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Perennial forage legume cultivation and their above-ground mass management methods for weed suppression in arable organic cropping systems

Aušra Arlauskienė¹, Danutė Jablonskytė-Raščė¹, Lina Šarūnaitė^{2*} , Monika Toleikienė², Laura Masilionytė¹, Viktorija Gecaitė¹ and Žydrė Kadžiulienė²

Abstract

Background: In organic crop farms, growing crop yields are limited by insufficient nitrogen supply to plants and crop weediness. In such farms, legume swards are proposed as a service crop to improve nitrogen cycling. However, a positive effect of nitrogen is not only on cereals but also on weeds. In crop rotation, legume swards can stimulate the competition of cereals using the above-ground mass of legume to control the spread of weeds.

The effects of the following methods for weeds control were analyzed: (i) forage legumes (*Trifolium pratense* L. and *T. repens*) undersown in cereals, (ii) forage legumes (*T. pratense* L., *Medicago sativa* L.) and their mixture with festulolium (*Festulolium*) and their above-ground mass management methods, and (iii) plant-based fertilizers (red clover above-ground mass fermented and composted).

Results: Oat with red clover undersown reduced weediness more than red clover monocrops, pea, and their mixture with oats. Incorporated undersown white clover mass increased spring barley competitiveness with weeds. When growing legume swards for a longer period of time (green fallow), red clover and their mixture with festulolium are the most suitable for this purpose. The lowest weed dry weight (average 34%, compared with the removal from the field) was obtained while using the mixed management. The cultivation of cereals after forage legumes and their mixtures with festulolium (as a preceding crop) increases its grain yield and competitive ability against weeds. Fermented red clover and fermented pea and spring wheat mixture mass, as a manure, did not increase weediness.

Conclusions: It was concluded that the effectiveness of the perennial forage legumes is determined by the uses of the above-ground mass: soil cover, mulching, application of green manure, and intensity of mass mineralization. Type of activity of forage legumes on weeds were competition for environmental resources, disruption/promotion of germination, destruction of above-ground mass, reduction of the amount of matured seeds, creation of a physical barrier (mulch), and increase of competitiveness of cereals. Growing forage legumes in pure crops usually leads to a loss of marketable production.

Keywords: Clover, Farmyard manure, Lucerne, Mulch, Plant-based organic manure, Undersown

Background

Crop rotation diversification has high value to control weed populations and maintain its effectiveness under different environmental conditions and management scenarios [48] Crop rotations of arable organic farms

*Correspondence: lina.sarunaite@lammlt

² Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Kėdainiai District, Akademija, Lithuania
Full list of author information is available at the end of the article

are dominated by a small variety of plants (mostly cereals). Grain legume (pea, faba bean, and other) serve as a source of nitrogen. Lundkvist et al. [23] and Sinchana et al. [39] indicated that including peas in a crop rotation increased the risk of weed problems in the long term. The cultivation and agrotechnics of grain legumes are very close to those of cereals and are favorable for the spread of the same weed species [4]. This fact indicates that crop type modulates weed community composition and diversity [44]. Annual weeds are well adapted to such crops (cereals, grain legumes), they grow well in partial shade, have similar growing needs to the crops, and are able to mature and spread the seeds before harvest [30]; they are commonly referred to as cereals weeds [19]. Homogeneous of crops, low diversity of legume crops and short crop rotations also result in the problems associated with plant nutrients supply, disease and pest control, and declining soil fertility. This weakens crop growth, productivity, and competitive ability against weeds [17]. Weed spread represents a major source of inefficiency, diverting scarce resources (nutrients, water, light, and labor) and has been reported to result in approximately one-third of yield losses in major crops [5]. Mechanical control of weeds is most commonly applied, but it increases energy and labor costs.

Kolb and Gallandt [17] point out that the main weed management mechanisms include increasing the competitive advantage of cereals through practices that reduce weed density and increase interspecies competition.

Rotating crops with different planting dates appears to be important for reducing the size of seedbanks, presumably because it changes the timing of field activities [31] and gives priority to depletion of seedbanks through interfering with dormancy or germination requirements [8]. Therefore, the cultivation of plants that differ in architecture, growth intensity, and duration at the time of harvest is of great value [21].

Perennial legumes are a good preceding crop, improving soil properties, ensuring nitrogen supply to crop of rotation [45]. A better use of resources (especially nitrogen) by cultivated plants and growth stimulation are key conditions for indirect weed control, and ensure plant competitiveness and productivity [35, 38]. However, nitrogen also influences germination, emergence, and competitiveness of weeds [7]. Studies show that the forage legume mass incorporated as green manure increases crop yields and weed biomass, as well as the highest crop N uptake reduction in the presence of weeds [10, 28]. Various studies have been carried out on the cultivation of forage legumes in organic crop rotation [25, 43, 47]. Studies show that the use of legume cover crops (grow for 1 year) generally enhances weed control without reducing crop yield [43, 45, 47]. However, the conditions

that determine the benefits should be further explored to encourage this practice among farmers. Perennial legumes are more effective if they grow more than a year in crop rotation. The cultivation of perennial legumes for a longer period of time is limited due to the above-ground mass utilization of perennial legumes is limited in arable farms. Melander et al. [25] argue that biennial leguminous green manure crop can mitigate weed problems in organic annual crops. Forage legumes ground mass can be used as needed, e.g., change the length of the vegetation period, suspension strategy, and competition. This can be done by mulching [6, 33, 34]. Mulching smothers the weeds by excluding light and providing a physical barrier to impede their emergence. The properties and functions of perennial legume mulch differ substantially from grasses swards [1]; therefore, the differences in their use for weed control are not clear. Combining different agronomic strategies helps to manipulate nutrient cycles, yield, and weed control [10].

