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Mineral nitrogen content in the soil and winter wheat productivity as influenced by the pre-crop grass species and their management

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Abstract

The current paper presents the effects of perennial grasses (*Trifolium pratense* L., *Medicago sativa* L., x *Festulolium)* as preceding crops for winter wheat and their aboveground biomass management methods (removed from the field, mixed, mulched) on mineral nitrogen (N_{inorg}) content and dynamics in the soil as well as on winter wheat grain yield and quality under organic farming conditions. Field experiments were carried out at the Joniškėlis Experimental Station of the Lithuanian Institute of Agriculture (LIA) on an *Endocalcari-Endohypogleyic Cambisol* (*CMg-n-w-can*). Chemical analyses were done at the LIA's Laboratory of Chemical Research. Experimental evidence indicated that under mixed management of the aboveground biomass of red clover and lucerne, when the herbage of the first cut had been used as forage and that of the second and third cuts as green manure, the soil received 208.0–215.8 kg ha^{-1} of nitrogen. When all the herbage had been used as green manure (mulching method), the soil received 266.6–298.0 kg ha^{-1} of nitrogen. The content of symbiotically fixed nitrogen in the biomass of legumes accounted for the largest share (60.8–83.6%) of the total nitrogen content, accumulated in the biomass. Incorporation of the biomass of perennial grasses influenced an increase in N_{inorg} content in the soil late in the autumn and was 1.2–2.0 times or 9.6–39.0 kg ha^{-1} higher compared to that prior to the ploughing in of grasses. In spring, in most cases, the content of N_{inorg} in the soil (having removed the herbage yield or having mulched all the herbage) tended to decrease compared with that measured late in the autumn. The highest N_{inorg} content was identified having used mixed management of the aboveground biomass of legumes or having mulched all biomass of legumes and that of legume/festulolium mixture. Winter wheat grain yield depended on the N_{inorg} content in the soil late in the autumn and early in spring ($r = 0.632$, $r = 0.783$ respectively; $P > 0.05$). Festulolium and its mixtures with legumes as pre-crops reduced winter wheat yield compared with red clover. The application of the total aboveground biomass as green manure (mulching) increased the grain yield by approximately 0.62 t ha^{-1}, while part of the aboveground biomass applied as green manure (mixed management) increased the grain yield by 0.30 t ha^{-1}, compared to the treatment with the total herbage yield removed from the field. Protein and gluten content in organically grown winter wheat grain as well as sedimentation index significantly depended on the perennial grass pre-crop species and the aboveground biomass management method. These parameters improved when wheat had been grown after a legume pre-crop and perennial grasses had been used as green manure. Mixed management for clover and mulching for lucerne were found to be the practices that secured a higher concentration of protein in winter wheat grain.

Key words: perennial grasses, green manure, N_{inorg}, winter wheat yield, grain quality.

Introduction

In recent years, the application of the EU and national support aids as well as consumers' interest in safe food, have stimulated the development of organic farming in Lithuania. Therefore, it is important to satisfy consumers' expectations and offer healthy products of the highest quality. Organic farming, unlike conventional farming, has complex relationships between different components of the agro-system and the quantity and quality of the end products depend on the functioning of the entire system (Watson et al., 2002). German researchers have reported that the base of organic farming should have multi-structural composition with legumes (N_2 fixation – 83 kg ha^{-1} per year), cattle (density – 1.4 LU ha^{-1}) and production, oriented towards the closed cycle of nutrient circulation (Kustermann et al., 2010). Organic farming with appropriate management sustained soil fertility, augmented soil C (36% increase), enhanced N retention (50% decrease in nitrate N leaching) and improved N use efficiency, compared to conventional or integrated farming (Snapp et al., 2010).

Some researchers suggest that legumes that can support biological N_2 fixation, offer a more environmentally sound and sustainable source of N to cropping systems (Jensen, Hauggaard-Nielsen, 2003; Herridge et al., 2008). Many researchers have indicated that one tone of biomass of dry legumes (aboveground and underground) can approximately bind 30–40 kg of nitrogen from the air (Peoples et al., 2009). This is one of the main nitrogen sources in organic farms. Perennial legume grasses overtake grain legumes by nitrogen fixation from the atmosphere, increase productivity of crop rotation plants, and improve soil properties (Watson et al., 2002). Hence, the application of biomass of perennial legumes in stockless organic farming, whose area is growing, has been limited. The greatest challenge for such farms is management of the nutrient supply (Watson et al., 2002). One of the nitrogen sources for crop rotation plants is the aboveground biomass used as green manure (Watson et al., 2002; Arlauskienė, Maikštėnienė, 2005).

Within the sequence of crops from legumes to non-legumes, it is important to assess the synchronic features of nitrogen release from the incorporated crops' residues or green manure and need for nitrogen of cultivated crops (Crews, Peoples, 2005). The intensity of the incorporated biomass mineralization is indicated by mineral nitrogen (N_{inorg}) content and dynamics in soil. The amount of nitrate accumulated in the soil during legume cropping is susceptible to agronomic management (Connor et al., 2010). Together with an increase in N_{inorg} content in the soil, the yield of crops increases (Crews, Peoples, 2005). The summarized data evidenced that crops can assimilate 4–33% of nitrogen from legume residues during the first year (Peoples et al., 2009). Micro-organisms also immobilize part of nitrogen (8.2–10.6% of total N accumulated in biomass) from the residues of legumes; nitrogen becomes available for plants after the wheat anthesis growth stage (Mayer et al., 2003).

The N content of grain grown without N fertiliser was highly significantly related to the reserves of available N in the soil. Growing cereals in a leguminous living mulch bicropping could potentially reduce the need for synthetic inputs in cereal production while preventing losses of nutrients and increasing soil biological activity (Burke et al., 1998). The protein content is a purely quantitative analysis that may or may not be indicative of protein quality. The quality of protein complex in view of baking utilisation is partly characterised by gluten quantity and quality and Zeleny's sedimentation test.

