

## Changes of lignin concentration and C:N in oilseed rape, wheat and clover residues during their decomposition in the soil

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**Abstract.** Field experiments were conducted in 2003–2005 at the Experimental Station of the Lithuanian University of Agriculture to study the changes of lignin concentration and C:N in roots and top residues of winter and spring oilseed rape (*Brassica napus* L.), winter wheat (*Triticum aestivum* L.) and red clover (*Trifolium pratense* L.) after 14, 33, 63, 85 and 116 weeks of decomposition in the soil. Correlation between lignin concentration and amount of dry matter as well as the ratio of carbon and nitrogen in investigated crop residues were estimated.

Investigation of crop residue decomposition during the period of 116 weeks showed that stubble and roots of winter and spring oilseed rape decompose more slowly than their threshing remains, or stubble, and roots of winter wheat and red clover. Dry matter and lignin decomposed and the ratio of carbon and nitrogen in winter oilseed rape residues decreased more slowly than that of spring oilseed rape residues.

The ratio of carbon and nitrogen in the decomposing crop residues decreased most intensively during the 33–63 week period. After that, the concentration of lignin started to decrease, but its significant decline in all investigated crop residues was estimated after 116 weeks of decomposition. Lignin was most decomposed from its concentration peak in the stubble of red clover (37.9%) and least in winter oilseed rape roots (12.8%).

Negative correlation between lignin concentration and dry matter amount and between lignin concentration and C:N was established in winter and spring oilseed rape, winter wheat and red clover top and root residues decomposing in the soil.

**Key words:** *Brassica napus*, crop top and root residue, decomposition, dry matter, organic carbon and nitrogen ratio, lignin, *Triticum aestivum*, *Trifolium pratense*.

### INTRODUCTION

An important parameter for estimation of soil fertility stability is the intensity of organic matter decomposition, including mineralization, humification, fixation and migration (Aleksandrova, 1980; Sollins et al., 1996). The intensity of these processes and their concatenation is determined by easily dissoluble components and lignin concentration and by their ratio in plant residues (Klimanek, 1988; Sollins et al., 1996; Kalbitz et al., 2000). Lignin is one of the main sources for humus formation and maintenance for soil fertility and ecology (Crawford, 1981; Tate, 1999).

The content and composition of lignin in plants may vary widely depending on the species. According to the investigation at the Lithuanian Institute of Botany, winter

wheat straw contains 51.4% cellulose and 14.3% lignin, rye straw – 36.8 and 11.7%, lucerne stems – 38.0 and 15.7%, respectively (Lugauskas, 1997). The composition of lignin in oilseed rape stems is typical for angiospermous wood: syringyl and coniferyl ratio varies slightly with plants' maturation (Evansa et al., 2003). The amount and chemical characteristics of lignin vary across the cell wall and among cell types (Gisi, 1990; Donaldson, 2001; Grabber, 2005). Lignin is a component of enclosed and water vascular bundle parenchyma of the plant cell walls (Kogel-Knaber, 2002; Grabber, 2005). The highest concentration of lignin is found in the middle lamella and in corner regions. From 30–50% of lignin is composed of macromolecular polysaccharides that are products of monosaccharides condensation (Tate, 1999). Lignin belongs to the group of nitrogen-free organic compounds (Donaldson, 2001; Kogel-Knaber, 2002; Sjoberg, 2003).

Lignin is a complex polymer resistant to exogenous factors. It is static and protects cell cytoplasm against microbiological decomposition (Tate, 1999; Blanchette, 2000). However, some soil fungi degrade lignin and use metabolites of lignin as the source of carbon (Lugauskas, 1997). Secondary metabolites containing sulphur, such as thioglucosides and glucosinolates, are found in crucial plants (Sanger et al., 1997). Glucosinolates stimulate some types of soil biota while inhibiting other types (Yourchac & Shrol, 1981). This affects the decomposition of oilseed rape residues.

