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IMPACT OF SHELTERBELTS ON OXIDATION-REDUCTION PROPERTIES AND GREENHOUSE GASES EMISSION FROM SOILS

WPLYW ZADRZEWIŃ NA WŁAŚCIWOŚCI UTLENIANIA-REDUKCJI I EMISJĘ GAZÓW CIEPLARNIANYCH Z GLEB

Abstract: The *Typic Hapludalfs* soils under two old shelterbelts (200 years old) *Robinia pseudacacia* and *Crataegus monogyna*, multi species of trees (young shelterbelt - 20 years old) and neighbouring cultivated fields were investigated. The function of shelterbelts of different age and plant composition in agricultural landscape and estimation of biochemical and chemical soil conditions for the decrease of greenhouse gases release from soil to the atmosphere was the aim of the research. In soils under shelterbelts were estimated activities of several enzymes participating in the oxidation-reduction processes, ferric and ferrous ions and the evolutions of gases like N₂, N₂O, CO₂, and CH₄. The soils under old shelterbelts characterized higher peroxidase activity than in young shelterbelt and adjoining cultivated fields. However, no significant differences were observed for nitrate reductase activity between old and young shelterbelts. There were proved differences between emission of N₂O in soils under shelterbelts and in adjoining cultivated fields. Furthermore, it was observed significant effect of the young shelterbelt on the decrease of carbon dioxide release than in the adjoining cultivated field. The manipulation of the landscape through the introduction of shelterbelts of different age and the composition of plants leads to the modification of biogeochemical soil conditions for N₂O and N₂ formation and finally decrease of the greenhouse gases evolution from soils to the atmosphere. Thus the creation of new shelterbelts is favourable factor for agricultural landscape.

Keywords: agricultural landscape, greenhouse gases, nitrate reductase, peroxidase, shelterbelts

Introduction

Agriculture is the main supplier of greenhouse gases like N₂O, CO₂, and CH₄ to the atmosphere. These gases are formed during several aerobic and anaerobic conversions and pathways controlled by the biogeochemical conditions of the sites. Therefore, the search for the methods which may significantly decrease the greenhouse gases evolution from soils to

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the atmosphere is needed. The introduction of shelterbelts (mid-field rows of trees afforestation or shrubs in linear configurations) into agricultural landscape may be such a possibility [1].

One of the most important functions of shelterbelts in agricultural landscape is water retention. Shelterbelts significantly affect the greater water storage capacity of their soil organic matter layer than in adjoining cultivated fields [2]. Plant cover increases infiltration rates by slowing runoff. Shelterbelts as biogeochemical barriers in agricultural landscape fulfil significant positive functions also by reducing soil erosion from wind and protecting plants from wind-related damage. In addition, they limit the spread of chemical compounds in agricultural landscape between ecosystems, control matter cycling and protect the accumulation of toxic chemicals and threats. Moreover, they improve microclimate for agricultural production and are able to counteract or minimize the effect of extreme climatic or weather phenomena (particularly low and high temperatures). However, the shelterbelts efficiency dependent of water flux intensity, soil permeability, meteorological and weather condition changes and the type and quantity of organic matter accumulated under the canopy. Institute for Agricultural and Forest Environment, Polish Academy of Sciences has a deep tradition of long-term researches under shelterbelts in many aspects: control of diffuse pollution, investigations of humic and fulvic acids, estimation of hydrophilic and hydrophobic properties of humic compounds, evaluation of amino acids and phytohormone - indole-3-acetic acids, enzymes activity, diversity of plants and animals, heat and water balance.

Taking into account all positive functions of shelterbelts in rural areas, the Council of Europe Committee of Ministers in Recommendation No. R(94)6 of the Committee of Ministers to Member States for Sustainable Development and use of the Countryside with the Particular Focus on the Safeguarding of Wildlife and Landscapes [3] suggested the limitation of pollutions concentration and their spread into natural habitats and control non-specific sources of pollution, especially through simple and inexpensive means such as windbreaks, natural meadows, ponds and buffer zones.

Oxidation-reduction reactions play a key role in ecologically important biogeochemical processes in soil and influence on soil chemical, biochemical and biological properties. The redox potential is a critical environmental factor because it develops the chemical and biochemical form of many compounds and their availability for soil microorganisms and plants and it also influences the products of microbial metabolism in soil. Moreover, redox gradient regulates the precipitation of phosphorus, and oxidation of sulphides, and the breakdown of toxic organic compounds in soils, and the ratio Fe(II)/Fe(III) responsible for the of iron oxidation. Iron forms stable compounds in both divalent and trivalent states depending on the environmental conditions. However, the complexation of iron in forest soils depends on several soil solution factors (redox potential, solution pH) [4].

In addition, redox gradients may be used as an indicator of potential nitrification-denitrification reactions, which governs the release of nitrous oxide (N_2O) and dinitrogen (N_2) from soils to the atmosphere, and the formation of carbon dioxide (CO_2), and methane (CH_4) from the degradation of organic matter. However, the proportion of these gases varies greatly with environmental conditions [5, 6].

Nitrification is an aerobic microbial process oxidation of ammonium to nitrate ions, performed both by autotrophs and heterotrophs microorganisms. Gaseous nitrogen compounds (NO , N_2O and N_2) can be produced as a by-product of nitrification. However,

this process depends on redox potential which is the aeration parameter characterizing the intensity of soil redox transformations. Therefore, one of the major factors controlling the magnitude of nitrification and denitrification in soils is the availability of O_2 . However, denitrification is defined as the “microbial reduction of nitrate or nitrite coupled to electron transport phosphorylation resulting in gaseous N either as molecular N_2 or as an oxide of N_2O ” [7, 8].

Denitrification is fundamentally important in the global biogeochemical N cycle because it is the major route by which inorganic oxidized N compounds in the soil return to the atmospheric N pool. Inorganic N transformations are also influenced by alternate aerobic and anaerobic conditions. Generally the transformation of nitrogen in soils depends on water-filled pore space (WFPS). The highest NO fluxes are expected in 30-60 % WFPS, when nitrification is most active. Furthermore, intensive production of N_2O is observed at above 70 % WFPS, whereas N_2 production occurs mainly in soils saturated with water [9]. In addition, other factors controlling denitrification are the content of nitrogen oxides, availability of electrons in organic carbon compounds, pH and temperature.

Soil enzymes are indicators of microbial activities in soil and are often considered as an index of soil health and fertility [10]. The role of soil enzymes is important in terms of ecosystem functioning and describes the relationship between soil enzymes and the environmental factors affecting their activities [11].

