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CHEMICAL COMPOSITION OF *TRIFOLIUM REPENS* L. FROM PERMANENT GRASSLANDS IN RELATION TO SOME CHEMICAL SOIL PROPERTIES

Jacek Alberski, Marzenna Olszewska

Department of Grassland and Green Space Management
University of Warmia and Mazury in Olsztyn
pl. Łódzki 1/17, 10-718 Olsztyn
e-mail: alberj@uwm.edu.pl

A b s t r a c t. The objective of this study was to determine the relationship between selected soil properties and the chemical composition of *Trifolium repens* in swards of permanent grasslands in the Olsztyn Lakeland. The study was carried out in 2005-2008 on permanent grasslands with a high share of legumes in the sward. A total of 26 meadow and pasture sites were investigated where the share of *Trifolium repens* in the sward ranged from 5% to 25% (2 points on the Braun-Blanquet cover scale). Phytosociological releves were done by the Braun-Blanquet method in selected grasslands in the most representative phytocenoses covering an estimated area of 25 m^2. Soil samples were collected at a depth of 5-15 cm for analyses of the physical and chemical properties of soil. Samples of plant material of *Trifolium repens* were also collected from each treatment for chemical analyses. Analyses of soil composition and nutrient content in plant material were carried out with the standard methods. Bulk density, actual moisture content and water-holding capacity were determined by the oven-drying and gravimetric method, acidity was measured in KCl solution with the concentration of 1 mol dm^{-3}, phosphorus and potassium content were determined by the Egner-Riehm method, magnesium content – by the Schachtschabel method, calcium and sodium content – by the universal method proposed by Nowosielski, and copper, zinc, manganese and iron content – by atomic absorption spectrometry (AAS). Plant material was analysed to determine the content of total nitrogen – by the Kjeldahl method, crude fibre – by the Henneberg-Stohman method, phosphorus – by the vanadium-molybdenum method, potassium, calcium and sodium – by flame photometry, magnesium and micronutrients by AAS. The highest number of communities with *Trifolium repens* were noted on light and medium textured soils with high and very high content of Mg, moderate abundance of Ca and micronutrients, moderate and low levels of P, low content of K and slightly acidic pH. Dry matter of *Trifolium repens* was characterised by high total protein content and optimal crude fibre content. A mineral composition analysis revealed optimal levels of phosphorus, magnesium and sodium, excessive content of potassium, calcium and manganese, and deficiency of copper, zinc and iron. A significant relationship between the chemical properties of soil and the content of total protein, crude fibre and mineral components in white clover dry matter was confirmed.

K e y w o r d s: chemical composition, grasslands, *Trifolium repens*, soil

INTRODUCTION

White clover is an important sward component of grazed grasslands. The plant is resistant to treading and chewing, it increases crop yields and improves the quality and palatability of feed (Goliński *et al.* 2006, Hennessy *et al.* 2012, Sarunaite *et al.* 2012, Trąba *et al.* 2012). White clover is characterised by high protein content and lower fibre content in comparison with other species of the legume family. It is a source of valuable macronutrients, micronutrients and vitamins, and it is willingly consumed by animals due to its high palatability (Grzegorczyk 1999). White clover establishes symbiotic relationships with rhizobia that fix free nitrogen from air, and the plant's significance has been recently recognised in sustainable agriculture. The species does not require nitrogen fertilisation. At an estimated 1% share of sward, white clover supplies the soil with approximately 3 kg N ha^{-1} per year. Decomposing root nodules and root secretions make nitrogen available to grasses, which reduces nitrogen fertilisation costs (Ledgard 1991, Ledgard and Steele 1992, Warda and Krzywiec 2000, Gaweł 2011, Phelan *et al.* 2012). Approximately 20% to 70% of released nitrogen can be fixed by legumes (Mallarino *et al.* 1990, Vance 1998). The transfer of nitrogen to grasses is determined by numerous factors, mostly habitat conditions. In the rapidly growing branch of sustainable agriculture, *Trifolium repens* will be sown on pastures for ruminants, it will be used on arable land as a cover crop, as well as on fallow land and in pastures for non-ruminants. Optimal habitat conditions for white clover and their impact on the species nutritive value should be investigated to contribute to the success of sustainable farming practices.

The objective of this study was to determine the relationship between selected soil properties and the chemical composition of *Trifolium repens* in swards of permanent grasslands in the Olsztyn Lakeland.