We hypothesized that cultivation of perennial forage legumes in crop rotation as a cover crop (legume swards infestation in cereals) or nitrogen supply and soil improvement (green fallow) can increase the competition of cereals, and control the spread of weeds by properly selecting the practice of using the above-ground mass of legume swards. The objective of these complex studies was to evaluate the effects of the cultivation methods of perennial forage legumes, using the above-ground mass, for the suppression of dicotyledonous weeds in organic cropping systems.

Materials and methods

Experimental site and soil

The study was carried out at the Joniškėlis Experimental Station of the Lithuanian Research Centre for Agriculture and Forestry, situated in the northern part of Central Lithuania's lowland region (56°21' N, 24°10' E) during the period 2008–2017. The soil of the experimental site is Endocalcari–Endohypogleyic Cambisol (CMg-n-w-can), with a clay–loam texture (27% clay, 50% silt, 23% sand). The topsoil (0–25 cm) pH was close to neutral (6.8), and had moderate phosphorus (150 mg kg⁻¹ of soil) and humus (2.5%) contents and a high (220 mg kg⁻¹ of soil) potassium content. The annual mean temperature and total amount of precipitation are 6.1 °C and 547.4 mm, respectively. The climate of Lithuania's territory is moderately cold. During the field experiments, the plants were grown according to organic farming standards.

Experimental treatments and design

Undersown clover (experiment I)

This study was conducted in 2013–2015 to determine the influence of forage legumes undersown in cereals

on the weediness and productivity of a crop rotation sequence compared to granulated cattle manure (GCM). Crop rotation and experimental design are presented in Table 1. The main crops included oats (*Avena sativa* L.) ‘Migla’ (seed rate 220 kg ha⁻¹), pea (*Pisum sativum* L.) ‘Simona’ (320 kg ha⁻¹), spring wheat (*Triticum aestivum* L.) ‘Vanek’ (250 kg ha⁻¹), red clover (*Trifolium pratense* L.) ‘Sadūnai’ (12 kg ha⁻¹), an oat–pea mixture (seed ratio 1:1), and spring barley (*Hordeum vulgare* L.) ‘Noja’ (250 kg ha⁻¹). Red clover (2013) and white clover (*Trifolium repens* L.) ‘Nemuniai’ (10 kg ha⁻¹) were undersown in cereals, with a seeding rate reduced by 10%. According to experimental design, GCM was applied at 2000 kg ha⁻¹ (N-55 kg ha⁻¹, P₂O₅—27 kg ha⁻¹ and K₂O—109 kg ha⁻¹), above-ground mass of red clover—2269 kg ha⁻¹ (N-76 kg ha⁻¹, P₂O₅—6 kg ha⁻¹ and K₂O—65 kg ha⁻¹), white clover—2023 kg ha⁻¹ (N-54 kg ha⁻¹, P₂O₅—6 kg ha⁻¹ and K₂O—75 kg ha⁻¹). The experimental plots, each of 5 m × 12 m, were laid out in a complete one-factor randomized block design with four replicates.

Perennial legumes and above-ground mass mulch (experiment II)

The aim of the experiment was to determine the influence of methods for using forage legumes and their above-ground mass (as mulch and forage) on the weediness and productivity of cereal crops. Three analogous field experiments were established and carried out (in 2007–2010, in 2008–2011, and in 2009–2012). The experiments were conducted in the following crop rotation sequence: barley + undersown perennial grasses (year of introduction) → perennial grasses → winter wheat → winter triticale. The experimental design is presented in Table 2. In the starting years of the experiments (2007, 2008 and 2009), spring barley (‘Ūla’, seed rate 250 kg ha⁻¹) was undersown with perennial grasses in compliance with the experimental design: intergeneric hybrid festulium (*x Festulolium*) ‘Punia’ (12 kg ha⁻¹), red clover ‘Vyliai’ (12 kg ha⁻¹), lucerne (*Medicago sativa* L.) ‘Birutė’ (8 kg ha⁻¹), and mixtures of forage legumes with festulium (seed ratio of legume to grass 2:1). The management methods for the above-ground biomass of perennial grasses can be explained as follows: (1) cut twice and removal from the field; (2) mixed management: the herbage of the first cut was removed from the field and the herbage of the second and third cuts was mulched; (3) mulching: herbage was cut four times and mulched. Perennial grasses were cut for forage at the flowering stage, and for mulching at the bud formation stage. In the second half of August, the plots of all treatments were disked and 2 weeks later were ploughed to a depth of 25 cm. The field was cultivated before wheat sowing. In the first year

(2009, 2010, and 2011) after perennial grasses and their mulch incorporation, winter wheat (‘Tauras’, 250 kg ha⁻¹) was grown, and in the second year (2010, 2011, and 2012) (winter triticale (*Triticum x secale*, ‘Woltario’ 250 kg ha⁻¹) was grown. The experimental plots were laid out in a randomized complete two-factor block design in four replicates. Individual plot size was 5 m × 16 m.

