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Spring rye as a source of biomass and carbon in the soil

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Abstract. The aim of the experiment was to determine the mass yield and amount of total carbon accumulated by spring rye biomass in individual stages of growth determined according to the BBCH scale (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie): BBCH 30–31 – leaves, BBCH 55–59 – whole plants, BBCH 89–92 – grain and straw). The required results were obtained by conducting a field experiment (2009-2011) which tested the effect of the application of nitrogen (0, 30, 60, 90 kg ha^{-1}) and sulphur (0, 40 kg ha^{-1}) on biomass yield, carbon content and accumulation, and also the C:N ratio. N application in the amount of 60 and 90 kg ha^{-1} was shown to have the most beneficial effect on biomass yield at each stage of growth. Carbon was accumulated in the amount of 1 294 kg ha^{-1} by the leaves (BBCH stage 30–31), 2 365 kg ha^{-1} by the whole plants (BBCH 55–59), 1 334 kg ha^{-1} by the grain (BBCH 89–92), and 2 062 kg ha^{-1} by the straw (BBCH 89-92). The total accumulation of carbon by the dry matter of grain + straw increased up to the application rate of 90 kg N ha^{-1} following the addition of sulphur. The average total accumulation of C was 3 408 kg ha^{-1}. The unit accumulation of carbon was reduced following the application of 30 kg N ha^{-1}, but increased significantly with the level of nitrogen applied, averaging 892.7 C t^{-1}. In general, it may be concluded that under conditions without manure application, ploughing the green matter and straw of spring rye is a good source of carbon in the soil, and is furthermore a technique aimed at limiting global warming by reducing greenhouse gases emissions.

Keywords: nitrogen, sulphur, rye biomass, carbon

1. INTRODUCTION

One of the most important crop plants in Poland is rye. As a weed in wheat, rye (*Secale cereale* L.) spread from Asia to Europe in the Neolithic age (Tarkowski, 1983). In the 16th and 17th centuries, rye became the basic cereal for consumption in Central and Eastern Europe, as wheat bread was scarcer and its price was 4-5 times that of rye bread (Li, 2012). In the 20th century, spring rye was cultivated quite widely in Poland (Dopka *et al.*, 2007). Before 1945, there were two spring rye cultivars registered. One of the cultivars - Strzekęcińskie was registered in 1964 and was cultivated in practice until 1981 (Grochowski, 1995). In 1999, two new cultivars, Abago and Bojko, were introduced. Similarly, in Germany, the new Ovid cultivar of spring rye was registered in 1995 (Galek and Grochowski, 1998). The countries with the highest production of rye are the Russian Federation, Poland and Germany, which account 57.66% of global rye production (Narolski, 2016) The average yield of spring rye grain obtained in the COBORU (Research Centre for Cultivar Testing) trials in Słupia Wielka (Poland) for the cultivars registered in 2022 amounted to 4.11 t ha^{-1} (COBORU, 2022). Poland, Belarus, Ukraine and Germany are the largest consumers of rye (Narolski, 2016; Podleśna *et al.*, 2018; Klikocka *et al.,* 2020, 2022).

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Spring rye has taken on great economic importance in recent years. Grown for grain, it can be used in bread production (Narolski, 2016). The Bojko cultivar can be a useful addition to animal feeds, this is due to its favourable amino acid composition and high protein content, *i.e.* 23.7-14.8% (Galek and Grochowski, 1998). The Bojko cultivar is also characterized by a large proportion of green matter and straw, which remains green practically until harvest, so that it may be used as a green fertilizer (Dopka *et al.*, 2012). Therefore, current forms of spring rye are used as a green fertilizer, green forage, and grain. Short-straw forms can be an excellent supporting species in mixed crops with legume plants (Galek and Grochowski, 1998). Apart from the traditional uses of rye (fodder, human consumption, and alcohol production), spring rye can be a very good substrate for the production of biogas. It can also be used as an important phytosanitary plant in increasingly simplified crop rotations. Moreover, it can be useful in cases where the re-sowing of winter wheat and winter rye is necessary if the crop is attacked by snow mould or completely frozen (Narolski, 2016).

In order to meet the goals of the Paris Agreement adopted during the 21st Conference of Parties (COP21) of the Parties to the United Nations Framework Convention on Climate Change (UNFCCC), it was assumed that in order to reduce global warming to below 2°C, greenhouse gas emissions (GHG) must be reduced by introducing different technologies of CO_2 removal from the atmosphere (UNFCCC, 2023). One of these is carbon capture by agricultural crops which can significantly reduce anthropogenic emissions of carbon dioxide (CO_2) to the atmosphere. Therefore, there is a need for joint efforts to reduce CO_2 emissions to the air and increase C sequestration in the soil. The agricultural sector currently has a major carbon (C) footprint and is responsible for more than 25% of global anthropogenic greenhouse gas emissions (Jat *et al.*, 2022). At the same time, agricultural soils have a substantial capacity to absorb CO_2. During their growth cycle, plants, including crop plants, accumulate large amounts of C and N from the atmosphere in terms of both yield as well as above-ground and underground residues (Klikocka and Cybulska, 2014).

Soil and the agrotechnical treatments performed on it play a fundamental role in the global carbon cycle. However, the C cycle together with changes in the concentration of greenhouse gases in the atmosphere can have a significant influence on the global biochemical cycle (Heikkinen *et al.*, 2013). Soils are the largest terrestrial reservoir of elements and could be the best means of removing carbon from the atmosphere (FAO, 2004). Therefore, in practice there is a need for technologies, methods, and tools which could potentially lead to the capture of CO_2 from the atmosphere, resulting in an increase in carbon (C) sequestration in the soil (Kalembasa *et al.,* 2023). The addition of organic matter from plants or their residues contributes to a significant extent to carbon storage and sequestration in the soil which can lead to a reduction in greenhouse gas emissions (Powlson *et al.,* 2011). The storage of organic matter in the soil is directly linked to the amount of C introduced to it by ploughing in straw, biomass, underground roots, and rhizomes (Pasricha, 2017; Dopka *et al.*, 2012). Stores of organic C in the soil undergo changes due to the biotic activity of plants, microbes (fungi and bacteria), and ‘ecosystem engineers’ *i.e.* earthworms, termites, and ants (Dignac *et al.*, 2017). C sequestration in agroecosystems can easily be increased by returning vegetation to the soil and incorporating organic soil components (Fang *et al.,* 2015). Depending on the type of land use and environmental conditions, as well as the agricultural practices applied, mineral soils can either be a carbon source or sink (FAO, 2004; Eglin *et al.*, 2010; Bardule *et al.*, 2017).

