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Soybean root traits after 24 years of different soil tillage and mineral phosphorus fertilization management

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Legume crops are widely used in conservation agricultural systems, which are associated with minimum soil tillage, due to their nitrogen-fixing capabilities. However, tillage and fertilization regimes may affect the vertical distribution of legume roots and root traits, hence nutrient and water uptake by altering soil properties in the long term. This study aimed to investigate how tillage and P fertilization affect soybean (*Glycine max*, L.) root distribution and morphology in a long-term experiment.

A 24-year rain-fed corn-soybean rotation was established in 1992 on a clay loam soil in L'Acadie, Quebec, Canada. The split-plot design (four replicates) comprised tillage systems [moldboard plough (MP) and no-till (NT)] as main plot factors and P fertilization [0 (0 P), 17.5 (0.5 P) and 35 (1 P) kg P ha^{-1} every two years during the corn (*Zea mays* L.) phase] as sub-plot factors. Soybean roots and shoots were sampled in 2015, after 24 years, at flowering stage. Root samples were taken by collecting 5.25-cm diameter cores to a depth of 60 cm at 5 cm, 15 cm and 25 cm perpendicular to crop row. Soil cores were cut into 0–5, 5–10, 10–20, 20–30, 30–40 and 40–60 cm layers. After washing and separating the soil and roots, root traits (biomass, length, surface and diameter, and the proportions of primary, secondary and tertiary roots) were quantified using the WinRHIZO software.

Tillage and P fertilization regimes showed no significant effect on soybean root traits. Roots under NT had a relatively higher root length density (RLD) of 1.95 cm cm^{-3} for a 60-cm soil profile compared to roots under MP (1.55 cm cm^{-3}); RLD was relatively smaller at the highest P rate (1.57 cm cm^{-3}) compared to the control and half rate treatment (1.82 and 1.86 cm cm^{-3}, respectively). However, the interaction between tillage and P fertilization significantly influenced the vertical distribution of soybean roots. Roots under NT primarily accumulated at 0–10 cm, containing 44% of the total root length (24% under MP); by contrast, 36% of root length under MP and 21% under NT accumulated at 10–20 cm. However, the difference in vertical root distribution between NT and MP was mitigated as P fertilization increased.

Soybean roots under NT showed higher RLD and greater root accumulation in the upper layers than MP possibly in response to nutrient availability and stratifications with higher nutrient contents in the top layers (0–10 cm) after 24 years of continuous NT practice.

Abbreviations: MP, Moldboard plough; NT, No-till; 0 P, 05 P and 1 P, P fertilization rates of 0 17.5 and 35 kg P ha^{-1} applied every two years on corn phase; RMD, Root mass density (cm cm^{-3}); RSD, Root surface density (cm^2 cm^{-3}); RLD, Root length density (cm cm^{-3}); RND, Root nodule density per root length (cm^{-1}); 3rdRLD, 2ndRLD 1stRLD root length densities of tertiary secondary and primary roots (cm cm^{-3}); 3rdRL%, 2ndRL% 1stRL% root length proportions of tertiary secondary and primary roots; RL*ilr1*, Isometric log ratio transformation of root length proportions (tertiary roots *vs.* secondary and primary roots); RL*ilr2*, Isometric log ratio transformation of root length proportions (secondary roots *vs.* primary roots); V*ilr1*, Isometric log ratio transformation of vertical root length proportions (root lengths at 0–20 cm *vs.* roots at 20–60 cm); V*ilr2*, Isometric log ratio transformation of vertical root length proportions (roots at 0–10 cm *vs.* roots at 10–20 cm); V*ilr3*, Isometric log ratio transformation of vertical root length proportions (roots at 0–5 cm *vs.* roots at 5–10 cm); V*ilr4*, Isometric log ratio transformation of vertical root length proportions (roots at 20–40 cm *vs.* roots at 40–60 cm); V*ilr5*, Isometric log ratio transformation of vertical root length proportions (roots at 20–30 cm *vs.* roots at 30–40 cm).

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1. Introduction

Conservation agriculture, as defined by Kassam et al. (2012), has been widely adopted to reduce soil erosion, decrease input costs, and sustain long-term crop productivity (Pittelkow et al., 2015; Soane et al., 2012). Legumes are typically implemented in conservation agricultural systems as row crops (Vanhie et al., 2015) or cover crops (Sainju et al., 2005) due to their roles in nitrogen fixation and weed control. Long-term conservation systems, especially no-till (NT) without soil inversion, result in the alteration of soil properties (Madejón et al., 2007) and the heterogonous distribution of relatively immobile nutrients (e.g., phosphorus (P)) compared to conventional tillage systems (Messiga et al., 2011). Consequently, root growth, crop yields and plant nutrition can be affected in legume crops (e.g., soybean) under NT.

Greater soil bulk density and resistance usually observed under NT (Gantzer and Blake, 1978; Guan et al., 2014; Javeed et al., 2013; Soane et al., 2012); such soil properties can hinder root penetration and reduce root length density (Nunes et al., 2015; Qin et al., 2005). Lal et al. (1989) reported a 45% reduction in soybean root length density (RLD) under NT compared with plow-till in a calcareous soil; this was a result of the greater soil bulk density and penetration resistance caused by wheel traffic. It was also reported that soil compaction could affect soybean root branching and diameter (Cesar Ramos et al., 2010; Colombi and Walter, 2016; de Assis Valadao et al., 2015). In contrast, Micucci and Taboada (2006) did not observe any differences in soybean RLD between conventional and NT systems. Therefore, there is a critical soil bulk density or resistance for successful soybean root growth (Keisuke Sato et al., 2015). No-till usually provides a stable soil environment for micro-organisms (Helgason et al., 2010; Scopel et al., 2013) that is favorable for nodulation and biological nitrogen fixation by soybean roots (Muchabi et al., 2014). Modified soil temperature, water availability and crop residues under NT could also influence soybean root growth and nutrient uptake (Farmaha et al., 2012; Vanhie et al., 2015).