Plant-based organic manure (experiment III)

Field experiments were established and carried out in 2015–2017 to determine the influence of differently treated (composted, fermented) fertilized forage legume mass on the weediness and productivity of cereals (design in Table 3). The experiments were conducted in the following crop rotation sequence: winter wheat ‘Ada’ (seed rate 250 kg ha⁻¹) → spring wheat ‘Vanek’ (250 kg ha⁻¹) → spring barley Noja (250 kg ha⁻¹) with undersown red clover ‘Vyliai’ (12 kg ha⁻¹). Organic manure was produced in summer of 2015 and applied in spring of 2016, excluding the treatment when green clovers had been mass cut and ploughed in the autumn of 2015. The fermentation (ensiling) of plant-based organic manure was produced from red clover mass (cut at the beginning of flowering stage, on 19 June 2015) and from pea and spring wheat (mass ratio 1:2) mass (cut at the beginning of the development of the wheat grain stage, on 24 July 2015). The fermentation of plant above-ground mass was performed as follows: the mass was cut, chopped, piled into a special trench, and then pressed well to minimize the contact with air as much as possible. Having completed the piling, the mass was sealed as hermetically as possible with a special white bale silage film (standard film thickness, 25 μm). The compost was piled on 22 June 2015 using two main components: the above-ground mass of red clover (three parts) and winter wheat straw (one part). Aerobic composting was used, and to stimulate decomposition, the pile was turned five times. After 9–10 months, in spring, the organic manure was used. After spreading, the manure was incorporated into the 0–10 cm soil layer. The amount of manure was calculated based on the nitrogen content of the manure, so that 1 ha would receive 50 kg ha⁻¹ N (with the exception of undersown red clover and fermented pea and wheat mass, which received 76 kg ha⁻¹ and 25 kg ha⁻¹ N, respectively). Individual plot size was 5 m × 12 m.

Experimental methods and assessments

The above-ground biomass of legumes and legume–festulium mixtures at each cut and belowground biomass before their ploughing-in were measured. Sampling of above-ground biomass occurred at four randomly chosen squares of 0.25 m² in each plot that were cut to ground level and weighed. After plant biomass determination,

Table 1 Design of field experiment I and fertilizing

| Rotation sequences | | | | | |
|--|----------------------|---|----------------------|--------------------|-------------|
| 2013 | | 2014 | | 2015 | |
| Crops | Fertilizing | Crops | Fertilizing | Crops | Fertilizing |
| Oats (Control) | | Spring wheat (SW) | | Spring barley (SB) | |
| Oats | GCM | Spring wheat (SW) | | Spring barley (SB) | |
| Oats | GCM | Spring wheat and undersown white clover (SW + WC) | WC mass ^a | Spring barley (SB) | |
| Oats and undersown red clover (O + RC) | RC mass ^a | Spring wheat (SW) | | Spring barley (SB) | |
| Mixture of oats and pea (O + P) | | Spring wheat (SW) | | Spring barley (SB) | |
| Pea (P) | | Spring wheat (SW) | | Spring barley (SB) | |
| Red clover (RC) | RC mass ^a | Spring wheat (SW) | | Spring barley (SB) | |

GCM granulated cattle manure

^a Incorporated RC or WC mass

the dry matter was determined after drying the samples to a constant weight at 105 °C, and the above-ground biomasses of the legumes and legume–festulolium mixtures were calculated. All plant samples were dried, milled, and analyzed for nitrogen (N) content, determined by the Kjeldahl method. Grain yield was harvested when the majority of crops had reached hard dough stage (BBCH 87). Each plot of the experiment was harvested with a small-size combine harvester. After threshing grain yields were reported at 14% moisture.

Weed incidence in cereals was assessed twice during the growing period, by measuring the number of weeds and species during the tillering stage (BBCH 27) and during the milk stage (BBCH 75) of cereals, while the dry biomass was only measured during the milk stage. Weed incidence in forage legumes was assessed in spring and after the last mulching (experiment II), or at the grass growth stage (BBCH 61). Measurements were made in 0.25 m² fixed plots, in four places per plot.

The data of Experiments I and III were statistically processed using a one-factor the data of Experiments II, a two-factor analysis of variance, as well as correlation and regression methods [36]. The data of the experiment were analyzed when the factual Fisher criterion ($F_{\text{fact.}}$) was higher than the theoretical one ($F_{\text{theor.}}$). The significance of differences between the means (control and individual treatments) was estimated according to the least significant difference (LSD) at the 0.05 and 0.01 probability levels. The data for the number and mass of weeds were transformed, according to the formula $\sqrt{x+1}$, for statistical analysis. Experiment II provided average data for the factors (perennial grasses and management methods of above-ground biomass) as it showed significant differences. Statistical analysis of the experimental data was performed using the ANOVA version 3.1 software and

STAT_ENG version 1.5 from the programme package SELEKCIJA [40], Lithuanian University of Agriculture, Lithuania).

Results and discussion

Undersown clover (experiment I).

Oats and other crops

In experimental plots among annual weeds, *Stellaria media* L., *Veronica arvensis* L., *Sinapis arvensis* L., *Polygonum convolvulus* L., and *Chenopodium album* L. prevailed. Red clover sown in oats suppressed weeds significantly (Table 4). During the growing season (from BBCH 27 to BBCH 75), oats reduced the number of weeds by 73%, and in the case when they were grown with undersown RC—by 84%. The number of weeds was greatest in crops where red clover was grown alone, and where peas and their mixture with oats. The significantly higher oat yield was determined by GCM fertilization compared to non-fertilized one. A strong, significant negative correlation was found between grain yield and weed weight ($r = -0.95$; $p < 0.01$). The competition of oats, peas, and pure red clover (without oats) depended on the ability of these plants to suppress weeds. Oats have outstanding tillering ability, peculiarities of nutrition: they grow tall and, therefore, their competitive properties are strong. Semi-leafless peas are classified as plants with weak weed-suppressing ability [4, 23].

