Effect of spring oilseed rape crop density on plant root biomass and soil enzymes activity

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Abstract. A field experiment was carried out at the Experimental Station of the Aleksandras Stulginskis University in 2011 with the objective to establish the influence of spring oilseed rape (Brassica napus L.) crop density (50–100, 100–150, 150–200, 200–250, 250–300, 300–350, 350–400, 400–450 plants m$^{-2}$) on plant root biomass and soil enzymes urease and saccharase activity. It was found that the highest plant root biomass in the 0–10 and 10–20 cm soil layers was formed at a plant density of 100–150 plants m$^{-2}$. With rape crop density increasing from 150 to 450 plants m$^{-2}$, a decreasing trend of plant root biomass in both soil layers was detected. Statistically significant dependencies were determined between the dry biomass of oilseed rape roots in the 0–10 cm soil layer and the content of potassium in the soil ($r = 0.76, P < 0.05$), and between the dry biomass of rape roots in the 10–20 cm soil layer and soil pH ($r = 0.74, P < 0.05$). With increasing rape crop density, compared with the thinnest crop, the activity of urease in the soil did not change significantly. At a rape crop density of more than 100 plants m$^{-2}$ the activity of saccharase significantly increased (by 31–56%) in comparison with saccharase activity in the thinnest crop. The soil urease activity depended on the spring rape crop density ($r = 0.81, P < 0.05$) and the content of available phosphorus ($r = 0.75, P < 0.05$). The soil saccharase activity was influenced by the rape crop density ($r = 0.79, P < 0.05$) and soil pH ($r = 0.72, P < 0.05$).

Key words: Brassica napus, crop density, root biomass, soil enzymes activity.

INTRODUCTION

Soil health is the capacity of soil to function as a vital living system within ecosystem and land-use boundaries, to sustain plant and animal productivity and water and air quality, and to promote plant and animal health. To evaluate sustainability of agricultural practices, assessment of soil health using various indicators of soil quality is needed (Doran & Zeiss, 2000). Soil enzyme activity can be used as an indicator of soil quality for assessing the sustainability of agricultural ecosystems (Gianfreda et al., 2005; Roldan et al., 2005). Soil enzymes are important in catalysing innumerable reactions involved in the decomposition of organic matter, cycling of nutrients, and formation of organic matter structure (Bandick & Dick, 1999; Kandeler et al., 1999; Liu et al., 2008; Melero et al., 2008). Enzyme activity is closely related to other important indicators of biological activity: respiration.
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intensity, nitrification ability, total amount of microorganisms, and even more strongly to soil organic carbon content, content of available P$_2$O$_5$ and K$_2$O, soil acidity, and crop yield (Schimner & Sonnleitner, 1996; Bandick & Dick, 1999; Svirskienė, 1999; Liu et al., 2008).

Enzymes that catalyse a wide range of soil biological processes offer a useful assessment of soil ‘function’, and common enzymes, such as urease and saccharase, fit into this category (Burns & Dick, 2005). Urease catalyses the hydrolysis of urea to CO$_2$ and NH$_3$, which is of particular interest because urea is an important nitrogen fertilizer. Urease is released from living and disintegrated microbial cells, and in the soil it can exist as an extracellular enzyme absorbed on clay particles or encapsulated in humic complexes (Nannipieri, 1994; Schimner & Sonnleitner, 1996). Saccharase catalyses the hydrolysis of saccharose into glucose and fructose and characterizes change processes of organic carbon compounds (Schimner & Sonnleitner, 1996).

Several studies show that enzyme activities can be used as early indicators of changes in soil properties originated by soil and crop management practices such as tillage, crop rotation, residue management, and fertilization (Bandick & Dick, 1999; Kandeler et al., 1999; Acosta-Martinez et al., 2003; Roldan et al., 2005; Melero et al., 2008; Zakarauskaitė et al., 2008; Wang et al., 2011). Acosta-Martinez et al. (2003) reported that the trends of the enzyme activities as affected by management depend on the soil, but in general crop rotation and conservation tillage increase enzyme activities. According to Bandick & Dick (1999), enzyme activities are generally higher in continuous grass fields than in cultivated fields. In cultivated systems, enzyme activity is higher where organic residues have been added as compared to treatments without organic amendments. Zakarauskaitė et al. (2005) found higher urease activity in the soil where cereals were grown and higher saccharase and dehydrogenase activity where perennial grasses were grown. Marcinkevičienė et al. (2011) established that with increasing spring rape crop density from 100 to 450 plants m$^{-2}$, compared with the thinnest crop (50–100 plants m$^{-2}$), the activity of urease in the soil does not change significantly, while the activity of saccharase significantly increases (by 31–53%). According to the data of Zakarauskaitė et al. (2008), long-term application of mineral fertilizers inhibits the activity of urease and saccharase.