In order to establish the amount of total carbon accumulated by the biomass of spring rye, the biomass yield, carbon content and its accumulation and also the C:N ratio, in individual stages of growth (BBCH 30-31: in leaves, BBCH 55-59: in whole plants, BBCH 89-92: in grain and straw), a field experiment was carried out using different doses of nitrogen (0, 30, 60, 90 kg ha^{-1}) and sulphur (0, 40 kg ha^{-1}).

2. MATERIAL AND METHODS

The experiment was located in the village Malice, which is situated in south-eastern Poland. Its precise location is defined by the following coordinates: east longitude 23º45’ E and north latitude from 50º42’ N. The following macroregions are distinguished in the Zamość region: the Lublin Upland, Roztocze, the Sandomierz Basin, Volhynian Polesie, the Western-Volhynian Upland and Pobuże (Kondracki, 1980).

Weather conditions were highly varied during the study period (2009-2011). The total precipitation during the growing season (March-July) in 2009 amounted to 300.2 mm, which was 12.8 mm lower than the long-term average (1971-2005: 313.0 mm). In the 2010 growing season, rainfall amounted to 357.3 mm and exceeded the long-term average by 44.3 mm. By contrast, in 2011, precipitation amounted to 261.0 mm and was 52.0 mm lower than the long-term average. In 2009, a particularly high level of precipitation was observed in May (102.6 mm) and June (124.4 mm), whereas July was dry (24.2 mm). In 2010, May (98.2 mm) and July (143.5 mm) were wet, while the amount of rainfall in June was optimal for rye vegetation (62.9 mm). In 2011, the rainfall distribution was optimal during the period from April to June, but July was very wet (148.0 mm). The air temperature sums during the 2009 growing season (March-July) amounted to 2 030ºC and were higher than the long-term average (1971-2005: 1 798ºC) by 232ºC. The air temperature sums during the 2 010 growing season amounted to 2 089ºC and were higher than the long-term

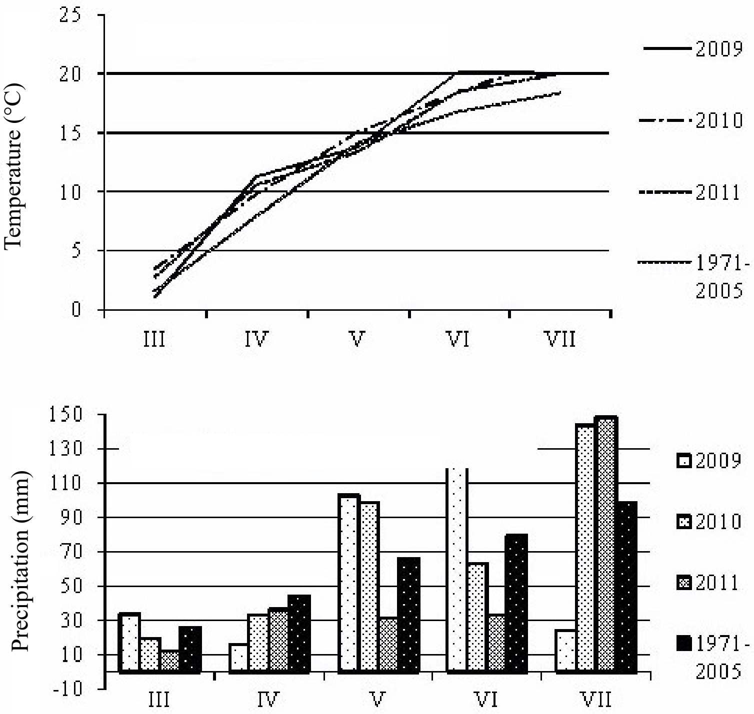

Fig. 1. Monthly precipitation totals (mm) and average air temperature in each month (°C) in the years 2009-2011 and the long-term averages (1971-2005) (Research Station in Zamość).

average (1971-2005) by 291°C. The air temperature sum during the 2011 growing season amounted to 1 995°C and was higher than the long-term average (1971-2005) by 197°C. It is worth emphasizing that in every month of the 3 years of the study period, the air temperature was higher than the long-term average (Fig. 1).

The subject of the study was the Bojko cultivar of spring rye (*Secale cereale* L.), it was fertilized with varying amounts of nitrogen (factor I) and sulphur (factor II). The paper presents the results of a three-year field experiment conducted in 2009-2011.

The experiment was set up on Cambisols (Ditzler *et al.*, 2017) consisting of a light silty sand (sand 68%, loam 31%, clay 1%), this has been classified as a good rye complex. The experiment was begun in the last 10 days of September 2008 and was set up in a split-plot design with four replicates. The soil in the experimental field was characterized by a slightly acidic reaction (pH_{KCl} = 5.6), a high content of available phosphorus (P_2O_5) (52.1 mg kg^{-1}; double-lactate extraction at pH 3.6 (1:50 m/v), a medium content of potassium (K_2O) (84.5 mg kg^{-1}; extracted as P_2O_5) and magnesium (Mg) (34.5 mg kg^{-1}; extracted with 0.0125 mol L^{-1} $CaCl_2$ (1:10 m/v ratio). The content of sulphate sulphur ($S-SO_4$) was found to be low (12.4 mg kg^{-1}; extracted with 0.025 mol L^{-1} KCl) (Narolski, 2016).

The forecrop for spring rye was potato, which was fertilized with cattle manure in the amount of 30 t ha^{-1}. After harvest, the potato medium ploughing was carried out at a depth of 20 cm.

The field experiment included 2 factors:

1. Nitrogen application at a dose: 0, 30, 60, 90 kg ha^{-1}.
2. Sulphur application at a dose: 0, 40 kg ha^{-1}.

The nitrogen was applied in the form of ammonium nitrate (34%). Nitrogen in the amount of 30 kg ha^{-1} was applied before sowing. In turn, a dose of 60 kg N ha^{-1} was applied in two equal portions – before sowing and as a top dressing at the start of stem elongation (BBCH 30–31). The highest nitrogen dose *i.e.* 90 kg ha^{-1} was applied in three equal portions – before sowing, as a top dressing at the start of stem elongation (BBCH 30–31), and as a top dressing between the middle and the end of heading (BBCH 55–59). Sulphur in the amount of 40 kg ha^{-1} was applied in two portions – *i.e.* 30 kg S ha^{-1} before sowing in the form of kieserite ($MgSO_4 \times H_2O$) and 10 kg S ha^{-1} as a foliar application, this was applied between the middle and the end of heading (BBCH 55–59) in the form of magnesium sulphate heptahydrate ($MgSO_4 \times 7H_2O$) (5% solution of SO_3 per 300 L H_2O ha^{-1}).