Research data on the effect of legume residues, incorporated into soil as well as aboveground biomass on nitrogen leaching are controversial. Compared with intensive application of mineral nitrogen fertilisers, inclusion of perennial legumes into a crop rotation reduces the risk of nitrogen leaching. Studies that have simultaneously compared the fate of both sources of N suggest that in rain-fed agricultures, crops recover more N from mineral fertiliser, but a higher proportion of the legume N is retained in the soil (Crews, Peoples, 2005). However, the findings of foreign and Lithuanian researchers indicate that incorporation of biomass of legumes for winter wheat can lead to mineral nitrogen leaching during the period of autumn–winter–early spring, due to a rapid mineralization of biomass, rich in nitrogen (Mayer et al., 2003; Tripolskaja, Šidlauskas, 2010). Austrian scientists suggest that under the conditions of warmer and longer autumn, N_{inorg} content in the 0–150 cm soil layer can reach 100–200 kg ha^{-1} (Farthofer et al., 2003). Studies, carried out in Lithuania on a sandy loam *Luvisol*, indicated that incorporation of green manure (lupine or clover aftermath) for winter cereals increased the decomposition rate of organic matter and nitrogen leaching to the subsoil (25–100 cm) in October and November by on average 24–63%, compared to stubble ploughing in (Tripolskaja, 2005). In heavy-textured soil, incorporation of aftermath of perennial grasses (lucerne and clover) as green manure did not increase N_{inorg} content in the 0–40 soil layer in spring, however, a significant grain yield increase was obtained (Arlauskienė, Maikštėnienė, 2005). This enables us to presume that mineralization of incorporated organic manures in clay loam soils is slower.

This article deals with the application of the biomass of perennial legumes as green manure using the mulch technology. The aboveground biomass of perennial legumes is mulched in the soil surface 2–4 times per growing season. The herbage is cut, chopped and spread in order to use the biologic nitrogen, bound by legumes more efficiently, and to protect the environment from pollution (Farthofer et al., 2003). Chopped biomass of nitrogen-rich legumes rapidly mineralises, the released nitrogen is bound by intensively growing perennial grasses or is incorporated into the soil organic matter (Schäfer et al., 2001), which prevents it from being leached. The present study was aimed to analyze the effects of perennial legumes and their aboveground biomass, managed as green manure on the change of soil N_{inorg} as well as the impact on winter wheat grain yield and quality under organic farming conditions.

Materials and methods

Site and soil. Experiments were carried out during 2007–2009 at the LIA's Joniškėlis Experimental Station on an *Endocalcari-Endohypogleyic Cambisol* (*CMg-n-w-can*), with a texture of clay loam (clay particles <0.002 mm in Ap horizon 0–30 cm make up 27.0%), on silty clay with deeper lying sandy loam. Parental rock is limnoglacial clay on morenic clay loam at the depth of 70–80 cm. Bulk density of the ploughlayer is 1.5 Mg m^{-3}, total porosity 41–43%, air-filled porosity 15–17%. Agrochemical characteristics of the ploughlayer: pH_{KCl} – 6.4, mobile P_2O_5 and K_2O – 154 and 224 mg kg^{-1} of soil respectively, N_{total} – 0.135%, C_{org} – 1.68%. Soil and plant analyses were done at the Laboratory of Chemical Research at LIA.

Experimental design and field management. Research was conducted in the following sequence of the crop rotation: barley + undersown perennial grasses → perennial grasses → winter wheat. Factor A: perennial grasses: 1) red clover (*Trifolium pratense* L.) (control, aboveground biomass removed from the field), 2) mixture of red clover and festulolium (x *Festulolium*), 3) lucerne (*Medicago sativa* L.), 4) mixture of lucerne and festulolium, 5) festulolium. Factor B: management methods of aboveground biomass of perennial grasses: 1) removed from the field, 2) mixed management, 3) mulching.

In the first experimental year (2007), spring barley (*Hordeum vulgare* L., cv. 'Ula') was undersown with perennial grasses in compliance with the experimental design: red clover (cv. 'Vyliai', at a seed rate of 7.5 million ha^{-1}), lucerne (cv. 'Birutė', at a seed rate of 7.5 million ha^{-1}), intergeneric hybrid festulolium (cv. 'Punia', at a seed rate of 6.2 million ha^{-1}) and mixtures of both legume grasses with festulolium (seed rate ratio of legume to grass 2:1). In the first treatment (B1) of application of the aboveground biomass of perennial grasses, the grass was cut twice at the beginning of flowering: on 10 06 2008 and 25 08 2008 and removed from field. In the second treatment (B2), the aboveground biomass was used under mixed management: the first grass was cut at the beginning of flowering (10 06 2008) and removed from the field, the second and third cuts were taken during perennial grass inflorescence growth stage (17 07 2008, 12 08 2008) and mulched on the soil surface. In the third treatment (B3), the grass was cut every 30–40 days four times (12 05 2008, 13 06 2008, 11 07 2008, 12 08 2008) and mulched on the soil surface. Aboveground biomass for green manure was cut by a self-propelled mower, equipped with a mulching device, then chopped and evenly spread on the plot. In the second half of August, the plots of all treatments were disked and 2 weeks later were ploughed at the 25 cm depth. Before sowing, the field was cultivated and harrowed at the same time. Winter wheat (*Triticum aestivum* L., cv. 'Tauras') was sown at a seed rate of 220 kg ha^{-1}. Organic wheat cultivation technology was applied.

Soil analyses. Soil samples for the determination of N_{inorg} ($N\text{-}NH_4 + N\text{-}NO_3$) content were collected from the 0–60 cm layer before ploughing in of perennial grasses (28 08 2008), late in the autumn (12 11 2008), early in spring after resumption of wheat growth (08 04 2009) and after wheat harvesting (11 08 2009). N_{inorg} was determined: $N\text{-}NH_4$ by spectrophotometric method, $N\text{-}NO_3$ by ionometric method (ISO/TS 14256-1:2033).

Plant analyses. After each cut, the aboveground biomass of perennial grasses was weighed. To determine the root macro-fraction biomass of plants, monoliths 0.25 x 0.25 x 0.24 m in size were dug out in the plots of each treatment replicated three times (Лапинскене, 1986). The roots were washed, dried, and air-dry weight was determined. Samples of the aboveground and underground biomass were taken for the determination of dry matter (dried to a constant weight at 105ºC), nitrogen and organic carbon. Elementar analyzer "Vario EL" and "Carry 50" were used for the determination of nitrogen and carbon in biomass. Having determined nitrogen concentration in the biomass, we calculated the amount of nitrogen, incorporated into the soil (kg ha^{-1}). Nitrogen content (N_2 kg ha^{-1}) fixed from the atmosphere in legume biomass was calculated using the method of difference according to the following formula: $N_2 = N_{legumes} - N_{festulolium}$ (Herridge et al., 2008).