According to the investigation carried out in 1958 by Kononova, the intensity of plant residue mineralization depends on concentrations of easily dissolvable components and lignin and on their ratio in decomposing material (Kononova, 1963). Decomposition of different plant residues was investigated under various soil and climatic conditions by other researchers as well (Jenkinson, 1965; Klimanek, 1988; Kalbitz et al., 2000). The study conducted in Slovak Agricultural University during 1998–1999 showed that according to the type and granulometric composition of the soil in the period of 360 days, 78.5–92.4% of winter rape straw (Jurcova & Tobiasova, 2002) decomposed.

Previous study showed that the positive influence of oilseed rape crop on the subsequent plants lasts for two years. There is more humus in the arable layer after growing oilseed rape than after wheat, but less than after clover (Velicka, 2002). Investigation of other parameters should be undertaken in order to understand the mechanism of oilseed rape's positive influence on subsequent crops.

A nitrogen ratio favorable for the decomposition of carbon in the oilseed rape residues should enable more intensive mineralization, but on the other hand, lignin concentration higher than in other crops could make this process slower. The aim of our research was to estimate the changes of lignin concentration and C:N in winter and spring oilseed rape residues during their decomposition in the soil and to compare them with residue in winter wheat and red clover.

## MATERIALS AND METHODS

The experiment of decomposition of oilseed rape and other crop residues was carried out in the Experimental Station of the Lithuanian University of Agriculture (54°53'N, 23°50'E) in model field experiment during the period of 2003–2005. The soil of experimental site – *Epihypogleyic Cambisol (Endocalcaric)* (artificial drainage);

silt loam (sand 33.8%, silt – 55.3%, clay – 10.9%) from loess over moraine clay loam (sand 36.5%, silt – 24.9%, clay – 38.6%). Humus content (0–25 cm layer) – 21.0 g kg<sup>-1</sup>, determined by the Tyurin method modified by Nikitin and calculated by multiplying C<sub>org.</sub> content by 1.724 (Nikitin, 1999); pH (KCl 1 M, w/v 1:2.5) – 6.7, determined using a pH-meter; total N – 1.47 g kg<sup>-1</sup>, determined by the Kjeldahl method; available phosphorus (P<sub>2</sub>O<sub>5</sub>) – 119 mg kg<sup>-1</sup> and available potassium (K<sub>2</sub>O) – 100 mg kg<sup>-1</sup>, determined by the Egner-Riem-Domingo (A-L) method (Egner et al., 1960).

The experiment involved a two-factor design: Factor A – crop residues: 1. Roots of winter oilseed rape; 2. Roots of spring oilseed rape; 3. Roots of winter wheat; 4. Roots of red clover; 5. Stubble of winter oilseed rape (30 cm from root collar); 6. Stubble of spring oilseed rape (30 cm from root collar); 7. Stubble of winter wheat (20 cm height); 8. Stubble of red clover (20 cm height); 9. Threshing remains of winter oilseed rape (stems with branches and siliques); 10. Threshing remains of spring oilseed rape (stems with branches and siliques). Factor B – decomposition periods: 1.0 weeks; 2.14 weeks; 3.33 weeks; 4.63 weeks; 5.85 weeks; 6.116 weeks. The experiment was performed with 4 replications.