Nitrate reductase is an enzyme involved in the process of denitrification. This enzyme participates in the process of nitrate reduction to nitrite. Next, the formed NO_2^- ions are reduced with the participation of nitrite reductase to N_2O . The reduction reaction of N_2O to N_2 is catalyzed by nitrous oxide reductase. Direct emissions of N_2O from soils occur via nitrification (ammonia oxidation) and nitrate dissimilation (denitrification and nitrate ammonification) pathways [12].

Peroxidase is an enzyme, which is involved in the formation of carbon dioxide (CO_2) from the degradation of organic matter in soils and from the internal conversions of organic compounds in soils. This enzyme catalyzes the oxidation of phenols and aromatic amines in the presence of hydrogen peroxide as an electron acceptor in the biochemical conversions. The release of carboxyl and methoxyl groups from phenolic substrates is ascribed to microbial activity and lead to CO_2 production in soil [13]. Besides nitrate reductase, peroxidase and forms of iron are sensitive factors of redox potential in soils.

The objective of our works was to assess the manipulation of the agricultural landscape, through the introduction of shelterbelts of different age and the composition of plants in agricultural landscape, modification of the biogeochemical soil conditions for N_2O and N_2 formation, and finally the decrease of the greenhouse gases evolution from soils to the atmosphere.

Materials and methods

The investigations were carried out in Dezydery Chlapowski Landscape Park in Turew (40 km South-West of Poznan, West Polish Lowlands, 16°45' E and 52°01' N). This area, from Polish climatic conditions, is warm, with an annual mean temperature of 9 °C. Therefore, thermal conditions are favourable for vegetation growth. The growing season, with air temperatures above 5 °C, lasts 225 days. The most advantageous component of the landscape shows its shelterbelts (rows or clumps of trees). They were planted in Turew due to initiative of Dezydery Chlapowski in 19th century. Shelterbelts show characteristic

features of this landscape. The system of shelterbelts established in Dezydery Chlapowski Landscape Park is unique and resembles a network. Total area of all shelterbelts located in Dezydery Chlapowski Landscape Park is equal to 560 ha. Shelterbelts and afforestations cover 14 % of the whole area. Although this area is characterized by different kind of shelterbelts: *Pinus sylvestris* (65.5 % of the total afforested area), *Quercus petraea* and *Quercus robur* (14.5 %), *Robinia pseudacacia* (5 %), *Betula pendula* (4.3 %) and other totaling 24 species. However, oaks, false acacias, maples, lindens, larch, poplars dominated. Shelterbelts and adjoining cultivated fields were introduced on *Typic Hapludalfs* soils (according to Food and Agriculture Organization of the United Nations classification). The soil samples were taken from three shelterbelts differing the age and the composition of trees:

- 1) first 200-years "old shelterbelt" consists mostly of *R. pseudoacacia* (99.5 %), *Q. robur* (0.4 %), *Larix decidua* (0.1 %); shrubs (represents 10 % of the area of shelterbelt) *Sambucus nigra* (20 %), *Prunus serotina* (20 %), *R. pseudoacacia* (40 %), *Q. robur* (20 %). The humus horizon layer of this shelterbelt reaches a depth of 15 cm. It is 2 km long and 36 m wide. This shelterbelt consists of multiple rows of trees occurring along the sandy way. In the *R. pseudoacacia* shelterbelt the mean tree total height is 13 m, the average diameter at breast height (DBH) is 21 cm and total volume is 350.8 m³·ha⁻¹;
- 2) the second 200-years "old shelterbelt" consists mostly of *Crataegus monogyna* (98 %), *Fraxinus excelsior* (0.2 %), *Q. petraea* (0.2 %), *Q. robur* (0.7 %), *R. pseudoacacia* (0.6 %) and *Ulmus laevis* (0.3 %); shrubs *Sambucus nigra* (46 %), *Rosa canina* (42 %), *Acer* sp. (0.3 %), *Fraxinus excelsior* (0.4 %) and *R. pseudoacacia* (0.5 %). The humus horizon layer of this shelterbelt reaches a depth of 10 cm. It is 300 m long and 10 m wide. The *Crataegus monogyna* shelterbelt is made of single row of trees. This is alley shelterbelt. The total height of this shelterbelt is 7 m and the DBH is 20.5 cm;
- 3) the third "young shelterbelt" was created in 1993, by the Institute for Agricultural and Forest Environment, Polish Academy of Sciences in Poznan, Poland. It includes the following species: *Tilia cordata* (10 %), *Larix decidua* (9 %), *Sorbus aucuparia* (9 %), *Populus nigra* (9 %), *Betula pendula* (8 %), *Pinus sylvestris* (8 %), *Acer pseudoplatanus* (8 %), *Fraxinus excelsior* (7 %), *Carpinus betulus* (5 %), *Q. robur* (5 %), *Sorbus intermedia* (5 %), *Picea abies* (5 %), *Acer campestre* (3 %), *Alnus glutinosa* (3 %), *Ulmus* sp. (3 %), *Q. petraea* (2 %), *Fagus sylvatica* (1 %); shrubs *R. canina* (95 %), *Prunus serotina* (5 %). It reveals a small humus layer. It is 340 m long and 17.5 m wide. The young shelterbelt consists of eleven rows. The highest trees in this shelterbelt are *Populus nigra* (height: 15 m, DBH: 25 cm and volume: 379 m³), and *B. pendula* (height: 11 m, DBH: 12 cm and volume: 144 m³). The *L. decidua* is 8 m height and has DBH of 14 cm and volume of 148 m³. The average height of *Ulmus* sp. is 8 m, DBH - 14 cm and volume - 133 m³, while *P. sylvestris* is 7.5 m height, and has DBH of 14 cm and volume 186 m³. The mean height of other trees in this shelterbelt is 6 m, whereas the average DBH is 9 cm. There are 397 trees with a total volume 104 498 m³·ha⁻¹ in the shelterbelt.

During the three years of research triticale was cultivated on the fields adjoining to *R. pseudoacacia* shelterbelt. The field was supplied with nitrogen, phosphorus and potassium fertilizers. The mean yearly amount of fertilizers (NPK) was 136 N, 30 P, 45 K kg·ha⁻¹ in 2007, 136 N, 50 P, 140 K kg·ha⁻¹ in 2008 and 161.4 N, 76 P, 96 K kg·ha⁻¹ in 2010. The crops in the field adjoining to *C. monogyna* shelterbelt were cultivated using a crop

rotation system. Triticale was grown in 2007, mixture (barley, oat, wheat) in 2008 and barley in 2010. The average yearly amount of fertilizers NPK used in the field were 206.3, 56.0, 88.0 kg·ha⁻¹; 113.3, 55.2, 151.2 kg·ha⁻¹; 137.0, 76.0, 96.0 kg·ha⁻¹ in 2007, 2008 and 2010 respectively. The crops cultivated on the field neighboring with the young shelterbelt were also grown using the crop rotation system (triticale, maize and winter wheat). The mean yearly amount of fertilizers was 112.0 N kg·ha⁻¹ in 2007; 183.6 N, 134.3 K kg·ha⁻¹ in 2008; and 39.5 N, 60.0 K kg·ha⁻¹ in 2010.