Before sowing the rye grain, phosphorus (39.6 kg P ha^{-1} in the form of granular triple superphosphate, 46%,) and potassium (83 kg K ha^{-1} as potassium chloride, 60%,) fertilizers were applied in all experimental plots. Magnesium lime and calcium carbonate were applied to the plots with various fertilizer combinations in order to balance the soil pH.

The area of the plots for sowing and observation was 30 m^2, while the area for harvest was 20 m^2 (4 × 5 m). The seeds of the Bojko cultivar of spring rye were sown in the third decade of March or in the first decade of April, depending on climate and soil conditions, and the grain harvest was performed by hand in the first decade of August. The sowing rate was 140 kg ha^{-1}, assuming a density of 350 germinating seeds per m^2.

Before sowing and before the growing season commenced, the spring rye grain was treated with Vitavax 200 FS (carboxin + thiram), in the amount of 300 ml per 100 kg of grain, in order to protect the plants from fungal diseases. During the growing season, the dicotyledonous and monocotyledonous weeds were controlled during tillering (BBCH 28) by using the herbicides Puma Uniwersal 069 EW (fenoxaprop-P-ethyl) in the amount of 1 L ha^{-1} + Granstar 75 WG (sulfmetmetonmethyl). In order to obtain shorter rye stems, the growth regulator Stabilan 750 SL (chlormequat chloride) was applied in the amount of 1.8 L ha^{-1} at the start of the stem elongation phase (BBCH 30–31). Disease and pest control agents were not applied in the growing season, as there were no agents recommended for this purpose by the Institute of Plant Protection in Poznań.

During the field experiment, the phaenological stages of the plants were noted (Witzenberg *et al.,* 1989) (Table 1).

The plant material was sampled during the three development stages, *i.e.* stem elongation to the first node stage (BBCH 30–31), the stage between the middle and the end of the heading process (BBCH 55–59), and the stage of fully ripened grain and straw (BBCH 89–92). In the development stages of BBCH 30–31 and 55–59, the plants were collected from an area of 1 m^2 in order to determine the fresh and air-dry weight. At the BBCH stage 89–92 samples of mature plants were collected for the assessment of the effect of nitrogen and sulphur fertilizers on the grain and straw yields. Fresh samples of plant material were weighed and then subsequently crushed and dried at 60°C. The dry matter content was determined by applying the oven-dry method at 105°C. The total carbon content in the dry matter of the samples (g kg^{-1} d.m.) was determined using a Carbon Elemental Analyser LECO CNS-2000 belonging to the Department of Forest Ecology, University of Agriculture in Kraków. The total carbon content was used to perform the calculation of its accumulation (uptake) in plants. The following calculation method and terminology were used according to Grzebisz (2009):

$$CA = C \times DMY, \tag{1}$$

where: CA – carbon accumulation (kg ha^{-1}), C – carbon content (g kg^{-1} d.m.), DMY – dry matter yield (t ha^{-1}).

For the statistical analysis, an analysis of variance with Snedecor's F-test was applied, this was followed by the calculation of its distribution. The significance of differences was determined using Tukey's test ($\alpha = 0.05$, $\alpha = 0.01$). The standard deviation was also determined. An Excel 7.0 spreadsheet and Statistica 12 software (StatSoft Inc., 2013) were used for the compilation and statistical analysis of the results.

3. RESULTS

An analysis of variance showed that for the fresh and air-dry yield of spring rye in the tillering (BBCH 30–31) and heading (BBCH 55–59) stages, its weight increased significantly following the application of the highest nitrogen dose *i.e.* 90 kg N ha^{-1} (Tables 2, 3). The addition of sulphur significantly increased the fresh and air-dry yield of the plants in both of these stages of growth. The fresh yield and air-dry weight of the plants following the addition of sulphur increased by 4.3 and 7.8% at BBCH 30–31 and by 5.0 and 4.6% at BBCH 55–59, respectively. The interaction of the nitrogen and sulphur applications stemmed from the fact that the nitrogen was applied three times (30 kg N ha^{-1} before sowing, 30 kg N ha^{-1} at BBCH 30–31, and 30 kg N ha^{-1} at BBCH 55–59), therefore up to the BBCH 30–31 stage at all tested doses of nitrogen (*i.e.* 30, 60 and 90 kg N ha^{-1}) only the first portion of N was applied, while for BBCH 50–59 only combinations with a total N dose of 60 and 90 kg N ha^{-1} were fertilized with the next 30 kg N ha^{-1}. This means that the increase in biomass in individual development stages and after the application of various fertilizer combinations was not uniformly proportional to the level of nitrogen application and the addition of sulphur. Moreover, the conditions which occurred during the study years influenced the fresh and air-dry yields of the plants, which were at their highest in 2011 and significantly lower in 2009-2010. In the presented study, the dry matter yield of the stems and leaves of spring rye was the lowest from the start of stem elongation to the first node stage (BBCH

Table 1. Dates of phenological stages of spring rye growth in the experiment

Year	BBCH 00*	BBCH 10	BBCH 23	BBCH 31	BBCH 51	BBCH 83	BBCH 99
2009	8 Apr	17 Apr	4 May	20 May	7 Jun	26 Jul	10 Aug
2010	3 Apr	12 Apr	30 Apr	18 May	3 Jun	23 Jul	5 Aug
2011	31 Mar	11 Apr	1 May	17 May	5 Jun	17 Jul	3 Aug

*Explanatory notes: BBCH 00 – Dry seed (caryopsis), BBCH 10 – First leaf through coleoptiles, BBCH 23 – 3 tillers detectable, BBCH 31 – First node at least 1 cm above tillering node, BBCH 51 – Beginning of heading: tip of inflorescence emerged from sheath, first spikelet just visible, BBCH 83 – Early dough, BBCH 99 – Harvested product.