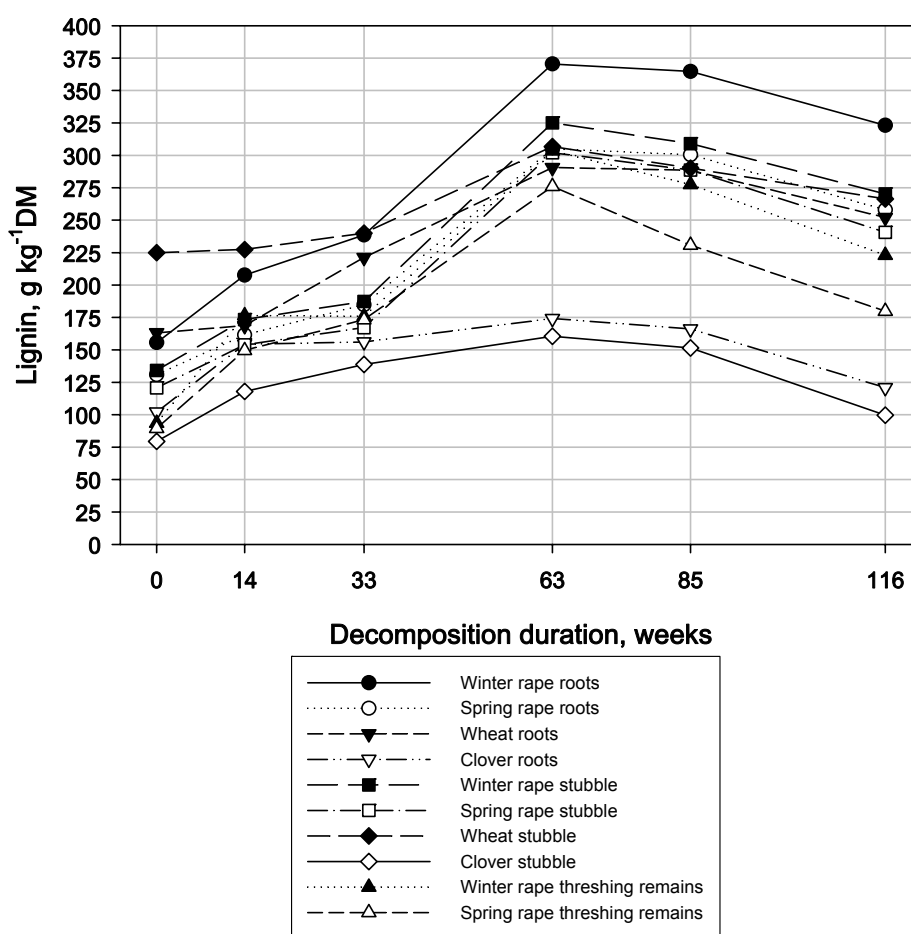
Samples of winter oilseed rape (*Brassica napus* L. ssp. *oleifera biennis* Metzg.), spring oilseed rape (*Brassica napus* L. ssp. *oleifera annua* Metzg.) and winter wheat (*Triticum aestivum* L.) residues were prepared after harvest. Samples of red clover (*Trifolium pratense* L.) residues were prepared after the first grass harvest of the second-year ley. Crop residues were chopped in 2–3 cm size chaffs and content of their dry matter was recorded. Natural humidity samples of 20 g were taken and put in the 9x12 cm size net polychlorvinyl bags. Diameter of net meshes was 0.05 mm. Bags with crops residues were put in ploughed-up furrows of bare fallow at 20 cm distances and ploughed in at the 20 cm depth. Five furrows with all crop residues in each were made to provide samples for experimental periods (factor B). Initiation and end datum-point of each period (except experiment starting time) was set up when soil average temperature at the depth of 20 cm during three successive days in spring reached  $\geq 5^{\circ}\text{C}$  and in autumn –  $\leq 5^{\circ}\text{C}$ . Bags with crop residues were dug out and the remaining soil was cleaned out at the end of decomposition period. First, dry matter content of crop residues was determined by drying in a thermostat at 105°C temperature. The decomposition rate of crop residues of natural humidity (which was determined before their incorporation into the soil) was calculated according to decreased amount of dry matter per particular investigation period. The content in the bag was dried out, grounded, sieved through a 1 mm separator and the following analyses performed: the lignin content by the Klason method (Yermacov et al., 1972), the content of organic carbon by the Tyurin method, the content of nitrogen, by the Kjeldahl method.

Experimental data were statistically evaluated using Fisher protected LSD test and correlation analyses.