Soil samples were collected once a month from the middle of each shelterbelt areas and from adjoining cultivated field - from surface (0-20 cm) of soils (humus horizon), and 100 m from the shelterbelts during the period of intensive plant growth in 10 replicates (for three years 2007, 2008 and 2010, once a month from April to October). These 10 sub-samples were mixed thoroughly to prepare a composite mixture. Plant materials and other debris were removed by hand from the samples and soil was sieved through a 5 mm mesh sieve. One part of soil material was air-dried and crushed to pass through a 1 mm mesh sieve the second part were stored at -20 °C until analysed. All chemicals used in this study were of analytical grade of purity.

Soil pH was measured in 1 N KCl from air dried soil samples using a 1:2.5 v/v soil solution suspension. Soil bulk density was determined in soil cores collected at depths of 0-10 cm and porosity calculated. The particle density was determined by water pycnometer method.

Water filled pore space (WFPS) refers to the volume of water filled pores present in the soil relative to the total volume of pores. The total organic carbon was analysed on Total Organic Carbon Analyzer (TOC 5050A) with Solid Sample Module (SSM-5000A) produced by Shimadzu (Japan). Hot water extractable organic carbon (C_{HWE}) was evaluated on TOC 5050A equipment produced by Shimadzu (Japan) [14]. Total nitrogen (TN) was estimated by the Kjeldahl method. Ammonium ions were assayed on ion chromatograph Waters 1515 (USA) equipped with a 1515 Isocratic HPLC pump, conductivity detector Waters 432, a rotary valve fitted with 20·10⁻⁶ dm³ sample loop and column PRP-X200 (150 × 4.1 mm I.D. - Internal Diameter) from Hamilton, protected with a guard column of the same material (25 × 2.3 mm I.D.). Nitrate ions were measured on ion chromatograph HIC-6A Shimadzu (Japan) equipped with a LP-6A Isocratic HPLC pump, conductivity detector CDD-6A, a rotary valve fitted with 20·10⁻⁶ dm³ sample loop and column PRP-X100 (150 × 4.1 mm I.D.) from Hamilton, protected with a guard column of the same material (25 × 2.3 mm I.D.) [1]. The ferric ions were estimated by thiocyanate technique. The ferrous ions in soils were determined by phenanthroline method [15].

Nitrate reductase activity was identify using potassium nitrate as a substrate and 2,4-dinitrophenol as inhibitor of nitrite reductase in field moist soils [1]. Peroxidase activity in soils was amounted by Bartha and Bordeleau method [15].

Factor of biochemical transformation may be used to estimate of the direction and the rate of biochemical conversion in the layers of soil taking into account the properties as bulk density and porosity. Thus, the factor reveals the efficiency of the transformation of the substrate of own enzyme in soil conditions. It may be expressed in different units of volume and time: [mol·m⁻³·h⁻¹], [mol·m⁻³·year⁻¹].

$$F = A_e \cdot Bd \cdot P \cdot C_c \quad (1)$$

where: F - factor of biochemical transformation; A_e - activity of enzyme [mmol·h⁻¹·g⁻¹]; Bd - bulk density [kg·m⁻³]; P - porosity [%]; C_c - volumetric coefficient.

The volumetric coefficient is calculated from the equation:

$$C_c = d \cdot l \cdot w \quad (2)$$

where: d - depth, l - length, w - width. For our investigation $C_c = 0.2 \text{ m} \cdot 100 \text{ m} \cdot 100 \text{ m} = 2000 \text{ m}^3$.

For measurement of gas flux rates soil cores (250 cm³) to a depth of 0-10 cm (5 replicates) were obtained from three different shelterbelts and adjoining cultivated fields from five randomly selected locations. The samples were taken in April 2010. Soil temperature during the sampling amounted to 7.3 °C under *Robinia pseudacacia* shelterbelt, 11.3 °C in adjoining cultivated field, 7.8 °C under *Crataegus monogyna* shelterbelt, 12.3 °C in adjoining cultivated field, 6.9 °C under young shelterbelt, and 8.9 °C in adjoining cultivated field. Emission of nitrous oxide, dinitrogen, methane and carbon dioxide was measured directly by a helium atmosphere incubation method which similarly works to the principle described by Butterbach et al. [16]. It is a steady state flow-through chamber system (incubation vessels) combined with gas analyses equipment.

All the experiments were run in triplicate, and the results were averaged. The confidence intervals were calculated using the following formula: $x \pm t_{\alpha(n-1)} SE$, where: x - mean, $t_{\alpha(n-1)}$ value of the Student test for $\alpha = 0.05$, and $n-1$ degree of freedom, SE - standard error. Linear correlations between the values were calculated.

Results and discussion

Significant differences were observed in biochemical, chemical and physical properties among soils under shelterbelts of different age and the content of plants and adjoining cultivated fields (Table 1).

Table 1
The physical and chemical properties of soils under three different shelterbelts and in adjoining cultivated fields

Site	pH [-]	Moisture [%]	Bulk density [g·cm ⁻³]	WFPS [%]	TN [g·kg ⁻¹]	N-NH ₄ ⁺ [mg·kg ⁻¹]	N-NO ₃ ⁻ [mg·kg ⁻¹]	TOC [g·kg ⁻¹]	C _{HWE} [g·kg ⁻¹]	C:N
<i>Robinia pseudacacia</i> shelterbelt	4.3	22.8	1.03 ^c ±0.20	46.9 ^a ±5.4	3.98 ^b ±0.37	5.9 ^a ±1.4	3.4 ^{bc} ±1.4	31.5 ^a ±6.1	2.46 ^d ±0.21	10.45
Cultivated field	6.8	10.8	1.49 ^{ab} ±0.14	40.3 ^a ±8.3	0.83 ^a ±0.24	4.9 ^{ab} ±2.0	1.14 ^a ±0.27	4.26 ^b ±0.24	0.28 ^a ±0.04	5.13
<i>Crataegus monogyna</i> shelterbelt	4.7	19.1	0.96 ^c ±0.13	46 ^a ±13	3.30 ^b ±0.37	7.9 ^a ±2.9	3.2 ^{ab} ±1.9	21.6 ^a ±6.4	1.84 ^c ±0.25	11.52
Cultivated field	5.3	10.9	1.63 ^b ±0.11	52.3 ^a ±8.2	0.84 ^a ±0.18	7.42 ^a ±0.98	1.33 ^a ±0.37	5.75 ^c ±0.30	0.31 ^a ±0.01	6.85
Young shelterbelt (20 years old)	5.9	11.2	1.39 ^a ±0.08	40.2 ^a ±6.7	1.50 ^c ±0.24	5.3 ^a ±1.3	1.99 ^{bc} ±0.65	9.35 ^d ±0.86	0.48 ^b ±0.06	8.35
Cultivated field	6.2	12.2	1.51 ^{ab} ±0.11	42.0 ^a ±7.8	0.90 ^a ±0.27	1.9 ^b ±1.6	3.8 ^b ±1.2	6.38 ^e ±0.15	0.27 ^a ±0.03	7.09

