Soybean root diseases are of concern in Manitoba as there have been increasing numbers of reports of significant losses in plant stand and yield caused by various root diseases. To evaluate the impact of rotation on soybean root rot, the effects of five crop rotations were evaluated near Brandon, MB, from 2014 to 2023. With the initial two years of stubble establishment, the current soybean-based rotation study consists of five crop rotations of soybean (S), canola (C) and wheat (W) with SC, SW, SWC, SCW and SSW, ranging in duration from two to three years in length. During the crop rotation study, soybean roots were collected and assessed for root disease incidence and severity. In the laboratory, root tissue was surface sterilized and processed for *Fusarium* spp. that are currently causing disease in economic crops in Manitoba including soybean. Throughout the study, soybean root rot was associated with all treatments and observed in all plots. The microorganisms most frequently isolated from roots of infected plants belonged to *Fusarium* spp., including *F. oxysporum*, *F. redolens*, *F. avenaceum*, *F. equiseti*, *F. acuminatum*, and *F. graminearum*. Based on the current study, the effect of rotation length and crop sequence on root rot suggests that the tight rotations and stacked soybean rotations show a trend of increased root rot severity in select years.