## RESULTS AND DISCUSSION

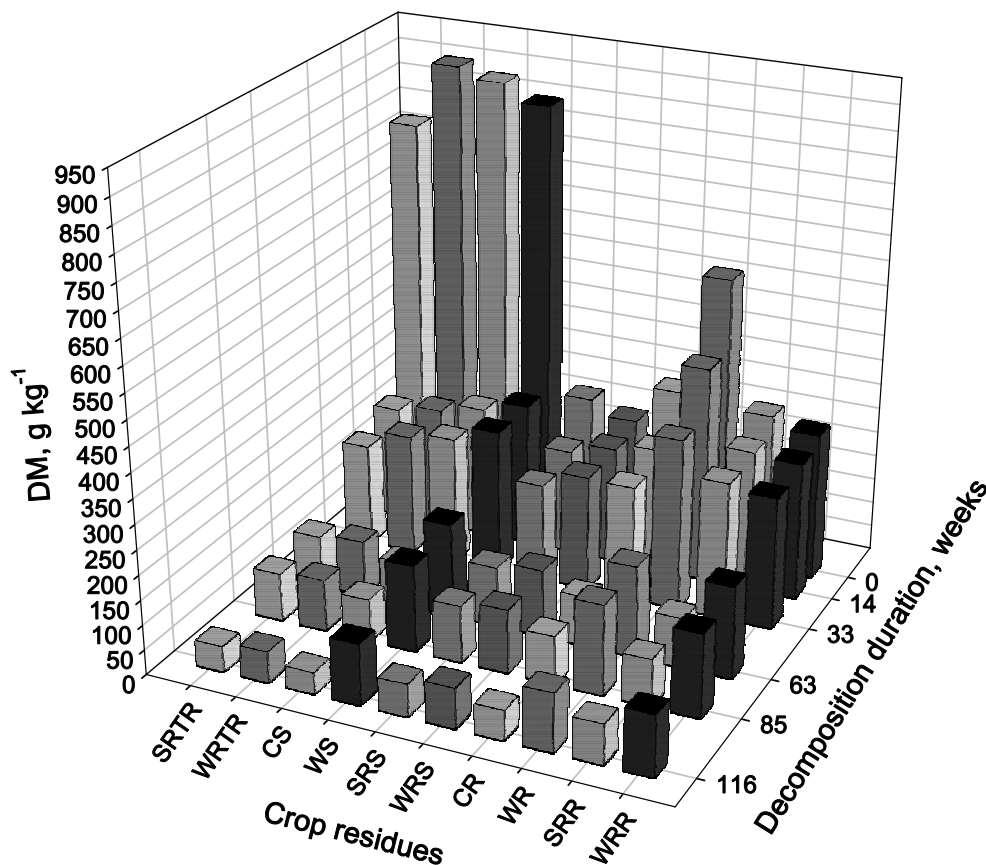
The changes of lignin concentration in investigated crop residues were determined by residue type and decomposition period. The significantly highest amount of lignin among post harvest residues was in the stubble of winter wheat (225.0 g kg<sup>-1</sup>), and the

lowest, in the stubble of red clover ( $79.4 \text{ g kg}^{-1}$ ) and threshing remains of spring and winter oilseed rape –  $89.5$  and  $93.5 \text{ g kg}^{-1}$ , respectively (Fig. 1). The concentration of lignin in the stubble of winter and spring oilseed rape was higher ( $P \leq 0.05$ ) than that in the stubble of red clover and lower ( $P \leq 0.05$ ) than that in winter wheat. The concentration of lignin in crop roots, except winter wheat, was higher than that in top residues. Higher lignin content was recorded in all residues of winter oilseed rape as compared with spring oilseed rape residues, but was significant only in the roots. After 63, 85 and 116 weeks of crop residue incubation in the soil, concentration of lignin was significantly higher in the roots of winter oilseed rape and lower in the roots and stubble of red clover in comparison with other residues. Other authors describe wide variation of lignin concentration in different plant species and also in particular morphological plant parts (Kononova, 1963; Jenkison, 1965; Klimanek, 1988).



**Fig. 1.** Lignin concentration in crop residues during their decomposition in the soil. DM – dry matter.  $LSD_{05} = 22.01$ .