Values in a column followed by the same subscript letter(s) are not significantly different at $\alpha = 0.05$; WFPS - water filled pore space, Mean ±95 % confidence interval

The chemical, biochemical, physical and biological processes in organic matter represent catalytic features. The pH was very acidic and acidic in soils under old

shelterbelts (4.3-4.7), but in adjoining cultivated fields and under young shelterbelt pH was slightly acidic and neutral (5.3-6.8) (Table 1). Therefore, those differences should influence many physical, chemical and biological properties, processes and mechanisms in these soils. Simek et al. [17] postulated neutral and basic conditions as an optimal for denitrification. However, the soil under old shelterbelt is much more acidic than young shelterbelt and in the corresponding cultivated fields.

Soil organic matter affects biochemical, chemical and physical properties that control soil microbial activity. It has been demonstrated that there is a correlation between the age of shelterbelts and the amount of total organic carbon (*TOC*) in research soils. Our investigations revealed that the quantity of *TOC* was significantly higher in soils under *Robinia pseudacacia* (31.45 g·kg⁻¹) and *Crataegus monogyna* (21.65 g·kg⁻¹) shelterbelts than in the soil under young shelterbelt (9.35 g·kg⁻¹) and adjoining cultivated fields. Thus, the accumulation of organic matter may proceed faster in shelterbelt soils than in the adjoining cultivated fields (Table 1). Partyka and Hamkalo [18] suggest that the predominance of more resistant to oxidation and accordingly stable part of soil organic matter is typical of the forest soil to the depth of 40 cm. Similar changes like *TOC* were measured for the hot water extractable organic carbon (*C_{HWE}*) in research soils. Higher contents of *C_{HWE}* were observed in soils under *R. pseudacacia* (2.46 g·kg⁻¹) and *C. monogyna* (1.84 g·kg⁻¹) shelterbelts than in soil under young shelterbelt 0.48 g·kg⁻¹. Furthermore, the concentration of *C_{HWE}* in soils under adjoining cultivated fields ranged from 0.27 to 0.31 g·kg⁻¹ (Table 1). 20 years old shelterbelt characterizes low contents of organic matter because process of withdrawal of new organic matter is shorter than in old shelterbelts (*R. pseudacacia* and *C. monogyna*) [7]. *C_{HWE}* is related to a microbial activity and it influences on enzymes activity. In addition, it may act as a substrate for microbial growth, but its production is also partly mediated by microbes. Therefore, higher contents of *C_{HWE}* in soils under *R. pseudacacia* and *C. monogyna* than in young shelterbelts and adjoining cultivated fields should indicate higher microbial and enzymatic activity in these soils. Remarkable differences of *TOC* under shelterbelts of different age and the composition of plants led to the high moisture content. The impact of the kind of shelterbelt on the moisture content in organic matter was observed. The moisture content of research soils was higher in *Robinia pseudacacia* (22.8 %) and *Crataegus monogyna* (19.1 %) shelterbelts than young shelterbelt and adjoining cultivated fields, where it ranged from 10.8 to 12.2 %.

Bulk density is a dynamic property that varies with the structural condition of the soil. Our results showed significantly lower soil bulk density 1.03 and 0.97 g·cm⁻¹ in soils under old shelterbelts (*R. pseudacacia* and *C. monogyna*) than in young shelterbelts and adjoining cultivated fields (1.39-1.63 g·cm⁻¹). This may suggest higher content of organic matter in soils under old shelterbelts than in young shelterbelt and adjoining cultivated fields. The type and frequency of tillage may influence soil properties. Fortunately bulk density is closely linked with soil organic matter. The increase in organic matter causes the decrease in bulk density [19]. Therefore, the results of soil bulk density are in line with that of total organic carbon. The WFPS is the factor affecting in the enzymatic activity and emission of gases from soils. The WFPS in soils under shelterbelts and adjoining cultivated fields ranged from 40.20 to 52.26 %. The highest (52.26 %) WFPS was observed in soil under cultivated field to *C. monogyna* shelterbelt. While the WFPS in soil under *R. pseudacacia* and *C. monogyna* shelterbelts were 46.95 and 45.61 %. In soils under young shelterbelt and in adjoining cultivated fields, and in adjoining cultivated fields to *R. pseudacacia*

shelterbelts the WFPS ranged from 40.20 to 41.99 % (Table 1). However, there were no significant differences in WFPS from the all research soils.

In addition, the effect of the age and the content of plants on the quantities of *TN* in soils under shelterbelts were visible. The concentrations of *TN* in soils under old shelterbelts (*R. pseudacacia* and *C. monogyna*) were significantly higher than in young shelterbelt and adjoining cultivated fields. The *TN* contents in soil under old shelterbelts ranged from 3.30 to 3.98 g·kg⁻¹, however, in adjoining cultivated fields to all shelterbelts (from 0.83 to 0.90 g·kg⁻¹), and under young shelterbelt 1.50 g·kg⁻¹ (Table 1). A *R. pseudacacia* is characterized by the presence of root nodules, which bind free nitrogen from the air. It was observed average 2.7 times higher content of *TN* in soil under *R. pseudacacia* than in young shelterbelt. Large differences in N and C content in the soil under young and old shelterbelts were recorded because of different rates of decomposition of organic matter. Our results showed also significant correlation coefficient between concentration of *TN* and *TOC* ($r = 0.85$, $\alpha = 0.05$).