Grain quality analyses. Grain protein content in dry matter was calculated by multiplying the corresponding total nitrogen (by Kjeldahl) content by a factor 5.7 (LST EN ISO 20483) using a semi-automated N analyser “Kjeltec system 1002” (“Foss Tecator” AB, Sweden). Sedimentation value was measured by Zeleny (LST ISO 5529). Wet gluten quantity (reported on a 14% grain moisture basis) was extracted from the whole meal flour in an automated gluten washer and gluten index determined by Perten (LST 1571 identical to ICC 155; “Glutomatic 2100”, “Centrifuge 2015”, “Perten Instruments”, Sweden). Falling number (α-amylase activity) was determined according to Hagberg-Perten method (LST ISO 3093), with the “Falling Number 1500” (“Perten Instruments”, Sweden).

Weather conditions. In 2008, during the plant growing season (May–August), the average daily air temperature and precipitation rate varied a little compared to the annual average, except for May, which was dry (precipitation level by 32.7 mm lower) and August, which was wet (precipitation level by 48.6 mm higher). Perennial grasses grew well. Dry weather (precipitation level 6.5 mm) settled in September. The month’s average daily temperature was by 0.6ºC lower compared to the annual average. Therefore, wheat, sown after ploughing in of grasses, emerged and developed slowly. October was warmer and wet (temperature was higher by 1.8ºC, precipitation rate exceeded annual level by 29.3 mm). High precipitation during this period (daily precipitation rate of 29.5 mm) might have influenced migration of nutrients from incorporated green manure into deeper soil layers. In 2009, the winter was warmer compared to the annual average: the temperature during all months was by 1.8–2.5ºC higher than the annual average. The highest precipitation was recorded in December. March was wet and cold. Last days of March had a positive daily air temperature, which was below +5ºC. Vegetation of winter wheat was resumed. It was warmer and dryer in April and May, the period when winter wheat reaches tillering and accumulates dry matter during the process of intensive growth. Average daily air temperature was by 2.2 and 0.6ºC higher than annual average; precipitation rate during the two months was 47.6 mm lower. June and July were warm and with excessive humidity (precipitation rate 80.9 and 107.6 mm, respectively); the mentioned above factors influenced winter wheat yield. Repetitive showery rains occurred in July; which might have been responsible for the lower quality of winter wheat.

Results and discussion

Nitrogen content. The content of nitrogen incorporated into the soil was significantly dependent on the species of perennial grasses and aboveground biomass management method. The soil received the highest nitrogen content with the ploughed in biomass of pure legumes (Table 1). Cultivation of legumes in mixtures with festulolium tended to reduce the nitrogen content, incorporated into the soil. Significantly (3.2 and 3.4 times) less nitrogen was incorporated into the soil with the biomass of pure festulolium compared to red clover and lucerne, respectively.

When the aboveground biomass of red clover had been used under mixed management, the total amount of nitrogen incorporated was 215.8 kg ha^{-1}, while during the process of mulching – 298.0 kg ha^{-1} N, respectively 8.5 and 11.7 times more, compared to the plot with removed herbage. With the aboveground biomass of red clover and festulolium mixture used under mixed management and mulching, the soil received less nitrogen compared to pure red clover sward with the same management of the aboveground biomass. Lucerne, during the first year of cultivation, formed a greater root biomass, though the aboveground biomass increased more rapidly only during the second half of summer. As a result, after lucerne cultivation, the incorporated nitrogen content was lower compared to that after red clover. Moreover, the effect of the biomass management methods on the incorporated nitrogen content was different. When the aboveground biomass of lucerne had been used under mixed management, the total incorporated N content was 208.0 kg ha^{-1}, while in mulching treatment 266.6 kg ha^{-1} N, or respectively 2.1 and 2.7 times more than in the treatments with all herbage yield of lucerne removed from the field. Cultivation of lucerne/festulolium mixtures and use of their aboveground biomass under mixed management influenced the fact, that the soil received by 36.2 kg ha^{-1} less nitrogen, while after mulching by 14.9 kg ha^{-1} more compared to pure lucerne sward with the same management of the aboveground biomass. When the aboveground biomass of festulolium had been used under mixed management and mulched, the soil received 63.8 and 81.4 kg ha^{-1}, or 2.8 and 3.6 times more nitrogen, respectively, compared with the treatment with all festulolium herbage removed from the field. However, the total nitrogen, accumulated in festulolium biomass, was removed from the soil.

Table 1. Nitrogen content, incorporated into the soil with the biomass of perennial grasses and biomass C:N ratio

Joniškėlis Experimental Station, 2008

Perennial grasses (factor A)		Management methods of perennial grasses (factor B)					
		removal from field		mixed		mulching	
		N kg ha^{-1}	C:N	N kg ha^{-1}	C:N	N kg ha^{-1}	C:N
RC	underground biomass	25.4	26.4	67.4	21.9	44.3	20.6
	aboveground biomass			148.4	18.1	253.7	17.1
RC + F	underground biomass	35.5	43.9	43.0	33.7	39.2	36.2
	aboveground biomass			113.6	25.6	219.1	22.5
L	underground biomass	99.5	24.5	59.0	28.0	51.6	28.7
	aboveground biomass			149.0	16.6	215.0	14.8
L + F	underground biomass	79.6	42.6	51.0	44.3	86.4	38.5
	aboveground biomass			120.8	25.0	195.1	23.3
F	underground biomass	22.5	83.2	32.8	79.9	26.0	71.8
	aboveground biomass			31.0	36.1	55.4	43.1

Note. RC – red clover, F – festulolium, RC + F – red clover + festulolium, L – lucerne, L + F – lucerne + festulolium.