Roots are the primary tools for the uptake of all mineral elements and water required for crop growth. Due to this fact, root growth and development are highly plastic (Neumann & Martinoia, 2002) and depend on climatic factors (Kjellström & Kirchmann, 1994), soil properties (Barraclough, 1989), crop density (Liakas et al., 2006), and fertilization (Govahi & Saffari, 2006). Roots store not only nutrients and carbohydrate reserves for plants, but they also host microbes (Chung et al., 2007) and add organic matter to the soil (Gale & Cambardella, 2000). Pietola & Alakukku (2005) found that the average total dry biomass of spring oilseed rape root (110 g DM m$^{-3}$) is less than that of barley (160 g DM m$^{-3}$), oats (260 g DM m$^{-3}$), and ryegrass (340 g DM m$^{-3}$). Plants can influence soil enzyme activity by excreting exogenous enzymes and can affect the species composition and diversity of microbes by releasing exudates and oxygen into the
rhizosphere, which indirectly affects enzyme activity (Kong et al., 2009). Niemi et al. (2005) found a positive correlation between root biomass and enzyme activity. Reboreda & Cacador (2008) also reported a positive correlation between root biomass and rhizosphere sediment enzyme activity of five hydrolases.

However, none of these studies examined relationships between root biomass, soil enzymes activity, and spring oilseed rape crop density. Therefore, the objective of this study was to evaluate the influence of spring oilseed rape crop density on plant root biomass and soil enzymes urease and saccharase activity.

**MATERIALS AND METHODS**

The field experiment was carried out at the Experimental Station of the Aleksandras Stulginskis University (54°53′N, 23°50′E) in 2011. The soil of the experimental area was *Calc(ar)i-Endohypogleyic Luvisol (LVg-n-w-cc)* and its texture was medium clay loam on heavy clay loam (according to the Katchinski method). Soil pH KCl was 6.50, content of total nitrogen 0.34%, available P2O5 426 mg kg⁻¹, and K2O 161 mg kg⁻¹. The agrochemical characteristics of the soil were determined as follows: pH KCl was measured potentiometrically, content of total nitrogen by the Kjeldahl method, content of available phosphorus and available potassium was determined by the ammonium lactate (AL) method.

Different crop densities of spring oilseed rape (*Brassica napus* L. subsp. *oleifera annua* Metzg.) were formed by a precision seed-drill with respect to rape ‘Sponsor’ seed germination rate and 1000 seed weight. The treatments of the experiment were as follows: (1) 2 kg ha⁻¹ (50–100 plants m⁻²), (2) 4 kg ha⁻¹ (100–150 plants m⁻²), (3) 6 kg ha⁻¹ (150–200 plants m⁻²), (4) 8 kg ha⁻¹ (200–250 plants m⁻²), (5) 10 kg ha⁻¹ (250–300 plants m⁻²), (6) 12 kg ha⁻¹ (300–350 plants m⁻²), (7) 14 kg ha⁻¹ (350–400 plants m⁻²), and (8) 16 kg ha⁻¹ (400–450 plants m⁻²).

The area of the sampling plots was 27.0 m². Three replications were made. Soil tillage: in autumn conventional ploughing at a depth of 23–25 cm, in spring cultivation (twice) and harrowing. Rape was sown after bare fallow. Rape was sprayed twice against pests with lambdachialotrin (0.0075 kg ha⁻¹). Fertilization: before rape sowing 64 kg ha⁻¹ N, 64 kg ha⁻¹ P2O5, 94 kg ha⁻¹ K2O (400 kg ha⁻¹ azophoska and 50 kg ha⁻¹ potassium chloride) and at the budding stage 70 kg ha⁻¹ N (200 kg ha⁻¹ ammonium nitrate).

Crop density of spring rape was determined in four points of each field by counting plants within a 0.25 m² frame.

Roots were studied in each plot at 0–10 and 10–20 cm soil depths using the small monolith (0.001 m³) method at spring rape flowering stage (Lapinskienė, 1993). In order to determine the dry biomass of rape root, roots from each monolith were dried in a thermostat at 105°C for 24 h and weighed. The dry biomass of rape root was expressed in g m⁻².

To estimate the activity of soil enzymes, soil samples were taken in 2011 from the soil layer of 0–25 cm at the spring rape flowering stage. The activity of soil urease (EC 3.5.1.5) was determined in dry samples according to the Hofmann &
Schmidt (1953) method and that of saccharase (invertase) (EC 3.2.1.26) according to the Hofmann & Seegerer (1950) method (both methods cited in and modified by Chunderova, 1973).