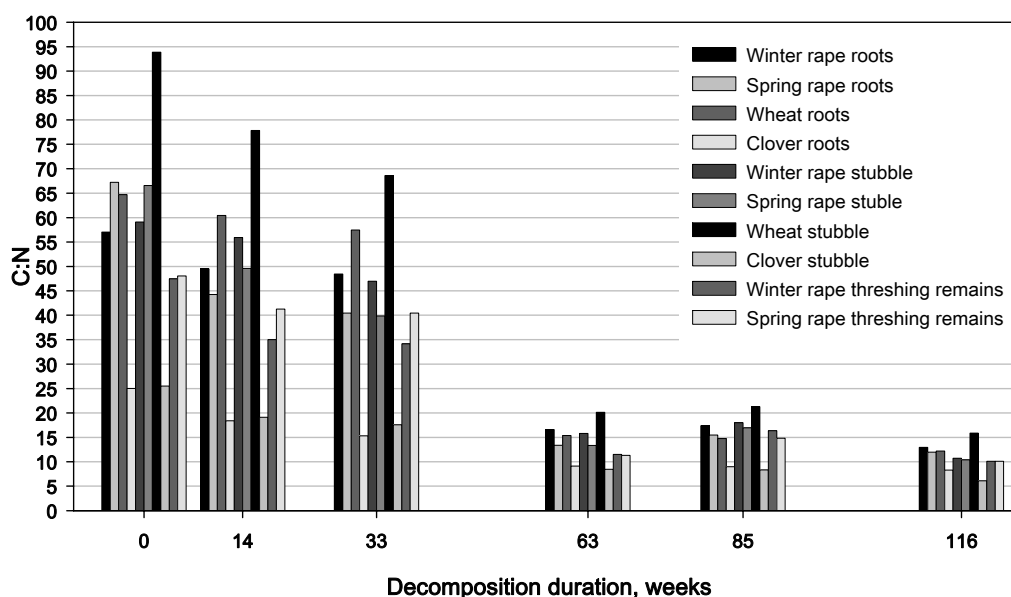
The concentration of lignin in all crop residues relatively increased till 63 weeks of their decomposition, because of intense degradation of easily soluble organic compounds and permanent decrease of the dry matter amount (Fig. 2). Afterwards, more intense degradation of lignin started and the decrease of its concentration was recorded after 85 weeks of crop residue incubation in the soil.



**Fig. 2.** Dry matter amount in the decomposing crop residues of natural humidity (as was determined before incorporation into the soil). WRR – winter rape roots, SRR – spring rape roots, WR – wheat roots, CR – clover roots, WRS – winter rape stubble, SRS – spring rape stubble, WS – wheat stubble, CS – clover stubble, WRTR – winter rape threshing remains, SRTR – spring rape threshing remains.  $LSD_{05} = 16.65$ .

Dry matter content in post-harvest residues varied from 255 g kg<sup>-1</sup> in the stubble of winter oilseed rape to 911 g kg<sup>-1</sup> in their threshing remains. After 14 weeks of crop residue decomposition the least decrease of dry matter content was in roots (6.4%,  $P \leq 0.05$ ) and stubble (7.6%,  $P \leq 0.05$ ) of winter oilseed rape, while lignin concentration increased significantly, by 33.5 and 29.3%, respectively. Roots of red

clover and winter wheat during this period were decomposed (accordingly 24.7 and 24.6%) significantly more than those of winter and spring oilseed rape (accordingly 6.4 and 10.6%). Despite the fact that decomposition intensity was of the same character in roots of red clover and winter wheat, the lignin concentration increased variably (by 51.4 and 3.4%, respectively). Threshing remains of winter and spring oilseed rape and stubble of red clover decomposed most intensively among residues; respectively 73.1, 70.7 and 70.3% of these residues were decomposed after the first incubation period of 14 weeks. The increase of lignin concentration in these residues was highest (88.7, 67.2 and 48.2%, respectively; Fig. 1). The ratio of C:N in all decomposing crop residues during this period decreased significantly (Fig. 3). Easily soluble organic compounds are decomposing and the content of C is decreasing whereas concentration of N is relatively increasing (Velicka et al., 2006). The increase of N concentration in the decomposing residues is also determined by the activity of soil biota (Hadas et al., 2004). The ratio of C:N decreased least in the stubble of winter oilseed rape (5.3%) and in the roots of winter wheat (6.6%). According to Klimanek (1988), the intensity of crop residue decomposition during the period of 90 days depends on the amount of lignin (41%) and on the ratio of carbon and nitrogen (15%).



