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Effect of crop rotation on mycorrhizal colonization and wheat yield under different fertilizer treatments

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ABSTRACT

Break crops are used in agriculture to reduce soil pathogens and improve yield of subsequent cereal crops. However, they can also affect soil microbial communities beneficial to plant growth including arbuscular mycorrhizal fungi (AMF). Two wheat genotypes (IAW2013 and 249) were planted after crop rotation with canola or chickpea with different nitrogen (N) and phosphorus (P) fertilizer treatments (0 and 100 kg N ha^{-1} and 0 or 20 kg P ha^{-1}) in the field. Plant and soil available N and P, AMF root colonization, shoot biomass, wheat yield and leaf $\delta^{13}C$ were examined. While crop rotation did not affect soil available N and P, AMF colonization in wheat was on average 60% higher after chickpea than after the canola rotation. Wheat yield after chickpea increased for genotype IAW2013, and was positively related to AMF colonization for both genotypes. N and P fertilization reduced AMF colonization and yield, but increased shoot biomass and leaf tissue N and P concentrations. Leaf $\delta^{13}C$ decreased with increased yield, suggesting that higher yielding and AMF colonized plants were less water stressed. In contrast to fertilization, cultivation of certain crops in the previous season, in our case chickpea, can promote AMF colonization of wheat roots, thereby increasing grain yield.

1. Introduction

Crop rotation is a soil management strategy designed to manage nutrient requirements, maintain healthy soil and minimize pests and disease, with the ultimate goal of sustainable high yields (Angus et al., 2015). Globally, wheat (*Triticum aestivum* L.) is an important food crop, widely grown under diverse climate conditions (FAO, 2015). Break crops, including canola (*Brassica napus* L.) and chickpea (*Cicer arietinum*) are vital components of the wheat industry, and may increase the yield of following wheat crops. Although the effect is dependent upon environmental conditions and host/pathogen interactions, canola may increase the yield through biofumigation of the soil and improve root penetration of the following wheat crop (Angus et al., 1991). Inclusion of legumes such as chickpea in rotation can also increase the yield as a result of a break in the life cycle of soil borne pathogens and fixation of atmospheric dinitrogen gas (N_2), thereby increasing residual soil N (López-Bellido et al., 1998) and reducing nitrogen (N) fertilizer requirements.

Application of N and phosphorous (P) fertilizers is another management practice to increase plant growth and crop yield. Large demand for food production has resulted in dramatic increases in the use of P and in particular N fertilizers (Tilman et al., 2001). However, usually no more than half of the nutrients of the applied chemical fertilizers are directly taken up by plant roots and therefore, large amounts of mobile nutrients such as nitrate (NO_3^-) can be easily leached below the root zone of plants (Cavagnaro et al., 2015) or lost from the soil surface as nitrous oxide (N_2O) and N_2 (Bender et al., 2015). Similarly, immobile nutrients such as P can bind to organic matter or colloids, which can be lost via leaching or runoff (Adesemoye and Kloepper, 2009). Leached nutrients can contaminate water supplies and pollute terrestrial ecosystems, so there is an urgent need for management practices that improve fertilizer use efficiency and reduce nutrient loss.

Soil microorganisms, and in particular arbuscular mycorrhizal fungi (AMF), may play a key role in crop rotation systems by affecting fertilizer use efficiency and therefore decreasing the dependency on fertilizers. AMF can form symbiotic associations with plants where AMF could supply up to 90% of plant P and 20% of plant N through hyphal

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networks in the soil in exchange for photosynthates from the host plant (Smith and Read, 2008). Through increased interception of nutrients by hyphae, AMF may also reduce soil nutrient loss (Cavagnaro et al., 2015). These symbiotic relationships have been shown to be particularly important in natural ecosystems under relatively nutrient poor conditions. For instance, low soil P availability stimulates colonization of roots by AMF, which in return could increase nutrient uptake and hence, reduce nutrient loss (Thompson, 1990; Collins and Foster, 2009). Conversely, agricultural practices including fertilizer application, the length of plant-free fallow periods, tillage and use of non-mycorrhizal crops in rotation have shown negative effects on AMF (Smith and Read, 2008).

Plants from the Brassica family, such as canola, do not host AMF (Vierheilig et al., 2000). Plants of this family release anti-microbial isothiocyanates (ITCs) in the soil as a result of glucosinolate degradation in root residues (Kirkegaard et al., 2000), which may have a negative effect on AMF (Paul Schreiner and Koide, 1993). The inclusion of non-mycorrhizal crops in rotations has been shown to decrease yield of subsequent crops (Arihara and Karasawa, 2000). On the other hand, mycorrhizal crops grown in rotation are more likely to benefit productivity and yield of the subsequent crop (Angus et al., 2015).

AMF associated with plant roots also influence plant resistance to drought through improved water relations (Rapparini and Penuelas, 2014). Root colonization by AMF can enhance leaf water and turgor potentials, increase stomatal conductance during water stress and hence increased transpiration rates (Augé et al., 2015), possibly because mycorrhizal hyphae can explore a greater volume of soil compared to roots, thereby enhancing water uptake (Augé, 2001; Allen et al., 2003). Plant associations with AMF could therefore play an important role in plant water uptake, particularly in rainfed agroecosystems. In wheat plants, the carbon isotope composition ($\delta^{13}C$) in plant tissue has been used to estimate water use efficiency (WUE), or the biomass produced per unit water transpired (Farquhar and Richards, 1984). Although, the relationship between wheat leaf $\delta^{13}C$ and WUE is often positive (Farquhar and Richards, 1984), the relationship between leaf $\delta^{13}C$ and grain yield may be either negative or positive, depending on environmental conditions (Araus et al., 2003; Condon et al., 2004). Currently, many studies about AMF functions have been done in laboratory experiments, in which plants were inoculated with different fungal species or isolates in sterilized soil (Klironomos, 2000; Munkvold et al., 2004). However, the impact of AMF under field conditions in agricultural systems has been little explored.