Spring wheat

Cultivating spring wheat as the second crop in a crop rotation resulted in more germinated weeds compared to oats. On average, the number of ephemeral weeds decreased by 51% during the growing season of spring wheat (compared its content in cereal BBCH 75 with BBCH 27). At the beginning of spring wheat maturity, a

Table 2 Crop rotation and design of field experiment II

| Years | Crops | Management methods of the above-ground mass* |
|------------------|---|--|
| 2007, 2008, 2009 | Spring barley with undersown (SB) | |
| 2008, 2009, 2010 | Festulolium (Fs) | Removed from the field Mixed management Mulching |
| | Red clover (RC) | Removed from the field Mixed management Mulching |
| | Mixture of red clover and festulolium (RC + Fs) | Removed from the field Mixed management Mulching |
| | Lucerne (L) | Removed from the field Mixed management Mulching |
| | Mixture of lucerne and festulolium (L + Fs) | Removed from the field Mixed management Mulching |
| 2009, 2010, 2011 | Winter wheat (WW) | |
| 2010, 2011, 2012 | Winter triticale (WT) | |

Note. Forage legumes and their mixture with festulolium mulched mass incorporated in autumn during ploughing, before winter wheat sowing

Table 3 Crop sequence and fertilization of field experiment III

| Years | Crops | Plant-based organic manure |
|-------|---|---|
| 2015 | Winter wheat (2 treatment undersown red clover) | No manured |
| 2016 | Spring wheat | Control with no manure (C) Green clovers (undersown in winter wheat) mass cut and ploughed in the fall of 2015 (RC) Red clover ground mass fermented under anaerobic conditions (FerRC) Fermented pea and spring wheat mass manure (FerP + W) Composted red clover and straw manure, in a ratio of 3: 1 (ComRC + S) Granulated cattle manure (GCM) |
| 2017 | Spring barley | No manured |

significantly lower density and dry weight of weeds were observed when the crop was grown together with undersown white clover. The presence of white clover in spring wheat reduced the number of weeds by 72% and weight by 67% compared to the control plot. The red clover mass (2269 kg ha⁻¹ DM, containing 76 kg ha⁻¹ N) that was incorporated in autumn significantly increased the grain yield of spring wheat, but it did not reduce weediness. Spring wheat grain yield increased (11–17%) using grown legume swards mass as fertilizer, growing grains legume or their mixtures with cereals fertilizing with organic fertilizers. When spring wheat was grown, there was no correlation between grain yield and weight of weeds ($r = 0.15$; $p > 0.05$).

Spring barley

In spring, the number of weeds was the highest in crop rotation sequence with peas, peas mixture with oats, and red clover (without oats) while growing spring barley as the third crop in rotation. A higher number of weeds was determined by sowing spring barley in a weedy crop plot (in crop rotation with red clover, pea, and mixture of pea and oat). During the growing season (from BBCH 27 to BBCH 75), the number of weeds increased in all plots (36% on average), except the barley crop after the incorporation of white clover mass. Contrary to spring wheat, the incorporated undersown white clover mass (2023 kg ha⁻¹ DM containing 54 kg ha⁻¹ N) increased spring barley competitiveness, and significantly reduced the number of weeds (44%) at the milk stage of cereals, compared to the control plot. Spring barley grain yield was found significantly higher after incorporation of

white clover mass compared to the control. The lowest grain yield was in the crop rotation sequence with peas.

Undersown clover establishes in the lower crop level and competes with weeds. This is especially important in the second half of summer when an undersown clover with drying crop leaves covers the soil surface [14, 49]. Plants and weeds compete for light, water, and nutrient resources [20, 22, 41]. Den Hollander et al. [12] state that the relative growth rate is determined by the characteristics of clover, such as light extinction coefficient, light use efficiency, and specific leaf area. It was determined that the fastest grow soil surface cover was demonstrated by annual clover *Trifolium resupinatum* L. [46]. Gaudin et al. [14] reported that those forage legume species that produced a high yield of above-ground mass were considered to be effective competitors for local resources. The competitive properties of forage legumes also depend on the size of their seeds, and the sowing rate and sowing time [12]. Forage legumes undersown in cereals can both suppress weeds; however, legume can compete with cereals, too [13, 33, 34]. Verret et al. [43] indicated that the use of legume companion plants generally seemed to enhance weed control without reducing crop yield.

Our results provide evidence that the undersowing of red clover in oats during the growing season reduced the number of weeds by 63.8%, and white clover undersown in spring wheat reduced the number of weeds by 72.3%, compared to the weeds present in the respective cereals without undersown. The presence of undersown forage legumes was more effective in spring barley that had lower suppression ability. The incorporation of legumes in crop rotation as a main or services crop (for one growing season) improves cereal nutrition and increases grain yield.

