ACCEPTED VERSION

Foyjunnessa, C, Ann McNeill, Ashlea Doolette, Sean Mason, and Mike J. McLaughlin
Use of 33P to trace in situ the fate of canola below-ground phosphorus, including wheat uptake in two contrasting soils

http://hdl.handle.net/2440/114548

Fate of canola below-ground phosphorus and subsequent availability to wheat uptake in two contrasting soils

Foyjunnessa [a,*] • **Ann McNeill** [a] • **Ashlea Doolette** [a] •
Sean Mason [a] • **Mike J. McLaughlin** [a,b]

[a] School of Agriculture, Food and Wine, University of Adelaide and The Waite Research Institute, SA 5064, Australia

[b] CSIRO Land and Water, PMB 2, Glen Osmond, SA 5064, Australia

[*]Corresponding author email: foyjunnessa@adelaide.edu.au; phone: +61 8 83138114; fax: +61 8 83136717; address: University of Adelaide, Waite Campus, Glen Osmond, SA 5064, Australia

Abstract

Background and Aims Current understanding regarding the contribution of crop root residues to phosphorus (P) cycling is mainly derived from studies using excavated roots re-introduced to soil whereas this study aims to quantify total below-ground (BG) P of mature canola *in situ* and directly estimate the proportion of this accessed by subsequent wheat.

Methods ^{33}P-labelled phosphoric acid was stem wick-fed to canola (*Brassica napus*) grown in sand or loam in pots. Shoots were removed from all plants at maturity. Half of the pots were destructively sampled. After a 3 week fallow wheat was grown for five weeks in the remaining undisturbed pots.

Results At canola maturity a large proportion of the fed ^{33}P, greater in the loam than the sand, was partitioned in recovered roots (23-36%) and soil (34-40%). Within the soil 6-10% of the fed ^{33}P was present in resin P and 3-5% was in hexanol-released P pools. Ratios for shoot P: BG P (8:1, sand and 15:1, loam) were much narrower than those for shoot P: recovered root P (17:1, sand and 39:1, loam). A greater proportion and amount of the mature canola BG^{33}P was recovered by wheat grown in the loam (26%; 2.6 mg/plant) than in the sand (21%; 1.5 mg/plant). The majority of canola BG^{33}P remained in the bulk soil.

Conclusion P input below-ground by mature canola and subsequent P benefit to wheat was greater in a loam than sand. P from canola BG residues contributed 20% of the P uptake in wheat during the first 5 weeks of growth. Longer term P benefits from BG residues require investigation.

Keywords: Below-ground P input, hexanol-released P, shoot P: total below-ground P, P benefits

Introduction

Canola (*Brassica napus* L) is a major oilseed crop grown across the world with large areas of production in Canada, China, France, Germany, Poland, India, United Kingdom, Australia and the United States (Rahman and McClean 2013). Currently canola is Australia's third largest broadacre crop after wheat and barley (ABS 2008), occupying 6% of the cropped area, and is considered one of the most profitable cropping options for southern Australian grain growers (Norton *et al.* 2013). Canola is an important crop in rotations (Angus *et al.* 1991; Kirkegaard *et al.* 2008a), particularly since it provides a disease break for cereals (Norton *et al.* 2013). It is also considered a phosphorus (P) efficient crop compared to wheat (Bolland and Brennan 2008) due to having an extensive root system with a high root hair density (Misra *et al.* 1988). Canola is reported to utilise both applied P and soil P effectively (Grant and Bailey 1993) which results in plants with relatively high P concentrations compared to cereals (Norton *et al.* 2013). Hence, canola is especially useful for low input and organic systems (Soon and Arshad 2002), not only because it can readily access P in soil but also because of the potential for return of high P content plant residues. Indeed, Jackson (2000) reported that 30% of P accumulated above-ground by a spring canola crop remained in the residues after harvest. Release of this P as the residues mineralise is a potential benefit to subsequently grown crops such as cereals.

However, information about P mineralisation of plant residues is mostly from incubation studies and relatively limited for canola. Published information in general suggests that the microbial P pool is rapidly influenced in the short term (Chauhan *et al.* 1979; McLaughlin and Alston 1986; White and Ayoub 1983), that rates of P mineralisation are faster for immature canola residues compared to more mature material (Iqbal 2009; Kirkegaard *et al.* 1994; Soon and Arshad 2002); that rates of P mineralisation for canola residues are faster than some other species (Soon and Arshad 2002); that P release is highly correlated to total P content of residues in general (Baggie *et al.* 2005; Kwabiah *et al.* 2003) and specifically for canola (Lupwayi *et al.* 2007), and that a high proportion of the P in stem residues of mature canola is orthophosphate (Noack *et al.* 2012). Furthermore, isotope P studies indicate wide variation in the measured amounts of P taken up from crop residues, ranging from 5-40% of the P input (Blair and Bolland 1978; Nachimuthu *et al.* 2009; Noack *et al.* 2014b). There is evidence that buried residues decompose faster than surface residues (Franzluebbers *et al.* 1996) and therefore in the no-till systems being widely adopted in Australia (Llewellyn *et al.* 2012), where a greater proportion of above-ground residues tend to remain on the soil surface, the contribution from root residues may become more significant. However, information on root mineralisation is scarce and is often for roots that have been recovered from soil, dried and reintroduced to the system (Friesen and Blair 1988; Martin and Cunningham

3

1973), which may not truly represent decomposition of roots *in situ* and will not account for inputs from unrecovered components of the root systems (e.g. fine roots). A recent isotope study showed that the total below-ground phosphorus (BG P) input by roots is much larger than the P measured in recovered roots alone (Foyjunnessa *et al.* 2014). This study examined plants at peak growth (flowering) and postulated that some of the BG P may be re-translocated to the shoot as the plants matured, since the developing grain is a sink for P (Batten and Khan 1987; Batten *et al.* 1986; Smith 1965). Hence, quantification of BG P at maturity is required to compare with previous estimates at peak growth. It is also widely believed that it is harder to recover roots from soils with a greater proportion of clay and so it would be expected that perhaps unrecovered BG P in finer textured soils would be greater than in coarse textured sandy soils, although this needs verification. Furthermore, as plants progress to maturity some leaves and roots senesce and thus any canola root-derived P in soil may move into labile pools which could make this P more susceptible to soil reactions that render it less available for the longer term, thus reducing the benefit of the BG P input from the crop.

This study uses a ^{33}P stem-wick feeding technique (Foyjunnessa *et al.* 2014) to label root systems of canola *in situ* to determine, for two soils of different texture: (a) quantities of root and root-derived below-ground P for canola at maturity, and (b) the proportion of canola below-ground P input accessed during early growth by subsequently grown wheat.

Materials and methods

Soil characteristics

As previously detailed (Foyjunnessa *et al.* 2015) bulk quantities of the top 10 cm of two agricultural soils were collected from South Australia; at Karoonda (36°04′S, 140°05′E) and Roseworthy (34°32′S, 138°45′E). The initial chemical properties of the soils were analysed after air drying and sieving (2 mm). The soil texture for Karoonda was a sand (< 5% clay) with pH_w 6.4 (1:5); and Roseworthy was a loam (about 25% clay) with pH_w 7.6 (1:5). A phosphorus buffering index (Moody 2007) of 4.2 and 61, a total P concentration of 114 and 827 mg/kg and a total C content of 0.33 and 1.78% were measured in the sand and loam respectively. The soils had sufficient P for plant growth as the measured initial resin P was 29 and 137 mg /kg, Colwell P was 32 and 163 mg/kg, and DGT-P was 330 and 466 C_{DGT} µg /L in the sand and loam respectively.