The aim of this study was to assess how growth of preceding crops (canola or chickpea) would affect AMF root colonization, soil available nutrients and grain yield of two wheat genotypes (IAW2013, 249) with different N and P fertilizer treatments (0 and 100 kg N ha^{-1} and 0 or 20 kg P ha^{-1}) in the field. We also assessed how AMF colonization relates to plant $\delta^{13}C$ (WUE). We hypothesized that crop rotation with chickpea would increase AMF colonization in wheat roots compared to the rotation with canola, and thus increase grain yield, particularly without N and P fertilization. To disentangle crop rotation and soil nutrient effects on AMF and wheat grain yield, we used a structural equation modelling (SEM) approach. We expected that variation in wheat root colonization by AMF caused by crop rotation and soil nutrients would be positively related to wheat grain yield.

2. Materials and methods

2.1. Experimental design

A field experiment was conducted in 2015 at I.A.Watson Grains Research Centre, Narrabri, NSW, Australia (149°48'15"E/30°16'23" S). The experiment was part of a bigger trial started in 2013 that covered an area of approximately 4.5 ha. The diagram of the trial is shown in the Supplementary material (Fig. S1, S2). The soil was a medium-clay grey Vertosol (Isbell, 2002) with a pH of 7.5, and total C and N contents of 10 g kg^{-1} and 0.6 g kg^{-1}, respectively. During the growing season (June-November 2015) 211 mm of precipitation were recorded, compared to an average of 244 mm recorded in the previous 5 years. In 2015 mean maximum temperature was 26.7 °C, while mean minimum temperature was 12.3 °C.

In 2013 the study area was sown with wheat followed in 2014 by canola (Pioneer hybrid 44Y84) or chickpea (HatTrick, an Ascochyta and Phytophthora resistant variety), thus creating pure and homogeneous monocultures. During the rotation in 2014, the study area was subdivided into four rotation areas (48 × 146 m, excluding buffers) and canola and chickpea were randomly allocated two rotation areas each. Each of the four rotation areas was then subdivided in two tillage sub-areas (24 × 146 m, excluding buffers), but for this study samples were collected from no-tilled sub-areas only. Each tillage sub-area was subdivided in 18 fertilization blocks (18 × 6 m, Fig. S1).

During the rotation in 2014, following common management practices 10 kg ha^{-1} of P and 5 kg ha^{-1} N were applied at sowing for both canola and chickpea as Granulock Cotton Sustain (5% N, 10% P, 21% K and 1% Zn). No further N was added with chickpea, as seeds were inoculated with Nodule N inoculant (New Edge Microbials Pty Ltd) following manufacturer recommendations to favour N-fixation by *Rhizobium* bacteria. Canola received another 100 kg ha^{-1} N in the form of urea. In 2015, a fertilizer treatment was applied to the wheat crop based on different combinations of N and P randomly assigned to blocks inside each rotation area. For this study, samples were collected from 32 blocks (18 × 6 m, 16 blocks for each rotation), which received a combination of different N and P fertilizer treatments (0 and 100 kg N ha^{-1} as urea and 0 or 20 kg P ha^{-1} as mono ammonium phosphate, 4 replicates for each fertilizer combination within each rotation).

Each of 32 fertilization blocks was sub-divided in 9 plots (6 × 2 m) and 9 different genotypes were randomly assigned to a plot. In this study two wheat genotypes, a synthetic genotype (IAW2013) and a wheat breeding genotype (249) were chosen. Each plot containing 5 plant rows, with 0.3 m spacing between rows (the remaining area of each block was allocated to other wheat genotypes, which were not considered in this study). During the previous study conducted in the same field area, IAW2013 and 249 showed significant differences in root architecture, soil microbial biomass (MB) and inorganic N availability (Corneo et al., 2016). In particular, at the vegetative stage, genotype 249 had higher specific root length (SRL) and was associated with higher MB and less inorganic N in the first 0–10 cm of the soil compared to genotype IAW2013. Water was supplied through sprinkler irrigation to reduce plant stress; 35 mm at 119 days after sowing (DAS) and 30 mm at 130 DAS.

2.2. Plant and soil analyses

Plant and soil samples were collected 104 DAS. At the time of sampling, genotype IAW2013 was at flowering stage, while genotype 249 was at heading stage (stage before flowering). Three rhizosphere soil cores (4.6 cm diameter × 10 cm deep) were collected in each plot using a soil corer, next to the plant stem bases. Two soil cores of each plot were pooled and mixed to obtain a homogeneous soil sample for determination of soil moisture, soil available N and soil available P (the third soil core was used for root sampling, see below). Soil moisture was measured gravimetrically by drying approximately 10 g of fresh soil at 105 °C for 24 h. For N analysis, the soil was completely mixed and a 5 g subsample of moist soil was extracted with 40 ml of 1 M KCl, filtered using Whatman No. 42 filter paper and frozen until analysis for NH_4^+ and NO_3^- on a flow injection analyser (FIA, Lachat Instrument, Loveland, Co, USA). For P analysis, a 0.5 g subsample was extracted with 40 ml of 0.5 M $NaHCO_3$, filtered, and extracts were analysed colorimetrically using the ammonium molybdate-tetrahydrate reagent (Murphy and Riley, 1962) at 890 nm on a UV–vis spectrophotometer (UVmini-1240, Shimadzu Scientific Instruments, Sydney, NSW,