Carbon to nitrogen (C:N) ratio, which is an indicator, describing mineralization of biomass of perennial grasses, incorporated into soil, was dependent on the species and plant part. Plant root biomass analysis indicates that the highest C:N ratio was in festulolium (71.8–83.2), the lowest in pure legume (red clover 20.6–26.4 and lucerne 24.5–28.7) roots, irrespective of the aboveground biomass management methods. Some researchers (Möller et al., 2008) suggest that nitrogen content in the biomass of plants, cultivated in mixtures with legumes, increased, while C:N ratio of the root biomass decreased. The C:N ratio of red clover mulch was 17.1–18.1 and that of lucerne mulch 14.8–16.6. When the mentioned legumes had been cultivated in mixtures with festulolium, the C:N ratio of the herbage mulch increased. This ratio in the mulch of festulolium was the highest (36.1–43.1).

Summarized data indicate that lucerne annually fixes from 300 to 600 kg ha^{-1}, red clover from 150 to 300 kg ha^{-1} of symbiotic nitrogen in their total biomass (Lapinskas, 2008). Experiments, conducted on a moderately heavy loam *Cambisol*, indicate that one ha of lucerne can fix 245 kg ha^{-1} of atmospheric nitrogen per year, while red clover up to 254 kg ha^{-1} (Kadžiulienė, 2001). Our research evidence suggests that symbiotic nitrogen content, fixed from the air by red clover was: if the aboveground biomass was removed from field – 137.9 kg ha^{-1}, if mixed management was applied – 182.4 kg ha^{-1}, if mulching was applied – 191.4 kg ha^{-1}; meanwhile lucerne – 206.5, 204.4 and 251.9 kg ha^{-1}, respectively. Depending on the aboveground biomass management method, symbiotic nitrogen (N_2) fixation efficiency in red clover biomass was 60.8–83.4%, in lucerne 68.1–83.6%. Assessment of management methods of the aboveground biomass of perennial grasses showed that the symbiotic nitrogen content in plant biomass was the highest when mulching of perennial legumes had been used. Hence, this method of the aboveground biomass management cannot be assessed unambiguously, because reoccurring perennial grasses could use mineralized nitrogen from previously spread mulch. According to Hatch et al. (2007), mulching of the aboveground biomass of clover/grass led to reduction of relative part of fixed nitrogen in biomass.

Dynamics of N_{inorg} change. At the end of summer, prior to the grass swards ploughing, the content of N_{inorg} was not high (27.1–40.6 kg ha^{-1}) in the soil layer 0–60 cm, while its highest content was in red clover and lucerne cultivation treatments (Table 2).

When legumes were cultivated in mixtures with festulolium, N_{inorg} tended to decrease. Significantly lower amount of N_{inorg} was found after festulolium, i.e. by on average 25.1% less compared to that in the soil after red clover sward. Comparison of individual perennial grasses' management methods revealed that if the whole aboveground biomass had been mulched in the soil surface, N_{inorg} tended to increase (except for the plots of red clover), compared to the respective data when herbage had been removed from the field.

Table 2. The amount of N_{inorg} in the 0–60 cm soil layer as affected by perennial grass species and aboveground biomass management methods before grass swards ploughing
Joniškėlis Experimental Station, 2008

Perennial grasses (factor A)	Management methods of perennial grasses (factor B)					
	removal from field		mixed		mulching	
	N_{inorg} kg ha^{-1}	NO_3 / NH_4	N_{inorg} kg ha^{-1}	NO_3 / NH_4	N_{inorg} kg ha^{-1}	NO_3 / NH_4
RC	40.6	3.2	43.2	2.6	38.1	3.2
RC + F	33.8	2.9	41.2	1.8*	37.7	1.7*
L	34.5	2.7	37.8	2.6	38.5	4.8*
L + F	33.9	2.6	35.3	2.7	38.6	3.4
F	27.1*	2.3	33.6	1.9*	30.6	2.3
LSD_{05} A					5.79	0.67
LSD_{05} B					4.48	0.52
LSD_{05} AB					10.03	1.15

Notes. RC – red clover, F – festulolium, RC + F – red clover + festulolium, L – lucerne, L + F – lucerne + festulolium.
* – significant at $p < 0.05$.

The nitrate to ammonium nitrogen ratio (NO_3 / NH_4) in the soil was significantly influenced by the species of perennial grasses, biomass management method and interaction between these factors. Averaged data indicated significantly lower NO_3 / NH_4 ratio when festulolium as well as its mixtures with red clover had been cultivated. The lowest ratio was recorded when the aboveground biomass had been used under mixed management, while when it had been mulched, an increase was observed. The highest nitrate (NO_3) nitrogen content accumulated in the soil when lucerne herbage biomass had been mulched.

Winter wheat was sown after perennial grasses' ploughing in. Late in the autumn (mid-November), N_{inorg} was measured in the 0–60 cm soil layer and the findings indicated that its content increased by 1.2–2.0 times or 9.6–39.0 kg ha^{-1}, compared to N_{inorg} content determined at the end of August (Table 3). The highest increase in N_{inorg} content, compared to that before perennial grasses' ploughing in, was recorded: when herbage yield had been removed from the field – after legumes; aboveground biomass had been used under mixed management – after mixture of festulolium and legume; the aboveground biomass had been mulched – in all treatments of legumes and their mixtures with festulolium. Research was performed on a silt loam gleyic *Cambisol* and it indicated that the most intensive decomposition during the first three months occurred in the plant residues containing more nitrogen (Velička et al., 2006). The data considerably varied between the treatments and replications, therefore only the trends of change were identified. Literature sources indicate that nitrogen content leached during the autumn period depends on the precipitation and soil type and fertility management, and ranges from 4.2–12 kg ha^{-1} in the soil with a high clay content (20–60%) (Smith et al., 1998) to 30–70 kg ha^{-1} (Thomsen et al., 1993) in sandy soils.

Averaged data show that the lowest N_{inorg} content in the soil was after festulolium 39.6 kg ha^{-1}, i.e. 33.7% or 37.2% less than after red clover or lucerne. When all aboveground herbage biomass had been removed from the field, the amount of N_{inorg} varied in a similar way to that obtained prior to ploughing of grasses (increased after legume crops), but when it had been used for green manure N_{inorg} content differed. If part of the aboveground biomass of perennial grasses had been used as green manure (mixed management), the highest N_{inorg} content was measured after red clover or its mixture with festulolium. When all aboveground biomass had been mulched, the highest N_{inorg} content accumulated after lucerne and its mixture with festulolium. When all aboveground biomass of perennial grasses, mentioned above, had been used under mixed management, N_{inorg} content was the lowest compared to that under other management methods.