Canola phase of the experiment

A complete nutrient solution without P was prepared. Nitrogen (N) as a combination of $Ca(NO_3)_2.4H_2O$, NH_4NO_3, and $Mg(NO_3)_2.6H_2O$ equivalent to an application rate of 90 kg N/ha, potassium (K) and sulphur (S) as K_2SO_4 at the rate of 50 kg K/ha and 20 kg S/ha, zinc (Zn) as $ZnSO_4.7H_2O$ at the rate of 15 kg/ha, copper (Cu) as $CuCl_2.2H_2O$ at the rate of 10 kg/ha and manganese (Mn) as $MnCl_2.4H_2O$ at the rate of 2 kg/ha. Nutrient solution was added to the dry soil and adjusted with deionised water to 70% of the maximum water holding capacity. Then the soil was packed sequentially (15cm by 10cm by 10cm), into a PVC cylinder that had been capped at one end (referred to as 'pots' from hereon), to a bulk density of 1.2 g/cm^3 (sand) and 1.1 g/cm^3 (loam). Under controlled glasshouse conditions with an average of 12 hours daylight, canola was grown in pots filled with either sand or loam with eight replicates (2 x 8) per soil type. Each pot was sown with four pre-germinated seeds of canola (*Brassica napus* L. cv. Tanami) and then thinned to one plant per pot after the first week. A complete randomised design was followed and the pots were re-randomised every week. During the first two weeks of canola growth the pots were watered daily with deionised water to maintain 70% of the maximum water holding capacity (WHC), after two weeks the pots were watered every second day. Watering was terminated one week prior to harvest to allow the soil to dry to 60% WHC. Glasshouse temperatures during the experiment ranged from a minimum of 16°C at night to a maximum of 28°C maximum during the day. Average relative humidity was 73 %.

^{33}P labelling of canola

Canola was labelled using *carrier free* ^{33}P (99% isotopic purity) as $H_3{}^{33}PO_4$ solution with an activity of 459 kBq /plant at 45 days after sowing (DAS) using a ^{33}P stem-wick feeding technique (Foyjunnessa *et al.* (2014). Briefly, a 5 ml vial was attached to the stem of the plant approximately 3 cm above the surface *via* a drilled hole (1 mm diameter) using a 100% cotton wick. A single dose of ^{33}P radioactive solution (1 ml of $H_3{}^{33}PO_4$ containing 0.0000746 µg P) was placed into the vial using a micro pipette. The cotton wick was passed through two pre-drilled holes (1.4 mm) on the vial cap and was encapsulated by 4 cm long plastic tubing (1.4 mm diameter) to minimize evaporation loss of the ^{33}P solution on the way from the vial to the stem. ^{33}P radioactive solution (1 ml) was then introduced to the vial by a third pre-drilled hole in the cap using a micro pipette. The uptake of ^{33}P solution by the plants took 2 to 3 days and 1 ml deionized water was then added to ensure any ^{33}P adhering to the vial and cotton wick was taken up by the plants. The vial and cotton wick were removed from all plants 10 days after the start of the feed and the radioactive cotton-wicks were stored for analysis. All canola

were then grown to maturity (105 DAS, 60 days after feeding commenced) and half of the replicates (4 per soil type) were destructively sampled.

Fallow & wheat phase of experiment

The above ground dry matter (canola shoots) was removed (1 cm above soil level) from the other 50% of the replicates (4) and the ^{33}P-labelled below ground systems (roots plus root derived (RD) P) left undisturbed in the PVC pots of soil for three weeks (fallow). Subsequently, four pre-germinated seeds of wheat (*Triticum aestivum L.,* cv Frame) were sown into each of these pots. During the wheat growing period, glasshouse temperatures ranged from 16°C to 24°C, relative humidity ranged from 25 to 82% and day length was 11h. Over the duration of the wheat phase (5 weeks) each pot received a single application (50 ml) of nutrient solution at 21 DAS equivalent to a total of K (25), S (20), Ca (5), Mg (5), Fe (0.3), Mn (0.5), Cu (1), Zn (1), N (75) and zero P (mg /pot). Pots were allowed to dry naturally for two weeks during the fallow period and resulted in the WHC decreasing to 30%. The pots were rewet to 60% WHC one week before planting of the wheat and maintained at 60% WHC throughout the remainder of the experiment. All wheat plants were harvested 35 days after sowing at booting, when the boot was just visibly swollen (GS43) (Zadoks *et al.* 1974).

*Sample harvesting, processing and analysis for both phases of the expe*riment

At canola maturity (105 days after sowing and 60 days after feeding), shoots were removed directly above the soil surface (1 cm) in the half of the pots assigned for destructive sampling. To ensure there was no input of above-ground plant material into the soil, any canola leaves, flowers and pods that matured and detached from the plant during the growth period were collected, stored in a paper bag and were added to the relevant above ground shoot dry matter at harvest. Sealed caps were removed from pots which were then placed in a plastic tray (60 cm long). A special piston shaped tool with a marginally smaller diameter than the pots (15 cm) was used to remove each intact root-soil column by pushing from the base of the pots. The intact root-soil column was then split into two depths: 0-10 cm and 10-35 cm using a sharp aluminium cutter. All visible roots and root fragments with adhering rhizosphere soil were recovered by hand from each soil depth separately and stored in plastic bags to freeze dry for further analysis and specific activity measurement (termed "recovered roots"). The remaining soil (termed "bulk" soil) from the two soil depths was then air dried at ambient temperature (glasshouse) for 24 hours and sieved using a 2 mm sieve. All root materials and fine roots >2 mm from each of the two soil depths

6

were considered as recovered roots. After sieving the soils from the two depths were mixed thoroughly and a 100 g subsample of the bulk soil from the entire core was collected for further analysis. All sample fractions (shoot, recovered roots plus rhizosphere soil and 1 x 100 g sub-sample of bulk soil) were frozen (-18°C) and subsequently freeze dried before processing and analysis. An additional 1 x 100 g fresh sub-sample of bulk soil was stored immediately after harvesting in a cool room (4°C) for 24 hours or less until microbial biomass P was determined. Following freeze drying, clean roots (recovered) were obtained by brushing off rhizosphere soil using a soft paint brush. Fresh and dry weight of shoots, dry weight of recovered roots, soil fractions (rhizosphere and bulk soil) were recorded and digested for chemical analysis (e.g. P concentration and ^{33}P activity). Subsequent wheat was harvested and samples processed similarly for chemical analysis.

All freeze dried plant samples (shoots and recovered roots) were finely ground and digested (1.0 g) using 5 ml concentrated HNO_3 (Zarcinas and Cartwright 1983). Bulk soil and rhizosphere soil was digested using 5 ml *aqua regia* (HNO_3:HCL 1:3) using the method of Zarcinas *et al.* (1996). The resulting digest solution was then used to determine total P concentration using inductively-coupled plasma atomic emission spectroscopy (ICP-AES) at 214.97 nm and ^{33}P activity (8 ml scintillate to a 2 ml digested aliquot) using a Rack Beta II Liquid Scintillation Counter. Each cotton-wick used to feed ^{33}P (sixteen) was also digested and analysed similarly for ^{33}P sorption to the wick. Recorded ^{33}P activities in each sample were then back corrected for decay and expressed as kBq. Resin (available) P and microbial P using the hexanol fumigation method proposed by McLaughlin and Alston (1986), were measured for fresh moist bulk soil. Briefly, duplicate soil samples (one with 1ml hexanol and one without hexanol) were extracted using distilled H_2O (soil: water ratio of 1:15) each containing one resin strip (BDH#55164) by simultaneous liquid fumigation and extraction for 16 hours (Kouno *et al.* 1995). The resin strips were then eluted with 0.1 M NaCl/HCl for at least 2 hours and the elution solution was then measured colorimetrically according to the molybdate blue method (Murphy and Riley 1962). Available P is presented as resin P for non-fumigated samples and microbial P (hexanol-released P) is presented as the difference in resin P between fumigated and non-fumigated samples without sorption correction since >90% of a P spike (10 mg P/kg) from non-fumigated samples was recovered in all cases.