MATERIALS AND METHODS

The study was carried out in 2005-2008 in the Olsztyn Lakeland on permanent grasslands with a high share of legume plants. The experiment covered 26 meadow and pasture sites where the share of *Trifolium repens* in the sward ranged from 5% to 25% (2 points on the Braun-Blanquet cover scale). The study was performed in spring during the first harvest. Phytosociological releves were done by the Braun-Blanquet method in selected grasslands in the most representative phytocenoses covering an estimated area of 25 m^2. Phytocenoses with *Trifolium repens* was analyzed on very light (3), light (9), medium (12) and heavy soils (2). Soil samples were collected at a depth of 5-15 cm for analyses of the physical and chemical properties of soil. Samples of plant material of *Trifolium repens* were also collected from each

treatment for chemical analyses. Analyses of soil composition and nutrient content in plant material were carried out with the standard methods. Bulk density, actual moisture content and water-holding capacity were determined by the oven-drying and gravimetric method, acidity was measured in KCl solution with the concentration of 1 mol dm^{-3}, phosphorus and potassium contents were determined by the Egner-Riehm method, magnesium content – by the Schachtschabel method, calcium and sodium content – by the universal method proposed by Nowosielski, and copper, zinc, manganese and iron content – by atomic absorption spectrometry (AAS). Plant material was analysed to determine the content of total nitrogen – by the Kjeldahl method, crude fibre – by the Henneberg-Stohman method, phosphorus – by the vanadium-molybdenum method, potassium, calcium and sodium – by flame photometry, magnesium and micronutrients by AAS. Coefficients of correlation between selected chemical properties of soil and the mineral composition of *Trifolium repens* dry matter were calculated. The statistical significance of correlation coefficients was evaluated based on the critical values of Spearman's rank correlation coefficient at $\alpha = 0.05$(*) and $\alpha = 0.01$(**).

RESULTS AND DISCUSSION

The evaluated communities occupied mineral soils belonging to different categories in the agronomic soil classification system. The highest number of phytocenoses with *Trifolium repens* was noted on light (9) and medium soils (12). White clover was less predominant on very light (3) and heavy soils (2). Regardless of soil class, *Trifolium repens* was encountered on slightly acidic soils with high content of Mg, moderate levels of P, Ca and micronutrients, and low abundance of K (Tab. 1). Numerous studies have demonstrated that soils overgrown by grasses with a high share of legumes are characterised by low content of phosphorus and potassium and high abundance of magnesium and micronutrients (Kryszak and Grynia 2001, Trąba and Wolański 2003, Trąba *et al.* 2012).

The evaluated treatments were characterised by high species diversity, and 91 species were identified in the sward of the analysed phytocenoses. The most common species in meadow-grassland phytocenoses with *Trifolium repens* were grasses – *Poa pratensis* L., *Dactylis glomerata* L., *Festuca pratensis* Huds., *Phleum pratense* L. and *Festuca rubra* L., legumes – *Trifolium pratense* L., herbaceous plants and weeds – *Taraxacum officinale* F. H. Wigg., *Achillea millefolium* L. and *Plantago lanceolata* L.

Protein content is one of the key criteria for evaluating the nutritional value of feed. A nutritionally balanced feed ration should have a total protein content of 125 g kg^{-1} dry matter (Falkowski *et al.* 2000). In this study, total protein content was estimated at 177 g kg^{-1} dry matter, and it remained fairly stable throughout the study. The coefficient of variation for total protein content was determined at 10-12%

(Tab. 2). Crude fibre content is also an important feed component that largely determines nutrient digestibility and utilisation. The average crude fibre content in white clover harvested from the analysed treatments was estimated at 264 g kg^{-1} dry matter, and it remained fairly stable throughout the study period (coefficient of variation – 11.4%). The noted crude fibre concentrations are regarded as optimal in cattle diets. Variations in total protein content and crude fibre content of legumes can be attributed to numerous factors, including the plant's development stage at the time of sampling, weather conditions, water and soil conditions, and the accompanying species which influence overall digestibility of legumes (Grzegorczyk 2000).