Table 2. Influence of nitrogen and sulphur application on the biomass of spring rye and carbon content at the BBCH 30–31 stage (2009-2011)

Fertilization / Year		Mass of stems and leaves (BBCH 30–31)		Total carbon	
		Fresh	Air-dry	Content	Accumulation
		(t ha^{-1})		(g kg^{-1} d.m.)	(kg ha^{-1})
Nitrogen	0 N	13.76 ± 2.47 d	2.34 ± 0.42 d	412.5 ± 3.2 a	965 ± 164 d
	30 N	16.78 ± 0.95 c	2.85 ± 0.17 c	407.0 ± 0.4 a	1 160 ± 67 c
	60 N	21.71 ± 1.50 b	3.71 ± 0.26 b	401.5 ± 2.3 a	1 490 ± 98 b
	90 N	22.57 ± 1.93 a	3.87 ± 0.34 a	403.5 ± 1.3 a	1 562 ± 134 a
Sulphur	0 S	18.31 ± 0.20 b	3.12 ± 0.03 b	403.8 ± 1.1 a	1 259 ± 17 b
	40 S	19.10 ± 0.19 a	3.26 ± 0.03 a	408.0 ± 0.9 a	1 330 ± 18 a
Year	2009	18.47 ± 0.12 b	3.15 ± 0.02 b	395.1 ± 5.5 b	1 240 ± 27 c
	2010	18.41 ± 0.15 b	3.15 ± 0.02 b	414.5 ± 4.2 a	1 303 ± 4 b
	2011	19.23 ± 0.26 a	3.28 ± 0.04 a	408.8 ± 1.3 a	1 340 ± 23 a
	Mean	18.71	3.19	406.1	1 294

Different letters denote significant differences ($p \leq 0.05$) between the treatments. SD ± standard deviation.

Table 3. Influence of nitrogen and sulphur application on the biomass of spring rye and carbon content at the stage BBCH 55–59 (2009-2011)

Fertilization / Years		Whole plant mass (BBCH 55–59)		Total carbon	
		Fresh	Air-dry	Content	Accumulation
		(t ha^{-1})		(g kg^{-1} d.m.)	(kg ha^{-1})
Nitrogen	0 N	20.04 ± 3.37 d	4.19 ± 0.70 d	418.9 ± 1,7 d	1 757 ± 304 d
	30 N	24.71 ± 1.03 c	5.13 ± 0.23 c	423.4 ± 0.5 b	2 175 ± 95 c
	60 N	29.70 ± 1.46 b	6.26 ± 0.33 b	422.3 ± 0.1 c	2 643 ± 139 b
	90 N	32.67 ± 2.94 a	6.78 ± 0.59 a	424.8 ± 1.2 a	2 883 ± 259 a
Sulphur	0 S	26.12 ± 0.33 b	5.47 ± 0.06 b	421.1 ± 0.6 a	2 305 ± 30 b
	40 S	27.4 ± 0.323 a	5.72 ± 0.06 a	423.7 ± 0.6 a	2 424 ± 29a
Years	2009	26.26 ± 0.26 b	5.51 ± 0.04 b	420.2 ± 1.1 b	2 315 ± 25 ab
	2010	26.48 ± 0.15 b	5.52 ± 0.03 b	415.4 ± 3.5 bc	2 299 ± 33 b
	2011	27.60 ± 0.41 a	5.74 ± 0.07 a	431.5 ± 4.5 a	2 479 ± 57 a
	Mean	26.78	5.59	422.4	2 365

Explanations as in Table 2.

30–31) and the highest between the middle and the end of heading (BBCH 55–59). The results confirm that dry matter yield increases with the progress of plant vegetation.

The carbon content at the BBCH 30–31 stage did not depend to a significant extent on nitrogen application (Table 2). However, a downward trend in total carbon content may be observed as N application increases. The addition of sulphur and the interaction between the nitrogen application level and sulphur fertilization did not cause significant changes in the total carbon content of spring rye leaves. The carbon content was positively influenced by the weather conditions in the years 2010 and 2011, while the biomass yield was significantly higher in 2011.

The highest carbon content in whole plants at BBCH 55–59 stage was recorded in the plots fertilized with a dose of 30 and 90 kg N ha^{-1}, while the lowest C content in the biomass was observed in the control treatments (without N) and in the plots fertilized with 60 kg N ha^{-1}. Sulphur application alone and its interaction with nitrogen application did not significantly affect the carbon content. The highest carbon content in leaves and ears of rye was found in 2011 (Table 3).

The total C accumulation by the rye dry matter at the BBCH 30–31 and BBCH 55–59 stages was higher in the conditions of each level of nitrogen application and with the addition of sulphur, but it was most favourable following the application of 60 and 90 kg N ha^{1} (Tables 2, 3). At BBCH 30–31 the leaves accumulated on average 1294 kg ha^{-1} of carbon, while the whole plants at BBCH 55–59 accumulated 2365 kg of carbon per hectare. The weather factor influenced the accumulation of total carbon; the highest level of its accumulation in biomass in both of the aforementioned development stages was noted in 2011.

An analysis of the results showed the significant beneficial effect of nitrogen (factor I) on the straw yield of spring rye. The straw yield increased significantly in proportion to the increase in the nitrogen dose application. A sulphur application (factor II) also had a beneficial impact on the straw yield. However, the interaction between the different nitrogen application rates and sulphur fertilization (factor II) was not apparent. Regardless of sulphur application, straw yield was most favourable on plots where 60 and 90 kg N ha^{-1} were applied. Similarly, irrespective of the nitrogen application rate, supplementation with sulphur in the amount of 40 kg ha^{-1} (factor II) improved the straw yield (although this was not confirmed statistically). In general, sulphur application enhanced the impact of nitrogen on straw yield. The greatest straw yield in spring rye was obtained in 2011 and it was lower by 3.5% on average in the years 2009-2010 (Table 4).

The carbon content in the straw was not significantly influenced by nitrogen application. However, it was found to be dependent on the addition of sulphur, which beneficially increased the content of this element in the straw. No interaction was noted between the nitrogen application rate and the addition of sulphur. The weather conditions also had no significant influence on the value of this feature. The average total carbon content in the straw was 474.3 g kg^{-1} d.m. Moreover, the accumulation of C by the straw increased in direct proportion to the level of nitrogen application and the addition of sulphur. The total carbon content was significantly higher in plants that were fertilized with the highest nitrogen dose, *i.e.* 90 kg N ha^{-1} (Table 4). The dry matter content of the straw in the fully matured stage (BBCH 92) accumulated on average 2062 kg ha^{-1} of total carbon. The weather factor did not influence the carbon content, but the high point of its accumulation was noted in 2011 (Tables 4, 5).