Calculations, assumptions and statistical analysis

Root-derived P (RDP) and total below-ground P (BGP$_{total}$) for canola were calculated using a modified approach to that used in ^{15}N studies to estimate total below-ground N for legumes (McNeill 2001).

RDP (equation I) was calculated as:

RDP (mg) = ^{33}P activity in soil (kBq ^{33}P /pot) / SA of clean roots (kBq ^{33}P /mg ^{31}P)…………………………. (I)

where SA is the specific activity of clean roots: ^{33}P in recovered roots (kBq) / dry weight recovered roots (mg).

RDP in the soil of pots where canola had been grown to maturity was considered to represent the sum of P in unrecovered roots plus any P-containing derivatives from roots in exudates, sloughed material or products of root decomposition, assuming all had the same SA.

Total BGP (Equation II) was calculated as the sum of P measured in recovered roots (P_{recrt}) and the estimated amounts of root-derived P in the bulk (RDP_{bulk}) and rhizosphere (RDP_{rh}) soils:

$$BGP_{total} = P_{recrt} + RDP_{bulk} + RDP_{rh} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \text{(II)}$$

An estimate of the dry mass of unrecovered root materials ($DW_{unrecrt}$) represented by the measured ^{33}P present in soil (assuming that all ^{33}P in bulk soil was in unrecovered roots apart from that measured in microbial and resin P pools), was also derived as follows (equation III):

$DW_{unrecrt}$ (g) = ^{33}P activity in soil (kBq)/ (^{33}P activity in recovered roots (kBq)/ Recovered roots wt (g))........ (III)

The distribution of canola BGP to subsequently grown wheat was calculated using equation IV since any ^{33}P activity detected in wheat after canola will have been derived from canola BG^{33}P:

Distribution of canola BG^{33}P to wheat (%) = ^{33}P activity in wheat (kBq /pot)/ Canola BG^{33}P activity in soil at maturity (kBq /pot) X 100...(IV)

Data were tested for normality and homogeneity of variance from each treatment. A one-way Analysis of Variance (ANOVA) was undertaken using the GENSTAT version 15 statistical package (VSN International, Rothamsted, UK). Least significance of variances (l.s.d) between treatments was determined at <5 % significance using Fisher's protected l.s.d.

Results:

Canola phase

Plant dry weight, P concentration and P content of mature canola plants

Shoot dry weight of mature canola (Table 1a) was significantly higher in the loam than the sand. The P concentration (mg /kg) of mature canola shoots and recovered roots was greater in the loam than the sand, but

due to no difference in the recovered root dry weights the P content (mg/plant) of recovered roots did not differ significantly between the soil textures (Table 1a).

^{33}P activity below-ground and specific activity of recovered roots at two soil depths (0-10 cm and 10-35 cm)

Partitioning of the activity detected below-ground was affected by soil texture; more ^{33}P was measured in the recovered roots than the bulk soil for the sand whereas for the loam a greater proportion of ^{33}P was measured in the bulk soil, indicating less root recovery in the loam (Table 2). However, ^{33}P activity of rhizosphere soil was similar for both soil textures and due to the small amounts of rhizosphere soil was much lower than activities for recovered root or bulk soil. No significant difference was found between the SA of recovered roots at the two different depths (0-10 cm and 10-35 cm) for canola grown in the loam even though amounts of ^{31}P and ^{33}P were very different (Table 3). However the SA of canola grown in the sand was greater in the 0-10 cm depth than that in the 10-35 cm depth.

*Estimates of RDP and DW$_{unrecrt}$ for canola at matur*ity

The RDP in the soil of pots containing canola at maturity (Figure 1) was significantly higher in the loam (6.12 mg /plant) than in the sand (3.96 mg /plant) with the amount of RDP in soil being 1.5 fold more than recovered root P for mature canola grown in loam, whereas RDP in soil was similar to recovered root P where canola was grown in sand. Estimates of DW$_{unrecrt}$ followed a similar pattern to RDP with more in loam than sand. Inclusion of these estimates of RDP and DW$_{unrecrt}$ in calculations of the shoot P content: root P content or DM ratios of mature canola results in substantially narrower ratios than if recovered root P or DM are used. Hence shoot P content: recovered root P content ratio is 17:1 in sand whereas shoot P: BG P$_{total}$ ratio is 8:1, and similarly for loam the ratios are 39:1 and 15:1 respectively.

Recovery and distribution of fed ^{33}P in the mature canola-soil system

At maturity mean recovery of fed ^{33}P (459 kBq /plant) in soil and plants was 96% (Figure 2) with sorption by the wick being only 7%. Distribution of ^{33}P in above-ground (shoot) and BG plant components was affected by soil texture. ^{33}P in the shoot was significantly higher in the loam than in the sand, whereas the distribution of ^{33}P in recovered roots was the opposite (Figure 2). Consequently the proportion of fed ^{33}P recovered in the soil (bulk plus rhizosphere) was significantly higher in the loam than in the sand. Furthermore, within the labile P fractions of the bulk soil 6-10% of fed ^{33}P was in the resin P pool and 3-5% was in the microbial (hexanol-

9

released) P pool (Figure 2), with significantly more in these pools in the loam than the sand. The proportion of fed ^{33}P in the bulk soil fraction present as less labile P, assumed to be associated with unrecovered fine roots and products of root turnover, was the same (25%) for both soil textures (Figure 2). In addition, on average 4% of the fed ^{33}P was 'lost' which was attributed to the fact that some of the collected fallen shoot materials (florets and senescent leaves) were not able to be assigned to a particular replicate and so were discarded rather than measured. Overall, the proportion of ^{33}P was greater in mature canola below-ground systems (recovered roots plus soil) than above-ground plant material (shoot) regardless of soil types (Figure 2).

Wheat phase

Plant dry weight, P concentration and P content of wheat after canola

Dry weight of shoot and recovered roots of wheat after canola followed similar trends to those of mature canola grown in sand and loam (Table 1b). Similarly, P concentration of shoot and recovered roots of wheat after canola was also affected by soil texture and was significantly higher in loam than sand (Table 1b). The bigger differences in wheat than for canola shoot and root P concentrations between the two soil textures, meant that recovered root P content (mg/plant) of wheat after canola (Table 1b) was also significantly greater in the loam (4.12 mg /plant) than in the sand (1.50 mg /plant).

Distribution & recovery of canola total BG^{33}P in the subsequent wheat-soil system

Recovery of canola BG ^{33}P in wheat shoot and recovered roots was affected by soil texture ($p<0.001$) and the proportion of canola BG ^{33}P in wheat shoot and recovered roots was significantly higher in the loam than sand (Figure 3). Although this represented a similar proportion (18-20%) of the total wheat P uptake in both soils the amounts of P were significantly different ($p=0.002$), being 1.56 mg P in the sand and 2.61 mg in the loam. However, the majority of canola BG^{33}P however remained in soil after the wheat was harvested (Figure 3) and was a significantly ($p<0.001$) greater proportion of the BG^{33}P in the sand (79%) than the loam (74%).