The concentration of nitrates and ammonium ions are another important regulator of nitrification and denitrification processes. We observed a disparity in the quantities of ammonium in soils under shelterbelts and adjoining cultivated fields. In soils under old and young shelterbelts the concentrations of ammonium ions were no significantly different and ranged from 5.25 to 7.93 mg·kg⁻¹. The significant differences in the concentration of ammonium were observed in the soil under young shelterbelt and adjoining cultivated field. The contents of nitrates in the soils under *C. monogyna*, *R. pseudacacia* shelterbelt and in the cultivated field neighbouring with the young shelterbelt were not significantly different and amounted to 3.21, 3.43 and 3.83 mg·kg⁻¹, respectively. However, these amounts were significantly higher than in the soil in adjoining in the cultivated field adjoining to *R. pseudacacia*. It was also observed that the concentration of nitrate ions in the soil under young shelterbelt was significantly lower than in the adjoining cultivated field (1.99 mg·kg⁻¹ under young shelterbelt, and 3.83 mg·kg⁻¹ in adjoining cultivated field) (Table 1).

Table 2

The contents of iron forms in soils under shelterbelts and adjoining cultivated fields

	Fe(II) [mg·kg ⁻¹]	Fe(III) [mg·kg ⁻¹]	Fe(II)/ Fe(III)	Fe _{total} [mg·kg ⁻¹]
<i>Robinia pseudacacia</i> shelterbelt	7.9 ^b ±2.9	8.9 ^b ±3.0	0.88	16.75
Cultivated field	2.42 ^c ±0.94	3.1 ^a ±1.3	0.77	5.56
<i>Crataegus monogyna</i> shelterbelt	5.9 ^{ab} ±2.2	6.7 ^{ab} ±2.7	0.88	12.50
Cultivated field	2.7 ^{ac} ±1.3	3.4 ^a ±1.9	0.79	6.13
Young shelterbelt (20 years old)	4.6 ^{abc} ±2.6	5.8 ^{ab} ±2.6	0.79	10.49
Cultivated field	3.2 ^{abc} ±1.5	4.4 ^{ab} ±2.3	0.74	7.60

Values followed by the same subscript letter(s) are not significantly different at $\alpha = 0.05$; Mean ± 95 % confidence interval

In flooded soils hydrates reduce Fe(III) oxide to Fe(II). Ferric iron ions and dissolved oxygen in soil solution are reactants in the redox reactions. It seems that the complexation of Fe with dissolved organic matter plays a fundamental part in acidification and pedogenesis and prevents immobilization by precipitation like inorganic metal complexes [20]. The pH and temperature are the factors affected the ferrous and ferric ions amounts in soils. Our research showed that there were no significant differences in the ferric ions concentration in soil under *C. monogyna* and young shelterbelts than in adjoining cultivated fields to these shelterbelts. However, the amounts of ferric ions were significantly higher in soils under *R. pseudacacia* shelterbelt than in neighbouring cultivated field (Table 2). Similar changes like in case of ferric ions were observed for the concentrations of ferrous ions in soils under *R. pseudacacia* shelterbelt. There were significant higher contents of ferrous ions than in adjoining cultivated fields (Table 2). Moreover, simultaneously total iron was the highest in soil under *R. pseudacacia* shelterbelt ($16.75 \text{ mg}\cdot\text{kg}^{-1}$) and the lowest in soil in adjoining cultivated field to *R. pseudacacia* shelterbelt ($5.56 \text{ mg}\cdot\text{kg}^{-1}$). Our investigations have shown that in research soils were more ferric than ferrous ions. This suggests that in the soils are favourable oxygen conditions.

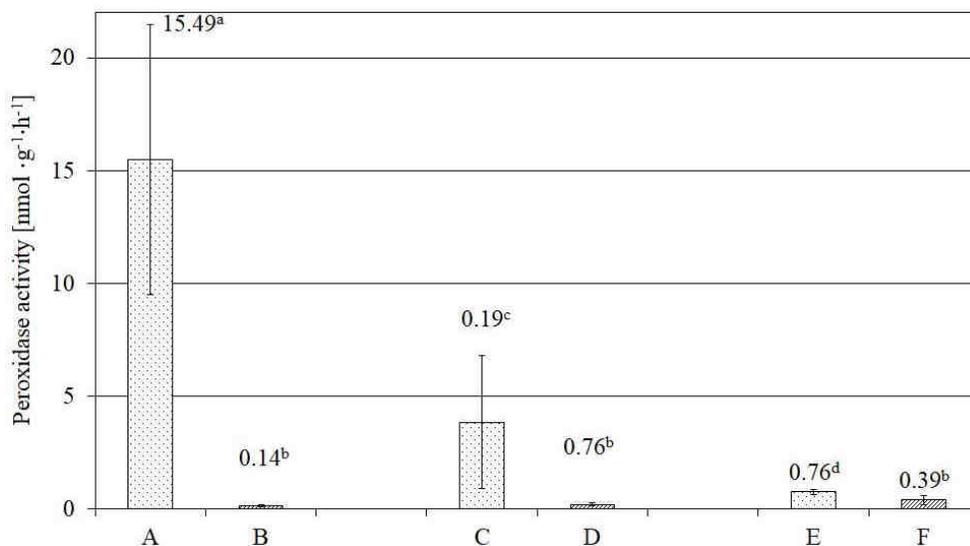


Fig. 1. The average value of peroxidase activity in soils under three different shelterbelts and in adjoining cultivated fields: A - *Robinia pseudacacia* shelterbelt, B - adjoining cultivated field to *Robinia pseudacacia* shelterbelt, C - *Crataegus monogyna* shelterbelt, D - adjoining cultivated field to *Crataegus monogyna* shelterbelt, E - young shelterbelt (20 years old), F - adjoining cultivated field to young shelterbelt

Soil enzymes are important for their role in nutrient cycling and were considered to be early indicators of specific biochemical reactions in soil, because of their relationship to soil biology, ease of measurement and rapid response to changes in soil managements. Peroxidase is the enzyme participating in oxidation-reduction processes in soils. Peroxidase has been the most studied because of their role in organic matter degradation and release of nutrients in the soils. Tian and Shi [21] hypothesized that peroxidase could enhance soil C

and N mineralization via improving the bioavailability of reducing sugars and amino acids. Their results indicated that the effects of peroxidase on organic matter degradation. Generally significantly higher activity of peroxidase in soils under old shelterbelts than in adjoining cultivated fields was observed. However, the highest activity of peroxidase was determined in soil under *Robinia pseudacacia* shelterbelt ($15.49 \text{ nmol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$). This value was significantly higher than in adjoining cultivated field ($0.14 \text{ nmol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) (Fig. 1). Moreover, the peroxidase activity in soil under *Crataegus monogyna* shelterbelt was 20 times higher than in adjoining cultivated field, however, in young shelterbelt 1.9 times higher than in adjoining cultivated field (Fig. 1). Previous studies Meysner and Szajdak [15] confirmed that the changes of peroxidase activity are in line with *TOC*.