An analysis of the results showed that the beneficial effects of nitrogen application occurred to a significant extent (factor I) on the grain yield of spring rye. After harvest, at the time of rye maturity, the grain yield had an average moisture content of 11.5% and was highest on the plot with the 60 kg N ha^{-1} (3.99 t ha^{-1}). Grain yield also increased significantly by a further 0.10 t ha^{-1} (2.8%) in a plot treated with 90 kg N ha^{-1} (Table 6). Sulphur application (factor II) had a beneficial influence on the spring rye yield, this was expressed as an increase in the grain yield by 2.53%. The interaction of various nitrogen rates (factor I) and sulphur application (factor II) in grain yield production by spring rye was not apparent. However, irrespective of the addition of sulphur, the grain yield directly after harvest and the dry matter yield of the grain were greatest after the application of 60 and 90 kg N ha^{-1}. Moreover, irrespective of the dose of nitrogen applied, sulphur supplementation in the amount of 40 kg ha^{-1} (factor II) improved the values of these features (although this was not confirmed

Table 4. Influence of nitrogen and sulphur application on the straw yield of spring rye and carbon content at the stage BBCH 89–92 (2009-2011)

Fertilization / Years		Straw mass (BBCH 89–92) Fresh (t ha^{-1})	Straw mass (BBCH 89–92) Air-dry (t ha^{-1})	Total carbon Content (g kg^{-1} d.m.)	Total carbon Accumulation (kg ha^{-1})
Nitrogen	0 N	3.70 ± 0.57 d	3.32 ± 0.51 d	474.4 ± 0.1 a	1574 ± 244 d
	30 N	4.27 ± 0.29 c	3.83 ± 0.26 c	473.5 ± 0.4 a	1812 ± 125 c
	60 N	5.49 ± 0.32 b	4.92 ± 0.28 b	474.9 ± 0.3 a	2335 ± 136 b
	90 N	5.94 ± 0.54 a	5.33 ± 0.49 a	474.4 ± 0.1 a	2529 ± 233 a
Sulphur	0 S	4.73 ± 0.06 b	4.24 ± 0.05 b	473.5 ± 0.4 b	2010 ± 26 b
	40 S	4.96 ± 0.05 a	4.45 ± 0.05 a	475.1 ± 0.4 a	2114 ± 26 a
Years	2009	4.76 ± 0.04 b	4.28 ± 0.03 b	474.0 ± 0.02 a	2030 ± 16 b
	2010	4.83 ± 0.01 b	4.32 ± 0.01 b	474.2 ± 0.01 a	2043 ± 9 b
	2011	4.97 ± 0.06 a	4.45 ± 0.05 a	474.7 ± 0.02 a	2114 ± 26 a
	Mean	4.85	4.35	474.3	2062

Explanations as in Table 2.

Table 5. Influence of nitrogen and sulphur application on the grain yield of spring rye and carbon content at the stage BBCH 89–92 (2009-2011)

Fertilization / Years		Grain mass (BBCH 89–92)		Total carbon	
		After harvest	Air-dry	Content	Accumulation
		(t ha^{-1})		(g kg^{-1} d.m.)	(kg ha^{-1})
Nitrogen	0 N	2.94 ± 0.31 d	2.64 ± 0.27 d	419.7 ± 0.6 a	1 106 ± 114 c
	30 N	3.19 ± 0.18 c	2.86 ± 0.16 c	419.1 ± 0.3 a	1 197 ± 68 b
	60 N	3.99 ± 0.21 b	3.58 ± 0.19 b	420.2 ± 0.9 a	1 504 ± 85 ab
	90 N	4.10 ± 0.27 a	3.68 ± 0.24 a	414.8 ± 1.8 a	1 528 ± 97 a
Sulphur	0 S	3.51 ± 0.02 b	3.15 ± 0.02 b	417.0 ± 0.7 a	1 312 ± 11 b
	40 S	3.61 ± 0.02 a	3.23 ± 0.02 a	419.8 ± 0.7 a	1 356 ± 11 a
Years	2009	3.46 ± 0.05 b	3.11 ± 0.04 b	418.4 ± 0.0 a	1 301 ± 16 b
	2010	3.51 ± 0.02 b	3.14 ± 0.02 b	418.1 ± 0.1 a	1 313 ± 10 b
	2011	3.60 ± 0.02 a	3.32 ± 0.06 a	418.8 ± 0.2 a	1 387 ± 26 a
	Mean	3.56	3.19	418.4	1 334

Explanations as in Table 2.

statistically). Results indicate that sulphur application can enhance the effect of nitrogen application on the grain yield of spring rye. The values of these traits increased at each level of N application, and the highly beneficial effect was found at 60 and 90 kg N ha^{-1} enriched with mineral sulphur applied in the amount of 40 kg ha^{-1} (Table 5). In spite of fertilization, the highest grain yield of spring rye was obtained in 2011 and in 2009-2010 it was lower, on average by 6.2%.

The carbon content in the grain was not determined by nitrogen and sulphur fertilization to any significant extent. However, the highest C content was found in the control treatments and those fertilized with 30 and 60 kg N ha^{-1}, whereas the highest nitrogen application (90 kg N ha^{-1}) exhibited a trend in the reduction of the carbon content, on average from 419.7 to 414.8 g kg^{-1} d.m. (Table 5). Sulphur application caused a minor trend to develop in terms of an increase in the C content in the grain of spring rye, from 417.0 to 419.8 g kg^{-1} d.m. The highest carbon content was found in the grain of spring rye harvested in 2009 and 2011, and the lowest in 2010. Also, the total C accumulation by the dry matter of the grain was favourable for each nitrogen application rate and with the addition of sulphur, but it was the greatest after the application of 60 and 90 kg N ha^{1} (Table 5). The rye grain accumulated on average 1 334 kg of carbon per hectare.