Available P in the bulk soil at harvest of wheat (Table 5) was affected by soil texture and was higher ($p<0.001$) in the loam than in the sand, probably due to the higher fertility of the loam as evidenced in the large difference in initial resin available P status of these soils (Table 4). ^{33}P detected in the resin P pool in bulk soil following wheat (Table 5) was lower than detected in the same pool after the canola (Table 4). Microbial P (hexanol-released P) was also significantly greater in the loam than the sand after wheat (Table 5), ^{33}P activity however was not detected in the microbial P (hexanol-released P) pool in bulk soil immediately after wheat (Table 5).

Discussion

Below-ground P input by canola at maturity

The first aim of this study was to quantify BG P of canola at maturity and determine any influence of soil texture on the amount of root and root-derived P BG. Irrespective of soil texture, the amount of P allocated BG by mature canola was greater than assessed by the P content of recovered coarse (>2 mm diameter) roots, which is perhaps not surprising given that canola root systems in particular have a high proportion of fine roots (Liu *et al.* 2010) with many fine root hairs (Brewster *et al.* 1976). The total amount of estimated BG P is similar to that recently reported for canola at late flowering stage (Foyjunnessa *et al.* 2015) although, in this present study of canola at maturity, there is less canola BG P accounted for as recovered roots and more as root-derived P, including some ^{33}P detected in resin and microbial P pools in the bulk soil. This latter point suggests that between peak growth and maturity there has been root senescence and subsequent cycling of root-derived P into labile P pools. Soil texture influenced the total quantity of canola P accumulated BG, which was greater in loam than sand, probably as a result of the overall greater plant dry matter and higher P concentration for plants grown in the greater yield potential loam.

Our results highlight that the quantitative contribution of the BG P component of crop plants to soil P pools is likely to be greater than inferred from studies to date where a proportion of the plant's roots have not been assessed as a consequence of the sampling techniques used, since these tend to be dictated by technical, time and labour cost constraints. Even in studies where rigorous attempts have been made to recover as many roots as possible from soil, for example using fine sieving (<0.5 mm mesh) and washing (Liu *et al.* 2010; Mayer *et al.* 2003; Soon and Arshad 2002) it is inevitable that a proportion of the roots will not be recovered. The difficulties of extracting roots from soil mean that reported measures of P accumulated in roots are extremely scarce, especially for canola (Iqbal 2009; Noack *et al.* 2014a; Soon and Arshad 2002). An alternative approach could be to estimate BG P from more readily assessed shoot P contents using the ratios generated in this present study, as has been done for estimating BG N of legumes (Peoples *et al.* 2001; Unkovich *et al.* 2010). Applying the ratios for shoot P content: total BG P content derived in this study to values for canola crop P content at maturity of 8-21 kg P /ha (Jackson 2000) it can be calculated that below-ground inputs of P by that canola crop would be 1.0-1.4 kg P /ha in a loam. Whereas if estimates were based only on recovered roots these values would be 0.47-

11

0.54 kg P /ha which fall within the range 0.35 to 0.84 kg P /ha derived from dry matter and P concentration data reported for recovered roots of field grown canola (Soon and Arshad 2002). Estimated BG P input of canola could be as large as 2.7 kg P /ha if the ratios from this study are applied to the highest value for a canola shoot P content of 40 kg P /ha reported for an irrigated field crop fertilised with a high rate of N (Jackson 2000). Indeed, measured amounts of P for recovered roots (<1 mm mesh) in the 0-0.15m soil depth sampled at maturity under a dryland canola crop, grown at the same site in South Australia where the sand used in this present study was collected, were in the order of 2.75 kg P /ha (Foyjunnessa, unpublished) which, assuming that 25% of the BGP was not recovered given the mesh size (1 mm) used in the field study compared to 52% of the unrecovered BG P (<2 mm) as shown in this study, translates to an estimated total BG P input of around 3.5 kg P /ha. Such quantities of P as BG P inputs may be important for long term sustainability of organic matter and nutrient supply in low fertility semi-arid farming systems.

As discussed in detail by previous studies (Foyjunnessa *et al.* 2014; Foyjunnessa *et al.* 2015) a high proportion of ^{33}P isotope fed to the plants in this study was allocated BG which may reflect the strong sink that growing roots present for P earlier in crop growth (Römer and Schilling 1986). The proportion of isotope detected BG at maturity was a similar proportion to that measured at peak growth of canola (Foyjunnessa *et al.* 2015), suggesting there was no re-allocation of P from roots to shoots of canola during plant maturation and senescence. This was somewhat unexpected given that maturing grain is considered a sink for P and that the P harvest index for canola is suggested to be 70-80% (Jackson 2000; Rose *et al.* 2007; Rose *et al.* 2008), although the partitioning of P between grain, stems and roots at maturity will also depend on environment (Damon *et al.* 2014) and the plants in this study were glasshouse grown. Furthermore, it is possible that grain demand for P was met by re-allocation of P from senescing leaves (Römer and Schilling 1986), since the P concentration of the shoots in this study was above that considered adequate for canola (Reuter and Robinson 1997).

Fate of mature canola BG P

The second aim of this study was to use the recently reported method for ^{33}P-labelling plant root systems 'in situ' (Foyjunnessa *et al.* 2014) to directly trace the fate of undisturbed BG P of mature canola plants in a subsequent canola-wheat rotation. The approach aimed to account for P derived from the intact entire plant root system and differed from previous studies of P release and mineralisation that have utilised roots extracted and re-introduced to the soil environment (Dalal 1979; Friesen and Blair 1988; Fuller *et al.* 1956; Lupwayi *et al.*

2007; Nachimuthu *et al.* 2009). Furthermore, P release from the canola BG residues was assessed in the presence of young wheat plants whereas other studies of P release from canola root residues have been undertaken without plants (Soon and Arshad 2002), even though biochemical and biological processes associated with P cycling in soils may be significantly altered by the presence of plant roots (Blair and Bolland 1978; Helal and Sauerbeck 1984; Richardson *et al.* 2009). The results from the study indicate that during a two month period following removal of the shoots of mature canola, up to one third of the canola residue BG P was readily accessible with 21-26% taken up by 5-week old wheat plants and 5-7% detected in the resin-extractable P pool. Soil texture influenced BG P decomposition with net release of P being a greater proportion of the canola BG P in the higher fertility loam than in the sand, and even though wheat dry matter was larger for the loam, the proportion of P in the wheat derived from the canola BG P was also greater. This greater P benefit from BG P in a loam soil is likely to be due to a combination of factors including greater BG P accumulation from canola, higher root P concentrations with the likelihood of greater surplus orthophosphate than roots in the sand, and more favourable edaphic conditions both for breakdown of this BG P and also for wheat roots to access any available P derived from decomposition of canola BG P. Furthermore, a loam can generally be considered to support more robust and vigorous plants than sand, which therefore implies a greater plant sink for P also, and indeed the wheat in the loam had a higher shoot P concentration than in the sand.