Table 1. Some physical and chemical properties of soils

Specification	Value min-max	Mean	Coefficient of variation
	g cm^{-3}		%
Bulk density	0.91-1.66	1.35	17.2
		%	
Actual moisture content	5.8-57.5	26.14	48.2
Capillary water capacity	22.2-66.4	42.21	28.1
Maximum water capacity	22.9-68.8	43.78	29.4
pH_{KCl}	4.4- 7.1	5.5	17.1
	g kg^{-1}		%
Humus	1.13-5.28	2.50	45.2
N	1.00-3.30	1.35	54.6
P_2O_5	0.01-0.23	0.06	96.6
K_2O	0.03-0.20	0.09	55.7
Mg	0.02-0.14	0.07	48.3
Ca	0.24-3.68	0.82	119.0
Na	0.08-0.15	0.12	118.0
	mg kg^{-1}		%
Cu	1.1-8.7	3.9	57.4
Mn	80.2-704.5	174.7	69.1
Zn	4.1-26.0	13.2	40.4
Fe	742.0-8858.0	1790.0	83.6

An analysis of the mineral composition of white clover dry matter revealed optimal content of phosphorus, magnesium and sodium, excessive levels of potassium, calcium and manganese, and deficiency of copper, zinc and iron (Tab. 2). Similar results were noted in a study of nutrient content in white clover harvested from semi-natural meadows and pastures (Trąba and Wolański 2003). Kitczak (2000) demonstrated high calcium and magnesium levels and low zinc content in *Trifolium repens*. Zinc deficiency often results from its low availability due to high soil pH, high phosphorus levels in the habitat and the biological characteristics of

various plant species (Bowszys *et al.* 2009). In this study, biomass samples differed significantly in their sodium, manganese and iron content, which was manifested by high coefficients of variation in those traits.

Table 2. Content of nutrients in dry matter of *Trifolium repens* L.

Specification	Value min-max	Mean	Coefficient of variation
	g kg^{-1}		%
Total protein	140.1-200.8	177.0	10.4
Crude fibre	206.4-314.3	264	11.4
P	1.5-5.6	3.6	30.6
K	15.4-44.6	30.4	21.8
Mg	1.7-3.0	2.0	16.2
Ca	12.5-26.0	19.0	19.6
Na	0.1-8.1	1.9	87.9
	mg kg^{-1}		%
Cu	4.0-10.4	7.1	20.0
Mn	30.4-185.8	84.5	57.1
Zn	20.3-58.9	30.7	28.2
Fe	60.6-633.3	201.3	56.1

The calculated coefficients of correlation between soil nutrient abundance and chemical composition of *Trifolium repens* biomass confirmed a significant positive relationship between soil pH, organic matter content and N, K, Mg, Ca and Cu content and protein accumulation in plants. Zinc levels in soil were negatively correlated with protein content of white clover, but contributed to the accumulation of crude fibre which, consequently, lowered dry matter digestibility (Tab. 3). Reverse relationships were noted for magnesium. High magnesium levels in soil contributed to protein accumulation, decreased fibre content and improved digestibility. The results of this study indicate that the chemical properties of soil had a varied influence on the macronutrient and micronutrient content in *Trifolium repens* dry matter (Tab. 4). White clover harvested from treatments with a higher soil pH was characterised by lower manganese content. Manganese is highly sensitive to changes in soil pH which influence the element's availability for plants. Plants growing on soils with a near-neutral pH can be deficient in manganese (Laser 2007, Sapek 2009). In this study, higher content of N, P, K, Mg, Ca, Na and Cu in soil also inhibited manganese accumulation in plants. The organic matter content of soil was positively correlated with sodium, copper and iron concentrations in plants, and the accumulation of those elements was also promoted by higher nitrogen levels in soil. A negative relationship between phosphorus and calcium and also between potassium and magnesium content in *Trifolium repens* plant material was confirmed. Those elements are mutually

antagonistic, and they can mutually block their uptake by plants under specific conditions. The abundance of Cu, Mn and Fe in soil contributed to the accumulation of copper and iron in plants, and iron also significantly influenced sodium levels. The results of this study indicate that habitat conditions have a significant influence on the chemical composition and nutritive value of white clover.

Table 3. The relationship between soil properties and total protein, crude fibre content and dry matter digestibility of *Trifolium repens* L.

Properties of soils	Total protein	Crude fibre	Digestibility
pH_{KCl}	0.476^{**}		
Humus	0.363^{*}		
N	0.421^{*}		
K_2O	0.363^{*}		
Mg	0.590^{**}	-0.580^{**}	0.610^{**}
Ca	0.366^{*}		
Cu	0.424^{*}		0.338^{*}
Zn	-0.386^{*}	0.606^{**}	-0.596^{**}

Table 4. Significant coefficients of correlation between chemical properties of soils and chemical composition of dry matter of *Trifolium repens* L.