Table 3. The amount of N_{inorg} in the 0–60 cm soil layer in autumn as influenced by perennial grass species and aboveground biomass management methods

Joniškėlis Experimental Station, 2008

Perennial grasses (factor A)	Management methods of perennial grasses (factor B)								
	removal from field			mixed			mulching		
	N_{inorg} kg ha^{-1}	difference[1] kg ha^{-1}	NO_3 / NH_4	N_{inorg} kg ha^{-1}	difference[1] kg ha^{-1}	NO_3 / NH_4	N_{inorg} kg ha^{-1}	difference[1] kg ha^{-1}	NO_3 / NH_4
RC	69.4	28.8	4.7	50.5	7.3	3.5	59.1	21.0	4.0
RC + F	43.4	9.6	2.9	69.2	27.9	5.6	68.7	31.0	7.6*
L	66.5	32.0	5.2	45.1	6.3	3.3	77.4	39.0	7.1
L + F	52.9	19.0	3.2	49.1	13.7	3.8	72.6	33.9	6.5
F	34.1	7.0	2.3	38.9	5.3	3.4	45.7	14.9	3.7
LSD_{05} A							20.52	17.77	1.60
LSD_{05} B							15.89	13.76	1.24
LSD_{05} AB							35.53	30.77	2.77

Notes. RC – red clover, F – festulolium, RC + F – red clover + festulolium, L – lucerne, L + F – lucerne + festulolium. * – significant at $p < 0.05$. [1] – difference, compared to the data prior to the ploughing in of grasses (28 08 2008).

With increasing N_{inorg} content in the soil, the relative part of nitrate nitrogen increased in most cases. The NO_3 / NH_4 ratio in the soil was significantly influenced by the methods of aboveground biomass management. Average data indicate that this ratio increased from 3.7 when herbage yield had been removed from the field to 5.8 when all aboveground biomass had been mulched. The highest nitrate nitrogen content was observed when red clover/festulolium, lucerne/festulolium as well as aboveground biomass of lucerne had been managed as mulch; the lowest – after festulolium (irrespective of the aboveground biomass management methods).

In spring, the amount of N_{inorg} in the 0–60 cm soil layer reduced (4.6–22.5%) in most cases, compared to its status in the autumn (Table 4). The greatest reduction in N_{inorg} content was noted in the treatments with the highest NO_3 to NH_4 ratio and the highest nitrate nitrogen content in the autumn (except for the plot with incorporated mulch of lucerne and festulolium mixture. Similar research conducted in Denmark showed that mulch, incorporated into a light-textured soil increased the risk of nitrogen leaching during the autumn-winter period (Olesen et al., 2009). Tripolskaja and Šidlauskas (2010) have indicated, that incorporation of red clover biomass as green manure in a sandy loam *Luvisol* significantly increased nitrogen leaching in spring (52.4%), and summer (52.0%) compared to the plot without organic manure.

Nevertheless, the change in N_{inorg} content in the soil was significantly influenced by the species of perennial grasses and the aboveground biomass management method. Comparison of perennial grass species evidenced significantly lower N_{inorg} content in the soil after cultivation of festulolium (on average 16.2 kg ha^{-1} or 29.1% lower) and its mixture with red clover (8.8 kg ha^{-1} or 15.9% lower), compared to pure red clover. Averaged data indicate that the effect of red clover, lucerne and their mixture with festulolium on N_{inorg} content was similar. When all aboveground biomass of perennial grasses had been used as green manure (mulching), N_{inorg} content in the soil significantly increased (by on average 13.4 kg ha^{-1} or 30.5%). When only part of the aboveground biomass had been used as green manure (mixed management), N_{inorg} content increased (by on average 6.2 kg ha^{-1} or 14.2%), compared to the plot with all herbage yield of perennial grasses removed from the field.

The NO_3 to NH_4 ratio in the soil in spring declined from 2.3–7.6 to 1.5–3.7 compared to that in the autumn. Incorporation of mulch of all perennial grasses (except for red clover/festulolium sward) had an effect on increase of nitrate content in N_{inorg} structure compared to the other aboveground biomass management methods. The lowest NO_3 content accumulated in the soil after festulolium herbage yield had been removed from the field or when part of it had been used as green manure (mixed management) compared to the control treatment.

Table 4. The amount of N_{inorg} in the 0–60 cm soil layer in spring as influenced by perennial grass species and aboveground biomass management methods

Joniškėlis Experimental Station, 2009

Perennial grasses (factor A)	Management methods of perennial grasses (factor B)								
	removal from field			mixed			mulching		
	N_{inorg} kg ha^{-1}	difference[1] kg ha^{-1}	NO_3 / NH_4	N_{inorg} kg ha^{-1}	difference[1] kg ha^{-1}	NO_3 / NH_4	N_{inorg} kg ha^{-1}	difference[1] kg ha^{-1}	NO_3 / NH_4
RC	49.3	−20.1	2.9	60.3	9.8	2.7	57.3	−1.9	3.5
RC + F	43.1	−0.3	2.2	46.7	−22.4	2.5	50.5	−18.2	2.3
L	46.4	−20.1	2.5	53.8	8.7	2.4	64.2	−13.3	3.7
L + F	43.4	−9.5	2.2	52.7	3.7	2.3	70.3*	−2.3	3.7
F	37.1	4.0	1.5*	37.0	−1.9	1.7*	44.1	−1.5	2.3
LSD_{05} A							8.76	22.19	0.59
LSD_{05} B							6.79	17.19	0.46
LSD_{05} AB							15.17	38.43	1.02

Notes. RC – red clover, F – festulolium, RC + F – red clover + festulolium, L – lucerne, L + F – lucerne + festulolium. * – significant at $p < 0.05$. [1] – difference, compared to the data late in the autumn (12 11 2009).

After winter wheat harvesting, soil N_{inorg} content in the 0–60 cm layer, compared to that in early spring, was a little higher when herbage yield of perennial grasses had been removed from the field. When the biomass had been managed as mulch, the N_{inorg} content was lower, and when it had been used under mixed management N_{inorg} varied (Table 5).