Phosphorus availability from decomposing residues has been described as occurring in three phases (Kwabiah *et al.* 2003) consisting of an initial rapid P release from sparingly soluble inorganic plant materials, a subsequent phase when P in solution comes from both soluble P and mineralisation of plant materials, and a final phase where P in solution is influenced by its equilibrium with P sorption processes. It is highly likely that much of the ^{33}P released from the decomposing roots in this present study was directly as soluble P since from 35-50 % of the P in the mature canola roots may have been soluble (Noack *et al.* 2014a). Rapid and substantial P release from the excised roots of wheat plants was demonstrated many years ago in a laboratory tracer study (Martin and Cunningham 1973) and, as it occurred from sterile roots, was attributed to autolytic degradation of organic phosphorus in the roots. Rapid P loss from clover root residues encased in mesh bags in the field was observed, especially where residues were buried rather than left on the surface (Buchanan and King 1993), and since initial losses of P were not correlated to C losses, it was concluded that they were from readily leached inorganic P in the residues. Indeed, early work measured high proportions (40-60%) of soluble inorganic P in whole plant residues of clover and phalaris (Jones and Bromfield 1969) and more recently this has been highlighted for other

agricultural species (Noack *et al.* 2012). During the 3 week fallow period it is possible that a lot of the soluble P may have diffused out of the decomposing roots into adjacent soil sorption sites, and the subsequent wheat roots are quite likely to have utilised these old root channels, which would increase the chance of access to that P derived from canola BG residues. There will have been competition from the microbial biomass for residue-released P (McLaughlin and Alston 1986), and hence some ^{33}P was detected in the microbial biomass. The ^{33}P in the resin extractable pool will also have been derived from the mature canola root residues via mineralisation of organic P, probably initially from more soluble organic forms (Friesen and Blair 1988). Although ^{33}P was present in the microbial biomass measured at canola maturity in this study, indicating that some BG P from roots had already been released and utilised as the plants senesced, there was no measurable ^{33}P in the microbial pool after 8 weeks of root decomposition even though the pool was the same size as at the start of the study. This suggests there has been microbial turnover and capture of that microbially-cycled ^{33}P by the wheat as the root systems of the seedlings increased in size and became more competitive. However, we would have expected additional ^{33}P to be detected in the microbial biomass derived from mineralisation of the remaining canola BG input. Indeed, a longer term study of the decomposition of ^{32}P labelled mature pea shoot residues and uptake by wheat detected up to 42% of the residue P in the microbial biomass after 80 days (Noack *et al.* 2014b). Factors other than P content suggested to be important in release of P from residues include polyphenol, lignin and cellulose contents (Baggie *et al.* 2005; Ha *et al.* 2008; Kwabiah *et al.* 2003a; Kwabiah *et al.* 2003b) and hence P release via the biological pathway may be reduced or slowed due to the remaining P in the root residues being associated with more recalcitrant organic matter. Since the lignin content of canola shoot residues has been reported to be higher than some other residues (Lupwayi *et al.* 2007) this may also be the case for canola roots.

A recent review of crop residue contributions to P pools in agricultural soils (Damon *et al.* 2014) highlighted the paucity of data regarding P content of crop roots and suggested that the root residue component of crop species could be assumed to have a comparable P release (per unit of biomass) to the shoot residue component. Indeed, the proportion of P (26-33%) released from the mature canola root BG P residues in this present study was slightly greater than that reported as released from mature canola above-ground residues (~20%) for an 8 week period following burial of litter bags in the field (Lupwayi *et al.* 2007). However, it was somewhat less than seen in early ^{32}P radiotracer studies using roots added to soils. For example, Dalal (1979) reported greater apparent mineralisation of P from clover root residues than shoots with 42% of root P used by subsequent oats

during ten weeks after residue addition, although the P concentration of the clover roots was 5 mg /g which is higher than the P concentrations of the roots in this present study. In another case more than 40% of P from oat root residues was measured as inorganic P forms in soil only 11 days after incorporation, and 30-40% of oat root residue P was taken up by the succeeding plants after 50 days (Friesen and Blair 1988) although growth period of the subsequent crop in that study was two weeks longer than our study. Such magnitude of P release, as pointed out by the authors, is likely to have occurred because these roots, unlike the intact root system in the present study, were finely ground to a powder and mixed into soil, thus increasing contact between residue particles and soil whilst also potentially altering forms of P in the residues. However, it may also be partly due to the fact that the roots were from young plants (only three weeks old), with a P concentration of 1.6 mg /g. The reported threshold for P concentration of residues above which net P mineralisation occurs is wide-ranging from 1-3 mg /g and investigations in both tropical and temperate soils have largely focussed on this parameter for shoot materials (Baggie *et al.* 2005; Floate 1970; Kaila 1949; Kwabiah *et al.* 2003a; Kwabiah *et al.* 2003b; White and Ayoub 1983) with fewer similarly focussed studies for roots (Fuller *et al.* 1956; Soon and Arshad 2002). Net mineralisation of canola and clover roots with total P concentrations from 1.22-1.44 mg /g has been demonstrated (Buchanan and King 1993; Soon and Arshad 2002) and so the net P release observed in this present study where root P concentrations were 2.2 - 2.7 mg /g was perhaps not surprising. Clearly, as mentioned earlier, factors other than just P concentration of residues are governing the rate of release of P.

Agronomic significance of canola BG P

The agronomic significance of the P contribution by crop residues in agricultural systems depends on whether only the immediate P benefit to a following crop is considered or if longer term P fertility benefits are included. Modelling undertaken by Damon *et al.* (2014) suggests that amounts of P likely to be released from canola shoot residues during the following cropping season are in the order of 0.2-2.4 kg P /ha, which the authors considered were 'not agronomically significant' for Australian systems; whereas modelling suggested that green manuring inputs, in some environments, have potential to release enough P (1.3-22 kg P /ha) to provide for the needs of a subsequent crop. Conversely, Noack *et al.* (2014b) concluded that the 0.6-0.7 kg P /ha contributed by surface residues of canola to following wheat was a small but 'agronomically significant' amount and we concur that this could be the case in the context of dryland cropping systems in southern Australia with a median P fertiliser rate of 12 kg P /ha /annum (Weaver and Wong 2011), a maximum (first year) fertiliser P use efficiency of 30%

(McBeath *et al.* 2012) and an average wheat crop P removal ranging from 2.5 to 15 kg P /ha assuming grain yields within 1-5 t /ha (Norton 2012). Other studies support the view that amounts of P released from canola residues will not provide a high proportion of the following crop's P requirement; for example, Lupwayi *et al.* (2007), using a litter bag decomposition method, estimated that P released by canola shoot residues in the cold semi-arid climate of Canada was in the order of 0.8 kg/ha in the year after incorporation which, according to our estimation, would only provide 3-4% of the P requirement of the following cereal crop in that environment. A radiotracer study using young (23 days old) legume root residues produced in solution culture concluded that they contributed less than 5% of the P uptake of 5 week old maize planted 10 days after residue incorporation (Nachimuthu *et al.* 2009), although this was surprising given the extraordinarily high P concentrations of the residues (11.4-14.1 mg /g). The present study suggests up to 20% of the P nutrition for a developing wheat crop can be contributed from decomposition of canola root residues which, given the importance of P availability during early growth of a wheat crop in Australia (Bolland 1997) may be considered valuable, particularly since it is possible that P uptake from fertiliser in the year of application can be similar to or less than that from plant residues (McBeath *et al.* 2012; Thibaud *et al.* 1988).