Perennial legumes and above-ground mass mulch (experiment II)

Perennial forage legumes and their mixture with festulolium

In spring, the highest number of weeds was observed in lucerne crop (Table 5). Among annual weeds, *Galium aparine* L., *Viola arvensis* Murr., *Chenopodium album* L., *Lamium purpureum* L., and *Thlapsi arvense* L. prevailed. At the end of summer (crops BBCH 61), species of legume and legume-festulolium mixture and their above-ground mass (herbage) management methods had a significant influence on the number of weeds. Lucerne increased the number of weeds (by 79%) compared to festulolium. The mixed management of the herbage of forage legumes and their mixtures with festulolium and mulching increased the number of weeds (mulching—significantly) compared to that removed from the field (by an average of 36% and by 60%, respectively). The highest number of weeds was observed when lucerne

herbage mass was used for mixed management or mulching, and the lowest number of weeds was observed when the whole herbage of red clover and mixture of red clover and festulolium was removed from the field (data not shown).

The dry weight of weeds was strongly influenced by perennial grasses and above-ground mass management. Both the removal of the herbage mass from the field and the whole of its mulching on the soil surface demonstrated the greatest weed dry weights of the forage legumes (Table 5). The conditions for weed growth in terms of higher light penetration, nutritional conditions, and lower competition were the most favorable in mulching treatment. The lowest weed dry weight was obtained while using the above-ground mass in mixed management (the difference was significantly). The species of forage legumes varied in their weed-suppression ability [2, 11]. Increased competitive properties were observed in forage legume and grass mixtures, which is consistent with previous research [37]. The highest weed mass was observed in lucerne; however, it depends on weed species, too. Meiss et al. [24] noted that lucerne had strong negative impacts on broad-leaved weeds with an upright morphology (e.g., *Chenopodium album* L.) and on species which climbed on neighbouring plants (e.g., *Galium aparine* L., *Polygonum convolvulus* L.).

When forage legumes are grown for fodder, they are harvested in later growth stages, and suppress weeds well during the growing season. On the contrary, for mulching, legumes and legume–festulolium mixture are cut in early stages, meaning that the soil surface is often uncovered, and the penetrating light causes weeds to germinate. Additionally, mulch degradation (forage legume mass is particularly intensively degrading) causes nitrogen release, which induces weed germination and increases their mass, unlike the twice cut grass for green feed in later stages [3]. The data show that the mixed management method for use of above-ground mass increased the legume and legume–festulolium mixture yields. The mulch of forage legumes with festulolium formed a more intensive cover on the soil surface due to a denser crop, a thicker mulch layer, and its slower degradation, and served as a physical barrier against weed growth and development [32]. Campiglia et al. [9] reported that the different types of organic mulches could control weeds effectively. In our research, there was no correlation between the mass of mulch and the weight of weeds. However, N of mulch directly correlated with the density and weight of weeds ($r=0.66$, $p<0.05$; $r=0.59$, $p<0.05$, respectively). Four time cutting and mulching can stop inhibited the maturation of weed seeds.

Table 4 Weed density, dry weight, and grain yield in different crop sequences, Experiment I

| Crops | The first crop in rotation, 2013 | | | | | | The second crop in rotation, 2014 | | | | | |
|----------------------------------|----------------------------------|-------|---------|----------------------------|-------|-------|-----------------------------------|-------|-------|-------------|--------|-------|
| | Cereal growth stage (BBCH) | | | | | | Crops | | | | | |
| | 27 | 75 | 75 | 75 | 75 | 75 | 27 | 75 | 75 | 75 | 75 | 75 |
| | Density, weeds m ⁻² | | | Weight gm ⁻² DM | | | Grain yield | | | Grain yield | | |
| | | | | | | | | | | | | |
| O | 172 | 47 | 5.2 | 5669 | 100 | 100 | SW | 204 | 112 | 18.5 | 3767 | 100 |
| O+GCM | 132 | 38 | 7.7 | 6322* | 112 | 112 | SW | 234 | 119 | 15.2 | 4281 | 114 |
| O+GCM | 152 | 48 | 11.1 | 6322* | 112 | 112 | SW+WC | 154* | 31** | 6.2** | 4162 | 110 |
| O+RC | 109** | 17* | 2.6 | 5988 | 106 | 106 | SW | 192 | 104 | 21.6 | 4420** | 117 |
| P+O | 133 | 78 | 37.4** | 5294 | 93 | 93 | SW | 207 | 87 | 16.0 | 4323 | 115 |
| P | 124 | 79 | 154.4** | 3051** | 54 | 54 | SW | 190 | 108 | 19.1 | 4308 | 114 |
| RC | 151 | 107* | 168.3** | 0 | 0 | 0 | SW | 205 | 119 | 14.9 | 3603 | 96 |
| Mean | 139 | 59 | 55.2 | 5441 | | | Mean | 198 | 97 | 15.9 | 4123 | |
| Statistics of ANOVA (p) | <0.01 | <0.01 | <0.01 | <0.05 | <0.05 | <0.05 | Statistics of ANOVA (p) | <0.05 | <0.01 | <0.05 | <0.01 | <0.01 |
| The third crop in rotation, 2015 | | | | | | | | | | | | |
| SB | 67 | 99 | 8.5 | 2491 | 100 | 100 | | | | | | |
| SB | 64 | 104 | 9.3 | 2683 | 108 | 108 | | | | | | |
| SB | 60 | 55* | 7.7 | 5016** | 201 | 201 | | | | | | |
| SB | 72 | 101 | 10.8 | 2456 | 99 | 99 | | | | | | |
| SB | 91** | 137** | 14.4* | 2536 | 102 | 102 | | | | | | |
| SB | 98** | 145** | 11.9 | 2286 | 92 | 92 | | | | | | |
| SB | 96** | 103 | 9.7 | 2405 | 97 | 97 | | | | | | |
| Mean | 78 | 106 | 10.3 | 2839 | | | | | | | | |
| Statistics of ANOVA (p) | <0.01 | <0.01 | <0.05 | <0.05 | <0.01 | <0.05 | | | | | | |