Table 5. The amount of N_{inorg} in the 0–60 cm soil layer after winter wheat harvesting as influenced by perennial grass species and aboveground biomass management methods

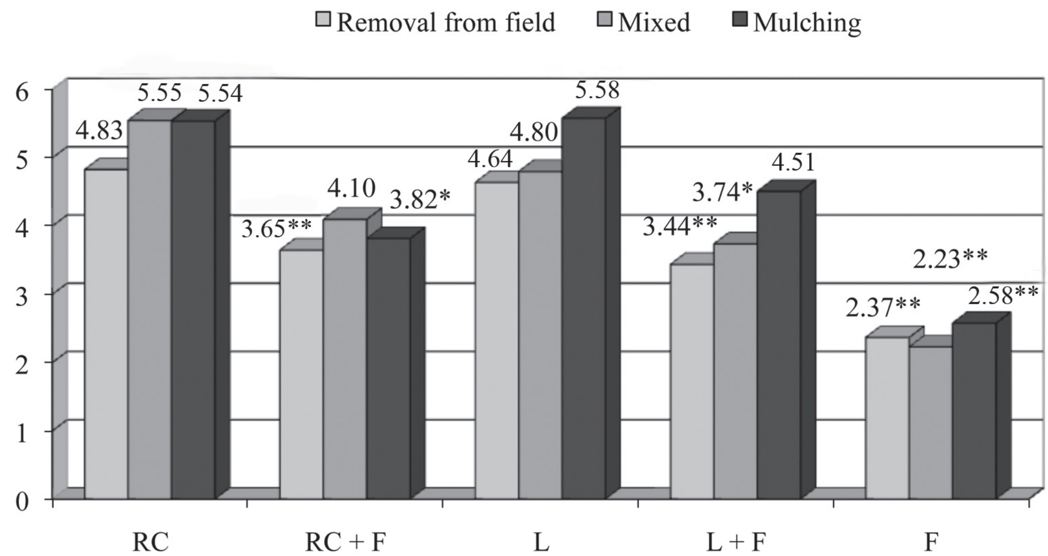
Joniškėlis Experimental Station, 2009

Perennial grasses (factor A)	Management methods of perennial grasses (factor B)								
	removal from field			mixed			mulching		
	N_{inorg} kg ha^{-1}	difference[1] kg ha^{-1}	NO_3 / NH_4	N_{inorg} kg ha^{-1}	difference[1] kg ha^{-1}	NO_3/ NH_4	N_{inorg} kg ha^{-1}	difference[1] kg ha^{-1}	NO_3 / NH_4
RC	51.3	2.0	1.0	42.1	–18.2	1.0	52.2	–5.0	1.1
RC + F	47.2	4.0	1.3	46.3	–0.4	1.2	41.2	–9.2	1.3
L	51.9	5.4	1.1	42.7	–11.1	1.0	51.0	–13.2	1.0
L + F	50.5	7.2	1.0	43.8	1.0	0.9	51.8	–18.5	1.1
F	50.2	13.1	1.0	47.6	10.6	1.1	42.1	–2.0	1.3
LSD_{05} A							10.05	14.29	0.23
LSD_{05} B							7.78	11.07	0.18
LSD_{05} AB							17.40	24.76	0.40

Notes. RC – red clover, F – festulolium, RC + F – red clover + festulolium, L – lucerne, L + F – lucerne + festulolium. [1] – difference, compared to the data early in spring (08 04 2009).

After winter wheat harvesting, soil N_{inorg} content was significantly influenced by the management methods of the aboveground biomass of perennial grasses. Soil N_{inorg} content was 42.1–52.2 kg ha^{-1} and varied a little between treatments. The highest content of plant-available nitrogen was after pure legumes and lucerne/festulolium. Comparison of the management methods of perennial grasses evidenced that when all aboveground biomass had been used for green manure or under mixed management N_{inorg} reduced, compared to its status in the plots, where all herbage yield of perennial grasses had been removed. The obtained data indicate that after harvesting of winter wheat, the nitrate nitrogen content was significantly lower, NO_3 / NH_4 ratio changed from 1.5–3.7 (in spring) to 0.9–1.3 (after harvesting) and varied a little between treatments. Winter wheat exhibited better assimilation of N_{inorg}, accumulated in the soil, because of longer growing season and length of roots to 2.2 m; moreover, much less of N_{inorg} migrates to deeper layers (Thorup-Kristensen et al., 2009).

Winter wheat yield. The productivity of winter wheat was significantly influenced by the pre-crops – perennial grasses as well as their aboveground biomass management method. Comparison of various perennial grasses, used as pre-crops, revealed that winter wheat grain yield was the lowest after festulolium, significantly by on average 55.0% or 2.92 t ha^{-1} lower compared to that after red clover (Fig.). Application of their aboveground biomass as green manure did not increase grain yield appreciably. The highest grain yield was produced when winter wheat had been cultivated after legumes. Legume/festulolium swards reduced winter wheat yield: red clover/festulolium by on average 27.3% or 1.45 t ha^{-1} and lucerne/festulolium by on average 26.6% or 1.11 t ha^{-1}, compared to respective pure legume sward.



Notes. RC – red clover, F – festulolium, RC + F – red clover + festulolium, L – lucerne, L + F – lucerne + festulolium. LSD_{05} A = 0.500, LSD_{05} B = 0.387, LSD_{05} AB = 0.865; * – significant at $p < 0.05$, ** – significant at $p < 0.01$.

Figure. The grain yield of winter wheat as influenced by perennial grass species and aboveground biomass management methods

Joniškėlis Experimental Station, 2009

Averaged data indicated that application of all herbage of perennial grasses as green manure (mulching) significantly increased the grain yield by 16.1% or 0.62 t ha^{-1}. When part of the aboveground biomass had been used as green manure (mixed management), the grain yield tended to increase, the increase amounted to on average 7.7% or 0.30 t ha^{-1}, compared to the treatment where all herbage had been removed from the field. Norwegian researches suggest that with the application of green manure for mulch (legume/non-legume) no significant increase in spring barley grain yield was obtained (Frøseth et al., 2008), meanwhile German researchers point out that red clover/grass mulch significantly increased wheat grain yield by 5.5%, compared to the treatments where all herbage had been removed from the field (Dreymann et al., 2003). The efficiency of nitrogen application from legume/festulolium mulch varied from 14% to 39% with the lowest value on the coarse sandy soil (Olesen et al., 2009).