The results of this isotope tracer study and others (McLaughlin *et al.* 1988) clearly indicate that up to 80% of the P in any crop is derived from residual P accumulated from prior inputs of fertiliser and organic matter as crop residues over the long term, although the relative contributions of these have not been clearly identified. Longer term soil P fertility benefits, from the contribution of P rich BG plant residues such as those of canola, may indirectly arise as a result of the concomitant increase in soil P status since it has been suggested that increasing P status of a soil can induce greater P mineralisation (Thibaud *et al.* 1988) as well as reduce residual and current fertiliser P sorption (Barrow and Debnath 2014). It has been said that another longer term potential value of BG P in root systems is that it will naturally be distributed to some depth through the soil profile (Read and Campbell 1981), in contrast to the stratification of P that tends to occur with retention of shoot residues on the surface under no-till management (Deubel *et al.* 2011) and applications of P fertiliser at shallow depths (~5 cm), and this may be advantageous for crops in dry land systems where the topsoil can be periodically too dry for nutrient uptake to occur (Armstrong *et al.* 2015). However, our study indicates that a large proportion of BG P is concentrated in the top 0.1m of soil depth. Nevertheless, these studies on the contribution of root residues *in situ* are highly relevant to conservation farming systems, especially in Australia with high rate of adoption of no-till (Llewellyn *et al.* 2012), where contribution from surface retention of above-ground residues to P nutrition

of crops is suggested to be less immediate and longer term (Noack *et al.* 2014b) so that by inference the relative importance of the BG residues for immediate P supply may increase.

Since in this study the wheat was sown only three weeks after harvest of the canola the results highlight a potential to capitalise on rapid P release from the decomposing canola root system. Agronomic significance therefore may relate more closely to relay or double cropping systems where successive crops are sown within relatively close timeframes, such as occurs more commonly in north eastern Australia (Birch and Bell 2011), although summer crops have been considered in some southern areas (Wilhelm 2001). However, rapid root P inputs are also highly relevant to the intercropping or pasture cropping systems that have recently been suggested as economically viable and environmentally sound options for cropping regions across Australia (Bengough *et al.* 2001; Craig *et al.* 2013) and would be important in dual-purpose systems where canola is utilised for both grazing and grain (Kirkegaard *et al.* 2008b). Where a length of time may elapse between harvest of canola and sowing of the subsequent crop, as occurs in areas with rainfed cropping in Mediterranean-type climates that often have up to 6 months fallow, opportunity for the following crop to capitalise on P released by decomposing roots may well be reduced by edaphic factors such as wetting and drying cycles that increase P availability in the short term (Butterly *et al.* 2009) but also can enhance soil P sorption reactions (Olsen and Court 1982). To trace the release of P from the canola BG residues over the long term using the technique described here is not possible as the 25 days half-life limits the time period that ^{33}P-isotope can be monitored, although developments using stable oxygen isotopes to trace P cycling in soils (Tamburini *et al.* 2014) may allow this to be explored in the future.

Overall it seems that opinion on the agronomic significance of P inputs from crop residues in Australian cropping systems is divergent, field data especially for BG residues is scarce, and the longer term fate of P from crop residues clearly requires further investigation. However, this study of the potential of canola BG residues to supply P provides information that may prompt growers to consider benefits from using canola in rotation additional to those of disease break or N supply (Kirkegaard *et al.* 2008a) and perhaps further to consider if there are management options for manipulation of fertiliser P applications in cereals following canola.

Although this study using ^{33}P isotope purports to provide improved estimation of BG P compared to coarse root recovery and undoubtedly enables *in situ* tracing of BG P dynamics, it is important to consider the validity of the assumptions inherent in the technique. Recently it has been suggested that substantial errors in estimation of root-derived N using ^{15}N isotope may occur because there may be immediate leakage of small amounts (0.5-1.0%) of highly enriched fed ^{15}N isotope from the roots (Gasser *et al.* 2015) which then are not representative of root- or root-derived N. Whilst the potential for leakage is attributed to *"the forced uptake of ^{15}N tracer"* the authors can only generalise about the likely driver for this, referring to artefacts caused by severe disturbance to the plant's metabolism as suggested in Chalk *et al.* (2014). The implication appears to be that the N in the fed solution may cause an increase in root cell N concentration in excess of plant demand and that this results in immediate excretion of N. However, another study which detected excess ^{15}N in soil 24 hours after feeding ^{15}N to the plant via the leaves suggested this could be attributed to ^{15}N in unrecovered very fine roots in soil and hence concluded that feeding did not induce substantial exudation (Gardner *et al.* 2012). Whether the fed P in this study can be assumed to act in a similar manner to fed N needs careful consideration as discussed next.

The P concentration of the canola roots and shoots in this study indicated that the plants were P sufficient (at least when sampled at 105 DAS although P status was not measured at 45 DAS when feeding occurred), and so any fed P could be considered as in excess of plant requirements. However, in soil studies using isotopic P the amounts of P introduced via small additions of *carrier free* isotope solution are extremely small in comparison to the P present in other P pools in the system (Bünemann *et al.* 2004), as is the case for the stem-fed P in this study in relation to the plant P pool. Nevertheless loss of P over a 22 day period has been reported from roots of plants fed [32]P (McLaughlin *et al.* 1987; McLaughlin *et al.* 1988) although it is suggested by these authors that the most likely fate of any exuded P would be uptake again by adjacent roots (Rovira and Bowen 1970), especially in a soil with inherently low P buffering capacity like the sand in this present study. Or, alternatively, assimilation by microbial biomass in the rhizosphere, as has been previously been reported for exudates from wheat fed [32]P (McLaughlin *et al.* 1987). In a previous study complementary to this current one, where plants fed [33]P 45 DAS were sampled at peak vegetative stage 57 DAS (Foyjunnessa *et al.* 2015) there was no ^{33}P detected in labile soil P pools such as microbial biomass or resin P which, in our opinion, supports the suggestion that rapid leakage of any fed P is unlikely to have occurred. However, we do concur that since these measures were

taken 11 days after the initiation of the feed, a sampling immediately after a feed to test the activity of P in the immediate rhizosphere soil would be required to rigorously test for any such 'leakage'.

We believe that a more important point to consider in relation to these results is the assumption inherent to the use of the isotope that all roots will be labelled uniformly by a single feed. It is possible that roots produced prior to feeding the isotope could remain unlabelled although given the inter-connectivity of the vascular system in plants this seems unlikely. Whilst this study provides some evidence that spatially there is some homogeneity in label distribution within the root system of canola grown in the loam as our SA data (Table 3) suggests but this was not the case in the sand. Furthermore we did not investigate different orders of roots. There is some evidence that different root cohorts may not necessarily be labelled uniformly, at least by leaf feeding isotope (Gasser *et al.* 2015), and any implications of this with regard to the estimates of BG P generated by this study need to be considered. Thus more work is warranted to test the SA for different root zones in more species and soil types as evident in some ^{15}N studies (McNeill and Fillery 2008; McNeill *et al.* 1997), to further clarify the uniformity of ^{33}P labelling across root systems.

Conclusion

This study confirms that the contribution of below-ground plant biomass to soil P pools is greater than estimated from studies to date using recovered root P contents. The work showed that canola BG P input and the subsequent P benefit to young wheat plants was greater in a high P fertility loam than a low P fertility sandy soil. Some cycling of root P occurs between flowering and break crop senescence. Phosphorus from canola BG residues contributed 20% of the P uptake of seedling wheat and further work is required to clarify the longer term P fertility benefits from BG residues.

Acknowledgments

References

ABS (2008) Agricultural Commodities: Small Area Data, Australia, 2005-06 (Reissue). In. Vol. RIRDC Publication No 10/113'. https://www.rirdc.infoservices.com.au/downloads/10-113)

Angus J, Herwaarden A, Howe G, Van HA (1991) Productivity and break crop effects of winter-growing oilseeds. *Australian Journal of Experimental Agriculture* **31**(5), 669-677.