Nitrogen and sulphur application influenced the C:N ratio in the grain and in the straw of rye for the BBCH 30–31 and BBCH 55–59 phases. Fertilization with nitrogen produced the following effects: As N application increased, the C:N ratio decreased (Table 6). In the case of sulphur application together with NPK fertilizers a reduction in the C:N ratio of the grain and in the straw occurred. However, it was not shown that the addition of sulphur influenced the N:S ratio in the rye biomass at the BBCH 30–31 or BBCH 55–59 stages. The weather factor influenced the C:N ratio at each of the aforementioned stages of growth *i.e.* in the biomass, grain and straw of rye. The interaction between the varied nitrogen rates and the sulphur dose in the formation of the C:N ratio values was only demonstrated in the case of BBCH 55–59 (Table 6).

The analysis of variance showed that the total carbon accumulation increased significantly in proportion to the nitrogen dose up to the highest level of 90 kg N ha^{-1} (Table 7). The addition of sulphur also had a significant positive influence on the total carbon accumulation in the grain and straw, although no statistically significant interaction of these factors was demonstrated. The passing of the years in the study did not have any significant influence over the total carbon accumulation, which amounted to an average of 3 408 kg ha^{-1}. The unit carbon accumulation decreased following the application of 30 kg N ha^{-1}, but this was significantly increased in plots with the use of 60 and 90 kg N ha^{-1}. Sulphur application also significantly increased the unit carbon accumulation, while the weather conditions occurring over the years of the study did not have any significant effect on this feature (Table 7).

Table 8 presents selected correlation coefficients between the biomass yield at various growth stages and carbon content, carbon accumulation, and the C:N ratio. A significant positive correlation was found between the biomass of the spring rye (determined at each stage) and carbon accumulation (uptake). No significant correlations were shown between the biomass and its carbon content. The grain and straw yield was negatively correlated with the C:N ratio.

Table 6. Influence of nitrogen and sulphur application on the C:N ratio (2009-2011)

Fertilization / Years		C:N ratio			
		Biomass yield			
		Stems and leaves BBCH 30–31	Whole plants BBCH 55–59	Grain (BBCH 89–92)	Straw (BBCH 89–92)
Nitrogen	0 N	15.84 ± 1.11 a	20.01 ± 1.07 a	20.13 ± 0.93 a	91.77 ± 1.99 a
	30 N	12.93 ± 0.34 b	17.32 ± 0.27 b	18.33 ± 0.03 b	90.15 ± 1.18 a
	60 N	12.83 ± 0.39 b	17.08 ± 0.39 b	17.87 ± 0.20 bc	85.97 ± 0.91 b
	90 N	12.86 ± 0.37 b	17.09 ± 0.39 b	16.74 ± 0.76 c	83.27 ± 2.26 c
Sulphur	0 S	13.56 ± 0.02 a	17.73 ± 0.07 a	18.70 ± 0.21 a	88.45 ± 0.33 a
	40 S	13.67 ± 0.03 a	18.01 ± 0.07 a	17.83 ± 0.22 b	86.08 ± 0.85 b
Years	2009	18.42 ± 2.40 a	18.76 ± 0.44 b	18.20 ± 0.03 a	87.26 ± 0.26 b
	2010	11.59 ± 1.01 b	19.95 ± 1.04 a	18.69 ± 0.21 a	89.84 ± 1.02 a
	2011	10.83 ± 1.39 b	14.90 ± 1.48 c	17.91 ± 0.18 b	86.26 ± 0.76 b
	Mean	13.61	17.87	18.27	87.79

Explanations as in Table 2.

Table 7. Influence nitrogen and sulphur application on total and unit carbon accumulation (grain + straw) (2009-2011)

Fertilization / Years		C:N ratio	
		Biomass yield	
		Stems and leaves BBCH 30–31	Whole plants BBCH 55–59
Nitrogen	0 N	2725 ± 341 d	894.1 ± 1.0 a
	30 N	3009 ± 199 c	892.6 ± 0.3 b
	60 N	3840 ± 216 b	895.0 ± 1.5 a
	90 N	4056 ± 324 a	889.2 ± 1.4 b
Sulphur	0 S	3323 ± 42 b	890.5 ± 0.7 b
	40 S	3493 ± 42 a	894.9 ± 1.4 a
Years	2009	3365 ± 21 a	892.3 ± 0.1 a
	2010	3356 ± 26 a	892.4 ± 0.2 a
	2011	3502 ± 47 a	893.5 ± 0.7 a
	Mean	3408	892.7

Explanations as in Table 2.

4. DISCUSSION

The results revealed a fairly complex picture of the response of spring rye to nitrogen and sulphur application, in relation to yield, carbon content and accumulation, and the carbon-to-nitrogen ratio (C:N) in the biomass. The search for optimal nitrogen application rates for the cultivation of spring rye has been the subject of research by the other authors (Kadłubiec and Bojarczuk, 2003). In these studies, spring rye fertilized with nitrogen in the amount of 80 kg N ha^{-1} on soil classified as a good rye complex produced a yield at the level of 5.1 t ha^{-1}. In the present study with spring rye, increasing the nitrogen doses also positively influenced the grain yield which was the highest in the plot with the highest level of nitrogen fertilization occurring at 90 kg N ha^{-1} which amounted to 4.08 t ha^{-1}.

In the presented study, sulphur application in the amount of 40 kg S ha^{-1} significantly increased the grain yield of spring rye on average by 0.10 t ha^{-1}, *i.e.* by 2.8%, in comparison with the control. Another study showed that the fertilization of spring barley with 40 kg S ha^{-1} also significantly increased grain yield, by about 11.9% (Klikocka, 2010; Klikocka *et al.*, 2014). No interaction between increasing the nitrogen application and the sulphur dose was obtained for grain yield or for the other features examined. Both of these factors, however, independently increased yields and the values of a number of other features. No interaction was observed in terms of the dry matter yield in phases BBCH 30–31 and BBCH 55–59 as well as for the carbon content in the rye biomass in stage BBCH 55–59 and in the grain. This type of sulphur influence as a yield-improving factor indicates its additive effect in relation to nitrogen. This type of response is revealed in the conditions of the moderately weak effect of the deficient factor and is called the law of diminishing increments of production, in scientific terms it is known as Mitscherlich's law (Grzebisz, 2009).