O oat (control), RC red clover, WC white clover, SW spring wheat, SB spring barley, GCM granulated cattle manure

Values in italic and followed by * or ** are significantly different than the control according to other variant at p<0.05 and p<0.01, respectively

Winter wheat

The use of lucerne and red clover as preceding crops for winter wheat created good growth conditions for both cereals and weeds. Also, this was demonstrated by the increase of soil mineral N in spring when the vegetation of wheat was renewed [3]. Following these preceding crops, the quantity of weeds which germinated in wheat was higher of 28% and 41% for red clover and lucerne (significantly) respectively, compared to festulolium (Table 6). The lowest number of weeds was observed in the winter wheat grown after festulolium had been used as a preceding crop. During the growing season (from cereal growth stages BBCH 27–BBCH 75), the winter wheat grown after festulolium was used as a preceding crop and had the lowest weed-suppression capacity: the number of weeds increased by 18%. Weeds were best suppressed by the winter wheat grown after red clover. The highest number of weeds was recorded in the winter wheat that was cultivated after lucerne. At the BBCH 75 growth stage of winter wheat, no significant influence of the perennial grasses and above-ground mass used on the dry weight of weed mass was determined. The herbage of lucerne and festulolium mixture reduced the dry weight of weeds (by 19%) compared to festulolium. A lower weed mass mostly corresponds to a lower number of matured seeds [26]. All forage legumes and legume–festulolium mixtures increased wheat grain yield (1220–2028 kg ha⁻¹ or 51–85%) compared to festulolium. Mulching increases the yield (454 kg ha⁻¹ or 23%) compared to the treatment when herbage was removed from the field.

Winter triticale

Cultivating winter triticale in the second year following ploughing of legumes, festulolium, and their mixture resulted in a higher number of germinated seeds in spring compared to winter wheat. The number of weeds was significantly reduced (by 13%) by the mulching of the herbage of legumes and legume–festulolium mixture. During the growing season, the number of weeds varied marginally. Forage legume and their above-ground mass management methods did not have any significant impact on the weed dry weight. A positive effect on the yield of legumes and their mixtures with festulolium (except mixture or lucerne and festulolium) was also found in the second year. The yield supplement of winter triticale grain was 395–482 kg ha⁻¹ or 15–19%, compared to control.

In our studies, the use of festulolium as a preceding crop did not increase the competitiveness of cereals. In contrast, crop residues may encourage weed emergence in certain circumstances, either when residues maintain soil moisture at a level suitable for germination during periods of drought or when germination-stimulating

compounds such as nitrates are released [22]. Red clover and lucerne induced the germination of weed seeds at the beginning of the growing season compared to the festulolium. In most cases, the above-ground mass of forage legumes increased cereal competitiveness against weeds, and the weed number decreased during the growing season (Table 6). The incorporated plant residues affect soil temperature, the quality and quantity of light, and biological activity at various trophic levels in the leaf canopy and underlying soil [41], with affect weed germination as well. Mulching of above-ground mass increased grain yield by 14% compared to non-mulched.

Our results provided the cultivation of perennial forage legumes in cereal crop rotation and the technique of using the above-ground mass for mulching changed the established life cycle of plants, which adversely affected the spread of weeds. Forage legumes stimulated weeds to germinate; after germination, repeated cutting and mulch spreading on the soil surface were performed, which prevented seed maturation. This reduced the spread of weeds via seeds.

Forage legumes and their combinations with festulolium as preceding crops provided favorable conditions for weed germination in the winter wheat crop. Cereal cultivation after forage legume cultivation strengthened the crop and its competitive ability. Winter cereals grown after festulolium cultivation demonstrated weak weed suppression.

Plant-based organic manure (experiment III)

Winter wheat (data not shown)

Plant-based organic manure was produced to preserve nitrogen in the plant mass, to reduce the intensity of mineralization and to extend their duration effect [42]. In the spring of the first (2015) research year, after the vegetative growth of winter wheat resumed, the weed number recorded was 54–88 plants m⁻². On average, at the end of summer, before wheat harvest, the number of weeds increased by 32% compared to the number of weeds in spring. Significantly lower weed dry weight was observed in cereals with undersown red clover. Among annual weeds, *Veronica arvensis* L., *Chenopodium album* L., and *Lamium purpureum* L. prevailed.

Spring wheat

In 2016, when organic manure was used, a significantly lower number (wheat growth stage BBCH 27 and 75) and dry weight of weeds (wheat growth stage BBCH 75) were observed in the plot with undersown red clover mass incorporated in the autumn (Table 7).