Properties of soils	K	Na	Ca	Mg	Cu	Mn	Fe
pH_{KCl}						-0.765^{**}	
Humus		0.569^{**}			0.440^{*}		0.481^{**}
N		0.510^{**}			0.413^{*}	-0.363^{*}	0.465^{**}
P_2O_5			-0.334^{*}			-0.506^{**}	
K_2O	0.366^{*}			-0.370^{*}		-0.472^{**}	
Mg					0.382^{*}	-0.408^{*}	
Ca						-0.520^{**}	
Na		0.696^{**}				-0.390^{*}	
Cu					0.429^{*}	-0.373^{*}	0.389^{*}
Mn					0.387^{*}		0.661^{**}
Fe		0.364^{*}			0.542^{**}		0.745^{**}

CONCLUSIONS

1. The highest number of plant communities with *Trifolium repens* were noted on light and medium soils characterised by high content of Mg, moderate abundance of Ca, P and micronutrients, low levels of K and slightly acidic pH.

2. Dry matter of *Trifolium repens* was characterised by high total protein content and optimal crude fibre content. Mineral composition analysis revealed optimal content of phosphorus, magnesium and sodium, excessive levels of potassium, calcium and manganese, and deficiency of copper, zinc and iron.

3. A significant relationship between the chemical properties of soil and the content of total protein, crude fibre and mineral components in white clover dry matter was confirmed.

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SKŁAD CHEMICZNY *TRIFOLIUM REPENS* L. Z TRWAŁYCH UŻYTKÓW ZIELONYCH W ZALEŻNOŚCI OD NIEKTÓRYCH WŁAŚCIWOŚCI GLEBY

Jacek Alberski, Marzenna Olszewska

Katedra Łąkarstwa i Urządzania Terenów Zieleni
Uniwersytet Warmińsko-Mazurski w Olsztynie
Pl. Łódzki 1/17, 10-718 Olsztyn
e-mail: alberj@uwm.edu.pl, olszewska.marzenna@uwm.edu.pl

S t r e s z c z e n i e. Celem podjętych badań było wykazanie zależności między niektórymi właściwościami gleby a składem chemicznym *Trifolium repens* z trwałych użytków zielonych Pojezierza Olsztyńskiego. Badania przeprowadzono w latach 2005-2008 na trwałych użytkach zielonych ze znacznym udziałem roślin motylkowatych w runi. Badaniami objęto 26 obiektów łąkowo – pastwiskowych, w runi których pokrycie powierzchni przez *Trifolium repens* wynosiło od 5 do 25% (2 – w skali Brauna-Blanqueta). Na wytypowanych użytkach zielonych, w najbardziej reprezentacyjnych płatach roślinnych, na powierzchni ok. 25 m^2 wykonano zdjęcia fitosocjologiczne metodą Brauna-Blanqueta. Z warstwy 5-15 cm pobrano próby glebowe do określenia właściwości fizycznych i chemicznych gleby. Z każdego obiektu pobrano również materiał roślinny *Trifolium repens* do analiz chemicznych. Analizy gleby i zawartości składników pokarmowych w roślinie wykonano według ogólnie przyjętych metod. Gęstość objętościową, wilgotność aktualną i pojemność wodną – metodą suszarkowo-wagową, kwasowość – w roztworze KCl o stężeniu 1 $mol \cdot dm^{-3}$, fosfor i potas – metodą Egnera-Riehma, magnez- metodą Schachtschabela, wapń i sód - metodą uniwersalną Nowosielskiego, miedź, cynk, mangan i żelazo – metodą absorpcyjnej spektrometrii atomowej (ASA). W materiale roślinnym oznaczono: azot ogólny – metodą Kiejdahla, włókno surowe – metodą Henneberga Stohmana, fosfor – metodą wanadowo-molibdenianową, potas, wapń i sód – metodą fotopłomieniową, natomiast magnez i mikroelementy – metodą ASA. Badania wykazały, że najwięcej zbiorowisk z udziałem *Trifolium repens* zanotowano na glebach lekkich i średnich o bardzo wysokiej i wysokiej zasobności w Mg, średniej w Ca i mikroelementy, średniej i niskiej zasobności P, niskiej zasobności K oraz lekko kwaśnym odczynie gleby. Sucha masa *Trifolium repens* charakteryzowała się wysoką zawartością białka ogólnego i optymalną zawartością włókna surowego. W składzie mineralnym stwierdzono optymalną zawartość fosforu, magnezu i sodu, zbyt dużą zawartość potasu, wapnia i manganu oraz niedobór miedzi, cynku i żelaza. Obliczone współczynniki korelacji świadczą o istotnej zależności między właściwościami chemicznymi gleby a zawartością białka ogólnego i włókna surowego oraz składników mineralnych w suchej masie koniczyny białej.

S ł o w a k l u c z o w e: skład chemiczny, użytki zielone, *Trifolium repens*, gleba