BIOLOGICAL AGRICULTURE & HORTICULTURE
https://doi.org/10.1080/01448765.2018.1509728

Leguminous pre-crops improved quality of organic winter and spring cereals

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ABSTRACT

The aim of this research was to assess the effect of leguminous pre-crop species on quality characteristics of subsequent spring and winter cereals. The experiment was carried out in an organic crop rotation in north-eastern Europe. The influence of biomass dry matter yield, carbon and nitrogen content of perennial red clover (*Trifolium pratense* L.), alsike clover (*Trifolium hybridum* L.), Washington lupin (*Lupinus polyphyllus* Lindl.), biennial white sweet clover (*Melilotus albus* L.), annual crimson clover (*Trifolium incarnatum* L.) and Alexandria clover (*Trifolium alexandrinum* L.) on protein concentration, bulk density and thousand grain weight of subsequent cereals were assessed. Barley, oats, winter rye, spring and winter wheat were grown in the first post-legume year and barley and oats in the second post-legume year. In the first year after perennial and biennial pre-crops, the protein concentration of the cereals, except for rye, increased by 0.8–2.6 percentage points compared with the control. The largest increases in protein concentration of the cereals were after red and alsike clovers. The legumes increased the bulk density of all of the cereals, while thousand kernel weights were increased only for barley, spring and winter wheat. All the legume species had a positive second-year after-effect on the protein concentration of barley and oats, with the largest effect after red and alsike clover at 1.0–1.3 percentage points. The results showed that all of the leguminous pre-crops were suitable for increasing the quality of cereals. The effect was greater after perennial and biennial species compared with the annual species.

ARTICLE HISTORY
Received 22 May 2018
Accepted 6 August 2018

KEYWORDS
Cereal quality; green manure; leguminous pre-crop; organic cultivation

Introduction

Green manuring has been an agricultural practice among European farmers for more than two thousand years (Pieters 1927). In recent years, the interest among organic as well as conventional stockless farmers in using alternative nutrient sources such as green manures has increased in popularity. Almost any crop can be used for green manuring, but legumes are often preferred because of their ability to fix atmospheric nitrogen (N). Biological N fixation is one of the primary sources of N in organic farming (Berry et al. 2002). Legumes provide significant amounts of N for the subsequent crop and are thus a valuable source of N in organic cultivation and they can also reduce the use of chemical fertilisers in conventional cultivation. Compared to N in mineral fertilisers, which is susceptible to nitrate leaching and denitrification, plant-derived N is relatively more stable (Drinkwater et al. 1998). However,

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some leaching may still occur depending on the soil type and the green manure crop used (Askegaard et al. 2005). This is an important consideration in cereal cultivation, which faces risks of increasing occurrence of adverse and extreme agro-climatic events (Trnka et al. 2014), such as intense rainfall causing N-leaching (Tosti et al. 2016).

The climatic conditions in north-eastern Europe are favourable for the production of leguminous species and also for cereal cultivation. One of the most important positive impacts of climate change is the prolongation of the growing season, which creates the need to test the potential of widely cultivated and new leguminous crop species for organic crop rotations in the region. There is a wide range of leguminous species that can be drawn upon, each with their particular traits.

The initiative for the experiments reported here came from organic farmers, aiming to diversify the selection of species and seasonal types of legumes suitable for green manure. A trial was established, which included six pure-sown leguminous pre-crop species and five spring and winter cereals.

Red clover (*Trifolium pratense* L.) is one of the most commonly grown leguminous green manures in northern Europe and countries with similar climate (Nykänen et al. 2008; Talgre et al. 2012). Some other clovers are also cultivated because they may offer particular advantages in specific circumstances. Alsike clover (*Trifolium hybridum* L.) is well adapted to wet acid soils (Döring and Boufartigue 2013) and white sweet clover (*Melilotus albus* L.) to wide range of soils (Döring 2013). Biennial white sweet clover is suitable as a medium-term N fixer. Washington lupin (*Lupinus polyphyllus* Lindl.) is relatively more tolerant to several abiotic stresses compared to other legumes (Kurlovich et al. 2008). The fertilisation value of Washington lupin fresh green mass was considered to be equal to the same weight of cattle manure (Bender and Tamm 2014). Short-term fertility building crops, such as crimson and Alexandria clover, are new species for cultivation in the Nordic latitudes and their use in the region has so far been insufficiently investigated (Coombs et al. 2017). In the northern regions crimson clover or Italian clover (*Trifolium incarnatum* L.) is cultivated as an annual crop (Clark 2007), suitable as a short-term N fixer that is commonly used as a break in intensive and organic systems. Alexandria clover (*Trifolium alexandrinum* L.), also called Egyptian or berseem clover, requires warmer temperatures although it may give reasonable yields in colder environments, where it is sown in spring (Piano and Pecetti 2010).

There is relatively modest amount of published information about cereal quality traits under organic management conditions, especially after different leguminous pre-crops.

Grain quality is a complex of quantitative characteristics, depending on the physical parameters of the grain, like bulk density and thousand kernel weight (TKW), and their chemical composition (protein concentration).

Organic methods of farming can have negative effects on the grain quality, especially in the case of protein concentration. The proportion of protein in grain is reduced because of the limited availability of soluble N (Krejčířová et al. 2006; Bilsborrow et al. 2013). According to a number of studies, protein concentration remained considerably lower in organic conditions compared with conventional farming (Talgre et al. 2009; Tamm et al. 2009; Jones et al. 2010; Bilsborrow et al. 2013).

Bulk density is an important criterion of cereal quality (Gaines et al. 1997). High bulk density values increase market grade and price for all the investigated cereal species (wheat, rye, oats, barley). Bulk density affects the productivity and efficiency of flour milling and therefore provides a good indication of grain quality of cereals for bread making (Halverson and Zeleny 1988).