The incorporation of red clover mass improved the nutrition and weed-competitiveness of cereals. This practice is widely used in other countries [15]. A significantly

Table 5 The effect of perennial forage legumes and their above-ground biomass management methods on the weed density and dry weight, Experiment II

| Treatments | Weeds | | Forage legumes and the mixture with festulolium | | | |
|--|--------------------------------------|--------------------------------|---|---------------------------|-----------------------------------|--|
| | Perennial swards growth stage (BBCH) | Density plants m ⁻² | Density plants m ⁻² | Yield kg ha ⁻¹ | Mass of mulch kg ha ⁻¹ | Applied N with mulch kg ha ⁻¹ |
| | 27 | 61 | | | | |
| Perennial legume and legume- grasses | | | | | | |
| Fs (control) | 30 | 47 | 8.1 | 4728 | 2030 | 286 |
| RC | 24 | 56 | 12.8 | 7866** | 4072 | 131.6 |
| Fs + RC | 24 | 55 | 7.7 | 8134** | 3979 | 106.8 |
| L | 39** | 84* | 14.5 | 7293** | 3754 | 129.7 |
| L + Fs | 31 | 56 | 5.9 | 8013** | 3836 | 103.7 |
| Statistics of ANOVA (p) | < 0.01 | < 0.05 | < 0.01 | < 0.01 | – | – |
| Management methods of above-ground biomass | | | | | | |
| Removal from field (control) | 30 | 45 | 11.0 | 7100 | 0 | 0.0 |
| Mixed | 31 | 61 | 7.3 | 7945** | 4028 | 115.4 |
| Mulching | 29 | 72** | 11.1 | 6575 | 6575 | 184.9 |
| Statistics of ANOVA (p) | n.s | < 0.01 | < 0.01 | < 0.01 | – | – |

Fs festulolium, RC red clover, L Lucerne, RC + Fs mixture of red clover and festulolium, L + Fs mixture lucerne and festulolium; Values in italic and followed by * or ** are significantly different than the control according to other variant at p < 0.05 and p < 0.01, respectively. n.s. indicate significant differences, value in brackets indicate standard error

lower dry weight of weeds (45% lower) was observed in the experiment that used fermented pea and spring wheat mass. The literature emphasizes that fermented plant mass contains phytoncides and acetic acid, which can inhibit the growth and development of weeds and contribute to a decline of the seed bank [16, 18]. Fermented red clover mass and composted red clover and straw manure have a tendency to reduce the weight of weeds (by 29% and 27%, respectively). The use of fertilizer GCM resulted in the highest number and dry weight of weeds. This could have been caused by uneven weed density, different species composition, and low competition for nutrients. Międzys et al. [27] found that cattle manure significantly increased the total number of productive stems per plant, making the crop denser and weed suppression stronger. Having compared the total weediness of spring wheat (2016) and winter wheat (2015), we found that the number of weeds (cereal growth stage BBCH 27 and 75) and the dry weight of weeds (cereal growth stage BBCH 75) decreased marginally after the application of plant-based manure. The spring wheat yield after the use of fermented and composted plant-based manure had a tendency to decrease.

Spring barley

In the second year (2017) of the use of organic manure, the number and the dry weight of weeds (cereal growth stage BBCH 75) increased on average by 22%, and by 36%, respectively, compared to spring wheat. A significantly higher dry weight of weeds before harvest was observed when red clover had been undersown. When using fermented red clover mass and fertilizer GCM, the grain yield increased by 13% and 17% respectively, compared to control. This had no significant effect on weediness. The results reported that spring barley grain yields inversely correlated with the weight of weeds ($r = -0.70$, $p < 0.05$).

The use of compost and manure amendment, cover crops, and other low-external-input management practices create a change in the physical, chemical and biological properties of soil [29]. Additionally, surface residues can affect seed germination via physical and chemical changes to the seed environment [31]. There is a paucity of research on the effects of plant-based organic manure on the incidence of weeds in organic farming.

Our results provide that fermented red clover mass and fermented pea and spring wheat mass low degree of decomposition and N release (C:N=21 and C:N=35, respectively) did not increase weed weight (with relatively high crop productivity of 3766–3774 kg ha⁻¹). These manure did not affect weeds in a less competitive spring barley.

Concluding remarks

Studies show that forage legumes are multifunctional plants, one of the functions of which is to control the spread of crop weed populations. The undersowing of red clover in oats during the growing season reduced the number of weeds by 63.8%, and white clover undersown in spring wheat reduced the number of weeds by 72.3%, compared to the weeds present in the respective cereals without a grass undersown. The presence of undersown forage legume was more effective in cereals that had lower suppression ability. White clover is valued for its ability to compete with cover crop. This type of clover has a creeping, rooted stem, making it the best 'carpet-forming' grass to cover the soil surface. In the case of short cultivation of legume swards (one vegetation period), their effect as a pre-sowing on cereal yield depends on the above-ground mass of legume swards.

The cultivation of perennial forage legumes in cereal crop rotation (for a longer period) and the technique of using the above-ground mass for mulching changed the established life cycle of plants, which adversely affected the spread of weeds. Forage legumes stimulated weeds to germinate; after germination, repeated cutting and mulch spreading on the soil surface were performed, which prevented seed maturation. This reduced the spread of weeds via seeds. Forage legumes, and their combinations with festulolium as preceding crops, provided favourable conditions for weed germination in the winter wheat crop. Cereal cultivation after forage legume (as a source of nitrogen) strengthened the crop and its competitive ability. The number of weeds in the winter wheat grown after red clover cultivation during the growing season decreased by 23% on average (while applying the above-ground mass in mixed ways and mulching); and after growing Lucerne, the number of weeds decreased by 14% (while using the above-ground mass in mixed ways). Winter cereals grown after festulolium cultivation demonstrated weak weed suppression. Red clover grows in almost all soils, competes well with weeds, and produces large above-ground mass, especially after harvesting cereals. It regrows quickly during mulching. During the sowing year, lucerne grows slowly and does not cover the soil surface as a rule. However, mixtures with grasses are ideal for growing for two years or more.