The highest winter wheat grain yield was produced when the crop had been cultivated after red clover with aboveground biomass used under mixed management or mulched. The grain yield increase was by 0.72 and 0.71 t ha^{-1} respectively, higher compared to that in the control plot. After lucerne pre-crop, the wheat grain yield was lower (0.37 t ha^{-1}) than that after red clover. It might have been influenced by higher root biomass and its C:N ratio. This fact influenced slower mineralization of organic matter and reduced N_{inorg} accumulation in the soil (except for aboveground biomass mulching), compared to red clover. After lucerne sward, the greatest wheat grain yield increase was obtained when the aboveground biomass was used for mulch.

When the aboveground biomass of red clover/festulolium was used under mixed management or mulched, the grain yield increased, but not as much as in the treatments where it was cultivated after red clover with all herbage removed from the field. When the aboveground biomass of lucerne/festulolium had been used as green manure, especially when all of it was used as mulch, the grain yield increased and was similar to that after lucerne with herbage yield removed from the field.

Winter wheat grain yield significantly ($P > 0.05$) correlated with soil inorganic nitrogen content. Grain yield relationship with N_{inorg} content in spring after resumption of winter wheat vegetation was strong ($r = 0.783$), with N_{inorg} determined late in the autumn the relationship was moderate ($r = 0.632$). There was no relationship between the grain yield and N_{inorg} determined after winter wheat harvesting.

Grain quality. It is difficult to estimate the baking potential of wheat samples with a protein content below 12% because dough mixing properties are non-linear (Fowler, Kovacs, 2004). Our research evidenced that winter wheat grain harvested in 2009 accumulated 10.4–11.9% protein in dry matter, its sedimentation index was 20.5–28.0 ml and the gluten content was 9.1–18.8% on a 14% grain moisture basis (Table 6). Accumulation of protein in winter wheat grain significantly depended on perennial grass species and aboveground biomass management method. Better values were determined for wheat grain indirect parameters, characterizing bread-making quality – higher values of crude protein (0.3–0.5% unit), wet gluten content (3.0–3.1% unit) and higher values of the sedimentation index by Zeleny (2.6–4.4 ml), when the aboveground biomass of herbage had been used under mixed management or all of it was mulched, compared with grain characteristics, when herbage had been removed from the field. Moreover, the grain quality was higher when wheat had been cultivated after monocrops, especially legume (clover and lucerne) swards.

Table 6. Winter wheat grain protein content, sedimentation and wet gluten content as influenced by perennial grass species and aboveground biomass management methods

Joniškėlis Experimental Station, 2009

Perennial grasses (factor A)	Management methods of perennial grasses (factor B)								
	removal from field			mixed			mulching		
	protein %	sedimentation ml	wet gluten %	protein %	sedimentation ml	wet gluten %	protein %	sedimentation ml	wet gluten %
RC	10.6	20.9	11.3	11.3**	28.0**	18.0**	11.4**	24.3	16.3
RC + F	10.4	20.5	9.1	10.5	23.5	15.5	10.6	22.9	15.0
L	10.8	22.8	14.8	11.3*	27.0**	17.7	11.9**	25.0	18.8**
L + F	11.2*	21.5	14.6	11.1	26.6**	16.1	11.2*	24.5	18.8**
F	11.0	20.9	17.0	11.2*	23.5	14.9	11.3**	22.6	13.8
LSD_{05} A							0.28	2.82	3.70
LSD_{05} B							0.22	2.18	2.87
LSD_{05} AB							0.48	4.88	6.41

Notes. RC – red clover, F – festulolium, RC + F– red clover + festulolium, L – lucerne, L + F – lucerne + festulolium. * – significant at $p < 0.05$, ** – at $p < 0.01$.

Depending on the pre-crop, the highest protein content in winter wheat grain was having used red clover biomass under mixed management and mulching and that of lucerne only mulching. Analysis of individual quality parameters showed that sedimentation index in grain increased more significantly with the application of the mixed management, while for protein and gluten content with mulching of the aboveground biomass.

Different pre-crops of perennial grasses and their management methods did not have any significant effect on wheat gluten quality. The variation range of gluten index, describing gluten quality, was very narrow, from 93 to 99 units (Table 7). This indicates that the tested cultivar had gluten with very strong properties and could be acceptable for bread making.

Table 7. Winter wheat grain gluten index and falling number as influenced by perennial grass species and aboveground biomass management methods

Joniškėlis Experimental Station, 2009

Perennial grasses (factor A)	Management methods of perennial grasses (factor B)					
	removal from field		mixed		mulching	
	gluten index (GI) unit	falling number sec	gluten index (GI) unit	falling number sec	gluten index (GI) unit	falling number sec
RC	96.5	361	94.5	365	95.0	364
RC + F	99.3	346	96.3	359	93.5	364
L	95.5	359	93.5	358	95.5	373
L + F	94.5	352	93.3	353	96.3	381
F	93.5	343	94.0	354	94.5	338
LSD_{05} A					2.05	20.3
LSD_{05} B					1.58	15.3
LSD_{05} AB					3.54	35.1

Notes. RC – red clover, F – festulolium, RC + F – red clover + festulolium, L – lucerne, L + F – lucerne + festulolium. * – significant at $p < 0.05$, ** – at $p < 0.01$.

Another important wheat grain quality parameter, falling number, indicates the activity of α-amylase, which is related with biochemical grain germination processes. Wheat grain with a falling number below 220 seconds has a limited use (LTS 1524). The activity of α-amylase significantly depended on the weather conditions, delayed harvest, cultivar properties and grain biology, as well as on fertilisation rate. The falling number of wheat which could intake higher content of nitrogen was higher than that when lower N intake was used (Kindred et al., 2005; Cesevičienė, Mašauskienė, 2007). Falling number was generally high (357 s) and tended to increase (from 348 to 361 seconds) with increasing aboveground biomass application. However, no significant changes in the falling number resulting from the pre-crop or aboveground biomass management method were established.