Kernel weight, usually expressed as grams per 1000 kernels (TKW), is a function of kernel size and density. Large and dense kernels normally have a higher ratio of endosperm (Andersson et al. 1999; Halverson and Zeleny 1988). TKW is a valuable physical indicator of grain for the processing industry (malting, milling etc.) (Nuttall et al. 2017). Bulk density and TKW have been found to be less dependent on the cultivation system and pre-crop and may be even higher in organic conditions (Talgre et al. 2009; Tamm et al. 2009; Bilsborrow et al. 2013).

For organic production to be competitive, the produce has to meet certain quality criteria, which do not vary between conventional and organic products. However, organic products that meet the same criteria receive a much higher premium. Currently, organic food is becoming more popular all over the world and consumers often value quality over cost (Crowder and Reganold 2015).

The aim of this research was to assess the effect of leguminous pre-crop species on quality characteristics of the subsequent spring and winter cereals. It was hypothesised that: (1) the preceding legume species, including the less commonly used annual Alexandria and crimson clovers, increase protein concentration, bulk density and TKW of the subsequent cereals in the organic crop rotation and (2) the increase in protein concentration, bulk density and TKW of the subsequent cereals depend on the species of the leguminous pre-crop. In relation to the second hypothesis, the aim was to identify, which of the six green manure pre-crops had the greatest effect on grain quality (protein concentration, bulk density and TKW) and was most suitable for the five subsequent cereals.

Materials and methods

The experiment was carried out during the period of 2011–2014 in an organic crop rotation at the Estonian Crop Research Institute at Jõgeva, Estonia, located in north-eastern Europe (58°45′N, 26°24′E) on clay loam (40–50% of clay) classified as Calcaric Cambic Phaeozem (Loamic) soil ([IUSS] Working Group WRB 2015). The trial field has been certified for organic agriculture since 2009. Cereals and legumes have been grown in crop rotation. A crop of oats was cultivated one year before the legume trial was started. The mean characteristics of the soil horizon were as follows: pH_{KCl} 6.7, P 65, K 101, Ca 3834 mg kg^{-1}, C_{org} 3.5% and total N 0.27%. Soil pH_{KCl} was determined by the ISO 10390 (International Standard 2005); P, K and Ca by Mehlich III (Mehlich 1984); C_{org} by the Tjurin method (Tjurin 1937) and N by ISO 11261 (International Standard 1995).

Six legume species followed by cereals in crop rotation were sown as sole crops (according to the common practice of farmers): early diploid red clover (*Trifolium pratense* L., perennial), cv. Jõgeva 433 (400 seeds m^{-2}), alsike clover (*T. hybridum* L., perennial) cv. Jõgeva 2 (700 seeds m^{-2}), Washington lupin (*Lupinus polyphyllus* Lindl., perennial), cv. Lupi (95 seeds m^{-2}), white sweet clover (*Melilotus albus* Medik., biennial), cv. Kuusiku 1 (950 seeds m^{-2}), crimson clover (*T. incarnatum* L., annual), cv. Contea (600 seeds m^{-2}) and Alexandria clover (*T. alexandrinum* L., annual), cv. Alex (600 seeds m^{-2}). Timothy (*Phleum pratense* L.), cv. Jõgeva 54 (1000 seeds m^{-2}), as a grass species with no N-fixing ability, was used as a control. All crops were drilled in rows. Five cereals were used to test the first-year after-effect (Year 1): winter rye (cv. Sangaste and Elvi; 500 seeds m^{-2}), winter wheat (cv. Ada and Skagen; 450 seeds m^{-2}), spring wheat (cv. Manu and Uffo; 600 seeds m^{-2}), barley (cv. Grace and Maali; 500 seeds m^{-2}) and oats (cv. Ivory and Kalle; 500 seeds m^{-2}). One variety of barley (cv. Maali) and one of oats (cv. Kalle) were used to test the second-year after-effect (Year 2). Each cereal plot in Year 1 was divided into two plots in Year 2 (barley and oats). The trial was arranged as a split-plot design with six legume pre-crops and a non-legume control as main plots (130 m^{-2}) followed by five cereals (two varieties per crop) on 5 m^{-2} subplots in three replicates. The experiment was divided into three consecutive phases – establishment of the legume stands in 2011 (perennials, biennial) and 2012 (annuals) and the first post-legume year in 2013 (Year 1) followed by the second post-legume year in 2014 (Year 2) (Figure 1 and Table 1).

No pest and disease control interventions were carried out and no additional fertilisers were applied; the plant nutrition sources were based on accumulated nutrients from the preceding leguminous crops and the natural soil nutrient pools. A detailed description of the yield of cereals and biomass of leguminous crops can be found in Tamm et al. (2016).

Red, alsike and white sweet clover plants were chopped and ploughed into the soil at the end of flowering at the beginning of August, before the sowing of winter cereals. Washington lupin started flowering in mid-May already and to avoid seed ripening, the first cut was made in June. The growth of Washington lupin plants was hampered by an infection of anthracnose (*Colletotrichum lupini*), influencing the results and its potential fertilisation value was not fully realized. Washington lupin regrowth was ploughed in at the same time as the other legumes. In the area for sowing of spring cereals, red, alsike and white sweet clover were cut, chopped and left to the trial area in the beginning