The morphological traits of soybean roots are also related to soil P status. Fernandez and Rubio (2015) reported higher specific root length and smaller average root diameter in soybean where P-uptake efficiency increased under P deficit. Higher P concentrations in the topsoil under NT results in P stratification, with a higher P concentration in the topsoil (Calegari et al., 2013; Costa et al., 2010; Lupwayi et al., 2006; Messiga et al., 2010). Such stratification could stimulate the growth of soybean roots (Holanda et al., 1998). It was also reported that soybean roots respond to local P fertility, but only under low soil P test levels (Farmaha et al., 2012).

The effects of tillage and P fertilization on soybean root growth are generally studied separately. In this study, however, we evaluated the combined effects of tillage [moldboard plough (MP) vs. no-till (NT)] and mineral P fertilization (0, 17.5 and 35 kg P ha^{-1} applied every two years as triple-superphosphate) on soybean root distribution and morphology at a long-term (24 years) corn-soybean rotation field experimental site in Eastern Canada. We hypothesized that soybean root distribution and morphology would respond to P fertilization and the subsequent effects of tillage practices on the vertical distribution of nutrients.

2. Materials and methods

2.1. Site description

The site was established in 1992 at the L'Acadie Experimental Farm (45°18′ N; 73°21′ W) of Agriculture and Agri-Food Canada. Details on this rain-fed field experiment are reported in Ziadi et al. (2014). Briefly, the soil is a deep clay loam soil (364 g kg^{-1} of clay and 204 g kg^{-1} of sand in the Ap horizon) and classified as Humic Orthic Gleysol, Typic Haplaquept. From 1992–1994, the site was planted with corn (*Zea mays* L.). The corn and soybean (*Glycine max* L.) rotation were initiated in 1995. The experimental set-up was a split-split-plot replicated four times with two tillage practices [Moldboard Plough (MP) and No-Till (NT)] randomized into main plots and nine combinations of nitrogen (N) and P levels randomized into subplots including three N (0, 80, 160 kg N ha^{-1}) and three P (0, 17.5, and 35 kg P ha^{-1}) regimes, which were applied every two years during the corn phase. The three P rates were referred to as 0P, 0.5 P and 1 P, which corresponded to approximately 0, 0.5 and 1 time(s) the P exported every two years by harvest, respectively. For the purpose of this study, we only considered the optimal N level of 160 kg N ha^{-1}, the two tillage practices (MP and NT) and the three P fertilization rates. We therefore considered a total of 24 field-plots measuring 25-m in length by 4.5-m in width. The moldboard plough operation to a depth of 20 cm occurred in the fall after crops were harvested. Each spring, the soil was tilled by disking and harrowing to 10 cm before seeding. For the NT treatment, plots were ridge-tilled from 1992 to 1997 and then flat direct-seeded starting in 1998. For all treatments, crop residues were left on the soil surface after harvest. At planting, fertilizers were banded-applied (5 cm from the seeding row with a commercial seeder). Whereas, side-dress N was applied using a disk opener (3–4 cm deep; CRAAQ, 2003) at approximately the 8-leaf stage of corn growth. The P fertilizers were applied as granules of commercial triple-superphosphate ($Ca(H_2PO_4)_2$, H_2O) during a single operation at seeding. Nitrogen at a rate of 160 kg ha^{-1} was applied first at seeding, as urea at 48 kg N ha^{-1}; this was followed by the addition of 112 kg N ha^{-1} side-dressed as ammonium nitrate. All plots received 50 kg K_2O ha^{-1}, band-applied at planting in 1992 and 2007; this was based on soil analyses and local recommendations (CRAAQ, 2003). Herbicides were applied based on provincial recommendations. In soybean years, plots were sprayed with a tank mix of bentazon (0.72 kg ai/ha) and imazethapyr (0.074 kg ai/ha) and 2 L/ha ammonium sulfate (28–0–0) (Légère et al., 2008). Soybean (Pioneer 2510RY) was sown with 75-cm inter-row at a plant density of 45×10^4 plants ha^{-1}. Soybean seeds were inoculated with a commercial formulation of *Bradyrhizobium japonicum* (Hi Coat N/TS225, Becker Underwood, Saskatoon, SK, Canada). Due to unfavorable climatic conditions, soybeans were sown on June 26, 2015; this was relatively late compared to previous growing seasons (in which sowing occurred in early June).

2.2. Root sampling and analysis

Root samples were collected on August 19–20, 2015 (54 days after seeding, at approximately the end of the soybean vegetative stage). Five consecutive soybean plants were randomly selected from the 3rd or 4th row of each field-plot to avoid side effects. The root samples were taken using the soil core method (Bohm, 1979) with Giddings soil coring sampler (5.25-cm inner diameter) (Giddings Machine Company, Inc.). In each field-plot, soil cores were sampled perpendicularly from one side of one chosen plant. Cores were taken up to a depth of 60 cm at three horizontal distances (5, 15 and 25 cm) perpendicular to the soybean row. Core samples were sliced as follows: 0–5, 5–10, 10–20, 20–30, 30–40 and 40–60 cm. A total of 432 root samples were collected. Root samples were placed in plastic bags and stored at 4 °C for a few days before soil-root separation was performed. Root samples were first soaked in a 1 M solution of NaCl (1 L per 5-cm soil core) for 16 h to disperse soil aggregates. Samples were then transferred to a hydro-pneumatic elutriation washing machine (Smucker et al., 1982). Cleaned roots were first collected on a 760 μm primary