The weather factor in the present study had a significant effect on the grain yield of spring rye. According to Gooding *et al.* (2003) high grain yields are generally dependent on low levels of precipitation in the winter and in April, but on a greater level of rainfall during the phases of stem elongation and flowering. In the research of Dopka *et al.* (2013), concerning good rye complex soil, the highest grain yield of spring rye was obtained when the total rainfall during the growing season was 254.3 mm. In the present study, the

Table 8. Selected correlation coefficients between biomass yield and carbon content, carbon accumulation, and the C:N ratio (2009-2011) (Variables n = 24)

Parameter (air DM)	C content	C accumulation	C:N ratio
Yield of grain in BBCH 89–92	-0.331	**0.999**	**-0.804**
Yield of straw in BBCH 89–92	0.225	**0.999**	**-0.790**
Yield of biomass in BBCH 30–31	-0.307	**0.524**	-0.315
Yield of biomass in BBCH 55–59	0.259	**0.928**	-0.397

Explanatory notes: significant for α = 0.05: **R = 0.406**, significant for α = 0.01: **R = 0.517**.

highest grain yield of spring rye, (on average 3.60 t ha^{-1}), was obtained in the 2011 growing season, in which total precipitation amounted to 261.0 mm. According to Rymuza *et al*. (2012) air temperature is the main factor influencing the rate of plant growth and development, while the water factor influences cereal yield, especially in the critical period between stem elongation and heading. However, in the present study the rainfall and temperature distribution did not influence the development stages of spring rye to a significant extent. However, the positive effect of all of the nitrogen application levels and the addition of sulphur on straw yield was noted. Another study conducted with oats showed that, in comparison with the control, sulphur application significantly increased the straw yield (Barczak and Nowak, 2010).

According to other authors, increasing the levels of nitrogen application increased the dry matter yield of the crop plants (Ercoli *et al*., 2009). A significant increase in the dry matter yield of wheat during the heading stage was also observed as a result of nitrogen application at a dose of 80 kg N ha^{-1} in comparison to the control (Jaśkiewicz, 2004).

The present study showed that sulphur application before the sowing of spring rye in the amount of 40 kg S ha^{-1} caused a significant increase in the dry matter yield of the stems and leaves from the start of stem elongation to the first node stage (BBCH 30–31) in comparison with the control (without S). The application of sulphur before sowing in the amount of 40 kg S ha^{-1} also significantly increased the dry matter yield of the whole plants at the stage between the middle and the end of heading (BBCH 55–59) in comparison to the control. These results indicate that sulphur application leads to an increase in the dry matter yield of the plant during the ontogenesis. The biomass yield was also influenced by the prevailing weather conditions. As noted by other authors, water deficiency during the growing period reduces the dry matter yield of plants (Grzebisz and Härdter, 2006).

The carbon content in the rye grain did not depend on the nitrogen fertilization level, and its average value amounted to 474.3 g kg^{-1} d.m. On the other hand, the addition of sulphur increased the carbon content by 1.6 g kg^{-1} d.m. However, these results suggest that carbon content is a constant feature which is only influenced to a minor extent by experimental factors and weather conditions. For comparison, in another study it was found that nitrogen application did not significantly increase the carbon content in the roots or in the vegetative parts of the rice, which is also a cereal plant (Ye *et al.,* 2014). Other research has also shown that a water deficit occurred after the flowering of the wheat decreased the total carbon content in the grain by 24% (Palta *et al*., 1994). Also, in the present study, somewhat less carbon was found in the grain yield from 2010 than in the other, wetter years, but the grain of spring rye accumulated on average 1334 kg of carbon per hectare. However, in the study of Klikocka and Cybulska (2014) it was found that the grain of spring wheat accumulated on average 1 510 kg ha^{-1} carbon.

According to Grzebisz and Härdter (2006) protein production in the plant is possibly an effect of carbon compound use in the process of protein synthesis. According to other researchers, plant growth is inhibited during carbon starvation, and its protein content is decreased (Sulpice *et al*., 2009). The results of the present study and previously published research (Klikocka and Cybulska, 2014) indicate that the natural carbon content in a plant varies depending on its development stage and increases together with the period of plant growth. Our research has shown that the total accumulation of carbon in the straw of spring rye was 13.4% higher than in the grain. Also, Povilaitis *et al*. (2011) found that carbon accumulation in wheat plants increased during the growing period, this was confirmed by other research. According to the other studies the average carbon content in straw was 475 g kg^{-1} d.m. for barley, 456 g kg^{-1} d.m. for wheat, 439 g kg^{-1} d.m. for triticale, and 466 g kg^{-1} d.m. for rye (Karcz *et al*., 2013). In the present study, it was found that the straw of spring rye contained on average 474.3 g of carbon per kg of d.m. which did not depend on nitrogen application. However, the addition of sulphur increased the carbon content in the straw by 1.6 g kg^{-1} d.m. This indicates that soil sulphur influences carbon metabolism in the plant. Our previously published research showed that spring wheat accumulated the least carbon at the stage from the start of stem elongation to the first node stage (BBCH 30–31), which amounted on average to 1 292 kg ha^{-1}. The greater accumulation of C (on average 2 363 kg ha^{-1}) took place at the stage between the middle and the end of heading (BBCH 55–59) (Klikocka and

Cybulska, 2014). Other authors have also observed a greater amount of carbon accumulation by plants fertilized with nitrogen in comparison with the controls (Ye *et al.*, 2014). The results of the presented study also showed a significant increase in carbon accumulation under the influence of nitrogen and sulphur application. However, carbon uptake resulted from significant increases in straw yield, this was possibly an effect of the aforementioned fertilization.

Some authors have drawn attention to the connection between C and N metabolism in the plant (Nunes-Nesi *et al.*, 2010). According to others, the products of C and N metabolism include not only trophic substances, but also compounds that provide information about the current status of the cells' carbon and nitrogen levels and the quantitative relationships between carbon and nitrogen compounds (mainly carbohydrates), *i.e.* the C:N ratio (Starck, 2006). According to other authors, the C:N ratio in the plant influences both the yield and the quality of the crop (Ghaley *et al.*, 2015). If the C:N ratio in the plant is too high, then yields are reduced (Lu *et al.*, 2009). This may be linked with other research which reports that the value of the C:N ratio in the plant is regulated by nitrogen accumulation (Starck, 2006).