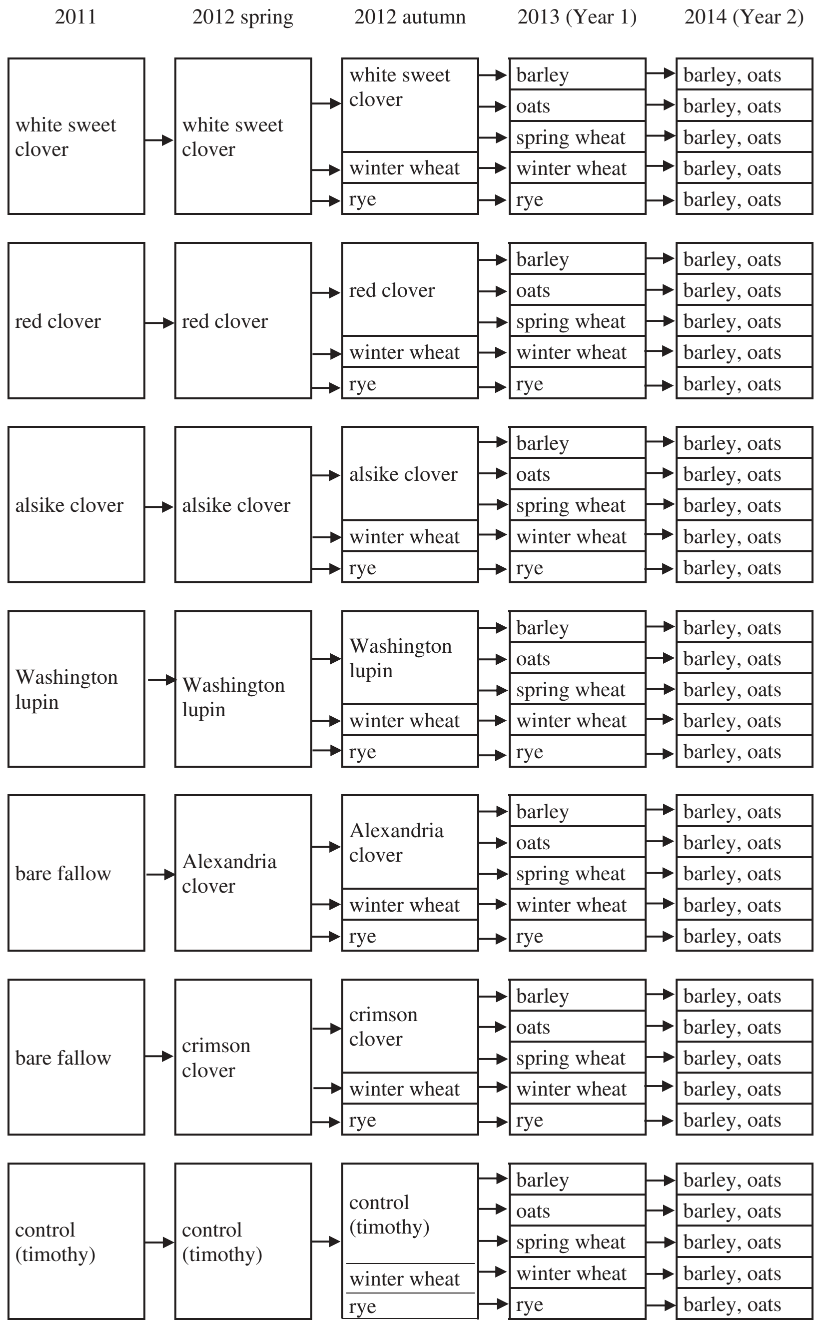

Figure 1. Crop sequence in the trial during 2011–2014.

of July. The same was done with Washington lupin in the end of June. The regrowth of Washington lupin and the primary growth of annual clovers were not cut in autumn and were ploughed in before sowing of the cereals in spring. The timothy grass in the control was sown and ploughed in at the same times as the perennial legumes.

In July (27 July) and October (3 October) 2012, before the incorporation of the green manures, a 6 m^2 area from each plot was cut to measure fresh above-ground biomass. One sample of approximately 1 kg per replicate plot was taken to measure dry matter yield. After weighing, the samples were dried at 105°C

Table 1. Sowing and incorporation dates of crops in the trial period of 2011–2014.

2011	2012 spring	2012 autumn	2013 (Year 1)	2014 (Year 2)
Sowing. 12 July 2011	7 May 2012	6 September 2012	9 May 2013	25 April 2014
Perennial and biennial legumes*: red clover alsike clover Washington lupin white sweet clover	**Annual legumes:** Alexandria clover crimson clover	**Winter cereals:** winter wheat rye	**Spring cereals:** spring wheat barley oats	**Spring cereals:** barley oats
Incorporation of legumes		1 August 2012	15 April 2013	

Note * – in the control, timothy was sown and incorporated at the same time.

to a constant weight and the dry matter yield (DMY) was calculated. To determine the below-ground biomass, rectangular soil blocks were taken from an area of 15 × 30 cm, in layers of 5 cm deep down to 25 cm (0–5 cm, 5–10 cm, 10–15 cm, 15–20 cm, 20–25 cm) by digging. One composite sample was taken in each replicate plot. The roots were washed on a sieve with a mesh size of 2 × 2 mm, dried as described above, and weighed (Vipper 1989). The same or similar methods have been used also by other researchers (Jensen et al. 2004; Talgre et al. 2010, 2012; Bender and Tamm 2014).

The cereal plots were harvested with a plot combine harvester (Hege 140). Plot yields were dried and cleaned. Cereal samples were taken from each plot and protein concentration was measured using near-infrared (NIR) transmittance technology. N and C concentrations of the biomass (above-ground and roots) of legumes were determined by the ISO/TC 16634–2:2009 method (Estonian Centre for Standardisation 2009). TKW of cereals was determined by EVS-EN ISO 520:2010 (Estonian Centre for Standardisation 2010a), bulk density by EVS-EN ISO 7971–3:2010 (Estonian Centre for Standardisation 2010b).

Statistical analyses were carried out by Agrobase Generation II SQL. One-way analysis of variance (ANOVA) was used to test the differences of DMY, C and N of leguminous pre-crops and multi-factorial ANOVA to test the first and second-year after-effect of leguminous pre-crops on cereal quality characteristics. The general linear mixed model of ANOVA was used for calculations of quality characteristics of cereals. Data for the different sampling times and years were analysed separately for quality characteristics of the green manures and the cereals. A Student t-test was used to determine significant effect of leguminous pre-crop life cycle type (perennial, biennial, annual) on DMY, C and N. Least significant differences were calculated for pre-crops DMY, C and N and cereal crop quality characteristics, in order to evaluate the statistical significance of differences. All quality characteristics of the cereal crops were calculated as the average of three replications of two varieties.