Conclusions

1. With the aboveground biomass of red clover and lucerne used under mixed management, the soil received 215.8 and 208.0 kg ha^{-1} of nitrogen, respectively, while in the mulching treatments – 298.0 and 266.6 kg ha^{-1}, respectively. Symbiotically fixed nitrogen content in the biomass of legumes accounted for the largest share (60.8–83.6%) of the total nitrogen content, accumulated in the biomass. When legume mulch had been used as green manure, its C:N ratio was the lowest and more favourable for a more rapid decomposition (14.8–18.1) compared with that of festulolium mulch (C:N – 36.1–43.1).

2. Late in the autumn, after perennial grasses, the amount N_{inorg} in the soil was 1.2–2.0 times or 9.6–39.0 kg ha^{-1} higher compared to that present in the soil prior to the sward ploughing. In spring, N_{inorg} content in the 0–60 cm soil layer in most cases tended to decrease compared to that late in the autumn. Nevertheless, when all aboveground bio-

mass of perennial grasses had been used as green manure (mulching), the amount of N_{inorg} in the soil was significantly higher (by on average 13.4 kg ha^{-1} or 30.5%); when it had been used under mixed management N_{inorg} content tended to increase compared with the treatments with all herbage removed from the field.

3. Festulolium and its mixtures with legumes as pre-crop reduced winter wheat grain yield, compared with red clover. Application of all aboveground biomass of perennial grasses as green manure (mulching) increased grain yield by on average 0.62 t ha^{-1}; when part of the aboveground biomass had been used as green manure (mixed management) – by 0.30 t ha^{-1}, compared with the treatments with herbage removed from the field. The highest winter wheat yield was obtained when it had been cultivated after red clover and its aboveground biomass had been used under mixed management or when all aboveground biomass had been mulched (5.55 and 5.54 t ha^{-1}, respectively) as well as after lucerne when all aboveground biomass had been mulched (5.58 t ha^{-1}).

4. Better protein and gluten content in organically grown winter wheat grain as well as sedimentation index were achieved when wheat had been cultivated after legume pre-crops and when perennial grasses were used as green manure. The highest protein accumulation in winter wheat grain was recorded in the treatments with the use of the aboveground biomass of red clover under mixed management and lucerne mulching.

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Mineralinio azoto kiekis dirvožemyje ir žieminių kviečių produktyvumas priklausomai nuo daugiamečių žolių priešsėlio rūšies ir jų naudojimo

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Santrauka

Straipsnyje pateikti daugiamečių žolių (*Trifolium pratense* L., *Medicago sativa* L., x *Festulolium)* kaip javų priešsėlių ir jų antžeminės masės naudojimo (derliui bei žaliajai trąšai) įtaka $N_{min.}$ kiekiui ir dinamikai dirvožemyje, žieminių kviečių grūdų derliui bei kokybei ekologinio ūkininkavimo sąlygomis. Lauko bandymai vykdyti Lietuvos žemdirbystės instituto (LŽI) Joniškėlio bandymų stotyje giliau karbonatingame giliau glėjiškame rudžemyje (RDg4-k2), *Endocalcari-Endohypogleyic Cambisol* (*CMg-n-w-can*), cheminės analizės atliktos LŽI Cheminių tyrimų laboratorijoje. Nustatyta, kad raudonųjų dobilų ir mėlynžiedžių liucernų antžeminę masę panaudojus kombinuotai (pirmo pjovimo žolę – derliui, antro ir trečio – žaliajai trąšai), iš viso įterpta atitinkamai 215,8 ir 208,0 kg ha^{-1}, o visą žaliajai trąšai – atitinkamai 298,0 ir 266,6 kg ha^{-1} azoto. Ankštinių žolių biomasėje simbiotinis azotas sudarė didžiąją dalį (60,8–83,6 %) viso biomasėje sukaupto azoto kiekio. Daugiamečių žolių biomasės įterpimas vėlai rudenį dirvožemyje padidino $N_{min.}$ kiekį. Jis buvo 1,2–2,0 karto, arba 9,6–39,0 kg ha^{1}, didesnis, palyginti su buvusiu prieš žolių užarimą. Pavasarį $N_{min.}$ kiekis daugeliu atvejų (žolės derlių išvežus iš lauko arba visą mulčiavus) turėjo tendenciją mažėti, palyginti su gautu vėlai rudenį. Didžiausias $N_{min.}$ kiekis nustatytas ankštinių žolių antžeminę masę panaudojus kombinuotai arba visą ankštinių ir ankštinių bei eraičinsvidrių mišinių biomasę mulčiavus. Žieminių kviečių derlius priklausė nuo $N_{min.}$ kiekio dirvožemyje vėlai rudenį ir anksti pavasarį (atitinkamai $r = 0{,}632$, $r = 0{,}783$; $P > 0{,}05$). Eraičinsvidrių priešsėlis ir jų mišiniai su ankštinėmis žolėmis žieminių kviečių grūdų derlių mažino, palyginti su raudonųjų dobilų priešsėliu. Visos antžeminės masės žaliajai trąšai panaudojimas (mulčiavimas) derlių padidino vidutiniškai 0,62 t ha^{-1}, o žaliajai trąšai panaudojus dalį antžeminės masės (kombinuotas būdas) – 0,30 t ha^{-1}, palyginti, kai visas žolės derlius išvežtas iš lauko. Ekologiškai išaugintų žieminių kviečių grūdų baltymų ir glitimo kiekis bei sedimentacijos indeksas iš esmės priklausė nuo daugiamečių žolių priešsėlio rūšies ir jų antžeminės masės panaudojimo būdo. Šie rodikliai pagerėjo, kai kviečiai buvo auginti atitinkamai po ankštinių žolių priešsėlio ir kai daugiametės žolės panaudotos žaliajai trąšai. Nustatyta, kad didesniam žieminių kviečių grūdų baltyminių medžiagų kiekiui sukaupti tinkamesnis dobilams taikytas kombinuotas, liucernoms – mulčio metodas.

Reikšminiai žodžiai: daugiametės žolės, žalioji trąša, $N_{min.}$, žieminių kviečių grūdų derlius ir kokybė.