In arable farms, legume sward can be processed into organic fertilizers. These manure were mainly affected by the years of fertilizer use. Studies show that the dry weight of weeds decreased (by 27–45%) when the crops were fertilized with fermented and composted organic fertilizers.

The choice of forage legume cultivation method (especially with the proliferation of weeds) is a trade-off

Table 6 The effect of the above-ground mass of perennial forage legumes, used as green manure, on annual weed density, dry weight, and grain yields of winter cereals, Experiment II

| Treatments | Winter wheat | | | | Winter triticale | | | |
|--|--------------------------------------|-------|----------------------------|-------|--------------------------------------|-------|----------------------------|--------|
| | Perennial swards growth stage (BBCH) | | | | Perennial swards growth stage (BBCH) | | | |
| | 27 | 75 | 75 | % | 27 | 75 | 75 | % |
| | Density weeds m ⁻² | | Weight gm ⁻² DM | | Density weeds m ⁻² | | Weight gm ⁻² DM | |
| Perennial legume and legume- grasses | | | | | | | | |
| Fs (control) | 39 | 46 | 20.7 | 100 | 69 | 54 | 19.9 | 2585 |
| RC | 50 | 43 | 18.8 | 185 | 69 | 62 | 21.4 | 2980** |
| Fs + RC | 47 | 47 | 19.0 | 155 | 64 | 57 | 21.4 | 3000** |
| L | 55** | 53 | 20.8 | 172 | 72 | 64 | 23.3 | 3067** |
| L + Fs | 44 | 43 | 16.7 | 151 | 62 | 62 | 26.2 | 2781 |
| Statistics of ANOVA (p) | <0.01 | <0.01 | n.s | <0.01 | n.s | <0.05 | n.s | <0.01 |
| Management methods of above-ground biomass | | | | | | | | |
| Removal from field (control) | 49 | 50 | 21.0 | 100 | 71 | 62 | 20.3 | 2712 |
| Mixed | 45 | 44 | 18.4 | 107 | 69 | 58 | 22.0 | 2840 |
| Mulching | 46 | 45 | 18.3 | 113 | 62* | 59 | 25.0 | 3096* |
| Statistics of ANOVA (p) | n.s | <0.01 | n.s | <0.01 | <0.05 | n.s | n.s | <0.05 |

Fs festulolium, RC red clover, L Lucerne, RC + Fs mixture of red clover and festulolium, L + Fs mixture lucerne and festulolium, K control
 Values in italic and followed by * or ** are significantly different than the control according to other variant at p < 0.05 and p < 0.01, respectively. n.s. indicate significant differences

Table 7 Influence of plant-based manure on weed density, dry weight, and grain yields of cereals, Experiment III

| Organic manure | Spring wheat | | | | | Spring barley + undersowing | | | | |
|-------------------------|----------------------------|-----------------|----------------------------|---------------------|-----|-----------------------------|-----|----------------------------|---------------------|-----|
| | Cereal growth stage (BBCH) | | | | | | | | | |
| | 27 | 75 | 75 | Grain yield | | 27 | 75 | 75 | Grain yield | |
| | number m ⁻² | | Weight gm ⁻² DM | kg ha ⁻¹ | % | number m ⁻² | | Weight gm ⁻² DM | kg ha ⁻¹ | % |
| No manure (control) | 70 | 67 | 23.2 | 4015 | 100 | 78 | 77 | 22.1 | 2450 | 100 |
| RC | 48* | 47* | 9.4* | 4237 | 108 | 115** | 90 | 29.0* | 2360 | 96 |
| FerRC | 63 | 66 | 16.4 | 3774 | 94 | 97 | 88 | 24.0 | 2777* | 113 |
| FerP + W | 57 | 52 | 12.7* | 3766 | 94 | 94 | 79 | 25.3 | 2496 | 102 |
| ComRC + S | 62 | 67 | 16.9 | 3818 | 95 | 95 | 70 | 20.2 | 2665 | 109 |
| GCM | 74 | 80 | 24.5 | 4297 | 107 | 86 | 60 | 19.7 | 2863* | 117 |
| Mean | 62 | 63 | 17.2 | 3985 | | 94 | 77 | 23.4 | 2602 | |
| Statistics of ANOVA (p) | <i>p</i> < 0.05 | <i>p</i> < 0.05 | <i>p</i> < 0.05 | n.s | | <i>p</i> < 0.01 | n.s | n.s | <i>p</i> < 0.05 | |

RC green clovers mass cut and ploughed in the fall, FerRC red clover ground mass fermented under anaerobic conditions, FerP + W fermented pea and spring wheat mass manure, ComRC + S composted red clover and straw manure, GCM granulated cattle manure

Values in italic and followed by * or ** are significantly different than the control according to other variant at *p* < 0.05 and *p* < 0.01, respectively

between the expected ecosystem services and the possibility to obtain commercial products each year.

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Authors' contributions

AA and LŠ—have drafted the work. VG—the acquisition, analyzed, and interpreted the data of Experiment I; LŠ and MT—the acquisition, analyzed, and interpreted the data of Experiment III; DR and LM—the acquisition, analyzed, and interpreted the data of Experiment II; ŽK and AA—developed a research scheme. All authors read and approved the final manuscript.

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Declarations

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Competing interests

The authors declare that they have no competing interests.

Author details

¹Lithuanian Research Centre for Agriculture and Forestry, Joniskelis Experimental Station, Pasvalys District, Joniškėlis, Lithuania. ²Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Kėdainiai District, Akademija, Lithuania.

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