Planting multiple crops in rotation with maize enables high maize grain production with smaller amounts of nitrogen fertilizer, a phenomenon referred to as the ‘rotation effect’. However, the factors that drive the rotation effect remain unclear, which could limit our ability to optimize the benefits of crop rotation. This research sought to understand if rooting characteristics contributed to the rotation effect by studying a long-term crop rotation experiment in Iowa, where a relatively short 2-year maize-soybean rotation was compared to a complex 4-year maize-soybean-oat/alfalfa-alfalfa rotation. The ‘rotation effect’ resulted in 8% higher maize grain yield in the complex rotation. Root measurements taken over several years showed the complex rotation maize had 11% deeper roots and also had less root mass at a shallow depth compared to the simple rotation. Additionally, soil measurements suggested soil was more compacted in the simple rotation which may have contributed to the differences in root distributions. The results suggest that the investment in deeper roots by the complex rotation maize may increase the crop’s ability to produce higher grain yields under unfavorable growing conditions. This study enhances our understanding of the ‘rotation effect’, and provides new data on yields and roots, both of which are highly relevant in this new era of soil carbon focus.