**Fig. 3.** The ratio of carbon and nitrogen in crop residues during their decomposition in the soil.  $LSD_{05} = 4.02$ .

Stubble of clover and wheat, and threshing remains of rape decomposed very intensively during the first investigation period (0–14 weeks), whereas, investigated crop root and rape stubble residues decomposed most intensively during the third warm investigation period (April–October, 63 weeks from the initiation of investigation). Still, after 63 weeks of incubation in the soil the stubble of red clover was decomposed

most (90.2%). The ratio of C:N decreased from 25.5–8.4 and was lowest among all crop residues (Fig. 3), due to intensive mineralization and increased concentration of nitrogen. Lignin concentration in stubble of red clover was also the lowest ( $160 \text{ g kg}^{-1}$ ) (Fig. 1). The roots of winter oilseed rape were decomposed least (37.6%); lignin concentration was significantly higher ( $P \leq 0.05$ ) than that in other residues. The concentration of lignin in residues of all morphological parts of winter oilseed rape was significantly higher than that in spring oilseed rape. During the period of 63 weeks of crop residue incubation in the soil the concentration of lignin in stubble of winter wheat relatively increased by 36.6%, which was 3.8 times less than that in the roots of winter oilseed rape. Therefore, after 63 weeks of residues' decomposition the highest lignin concentration among examined crop residues was in the roots of winter oilseed rape ( $370 \text{ g kg}^{-1}$ ). These alterations were determined by the complex of factors, and, as the most important, should be distinguished: chemical composition of crop residues, molecular structure of lignin, soil biodiversity, plurality and activity of soil biota (Lugauskas, 1997; Tate, 1999; Grabber et al., 2004).

The fourth investigation period (63–85 weeks) of crop residue decomposition took place during cold weather (October–April). It is indicated in literature that some microorganisms decompose organic matter at  $0^\circ\text{C}$  temperature (Kononova, 1963). Results of our investigation showed that lignin concentration decreased in all crop residues during this period, despite the fact that the decomposition of crop residues was not intensive (only 3.8–7.7% of crop top residues and 1.4–9.8% of roots decomposed). Significant decrease of lignin concentration was observed only in the threshing remains of winter and spring oilseed rape – 26.0 and  $45.3 \text{ g kg}^{-1}$ , respectively (Fig. 1).

After 116 weeks of crop residue incubation in the soil the stubble and roots of red clover were decomposed the most (accordingly 95.2% and 94.6%) and those residues of winter oilseed rape – least (accordingly 68.3 and 58.9%). After this period, the concentration of lignin decreased significantly in all crop residues as compared with that after 63 and 85 weeks. During the period of 63–116 weeks lignin degraded least in the roots of winter oilseed rape (12.8%) and in winter wheat residues (13.2%). Within this period lignin degraded most (37.9%) in the stubble of red clover; the C:N ratio was the lowest (6.1). This shows the intensive mineralization of red clover residues which is followed by the higher concentration of labile compounds that is typical of leguminous crop residues (Klimanek, 1988). During this last period lignin degraded slower in all winter oilseed rape residues as compared with that in spring oilseed rape: by 2.6 percentage units in roots, 3.4 in stubble and 8.4 in threshing remains.

During crop residue decomposition and decrease of dry matter and C:N the concentration of lignin was relatively increasing. It was estimated that during the period of investigation, concentration of lignin in winter oilseed rape roots was in strong negative correlation with the amount of dry matter and in very strong negative with the C:N ratio (Table 1). Very strong negative correlation between lignin concentration and the amount of dry matter as well as C:N was shown in the roots of spring oilseed rape and winter wheat.