The means were compared using the $F$ criterion and least significant differences (LSD) test at $P_{(level)} < 0.05$ with ANOVA. Correlations were established using STATENG (software package SELEKCIJA) (Tarakanovas & Raudonius, 2003).

**RESULTS AND DISCUSSION**

The highest dry biomass of spring rape root was accumulated in the 0–10 cm soil layer (Fig. 1). The root biomass in the 10–20 cm soil layer (Fig. 2) was found to be 8.2 to 13.2 times lower than that in the 0–10 cm soil layer. Barraclough (1989) and Pietola & Alakukku (2005) reported that most rape root growth occurs in the top 20 cm of soil: from 66% to 80% of total root length is there.

It was established that the highest root biomass in the 0–10 cm soil layer was formed at a crop density of 100–150 plants m$^{-2}$ (Fig. 1). With the increasing spring rape crop density from 150 to 300 plants m$^{-2}$ the root biomass decreased; however, no significant differences were established. At a crop density of 300–350 plants m$^{-2}$ the decrease of root biomass was significant (38.3%) compared with the crop density of 100–150 plants m$^{-2}$. A negative statistically significant relationship was established between spring rape crop density and dry biomass of roots in the 0–10 cm soil layer ($y = 353.21 – 0.26x, r = –0.73, P < 0.05$). Liakas et al. (2006) also reported that with the increasing rape crop density the root biomass decreases.

The highest root biomass in the 10–20 cm soil layer was formed at a crop density of 100–150 plants m$^{-2}$ (Fig. 2). With the increasing spring rape crop density from 150 to 350 plants m$^{-2}$ the root biomass decreased; however, no

**Fig. 1.** The effect of crop density on dry biomass of spring oilseed rape roots in the 0–10 cm soil layer at the flowering stage, 2011. Means not having a common letter (a, b) are significantly different ($P < 0.05$).
significant differences were established. At crop densities of 50–100 and 350–400 plants m\(^{-2}\) the decrease of root biomass was significant (by 37–40\%) compared with the crop density of 100–150 plants m\(^{-2}\). Due to deficit of mineral nutrients in the thickest spring rape crop (400–450 plants m\(^{-2}\)) roots of plants penetrate deeper into soil in search for nutrients.

Mineral nutrients supply greatly affects both the size and morphology of root systems (Durieux et al., 1994). Among nutrients nitrogen (Durieux et al., 1994) and phosphorus (Kuang et al., 2005) have great influence on root growth, while the effect of potassium is not well documented (Robinson & Vuuren, 1998). Correlation and regression analyses of our data revealed statistically significant dependencies between the dry biomass of oilseed rape roots in the 0–10 cm soil layer and the content of potassium in the soil \((y = -938.85 + 13.93x – 0.04x^2, r = 0.76, P < 0.05)\) and between the dry biomass of rape roots in the 10–20 cm soil layer and the soil pH \((y = -222.24 + 38.73x, r = 0.74, P < 0.05)\).

With increasing spring rape crop density, compared with the thinnest crop, a trend of increased activity of urease was observed (Fig. 3), but the differences were not significant. This can be explained by the fact that during the intensive growing period rape uses a lot of mineral nutrients, especially nitrogen. A positive and statistically significant relationship was established between spring rape crop density and soil urease activity \((y = 0.42 + 0.001x, r = 0.81, P < 0.05)\).

Significantly lower activity of soil saccharase (by 24–36\%) was observed in the case of the thinnest spring rape crop density (50–100 plants m\(^{-2}\)) compared with the crop density of 100–450 plants m\(^{-2}\) (Fig. 4). The highest activity of saccharase was found at a crop density of 100–150 plants m\(^{-2}\), where the dry biomass of rape root was the highest. With the increasing spring rape crop density from 150 to 450 plants m\(^{-2}\) saccharase activity showed a decreasing trend. According to the data of Švirskienë et al. (1997), soil saccharase activity depends on the plant root and stubble biomass and the content of humus in the soil. A parabolic statistically
significant relationship was established between spring rape crop density and soil saccharase activity ($y = 18.07 + 0.12x - 0.01x^2$, $r = 0.79$, $P < 0.05$).