The factor of biochemical transformation is a useful tool for the estimation of the rate of biochemical conversion based on the enzymes activity in the layers of soil taking into account the properties as bulk density and porosity. These factors calculated for peroxidase activity were higher in soils under shelterbelts than adjoining cultivated fields. The number of moles of phenols and aromatic amines converted by peroxidase in the volume unit and in the unit of time was from 1.00 to $17.91 \text{ mol}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$ under shelterbelts and from 0.14 to $0.51 \text{ mol}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$ in adjoining cultivated fields (Fig. 2). However, in soil under young shelterbelt the factor of biochemical transformation was significantly lower than under old shelterbelts, where contents of *TOC* and C_{HWE} were higher.

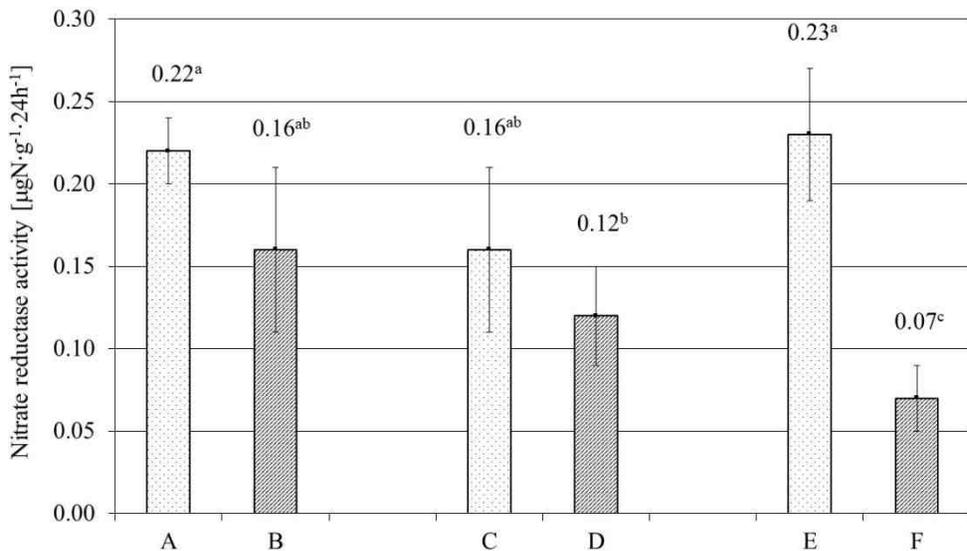


Fig. 2. Factors of biochemical transformations for peroxidase activity in soils under shelterbelts and adjoining cultivated fields: A - *Robinia pseudacacia* shelterbelt, B - adjoining cultivated field to *Robinia pseudacacia* shelterbelt, C - *Crataegus monogyna* shelterbelt, D - adjoining cultivated field to *Crataegus monogyna* shelterbelt, E - young shelterbelt (20 years old), F - adjoining cultivated field to young shelterbelt

Nitrate reductase is an enzyme participating in the reduction of nitrate to nitrite. This is one of the steps of dissimilatory reduction by essentially aerobic bacteria. A higher activity of this enzyme was discovered in the soils under shelterbelts than in adjoining cultivated

fields. The yearly mean value of nitrate reductase activity of in soil under young, *Robinia pseudacacia* and *Crataegus monogyna* shelterbelts were 0.22, 0.23 and 0.16 $\mu\text{g N}\cdot\text{g}^{-1}\cdot\text{d.m.}\cdot 24\text{ h}^{-1}$, respectively. There was no significant difference in the activity of this enzyme in all researched shelterbelts. Significant differences in nitrate reductase activity were observed in soil under young shelterbelt and adjoining cultivated field. However, the lowest value of nitrate reductase's activity was found in the soil in the cultivated field adjoining to young shelterbelt (0.07 $\mu\text{g N}\cdot\text{g}^{-1}\cdot\text{d.m.}\cdot 24\text{ h}^{-1}$) (Fig. 3). The nitrate reductase's activity was positively correlated with concentration *TOC*, *C_{HWE}* and *TN* ($r = 0.32$, $r = 0.30$ and $r = 0.30$). The factors of biochemical transformation calculated for nitrate reductase activity were significantly higher in soils under shelterbelts than in adjoining cultivated fields except field neighboring to *Robinia pseudacacia* shelterbelt. The number of moles nitrate converted to nitrite by nitrate reductase in volume of soil and in time was from 0.64 to 0.92 $\text{mol}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$ under all shelterbelts and from 0.22 to 0.70 $\text{mol}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$ in adjoining cultivated fields (Fig. 4). This factor may wide range than the same enzyme activity explain about activity of nitrate reductase in the volume of soil (one ha area and the depth of 20 cm) taking into account also the porosity and bulk density of research soils. However, bulk density and porosity of soils characterizes the availability of the substrate to enzyme. Thus, the factor of biochemical transformation reveals the efficiency of the transformation of the substrate of the own enzyme in specific soil conditions. Askin and Kizilkaya [22] demonstrated the mid-sized macroaggregates had enhanced microbiological properties and organic carbon contents. The main effect of the macroaggregates on the microbiological properties may be arisen by the accumulation or decomposition of organic matter erosion and deposition.