The previous research of Klikocka and Cybulska (2014) was initiated in the control plot through measuring the natural content of nitrogen and sulphur in the topsoil and determining that the C:N ratio in the grain of spring wheat was 16.7:1. In the present study, this ratio in the grain of spring rye in the control, *i.e.* with a natural content of nitrogen and sulphur in the topsoil, was 20.1:1. Fertilization with nitrogen and the addition of sulphur significantly reduced the C:N ratio. In previous research, it was found that the C:N ratio in the grain was also reduced by nitrogen and sulphur application (Klikocka and Cybulska, 2014). According to other authors, the carbon-to-nitrogen ratio in plants varies widely, from about 5 in algae to more than 100 in trees (Raven *et al.*, 2004). Other authors observed in a field experiment that during the tillering stage the C:N ratio ranged from 9:1 to 26:1, and its average value amounted 13:1 (Ort *et al.*, 2013). In the case of wheat stems, the C:N ratio after flowering was 17:1 (Simpson *et al.*, 1983). In the present study, the C:N ratio in the straw of the matured rye (BBCH 92) was much higher than in the grain (about 87.8:1), and was reduced through nitrogen and sulphur fertilization. Similarly, other authors have observed an increase in the C:N ratio in rice from emergence to harvest (Ye *et al.*, 2014). According to Sardans *et al.* (2012) nitrogen application, a lower or moderate air temperature and a sufficient water supply ~~reduces~~ the C:N ratio in the biomass. Other researchers also reported a high C:N ratio, at the level of 100:1, in the straw of cereals (Król *et al.*, 2007). Then, Kuś *et al.* (2008) found that the C:N ratio in the straw ranges from 60:1 in spring barley to 100:1 in winter wheat. These results reflect the data obtained in the present study. Larsen *et al.* (2011) observed that in conditions of drought and moderate temperatures, the C:N ratio in the plant increases. The present study also showed that the air temperature and precipitation significantly influenced the C:N ratio in the grain and in the straw. The chemical composition of the plant residues (C:N ratio and lignin content) determines their decomposition rate and the predominant type of product generated by the transformation of plant residues, straw, as well as natural and organic fertilizers, which together are referred to as fresh organic matter (FOM). Depending on the C:N ratio, the following fate of soil nitrogen and its availability to plants can be distinguished:

1. C:N ratio higher than 33.3:1 – permanent immobilization of soil mineral nitrogen,
2. C:N ratio in the range of 22.2–33.3:1 – temporary immobilization of soil mineral nitrogen,
3. C:N ratio less than 22.2 – direct mineralization of orga-nic nitrogen.

In the present study, a C:N ratio of over 22.2 was only noted in the straw (average 87.79:1). For this reason, during the ploughing of the straw in the field, a compensatory portion of the nitrogen should be applied. Its amount should be determined according to the following formula (Grzebisz, 2009):

$$D_{kN} = P_{no}\,(12 - N_{no}), \tag{2}$$

where: D_{kN} – compensatory portion of nitrogen (kg N ha^{-1}), P_{no} – weight of organic fertilizer (t ha^{-1}), N_{no} – current nitrogen content in fertilizer (kg t^{-1}).

Numerous experiments have shown that from the entire weight of carbon applied to the soil as FOM, 20–25% remains in the form of humic compounds after a 4(5)-year rotation. For example, when young plants rich in labile compounds and nitrogen but poor in compounds resistant to microbial degradation are introduced to the soil, it may be expected that 50% of the mineral compounds will be released after three months. For example, when a green fertilizer rich in carbohydrates and nitrogen is applied to the soil, the ploughing date must be effectively controlled, because the leaching of nitrate nitrogen (NO_3^-) takes place during mild, rainy winters (Grzebisz, 2009).

5. CONCLUSIONS

In the present study, the Bojko cultivar of spring rye was grown on Cambisols consisting of light silty sand, it is classified as a good rye complex and showed a positive response to nitrogen and sulphur application, this was expressed in the form of the carbon content in its vegetative and generative parts.

1. The grain and straw yield of the spring rye as well as its carbon content and accumulation were the factors most positively influenced by the application of nitrogen (factor I) in the amount of 60 and 90 kg N ha^{-1} and sulphur (factor II) in the amount of 40 kg S ha^{-1}. The biomass yield of spring rye at the BBCH 30–31 and BBCH 55–59 stages increased in proportion to the level of nitrogen application and the

addition of sulphur. The average fresh matter yield for these stages was 18.71 and 26.78 t ha^{-1} while the grain and straw yield amounted to 3.56 and 4.85 t ha^{-1}, respectively.

2. The carbon content at the BBCH 30–31 stage did not depend on the experimental factors but they influenced the C content at the BBCH 55–59 phase to a significant extent. The accumulation of C in the plants increased significantly with the level of nitrogen applied. In the BBCH 30–31 stage the rye leaves accumulated on average 1294 kg C ha^{-1}, while at the BBCH 55–59 stage, the whole plants accumulated 2365 kg C ha^{-1}. The average C:N ratio in the BBCH 30–31 and BBCH 55–59 stages amounted to 13.61:1 and 17.87:1, respectively.

3. The carbon content in the grain decreased significantly due to the effect of nitrogen application at a dose of 90 kg ha^{-1} but it increased in plants that were also fertilized with sulphur. Carbon accumulation increased due to the effect of nitrogen and sulphur fertilization. The average C uptake by the dry matter of the grain at the fully ripe stage (BBCH 89–92) was 1 334 kg ha^{-1}. The C:N ratio in rye plants decreased due to the application of nitrogen and sulphur.

4. Nitrogen fertilization and supplementation with sulphur did not affect the carbon content in the straw. The accumulation of C in straw increased in direct proportion to the level of the nitrogen dose and the addition of sulphur. The average C accumulation in the dry matter of straw in the over-ripe stage (BBCH 89–92) was 2 062 kg ha^{-1}.

5. The total accumulation of carbon by the dry matter of grain and straw increased up to a rate of 90 kg N ha^{-1} with the addition of sulphur. The average total accumulation of C in rye plants was 3 408 kg ha^{-1}. The unit accumulation of carbon was reduced following the application of 30 kg N ha^{-1}, but it increased significantly with the increase in the nitrogen fertilization level. Its mean accumulation in the rye amounted to 892.7 C t^{-1}.

6. In cultivation conditions without manure application the green matter and straw of the spring rye ploughed under the soil are a good source of carbon for successive plants. Furthermore, this treatment is an important factor with the potential to decrease the level of global warming by reducing greenhouse gas emissions.

Conflict of interest: The authors declare no conflict of interest.

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