Results and discussion

The temperature and humidity conditions for germination of legumes were satisfactory despite of the above-average temperatures and shortage of precipitation in 2011 (Table 2). Higher than average precipitation and average temperatures were favourable for production of biomass of the legumes in 2012. The weather conditions of the main trial year 2013 (Year 1) were favourable as well, although periodic shortage of moisture occurred, especially in the first half of the vegetation period, thus decreasing N uptake by cereals. In 2014 (Year 2) the first part of the growing season was cool, encouraging formation of higher yields and bigger grains but the protein concentrations of the cereals remained low.

Dry matter yield, amount of C and N in leguminous pre-crops (2012)

In this investigation, the dry matter yield (DMY), C and N left in the soil by leguminous pre-crops, except for crimson and Alexandria clovers, was significantly higher compared with the control (Table 3). Average DMY and amounts of C and N in the perennial (red clover, alsike

Table 2. Weather data from 2011–2014 and long-term means (1922–2015) at Jõgeva, Estonia.

	Temperature (°C)					Precipitation (mm)				
Month	2011	2012	2013	2014	Long term mean	2011	2012	2013	2014	Long term mean
January	−4.9	−5.4	−7.0	−7.9	−6.4	72	82	41	35	41
February	−12.3	−11.3	−4.1	−0.4	−6.9	29	47	35	35	31
March	−2.2	−1.0	−8.7	1.9	−3.2	23	48	13	34	31
April	6.0	4.6	2.9	5.9	3.7	10	53	38	10	36
May	11.0	11.5	14.3	11.5	10.3	35	62	83	64	50
June	17.4	13.4	17.7	13.1	14.5	38	110	37	157	69
July	20.5	17.9	17.6	19.2	16.8	34	85	35	48	79
August	16.3	14.8	16.7	16.6	15.4	75	130	70	123	89
September	12.6	12.1	10.9	11.5	10.6	53	59	32	27	66
October	7.3	5.8	6.6	5.2	5.3	73	72	58	48	66
November	3.7	2.4	4.0	1.1	0.3	36	76	82	18	56
December	1.4	−7.2	1.2	−1.7	−3.8	118	51	56	63	47

Table 3. Dry matter yield (DMY) (t ha^{-1}) and amount of C and N (kg ha^{-1}) in biomass (green mass + roots) of pre-crops measured before winter (BWC) and spring (BSC) cereals.

	DMY (t ha^{-1})		C (kg ha^{-1})		N (kg ha^{-1})	
Pre-crop	BWC*	BSC**	BWC	BSC	BWC	BSC
Red clover	14.7 ab	13.9 a	6233 ac	6474 a	319 a	322 a
Alsike clover	13.8 b	13.4 a	5882 a	5993 b	246 b	323 a
Washington lupin	10.3 c	12.0 b	3975 b	4871 c	159 c	268 b
White sweet clover	14.9 ab	11.8 b	6503 c	5302 d	327 a	275 bc
Crimson clover	6.4 d	4.6 c	2666 d	2004 e	111 d	87 d
Alexandria clover	6.3 d	10.6 d	2591 d	3336 f	125 cd	161 e
Control	5.5 d	5.4 c	1828 e	1667 e	38 e	38 f
$LSD_{0.05}$	1.0	0.8	485	374	40	23

Notes: * – green mass + roots sampling 27 July 2012 (for lupin 20 June + 27 July).
** – green mass + roots sampling 3 October 2012.
LSD – least significant difference.
Within columns, mean values followed by the same letter are not significantly different at $p \leq 0.05$.

clover and Washington lupin) and biennial (white sweet clover) leguminous species were, in most cases, considerably higher than that of the annual clovers (crimson clover and Alexandria clover). Other studies have found similar high amounts of DMY, C and N of perennial leguminous species (Skuodiene and Nekrošiene 2009; Lauringson et al. 2013; Bender and Tamm 2014). Red and alsike clover showed relatively high comparable levels of DMY, C and N in the samples taken in July before sowing of winter cereals (BWC) as well as in the samples taken in October before spring cereals (BSC). Although white sweet clover showed the highest levels of DMY, C and N in July, there was a relatively larger decrease of the measured characteristics compared to other legumes by October. Washington lupin had relatively smaller DMY and amounts of nutrients in this experiment, especially in the samples taken in July (BWC). This may be related to the poorer initial development of the stand of this legume crop. These results indicated that red and alsike clover can be suitable for production of green manure as preceding crops for both winter and spring cereals, and white sweet clover primarily for winter cereals.

Annual crimson and Alexandria clover produced lower DMY and amounts of C and N, indicating less potential as green manure crops compared with the other legumes. One of the possible reasons may be lower biomass production potential due to the shorter life cycle of annual species. Previous research confirmed significantly lower biomass production of crimson clover compared with red clover (Coombs et al. 2017). The DMY, amounts of C and N in crimson clover produced in July BWC was higher than that in October BSC (the differences between the dates were not assessed statistically). At the end of August and in September crimson clover seeds mature, after which the plants die (Bender and Tamm 2014). Therefore, the optimum ploughing time for this green manure crop is at the full flowering stage which occurs in the second half of July in north-eastern Europe. Thus, crimson clover can be considered to be more suitable for the fertilisation of winter crops. The N produced by crimson clover before winter crops was the lowest compared to other legumes, but still reached up to 110 kg N ha^{-1} which was sufficient to increase cereal yield and quality. The effect of crimson clover as a green manure crop was also shown by Tamm et al. (2016). In contrast to crimson clover, the DMY and amounts of C and N of Alexandria clover increased during the growing season and were lower in July BWC and higher in October BSC (the differences between the dates were not assessed statistically), since it continued growing until the end of the season. Therefore, Alexandria clover can be more suitable for green manure production for spring cereals than for winter cereals. However, caution should be taken, as the species is sensitive to temperatures below – 6°C (Knight 1985). Annual, biennial and perennial legumes can fit in different niches in diverse crop rotations. Annual clover green manures may provide advantages in crop tillage systems where continuation of a legume beyond one year is undesirable (Ross et al. 2009). Annual legume crops as green manures can add diversity to temperate cropping systems where cereals and oilseeds are predominate (Rice et al. 1993).