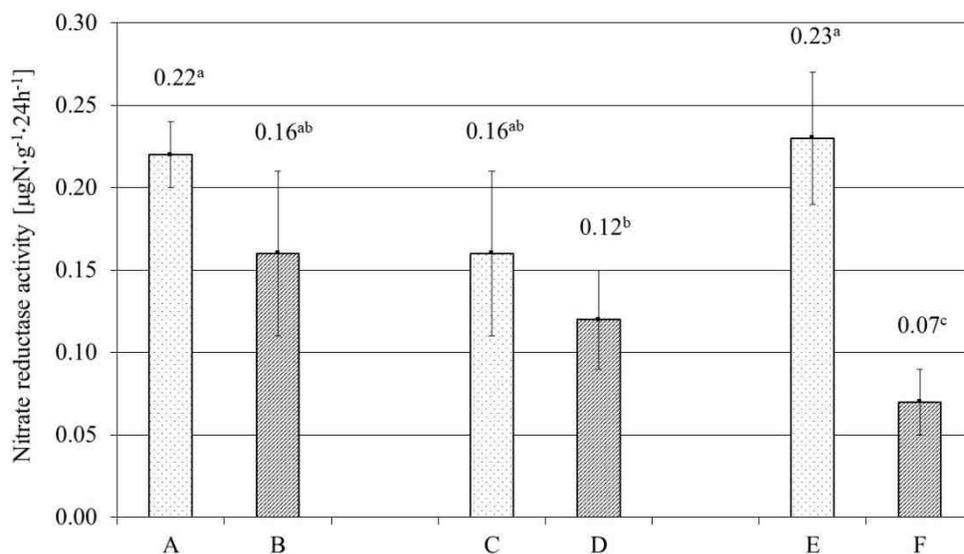


Fig. 3. The mean value of nitrate reductase activity in soils under three different shelterbelts and in adjoining cultivated fields in 2007, 2008 and 2010: A - *Robinia pseudacacia* shelterbelt, B - adjoining cultivated field to *Robinia pseudacacia* shelterbelt, C - *Crataegus monogyna* shelterbelt, D - adjoining cultivated field to *Crataegus monogyna* shelterbelt, E - young shelterbelt (20 years old), F - adjoining cultivated field to young shelterbelt

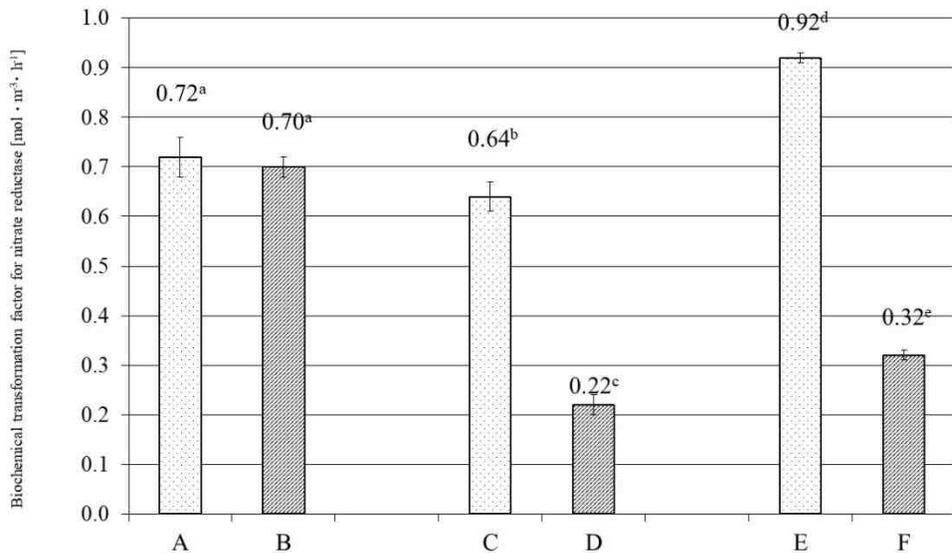


Fig. 4. Factors of biochemical transformations for nitrate reductase activity in soils under shelterbelts and adjoining cultivated fields: A - *Robinia pseudacacia* shelterbelt, B - adjoining cultivated field to *Robinia pseudacacia* shelterbelt, C - *Crataegus monogyna* shelterbelt, D - adjoining cultivated field to *Crataegus monogyna* shelterbelt, E - young shelterbelt (20 years old), F - adjoining cultivated field to young shelterbelt

The evolution of nitrous oxide from soils is an effect of the soil denitrification and nitrification processes. Our results revealed significant lower values of nitrous oxide evaluated from soils under *Robinia pseudacacia* and young shelterbelts than from adjoining cultivated fields (from 1.4 to 18.3 times lower) (Table 3). It was observed the relationship between the age of shelterbelt and the amount of the nitrous oxide emission. The lowest amount nitrous oxide evolution was determined from soils under young shelterbelt ($19.2 \mu\text{g N-N}_2\text{O}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$). The highest evolution was observed from soil under adjoining cultivated field to young shelterbelt ($351.0 \mu\text{g N-N}_2\text{O}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$). These results suggest positive effect of shelterbelt on the lowering emission of nitrous oxide from soils. Kesik et al. [23] also showed that the N_2O emissions from agricultural soils are average ten times stronger compared to the forest soils. Furthermore, there were no significant correlations between nitrous oxide emission and WFPS. The magnitude N_2O emission increased with increasing WFPS, in agreement with studies Abbasi and Adams [24]. Diffusion of oxygen in soil is limited by increasing of soil water content and anaerobic conditions predominate [9]. According these authors emissions of N_2O increase after fertilizer N application with increasing soil water content and most rapidly above 70 % WFPS where denitrification is higher. Bateman and Bags [5] proved that the autotrophic nitrification was the main source of N_2O in soils at 35-40 % WFPS, while at 70 % WFPS N_2O was produced during denitrification. In the research soils under shelterbelts and the adjoining cultivated fields the WFPS were from 40 to 50 %. These data may suggest evolution N_2O by autotrophic nitrification. Furthermore, no significant correlation was found between nitrate reductase's activity and the evolution of nitrous oxide. Kool et al. [12] hypothesized that nitrifier

denitrification is an important source of N_2O from the drier soil, whereas denitrifiers were identified as the dominant producers of N_2O from the wetter soil.

Table 3

The evolutions and accumulation of greenhouse gases from soils under shelterbelts and adjoining cultivated fields

Gasses	<i>Robinia pseudaccacia</i>		<i>Crataegus monogyna</i>		Young	
	shelterbelt	cultivated field	shelterbelt	cultivated field	shelterbelt	cultivated field
N_2 [mg $N-N_2 \cdot m^{-2} \cdot h^{-1}$]	0.0 ^a ±0.0	0.0 ^a ±0.0	0.0 ^a ±0.0	0.3 ^a ±0.4	0.0 ^a ±0.0	0.4 ^a ±1.1
N_2O [$\mu g N-N_2O \cdot m^{-2} \cdot h^{-1}$]	37.6 ^a ±7.4	50.8 ^b ±4.8	37 ^{abc} ±21	66 ^{ab} ±24	19.2 ^c ±9.1	351 ^d ±114
CH_4 [$\mu g C-CH_4 \cdot m^{-2} \cdot h^{-1}$]	1.2 ^a ±0.6	-5.2 ^{bc} ±3.0	-2.5 ^b ±1.2	-5.2 ^c ±1.3	-3.2 ^{bc} ±1.7	-2.5 ^{bc} ±4.3
CO_2 [mg $C-CO_2 \cdot m^{-2} \cdot h^{-1}$]	20.4 ^b ±5.5	26.5 ^{bc} ±5.7	28 ^{bc} ±10	12.1 ^a ±1.4	14.9 ^{ab} ±6.6	54 ^c ±24

Note. Plus indicates gas release from the soil and minus gas uptake by the soil, Mean \pm 95 % confidence interval. Values followed by the same subscript letter(s) are not significantly different at $\alpha = 0.05$.