Armstrong RD, Dunsford K, McLaughlin MJ, McBeath T, Mason S, Dunbabin VM (2015) Phosphorus and nitrogen fertiliser use efficiency of wheat seedlings grown in soils from contrasting tillage systems. *Plant Soil*, 1-13.

Baggie I, Rowell DL, Robinson JS, Warren GP (2005) Decomposition and phosphorus release from organic residues as affected by residue quality and added inorganic phosphorus. *Agroforestry Systems* **63**(2), 125-131.

Barrow N, Debnath A (2014) Effect of phosphate status on the sorption and desorption properties of some soils of northern India. *Plant Soil* **378**(1-2), 383-395.

Batten G, Khan M (1987) Uptake and utilisation of phosphorus and nitrogen by bread wheats grown under natural rainfall. *Australian Journal of Experimental Agriculture* **27**(3), 405-410.

Batten G, Wardlaw I, Aston M (1986) Growth and the distribution of phosphorus in wheat developed under various phosphorus and temperature regimes. *Australian Journal of Agricultural Research* **37**(5), 459-469.

Bengough AG, Lijima M, Barlow PW (2001) Image Analysis of Maize Root Caps—Estimating Cell Numbers from 2-D Longitudinal Sections. *Annals of Botany* **87**(5), 693-698.

Birch C, Bell L (2011) Rainfed farming systems of North-Eastern Australia. In 'Rainfed Farming Systems.' (Eds P Tow, C Cooper, I Partridge and C Birch) pp. 691-713. (Sringer Netherlands)

Blair GJ, Bolland OW (1978) The release of phosphorus from plant material added to soil. *Australian Journal of Soil Research* **16**, 101-111.

Bolland MDA (1997) Comparative phosphorus requirement of canola and wheat. *Journal of Plant Nutrition* **20**(7-8), 813-829.

Bolland MDA, Brennan RF (2008) Comparing the phosphorus requirements of wheat, lupin, and canola. *Australian Journal of Agricultural Research* **59**(11), 983-998.

Brewster JL, Bhat KKS, Nye PH (1976) The possibility of predicting solute uptake and plant growth response from independently measured soil and plant characteristics: IV. The growth and uptake of rape in solutions of different phosphorus concentration. *Plant Soil* **44**(2), 279-293.

Buchanan M, King LD (1993) Carbon and phosphorus losses from decomposing crop residues in no-till and conventional till agroecosystems. *Agronomy Journal* **85**(3), 631-638.

Bünemann EK, Bossio DA, Smithson PC, Frossard E, Oberson A (2004) Microbial community composition and substrate use in a highly weathered soil as affected by crop rotation and P fertilization. *Soil Biology and Biochemistry* **36**(6), 889-901.

Butterly CR, Bünemann EK, McNeill AM, Baldock JA, Marschner P (2009) Carbon pulses but not phosphorus pulses are related to decreases in microbial biomass during repeated drying and rewetting of soils. *Soil Biology and Biochemistry* **41**(7), 1406-1416.

Chalk PM, Peoples MB, McNeill AM, Boddey RM, Unkovich MJ, Gardener MJ, Silva CF, Chen D (2014) Methodologies for estimating nitrogen transfer between legumes and companion species in agro-ecosystems: A review of ^{15}N-enriched techniques. *Soil Biology and Biochemistry* **73**(0), 10-21.

Chauhan BS, Stewart JWB, Paul EA (1979) Effect of carbon additions on soil labile inorganic, organic and microbially held phosphate. *Canadian Journal of Soil Science* **59**(4), 387-396.

Craig PR, Coventry D, Edwards JH (2013) Productivity advantage of crop–perennial pasture intercropping in Southeastern Australia. *Agronomy Journal* **105**(6), 1588-1596.

Dalal RC (1979) Mineralization of Carbon and Phosphorus from Carbon-^{14} and Phosphorus-^{32} Labelled Plant Material Added to Soil1. *Soil Science Society of America Journal* **43**(5), 913-916.

Damon PM, Bowden B, Rose T, Rengel Z (2014) Crop residue contributions to phosphorus pools in agricultural soils: A review. *Soil Biology and Biochemistry* **74**, 127-137.

Deubel A, Hofmann B, Orzessek D (2011) Long-term effects of tillage on stratification and plant availability of phosphate and potassium in a loess chernozem. *Soil and Tillage Research* **117**, 85-92.

Floate MJS (1970) Decomposition of organic materials from hill soils and pastures: II. Comparative studies on the mineralization of carbon, nitrogen and phosphorus from plant materials and sheep faeces. *Soil Biology and Biochemistry* **2**(3), 173-185.

Foyjunnessa, McNeill A, Doolette A, Mason S, McLaughlin M (2014) In situ ^{33}P-labelling of canola and lupin to estimate total phosphorus accumulation in the root system. *Plant Soil* **382**(1-2), 291-299.

Foyjunnessa, McNeill A, Doolette A, Mason S, McLaughlin M (2015) Quantifying total phosphorus accumulation below-ground by canola and lupin plants using ^{33}P-labelling. *Plant Soil*, 10.1007/s11104-015-2545-y.

Franzluebbers AJ, Arshad MA, Ripmeester JA (1996) Alterations in canola residue composition during decomposition. *Soil Biology and Biochemistry* **28**(10–11), 1289-1295.

Friesen D, Blair G (1988) A dual radiotracer study of transformations of organic, inorganic and plant residue phosphorus in soil in the presence and absence of plants. *Soil Research* **26**(2), 355-366.

Fuller WH, Nielsen DR, Miller RW (1956) Some Factors Influencing the Utilization of Phosphorus from Crop Residues1. *Soil Science Society of America Journal* **20**(2), 218-224.

Gardner M, Peoples M, Condon J, Li G, Conyers M, Dear B Evaluating the importance of a potential source of error when applying shoot ^{15}N labelling techniques to legumes to quantify the below-ground

transfer of nitrogen to other species. In 'Proceedings of the 16th Australian Agronomy Conference', 2012, Armidale, Australia,

Gasser M, Hammelehle A, Oberson A, Frossard E, Mayer J (2015) Quantitative evidence of overestimated rhizodeposition using ^{15}N leaf-labelling. *Soil Biology and Biochemistry* **85**, 10-20.

Grant CA, Bailey LD (1993) Fertility management in canola production. *Canadian Journal of Plant Science* **73**(3), 651-670.

Ha KV, Marschner P, Bünemann EK (2008) Dynamics of C, N, P and microbial community composition in particulate soil organic matter during residue decomposition. *Plant Soil* **303**(1/2), 253-264.

Helal HM, Sauerbeck DR (1984) Influence of plant roots on C and P metabolism in soil. *Plant Soil* **76**(1-3), 175-182.

Iqbal SM (2009) Effect of crop residue qualities on decomposition rates, soil phosphorus dynamics and plant phosphorus uptake. The University of Adelaide, Adelaide

Jackson GD (2000) Effects of nitrogen and sulfur on canola yield and nutrient uptake. *Agronomy Journal* **92**(4), 644-649.

Jones O, Bromfield S (1969) Phosphorus changes during the leaching and decomposition of hayed-off pasture plants. *Australian Journal of Agricultural Research* **20**(4), 653-663.

Kaila A (1949) Biological absorption of phosphorus. *Soil Science* **68**(4), 279-290.

Kirkegaard J, Christen O, Krupinsky J, Layzell D (2008a) Break crop benefits in temperate wheat production. *Field Crops Research* **107**(3), 185-195.

