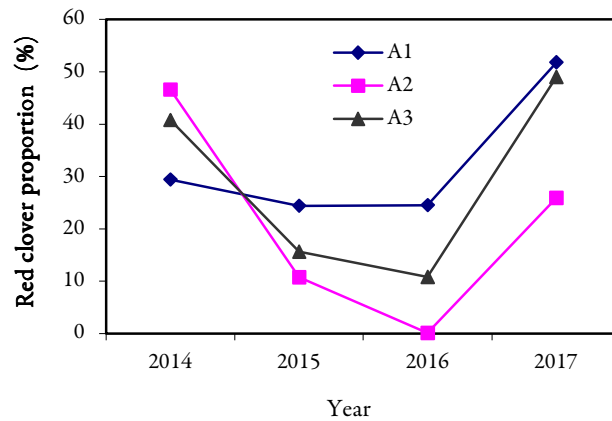


Figure 5. Clover proportion in the second cut in the period 2014-2020

The proportion of weeds in both cuts also followed the proportion of red clover. In the year of establishment of the crop, in all treatments, a generally high proportion of weeds was recorded in all cuts (Figures 6 and 7).

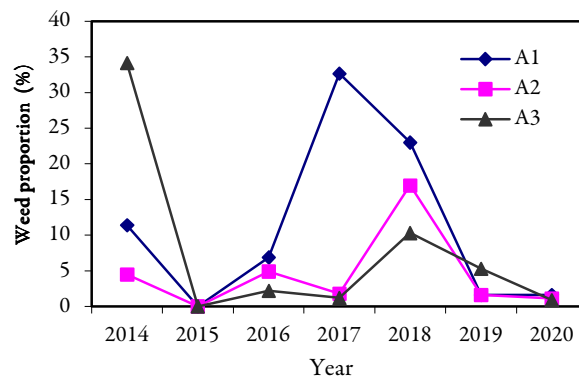


Figure 6. Weed proportion in the first cut in the period 2014-2020

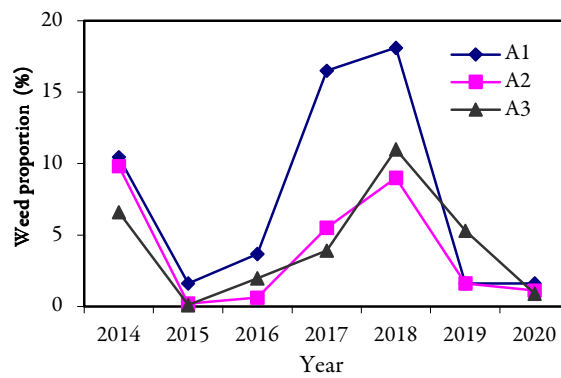


Figure 7. Weed proportion in the second cut in the period 2014-2020

However, already in the second and third year of production, the weed proportion in both cuts was minimal in all treatments thanks to the strong competition from tall oat grass and red clover. Thereafter, the weed proportion increased in both cuts until 2018 due to the gradual disappearance of red clover, followed by a gradual decrease in weed proportion until the end of the trial. The highest weed proportion in most years in both cuts was recorded in the control treatment. Liu *et al.* (2022) state that the application of soil liming leads to changes in the floristic composition of plant communities. The authors found that the highest weed presence in barley occurred consistently in the control variant without soil liming, in which the increased acidity of the soil and the negative effects of the increased content of slightly mobile Al led to a massive decline in the plants and a decrease in their competitiveness. According to the same authors, the change in soil pH had no significant effect on its fertility but did have a significant effect on the above-ground biomass, species richness, diversity, and biomass of the dominant species.

Conclusions

The response of the crop to soil liming was due to the interaction between the amount of lime applied, the amount of rainfall, and the stage of development of tall oat grass and red clover during the life cycle. The positive effect of soil liming on yield was more pronounced under more favourable growing conditions, i.e. when the plants had a better water supply. Red clover responded better to soil liming in the first year and with sufficient water supply, and tall oat grass in dry conditions. Under concrete conditions, the greatest positive effects of soil liming were recorded in the second and third years of production. The residual effects of soil liming lasted for five to seven years. Although it was expected that clover percentage and nitrogen fixation intensity would generally increase with the application of soil liming, this study showed that in some cases they responded differently and non-linearly to the application of lime. Soil liming generally contributed to reducing the proportion of weeds in the total dry matter yield. By applying soil liming and growing red clover in a mixture with fast growing grasses such as tall oat grass, with properly utilized and crop care measures, a stable production of forage can be achieved. Considering also that such grasslands are minimally fertilized and contribute to the recovery of the soil from intensive use, it can be concluded that they can be a very important component in the development of a sustainable farming system.

Authors' Contributions

Conceptualization: Dalibor Tomić, Vladeta Stevović, Vladimir Zornić. Methodology: Dalibor Tomić, Dragan Đurović. Writing-review and editing: Dalibor Tomić, Vladeta Stevović, Milomirka Madić. Resources: Miloš Marjanović, Đorđe Lazarević, Nenad Pavlović. Supervision: Dalibor Tomić, Vladeta Stevović. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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