Our results on the evolution of nitrous oxide are in line with the data of Ullah et al. [25]. They investigated the evolution of N_2O from forest soil and cultivated soil. They measured lower emission of nitrous oxide from forest soils than from cultivated fields. They suggests the following explanations of this process: the delay of gas flow at moisture states above field capacity, an increase non-dissimilatory nitrate reduction and an alternation at higher moisture states in the type and amount of available C, which is essential to the denitrification process and also there appears to be critical stage in moisture beyond which the process of denitrification slows down, thus altering the timing of peak denitrification with respect to a typical agricultural soils. In addition, no emission of dinitrogen from soils under all shelterbelts was observed. However, the evolution of dinitrogen from soils under adjoining cultivated field to *Crataegus monogyna* and young shelterbelt were observed 0.3 and 0.4 mg $N-N_2 \cdot m^{-2} \cdot h^{-1}$, respectively (Table 3). Significant correlation coefficient were found between N_2O and N_2 concentrations ($r = 0.86$, $\alpha = 0.05$).

Both denitrification and methane formation depends on the oxygen status of the soil. Khalil and Baggs [9] found positive correlations between $^{13}C-CH_4$ and NH_4^+ concentrations, indicating possible temporary inhibition of CH_4 oxidation. Methane fluxes from or to soils result from the interaction of several biological and physical processes in the soil. Our results have shown remarkable negative correlations between CH_4 concentrations and bulk density ($r = -0.51$). We did not observe any emission of methane from soils under all adjoining cultivated fields to shelterbelts and from soils under *Crataegus monogyna* shelterbelt and young shelterbelt. However, the evolution of methane was only determined from soil under *Robinia pseudaccacia* shelterbelt and was equal to 1.22 mg $C-CH_4 \cdot m^{-2} \cdot h^{-1}$ (Table 3).

Opposite to this, in other soils of shelterbelts (young and *C. monogyna*) and adjoining cultivated fields the consumption of methane was measured and ranged from -5.2 to -2.5 mg $C-CH_4 \cdot m^{-2} \cdot h^{-1}$ (Table 3). These results may suggest that in research soils under shelterbelts and adjoining cultivated fields the condition to formation of methane are unfavourable. It may suggest that in research soils (WFPS were from 40 to 52 %) are preferential treatment conditions to oxidation CH_4 . Khalil and Baggs [9] showed that most rapid oxidation rate CH_4 was measured at 45 % WFPS but the lowest at 30 % WFPS.

Luyssaert et al. [26] studies consider emissions and sequestration of carbon dioxide (CO₂) in forest ecosystems. Although, the majority of studies to date have been focused on soils as sources, soils can also however act as CO₂ sinks. Our results have shown significant lower 3.6 times emission of CO₂ from soils under young shelterbelt than adjoining cultivated fields. However, soil under *C. monogyna* revealed higher 2.3 times than adjoining cultivated field the evolution of CO₂. Furthermore, soil under young shelterbelt showed the lowest evolution of CO₂ in comparison to all shelterbelts. The smallest evolution of CO₂ from soils under young shelterbelt suggests low rate of mineralization and high rate of humification. This process can lead to the formation of humic and fulvic acids, which representing the main fraction of humic substances. It may show that in soil under young shelterbelts the anabolic process is dominated by catabolic. Maryganowa et al. [27] and Szajdak et al. [28] have proved significant differences of the humic (HA) and fulvic (FA) acids properties estimated by optical, thermal and molecular weight distribution extracted from soil under *R. pseudacacia* and young shelterbelt. They suggest that the HA from the soil under the old shelterbelt exhibited a higher degree of aromatic condensation and polyconjugation, i.e. was more humified and chemically mature compared to the reference HA. The HA from the young shelterbelt had a lower degree of humification, i.e. was younger compared to the HA from the adjoining field. This indicates that the processes of humification with the formation of the young immature humic molecules in the soil under the young shelterbelt were more intensive than in the soil of adjoining cultivated field and in the soil under the old shelterbelt. This fact shows the presence of easily degradable organic matter in soil under young shelterbelt.

Furthermore, our investigation showed different properties of soil under young shelterbelt than under old shelterbelts (*R. pseudacacia* and *C. monogyna*). In soil under young shelterbelt the opposite to the soils under old shelterbelts lower emissions of N₂O were showed. Soil under young shelterbelt was characterized quite the contrary properties (lower: organic matter content, N₂O emission, *TN* and bulk density) than soils under old shelterbelts. This lower content of organic matter in soil under young shelterbelt may contribute to the lower emissions of gases from this soil. It is assumed that the availability of soil organic carbon limits microbiological growth and enzymes activity in soils.

We observed significant correlation between enzymatic activity and hot water extractable organic carbon. C_{HWE} can be a substrate for microbial growth, but its production is also partly mediated by microbes. This fraction is responsible for microbiological activity [14]. These authors proved that the molecular size distribution and chemical composition of dissolved organic matter were similar in the soils under three tree species.

The results show that manipulation of plant cover in agricultural landscape is an important factor to control the quality and quantity of chemicals in soils that can appear some unwanted effects on the evolution of greenhouse gases from soils into the atmosphere. Our results suggest that the creation of new shelterbelts is one of the positive factors restrict the evolution of greenhouse gases from agricultural landscape.

Conclusions

1. Significant higher peroxidase activity was observed in soil under old than young shelterbelts and adjoining cultivated fields. These differences may affect organic matter contents. In soils under old shelterbelts the total organic carbon and hot water

- extractable organic carbon were significantly higher than in young shelterbelts and adjoining cultivated fields.
2. Our research showed no significant differences between old and young shelterbelts for nitrate reductase activity. There was no impact of the age and plant composition on the activity of this enzyme. Significant differences were, however, found between the soils under the young shelterbelt and in the adjoining cultivated field.
 3. The total nitrogen, total organic carbon, hot water extractable organic carbon and peroxidase activity in the soil under the young were significantly lower than in the soils under both old shelterbelts. The evolution of nitrous oxide was significantly higher under *Robinia pseudacacia* shelterbelt than under the young shelterbelt. However, there were no significant differences in the emission of carbon dioxide from soils under the three researched shelterbelts.
 4. Creating new shelterbelts is important element in agricultural landscape not only for its structure, as well as improving physical, chemical and biochemical properties of soils and restricting fluxes of greenhouse gases.

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