Average DMY, C and N content of annual legumes were significantly lower than those of perennial and biennial leguminous pre-crops (Table 4). As DMY consisted of above- and below-ground biomass, the relative proportions of shoots and roots can be important in assessing the potential of legumes for producing green manure. While most legume species produced relatively smaller amounts of roots compared to shoots, Washington lupin differed in this respect from other legumes by producing relatively higher proportion of roots (Figure 2). The results of Lauringson et al. (2013) showed similar proportions of roots (54%) in the total biomass of pure sown Washington lupin. All the legumes showed a decrease in the proportion of roots by October. Crimson clover, the annual species, that produced the smallest biomass and finished growth earlier, had the smallest proportion of roots from the total biomass in the October samples. The other annual, Alexandria clover has a longer vegetation period and it continued active growth and flowering even in October.

The first-year after-effect of legumes (Year 1)

Protein concentration

The variation in protein concentration of all the tested cereals was significantly influenced by the pre-crop (A) (Table 5). The influence of variety (B) was significant for all the cereals, except rye. A x B interaction remained non-significant for most of the cereals, except barley, showing a similar reaction of cereal varieties to the pre-crop.

The weather conditions during the year of the first trial were quite favourable for comparatively high yield and the average of all the cereals was 4.8 t ha^{-1} (Tamm et al. 2016). Grain yield and protein concentration are often inversely related. Therefore, the protein concentration of the cereals remained moderate after all the legumes (Table 6). In general, protein concentration in organic cultivation is estimated to be 2–3 percentage points lower compared to conventional cultivation (Bilsborrow et al. 2013). The highest average protein was accumulated by winter wheat (11.1%) and the lowest by rye (8.5%). The protein concentration of all the cereals, except rye, increased significantly after perennial leguminous pre-crops by 0.8 to 2.6 percentage points

Table 4. Average dry matter yield (DMY) (t ha^{-1}) and amount of C and N (kg ha^{-1}) in biomass (green mass + roots) of perennial, biennial and annual pre-crops measured before winter (BWC) and spring (BSC) cereals.

	DMY (t ha^{-1})		C (kg ha^{-1})		N (kg ha^{-1})	
Life cycle type of pre-crop	BWC	BSC	BWC	BSC	BWC	BSC
Perennial	12.9 a	13.1 a	5363 a	5779 a	241 a	304 a
Biennial	14.9 a	11.8 a	6503 a	5302 a	327 a	275 a
Annual	6.4 b	7.6 b	2629 b	2670 b	118 b	124 b

Within columns, mean values followed by the same letter are not significantly different at $p \leq 0.05$ (t-test).

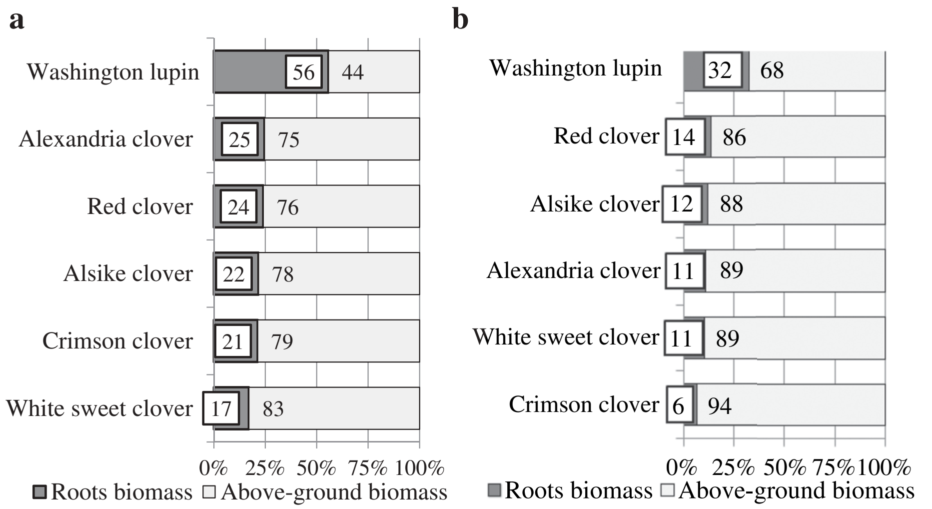

Figure 2. Proportions of roots and above-ground biomass from total dry matter yield of the leguminous pre-crops in July 2012, before of the winter cereals (a) and in October 2012, before spring cereals (b).

Table 5. Analyses of variance for protein concentration of cereals depending on leguminous pre-crop species (A), proceeding cereal variety (B) and their interaction (A x B) in Year 1.