**Table 1.** Correlation between lignin concentration and amount of dry matter also C : N in crop residues decomposing in soil.

| LUA Experimental Station, 2003–2005 |                    |                                         |                                         |                          |
|-------------------------------------|--------------------|-----------------------------------------|-----------------------------------------|--------------------------|
| Crop residues                       | Index ( <i>x</i> ) | Lignin, g kg <sup>-1</sup> ( <i>y</i> ) |                                         | Probability ( <i>P</i> ) |
|                                     |                    | equation of regression                  | coefficient of correlation ( <i>r</i> ) |                          |
| Winter rape roots                   | DM                 | $y = 518.66 - 1.10x$                    | -0.88                                   | $P \leq 0.05$            |
|                                     | C : N              | $y = 419.25 - 4.24x$                    | -0.95                                   | $P \leq 0.01$            |
| Spring rape roots                   | DM                 | $y = 346.48 - 0.64x$                    | -0.95                                   | $P \leq 0.01$            |
|                                     | C : N              | $y = 324.90 - 3.16x$                    | -0.94                                   | $P \leq 0.01$            |
| Winter wheat roots                  | DM                 | $y = 318.60 - 0.29x$                    | -0.90                                   | $P \leq 0.05$            |
|                                     | C : N              | $y = 303.92 - 1.86x$                    | -0.93                                   | $P \leq 0.01$            |
| Red clover roots                    | DM                 | $y = 142.64 - 0.07x$                    | -0.64                                   | $P \leq 0.05$            |
|                                     | C : N              | $y = 148.68 - 0.49x$                    | -0.50                                   | $P \leq 0.05$            |
| Winter rape stubble                 | DM                 | $y = 403.52 - 0.96x$                    | -0.88                                   | $P \leq 0.05$            |
|                                     | C : N              | $y = 349.51 - 3.38x$                    | -0.94                                   | $P \leq 0.01$            |
| Spring rape stubble                 | DM                 | $y = 346.60 - 0.81x$                    | -0.86                                   | $P \leq 0.05$            |
|                                     | C : N              | $y = 312.82 - 3.07x$                    | -0.93                                   | $P \leq 0.01$            |
| Winter wheat stubble                | DM                 | $y = 284.31 - 0.08x$                    | -0.52                                   | $P \leq 0.05$            |
|                                     | C : N              | $y = 308.65 - 1.79x$                    | -0.77                                   | $P \leq 0.01$            |
| Red clover stubble                  | DM                 | $y = 142.64 - 0.07x$                    | -0.64                                   | $P \leq 0.05$            |
|                                     | C : N              | $y = 148.68 - 0.49x$                    | -0.50                                   | $P \leq 0.05$            |
| Winter rape threshing remains       | DM                 | $y = 263.92 - 0.20x$                    | -0.81                                   | $P \leq 0.01$            |
|                                     | C : N              | $y = 325.54 - 4.55x$                    | -0.91                                   | $P \leq 0.05$            |
| Spring rape threshing remains       | DM                 | $y = 227.90 - 0.18x$                    | -0.78                                   | $P \leq 0.01$            |
|                                     | C : N              | $y = 266.93 - 3.03x$                    | -0.81                                   | $P \leq 0.05$            |

The correlation of lignin concentration in winter and spring rape stubble and threshing remains same as in winter wheat stubble was stronger with C:N than that with DM. Medium correlation between these indices was established in the roots and stubble of red clover.

## CONCLUSIONS

Investigation of crop residues decomposition in the soil during the 116-week period showed that stubble and roots of winter and spring oilseed rape decompose more slowly than their threshing remains, or stubble and roots of winter wheat and red clover.

The ratio of carbon and nitrogen in the decomposing crop residues decreases most intensively during the 33–63 week period. After that (during the 63–85 week period) intensive lignin degradation takes place. During this period the amount of lignin decreases in all crop residues, but significant decline takes place after 116 weeks. Lignin in winter oilseed rape residues, especially in roots, is more recalcitrant and therefore the decomposition of crop residues lasts longer.

The concentration of lignin in the decomposing winter and spring oilseed rape, winter wheat and red clover residues was in negative correlation ( $P \leq 0.05$ ) with the amount of dry matter and C:N.

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