Soil enzyme activities are usually correlated either with their organic carbon and/or total nitrogen contents (Gianfreda et al., 2005; Kheyrodin & Antoun, 2008; Melero et al., 2008). According to Liu et al. (2008), soil saccharase activity is positively correlated with total nitrogen and available phosphorus, and urease activity is negatively correlated with the soil pH. Zakarauskaitė et al. (2005) obtained a strong relationship between urease and saccharase activity and total nitrogen and between available potassium and saccharase activity. In the present study correlative–regressive analysis of the data revealed statistically significant dependencies between soil urease activity and the content of available phosphorus ($y = 0.38 + 0.001x$, $r = 0.75$, $P < 0.05$) and between soil saccharase activity and the soil pH ($y = -152.94 + 28.10x$, $r = 0.72$, $P < 0.05$).

Fig. 3. The effect of crop density on soil urease activity at the spring oilseed rape flowering stage, 2011 ($P > 0.05$).

Fig. 4. The effect of crop density on soil saccharase activity at the spring oilseed rape flowering stage, 2011. Means not having a common letter (a, b, c) are significantly different ($P < 0.05$).
According to the data of Kong et al. (2009), root activity shows a stronger correlation with enzyme activity, while root biomass shows a weaker correlation. These results suggest that root activity and root size (root surface area) are more important than root biomass in affecting enzyme activity. Also the results of our investigation did not show any significant effect of rape root biomass on urease and saccharase activity.

CONCLUSIONS

The present study showed that the highest plant root biomass in the 0–10 and 10–20 cm soil layers was formed at a plant density of 100–150 plants m⁻². With increasing rape crop density from 150 to 450 plants m⁻², a decreasing trend of plant root biomass was observed in both soil layers. Statistically significant dependencies existed between the dry biomass of oilseed rape roots in the 0–10 cm soil layer and the content of potassium in the soil \( (r = 0.76, P < 0.05) \) and between the dry biomass of rape roots in the 10–20 cm soil layer and the soil pH \( (r = 0.74, P < 0.05) \). With increasing rape crop density, compared with the thinnest crop, the activity of urease in the soil did not change significantly. At a rape crop density of over 100 plants m⁻² the activity of saccharase significantly increased (by 31–56\%) in comparison with the saccharase activity in the thinnest crop. The soil urease activity depended on the spring rape crop density \( (r = 0.81, P < 0.05) \) and the content of available phosphorus \( (r = 0.75, P < 0.05) \). The soil saccharase activity was influenced by the rape crop density \( (r = 0.79, P < 0.05) \) and the soil pH \( (r = 0.72, P < 0.05) \). The study revealed correlations between several indicators: soil enzymes activity and oilseed rape crop density, dry biomass of rape root and soil agrochemical properties, and soil enzymes activity and soil agrochemical properties.

REFERENCES


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Suvirapsi taimiku tiheduse mõju taimejuurte biomassile ja mulla ensüümide aktiivsusele

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Põldkatsed viidi läbi 2011. aastal Aleksandras Stulginskise Ülikooli katsejaamas. Katse eesmärgiks oli uurida suvirapsi (Brassica napus L.) taimiku tiheduse (50–100, 100–150, 150–200, 200–250, 250–300, 300–350, 350–400, 400–450 taim/m²) mõju taimejuurte biomassile ja mulla ensüümide ureaasi ning sähharaaasi aktiivsu- sele. Selgus, et suurim taimejuurte öitsemise aegne biomass 0–10 ja 10–20 cm mullakihis formeerub kultuuri tiheduse 100–150 taim/m² korral. Taimiku tiheduse suurenemisega 150–lt 450 taimeni ruutmeetri kohta kaasneb taimejuurte biomassi vähenemine mõlemas eelnimetatud mullakihis. Statistiliselt usaldusväärseid seosed esinesid suvirapsi 0–10 cm mullakihis juurte kuiva biomassi ja mulla kaaliumsisalduse vahel (r = 0,76, P < 0,05) ning 10–20 cm mullakihis juurte kuiva biomassi ja mulla pH vahel (r = 0,74, P < 0,05). Seoses suvirapsi taimiku tiheduse suurenemisega alates kõige hõredamast (50 taim/m²) kuni kõige tihedamani (450 taim/m²) ureaasi aktiivsuses statistiliselt olulisid muutusi ei täheldatud. Suvirapsi taimiku tihedusel üle 100 taimüri ruutmeetri kohta suureneb oluliselt (31–56% ulatuses) sähharaaas aktiivsus nullas. Mulla ureaasi aktiivsus sõltub taimedele omastatavast fosforisisaldusest (r = 0,75, P < 0,05). Mulla sähharaaasi aktiivsus mõjutavad enam suvirapsi taimiku tihedus (r = 0,79, P < 0,05) ja mulla pH (r = 0,72, P < 0,05).