Cereal	Source of the variation	df	SS	MS	F	*p*
Barley	Pre-crop (A)	6	6.478	1.080	15.91	0.000***
	Variety (B)	1	4.937	4.937	72.74	0.000***
	A x B	6	1.443	0.240	3.54	0.011*
Spring wheat	Pre-crop (A)	6	41.571	6.929	48.47	0.000***
	Variety (B)	1	16.594	16.594	116.09	0.000***
	A x B	6	0.439	0.073	0.51	0.794ns
Oats	Pre-crop (A)	6	10.672	1.779	3.21	0.017*
	Variety (B)	1	2.881	2.881	5.19	0.031*
	A x B	6	1.166	0.194	0.35	0.903ns
Rye	Pre-crop (A)	6	3.242	0.540	2.94	0.025*
	Variety (B)	1	0.002	0.002	0.01	0.915ns
	A x B	6	1.240	0.207	1.12	0.377ns
Winter wheat	Pre-crop (A)	6	8.451	1.409	17.36	0.000***
	Variety (B)	1	0.420	0.420	5.18	0.031*
	A x B	6	0.880	0.147	1.81	0.137ns

Notes: df – degrees of freedom; SS – sums of squares; MS – mean squares.
F – treatment mean square/error mean square.
p – significance probability value.
* – significant at $p < 0.05$; ** – significant at $p < 0.01$;*** – significant at $p < 0.001$.
ns – not significant.
Year 1 – the first-year after-effect of legumes.

compared with the control, whereas annual leguminous pre-crops had the most moderate effect on the protein concentration of the cereals. The results from this study confirmed earlier findings of Doltra et al. (2011) that leguminous green manure species had a positive effect on protein concentration of cereals in organic arable systems. The highest proteins as an average of all the cereals were formed after red and alsike clovers and the lowest after annual clovers.

In Estonia, the commonly used criterion for protein for marketing of high quality baking wheat is 13.0–14.0%. The protein concentration of spring wheat was in the range of 9.3–12.2% and increased the most after red and alsike clover, by 1.8 and 2.6 percentage points respectively, compared to the control. Protein concentration of winter wheat remained between 10.2–11.5%. In contrast to the

Table 6. Protein concentration (%) of cereals after different legumes in Year 1.

Pre-crop	Barley	Spring wheat	Oats	Rye	Winter wheat
Red clover	10.4 a	11.4 a	11.3 a	8.8 a	11.4 ab
Alsike clover	10.2 a	12.2 b	11.1 ab	8.2 b	11.3 ab
Washington lupin	10.2 a	10.8 c	11.0 abc	8.8 a	11.4 ab
White sweet clover	10.5 a	9.9 d	10.3 cd	8.9 a	11.5 b
Crimson clover	9.7 b	9.8 d	10.5 bcd	8.2 b	11.1 b
Alexandria clover	9.6 b	9.3 e	10.4 bcd	8.3 b	11.1 b
Control	9.4 b	9.6 de	9.8 d	8.6 ab	10.2 c
$LSD_{0.05}$	0.3	0.4	0.7	0.4	0.3

Notes: LSD – least significant difference.
Within columns, mean values followed by the same letter are not significantly different at p ≤ 0.05.
Year 1 – the first-year after-effect of legumes.
Within columns, mean values followed by the same letter are not significantly different at $p \leq 0.05$.

other cereals, winter wheat had significant increase in protein concentration (0.9–1.3 percentage points) after all the leguminous pre-crops. Maiksteniene and Arlauskiene (2004) reported lower (10.6%) and Talgre et al. (2010) higher (12.2%) protein concentration of winter wheat after red clover compared to the results reported here (11.4%). The differences could be explained by different weather conditions. The protein concentrations of winter wheat remained the lowest in the Lithuanian trial with moderate temperatures and optimal precipitation, whereas it was highest in Estonia in drought conditions. Although protein concentration of wheat remains generally lower in organic compared to conventional farming, it is quite usual for organic farmers to gain an increase in selling price for a slight increase of the protein concentration (Mazzoncini et al. 2015) and therefore even small improvement should be considered significant (Tosti et al. 2016).

Bulk density

Bulk density is influenced by many factors, including agronomic practice and weather conditions, disease infection, kernel shape and density (Gaines et al. 1997; Ingver 2007). Pre-crop and variety had a significant impact on the variation of bulk density of all the cereals (Table 7). A x B interaction remained non-significant for most of the cereals except spring wheat.

First-year after-effect of all the leguminous pre-crops on bulk density was significantly positive compared to the control in most cases but there were no clear trends (Table 8). Compared to the control species, the legume pre-crops resulted in the largest increases in bulk density in rye and oats. The bulk density of barley and rye increased the most after alsike clover, whereas, spring wheat achieved its highest bulk density after Washington lupin. However, the lowest bulk densities were obtained when winter wheat was grown after red clover, barley and spring wheat after crimson clover and oats after Washington lupin and white sweet clover.

In this study, winter wheat had higher (not assessed statistically) bulk density in Year 1 compared to spring wheat, since winter wheat suffered less from the early periodic shortage of moisture (Table 8). The negative influence of unfavourable growing conditions on the bulk density of wheat is a well-known phenomenon (Tipples 1986). However, both wheat types achieved the necessary quality requirements of the food industry (75.0 kg hL^{-1}). The marketing minimum for barley bulk density is 64.0 kg hL^{-1}. In Year 1, bulk density of barley was high after all the legumes. The recommended bulk density of oats for food purposes is 49.0 kg hL^{-1} or higher (Ganßmann and Vorwerck 1995). The bulk density values of the oats and the rye, following all of the legume species, fulfilled the minimum requirements.

Thousand kernel weight

Variation of TKW of the tested cereals, except oats, was significantly influenced by the pre-crop (A) (Table 9). The influence of variety (B) was significant for all the cereals. A x B interaction remained non-significant for most of the cereals except winter wheat.

Table 7. Analyses of variance for bulk density of cereals depending on leguminous pre-crop species (A), proceeding cereal variety (B) and their interaction (A x B) in Year 1.

Cereal	Source of the variation	df	SS	MS	F	*p*
Barley	Pre-crop (A)	6	28.876	4.813	15.81	0.000***
	Variety (B)	1	11.006	11.006	36.15	0.011*
	A x B	6	3.632	0.605	1.99	0.100ns
Spring wheat	Pre-crop (A)	6	45.983	7.664	22.10	0.000***
	Variety (B)	1	5.429	5.429	15.65	0.001***
	A x B	6	10.623	1.770	5.10	0.001**
Oats	Pre-crop (A)	6	74.909	12.485	7.92	0.000***
	Variety (B)	1	168.000	168.000	106.57	0.000***
	A x B	6	5.883	0.981	0.62	0.711ns
Rye	Pre-crop (A)	6	86.949	14.492	24.11	0.000***
	Variety (B)	1	152.381	152.381	253.53	0.000***
	A x B	6	3.749	0.625	1.04	0.423ns
Winter wheat	Pre-crop (A)	6	38.476	6.413	16.09	0.000***
	Variety (B)	1	108.804	108.804	272.92	0.000***
	A x B	6	3.916	0.653	1.64	0.177ns

Notes: df – degrees of freedom; SS – sums of squares; MS – mean squares.
F – treatment mean square/error mean square.
p – significance probability value.
* – significant at $p < 0.05$; ** – significant at $p < 0.01$;*** – significant at $p < 0.001$.
ns – not significant.
Year 1 – the first-year after-effect of legumes.

Table 8. Bulk density (kg hL^{-1}) of cereals after different legumes in Year 1.

Pre-crop	Barley	Spring wheat	Oats	Rye	Winter wheat
Red clover	69.4 a	78.1 a	51.6 a	69.3 a	79.3 a
Alsike clover	70.3 b	78.7 ac	52.4 ab	71.0 b	81.5 b
Washington lupin	69.5 a	79.5 bc	51.2 a	69.5 a	81.7 b
White sweet clover	68.6 cde	78.2 a	51.2 a	69.4 a	81.7 b
Crimson clover	68.3 cd	76.9 d	53.7 b	70.0 ac	81.2 b
Alexandria clover	69.0 ae	79.2 c	53.0 b	70.5 bc	81.1 b
Control	67.6 f	76.5 d	49.3 c	66.2 d	79.5 a
$LSD_{0.05}$	0.5	0.6	1.3	0.8	0.6

Notes: LSD – least significant difference.
Within columns, mean values followed by the same letter are not significantly different at $p \le 0.05$.
Year 1 – the first-year after-effect of legumes.

The weather conditions were quite favourable for the formation of heavier than average grains. All the green manure pre-crops increased significantly TKW of barley, spring and winter wheat (Table 10). However, the effect of leguminous pre-crops on TKW of rye was negative and there was no significant effect of the legumes on the oats. Previous experiments with red clover have found positive effects on TKW of winter and spring wheat and winter rye (Skuodiene and Nekrošiene 2009; Talgre et al. 2009, 2010), thus partly confirming the findings of this study. TKW (as the mean of all the leguminous pre-crops) was the highest for barley (50.0 g) followed by winter wheat (46.2 g), oats (44.0 g), spring wheat (37.2 g) and rye (36.0 g). For barley and spring wheat, the grains with the highest TKW were measured after alsike clover and Washington lupin. Oats and rye are naturally less intensive types of cereals compared to barley and wheat, with a lower demand for nutrients.

The second-year after-effect of legumes (Year 2)

The benefits of legumes were carried over into the second year. All the legume species had a positive second-year after-effect on the protein concentration of barley and oats (Table 11), with the largest after-effect after red and alsike clover, which exceeded the control by 1.0–1.3 percentage points. The larger second-year positive after-effect of red and alsike clover on protein

Table 9. Analyses of variance for thousand kernel weight of cereals depending on leguminous pre-crop species (A), proceeding cereal variety (B) and their interaction (A x B) in Year 1.

Cereal	Source of the variation	df	SS	MS	F	*p*
Barley	Pre-crop (A)	6	89.956	14.993	13.16	0.000***
	Variety (B)	1	8.595	8.595	7.54	0.011*
	A x B	6	4.265	0.711	0.62	0.710ns
Spring wheat	Pre-crop (A)	6	80.451	13.409	8.33	0.000***
	Variety (B)	1	92.115	92.115	57.26	0.000***
	A x B	6	6.665	1.111	0.69	0.659ns
Oats	Pre-crop (A)	6	2.570	0.428	0.34	0.907ns
	Variety (B)	1	46.515	46.515	37.28	0.000***
	A x B	6	5.945	0.991	0.79	0.583ns
Rye	Pre-crop (A)	6	73.966	12.328	10.96	0.000***
	Variety (B)	1	306.180	306.180	272.21	0.000***
	A x B	6	4.357	0.726	0.65	0.693ns
Winter wheat	Pre-crop (A)	6	48.841	8.140	21.51	0.000***
	Variety (B)	1	560.275	560.275	1480.19	0.000***
	A x B	6	6.815	1.136	3.00	0.023*

Notes: df – degrees of freedom; SS – sums of squares; MS – mean squares.
F – treatment mean square/error mean square.
p – significance probability value.
* – significant at $p < 0.05$; ** – significant at $p < 0.01$;*** – significant at $p < 0.001$.
ns – not significant.

Table 10. Thousand kernel weight (g) of cereals after different legumes in Year 1.

Pre-crop	Barley	Spring wheat	Oats	Rye	Winter wheat
Red clover	49.8 a	37.7 ab	44.0 a	37.1 a	45.8 a
Alsike clover	51.1 b	38.7 b	43.9 a	34.2 b	45.9 a
Washington lupin	51.1 b	37.3 ac	43.7 a	37.3 a	46.5 b
White sweet clover	50.3 ab	36.3 c	44.3 a	36.9 ad	47.1 c
Crimson clover	48.1 c	36.9 ac	43.9 a	34.8 bcd	45.8 a
Alexandria clover	49.8 a	36.5 ac	43.9 a	35.8 d	45.9 ab
Control	46.8 d	33.9 d	44.5 a	38.2 a	43.3 d
$LSD_{0.05}$	1.0	1.3	1.1	1.1	0.6

Notes: LSD – least significant difference.
Within columns, mean values followed by the same letter are not significantly different at $p \leq 0.05$.
Year 1 – the first-year after-effect of legumes.

Table 11. The protein concentration, thousand kernel weight and bulk density of barley and oats in Year 2.

	Protein (%)		Thousand kernel weight (g)		Bulk density (kg hL^{-1})	
Pre-crop in 2012	Barley	Oats	Barley	Oats	Barley	Oats
Red clover	10.5 a	10.4 a	44.4 a	43.8 ab	67.9 a	48.8 ac
Alsike clover	10.5 a	10.3 a	44.3 a	43.8 ab	67.3 b	49.4 ab
Washington lupin	10.0 b	9.6 b	43.7 b	44.2 a	66.5 c	50.0 b
White sweet clover	10.2 ab	9.7 b	43.9 ab	44.3 a	66.8 bc	48.4 c
Crimson clover	10.0 b	9.6 b	44.1 ab	43.6 b	67.0 bc	49.6 b
Alexandria clover	10.0 b	9.4 bc	43.6 b	44.1 ab	66.7 cd	49.3 ab
Control	9.5 c	9.1 c	44.1 ab	43.8 ab	67.1 bc	47.9 c
$LSD_{0.05}$	0.4	0.3	0.5	0.5	0.5	0.7

LSD – least significant difference.
Within columns, mean values followed by the same letter are not significantly different at $p \leq 0.05$.
Year 2 – the second-year after-effect of legumes.

concentration of barley and oats could be explained by the high DMY and N content of these legumes. There was an increase in the protein concentrations of barley and oats also after all other legumes, except for oats after Alexandria clover. In the second post-legume year, Bender and

Table 12. Quality characteristics of barley and oats after cereal pre-crops in Year 2.

	Protein(%)		Thousand kernel weight (g)		Bulk density(kg hL^{-1})	
Pre-crop in 2013	Barley	Oats	Barley	Oats	Barley	Oats
Barley	10.2 a	9.8 a	43.9 a	43.6 a	66.4 a	48.5 a
Oats	10.1 a	9.6 a	44.1 a	44.1 b	66.3 a	48.3 a
Spring wheat	10.2 a	9.7 a	44.0 a	43.8 ab	66.5 a	48.7 a
Winter wheat	9.9 a	9.6 a	44.0 a	43.9 ab	67.6 b	49.5 b
Rye	10.0 a	9.8 a	44.1 a	44.2 b	68.3 c	49.7 b
$LSD_{0.05}$	0.3	0.3	0.4	0.4	0.4	0.6

Notes: LSD – least significant difference.
Within columns, mean values followed by the same letter are not significantly different at $p \le 0.05$.

Tamm (2014) found a positive effect of Washington lupin on the protein of barley, while red and crimson clover had no significant effect.

In the second year, the average bulk density of barley and oats decreased by 3.1% and 5.6%, respectively compared to that in Year 1 (though the significance of these differences were not statistically analysed). Most of the legume species had a positive significant second-year after-effect on oats bulk density (except white sweet clover) ranging from 48.4–50.0 kg hL^{-1}. Bulk density of barley increased only after red clover, remaining between 66.5–67.9 kg hL^{-1}. Barley also had the highest yields after red clover as shown in Tamm et al. (2016). Talgre et al. (2010) also detected a positive effect of red clover on barley bulk density.

The preceding legume crops had no significant effect on TKW of barley and oats in Year 2 compared to the control. The same tendency regarding barley was described in the study of Bender and Tamm (2014) and no effect of crimson clover to TKW of barley and spring wheat in the second year was found. In contrast to the findings reported here, Talgre et al. (2010) found a positive second-year after-effect of red and white sweet clover on barley TKW.

The winter and spring cereals (Year 1) sown after legumes, had no significant effect on the protein accumulation of the succeeding barley and oats (Year 2) (Table 12). Bulk density of oats and barley was higher after winter cereals compared to the spring cereals. The preceding cereals had no significant influence on TKW of barley, the differences remained modest also for oats.

Conclusions

The results of this study confirmed the hypotheses that various legume species were good pre-crops, offering improvement of the quality characteristics (protein concentration, bulk density and TKW) of the subsequent cereals. There were clear differences in the fertilisation value of the legumes, depending whether they were perennial, biennial or annual species. The N yield produced by perennial and biennial species was high, providing significant benefit to the quality of subsequent cereals. Even the short growing annual species (crimson and Alexandria clover) were able to accumulate considerable amounts of N, though it was not sufficient to increase the quality characteristics of most of the subsequent cereals. The average dry matter yield and amounts of C and N in the legume species were significantly higher compared to the non-legume control.

The first- and second-year after-effect of leguminous pre-crops on grain quality varied depending on the preceding legume and following cereal species. Perennial red and alsike clover proved to be suitable as green manure pre-crops for all the cereals tested. Biennial white sweet clover has the potential to produce a large amount of biomass and can be used as pre-crop for cereals similar to the perennial legumes, but it may be more suitable for winter cereals due to its intensive growth in the first part of the season. The results of this study on the potential of annual crimson and Alexandria clovers as green-manures are valuable, since there is almost no previous research on these species in the northern part of Europe. Out of all the legume species, perennial red and alsike clover had the most positive second-year after-effect on protein concentration and bulk density of barley and oats. The benefits of the legumes on the protein concentration were carried over to the second year.

Green manure leguminous species with high ability to increase soil fertility are key components in organic crop rotations and are important in order to maintain and improve grain quality in sustainable agriculture. This study demonstrated that cultivation of effective leguminous green manure species improved the grain quality of subsequent winter and spring cereals in an organic crop rotation. This study offered practical recommendations for diversifying the selection of leguminous pre-crop species for organic producers in conditions of climate change and prolongation of the growing season in north-east European areas.

Acknowledgments

This research was funded by the Estonian Rural Development Plan sub-measure: Cooperation in the development of new products, processes and technologies in the sector of agriculture, food and forestry. The project was named: "Applied research to increase the effectiveness and sustainability of organic production and improvement of food cereals quality".

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Ministry of Rural Affairs of the Republic of Estonia by the Estonian Rural Development Plan sub-measure: Cooperation in the development of new products, processes and technologies in the sector of agriculture, food and forestry [grant number 1710011780024].

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