Kirkegaard J, Gardner P, Angus J, Koetz E (1994) Effect of Brassica break crops on the growth and yield of wheat. *Australian Journal of Agricultural Research* **45**(3), 529-545.

Kirkegaard JA, Sprague SJ, Dove H, Kelman WM, Marcroft SJ, Lieschke A, Howe GN, Graham JM (2008b) Dual-purpose canola—a new opportunity in mixed farming systems. *Australian Journal of Agricultural Research* **59**(4), 291-302.

Kouno K, Tuchiya Y, Ando T (1995) Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. *Soil Biology and Biochemistry* **27**(10), 1353-1357.

Kwabiah AB, Palm CA, Stoskopf NC, Voroney RP (2003a) Response of soil microbial biomass dynamics to quality of plant materials with emphasis on P availability. *Soil Biology and Biochemistry* **35**(2), 207-216.

Kwabiah AB, Stoskopf NC, Palm CA, Voroney RP (2003b) Soil P availability as affected by the chemical composition of plant materials: implications for P-limiting agriculture in tropical Africa. *Agriculture, Ecosystems & Environment* **100**(1), 53-61.

Kwabiah AB, Stoskopf NC, Palm CA, Voroney RP, Rao MR, Gacheru E (2003) Phosphorus availability and maize response to organic and inorganic fertilizer inputs in a short term study in western Kenya. *Agriculture, Ecosystems & Environment* **95**(1), 49-59.

Liu L, Gan Y, Bueckert R, Van Rees K, Warkentin T (2010) Fine root distributions in oilseed and pulse crops. *Crop Science* **50**(1), 222-226.

Llewellyn RS, D'Emden FH, Kuehne G (2012) Extensive use of no-tillage in grain growing regions of Australia. *Field Crops Research* **132**, 204-212.

Lupwayi NZ, Clayton GW, O'Donovan JT, Harker KN, Turkington TK, Soon YK (2007) Phosphorus release during decomposition of crop residues under conventional and zero tillage. *Soil and Tillage Research* **95**(1–2), 231-239.

Martin J, Cunningham R (1973) Factors controlling the release of phosphorus from decomposing wheat roots. *Australian Journal of Biological Sciences* **26**(4), 715-728.

Mayer J, Buegger F, Jensen ES, Schloter M, Heß J (2003) Estimating N rhizodeposition of grain legumes using a ^{15}N in situ stem labelling method. *Soil Biology and Biochemistry* **35**(1), 21-28.

McBeath TM, McLaughlin MJ, Kirby JK, Armstrong RD (2012) The effect of soil water status on fertiliser, topsoil and subsoil phosphorus utilisation by wheat. *Plant Soil* **358**(1-2), 337-348.

McLaughlin M, Alston A (1986) The relative contribution of plant residues and fertilizer to the phosphorus nutrition of wheat in a pasture cereal system. *Soil Research* **24**(4), 517-526.

McLaughlin M, Alston A, Martin J (1987) Transformations and movement of P in the rhizosphere. *Plant Soil* **97**(3), 391-399.

McLaughlin M, Alston A, Martin J (1988) Phosphorus cycling in wheat pasture rotations .I. The source of phosphorus taken up by wheat. *Soil Research* **26**(2), 323-331.

McNeill A (2001) Stable isotope techniques using enriched ^{15}N and ^{13}C for studies of soil organic matter accumulation and decomposition in agricultural systems. In 'Stable Isotope Techniques in the Study of Biological Processes and Functioning of Ecosystems. Vol. 40.' (Eds M Unkovich, J Pate, A McNeill and DJ Gibbs) pp. 195-218. (Springer Netherlands)

McNeill A, Fillery I (2008) Field measurement of lupin belowground nitrogen accumulation and recovery in the subsequent cereal-soil system in a semi-arid Mediterranean-type climate. *Plant Soil* **302**(1), 297-316.

McNeill AM, Zhu C, Fillery IRP (1997) Use of ^{15}N-labelling to estimate the total below-ground nitrogen of pasture legumes in intact soil-plant systems. *Australian Journal of Agricultural Research* **48**(3), 295-304.

Misra RK, Alston AM, Dexter AR (1988) Role of root hairs in phosphorus depletion from a macrostructured soil. *Plant Soil* **107**(1), 11-18.

Moody PW (2007) Interpretation of a single-point P buffering index for adjusting critical levels of the Colwell soil P test. *Soil Research* **45**(1), 55-62.

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* **27**(0), 31-36.

Nachimuthu G, Guppy C, Kristiansen P, Lockwood P (2009) Isotopic tracing of phosphorus uptake in corn from ^{33}P labelled legume residues and ^{32}P labelled fertilisers applied to a sandy loam soil. *Plant Soil* **314**(1), 303-310.

Noack S, McLaughlin M, Smernik R, McBeath T, Armstrong R (2012) Crop residue phosphorus: speciation and potential bio-availability. *Plant Soil* **359**(1-2), 375-385.

Noack S, McLaughlin M, Smernik R, McBeath T, Armstrong R (2014a) Phosphorus speciation in mature wheat and canola plants as affected by phosphorus supply. *Plant Soil* **378**(1-2), 125-137.

Noack SR, McBeath TM, McLaughlin MJ, Smernik RJ, Armstrong RD (2014b) Management of crop residues affects the transfer of phosphorus to plant and soil pools: Results from a dual-labelling experiment. *Soil Biology and Biochemistry* **71**(0), 31-39.

Norton A, Kirkegaard J, Angus J, T P (2013) Canola in Rotations 7. In. ' (The Regional Institute: http://www.regional.org.au/au/gcirc/canola/p-06.htm)

Norton RM (2012) Wheat grain nutrient concentrations for south-eastern Australia. In 'Australian Agronomy Conference. ' Armidale, NSW)

Olsen RG, Court MN (1982) Effect of wetting and drying of soils on phosphate adsorption and resin extraction of soil phosphate. *Journal of Soil Science* **33**(4), 709-717.

Peoples MB, Bowman AM*, et al.* (2001) Factors regulating the contributions of fixed nitrogen by pasture and crop legumes to different farming systems of eastern Australia. *Plant Soil* **228**(1), 29-41.

Rahman M, McClean P (2013) Genetic Analysis on Flowering Time and Root System in Brassica napus L. *Crop Science* **53**(1), 141-147.

Read DWL, Campbell CA (1981) Bio-cycling of phosphorus in soil by plant roots. *Canadian Journal of Soil Science* **61**(4), 587-589.

Reuter DJ, Robinson BJ (1997) 'Plant Analysis: an Interpretation Manual.' (CSIRO: Collingwood, Australia)

Richardson A, Barea J-M, McNeill A, Prigent-Combaret C (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil* **321**(1), 305-339.

Römer W, Schilling G (1986) Phosphorus requirements of the wheat plant in various stages of its life cycle. *Plant Soil* **91**(2), 221-229.

Rose TJ, Rengel Z, Ma Q, Bowden JW (2007) Differential accumulation patterns of phosphorus and potassium by canola cultivars compared to wheat. *Journal of Plant Nutrition and Soil Science* **170**(3), 404-411.

Rose TJ, Rengel Z, Ma Q, Bowden JW (2008) Post-flowering supply of P, but not K, is required for maximum canola seed yields. *European Journal of Agronomy* **28**(3), 371-379.

Rovira AD, Bowen GD (1970) Translocation and loss of phosphate along roots of wheat seedlings. *Planta* **93**(1), 15-25.

Smith A (1965) The influence of superphosphate fertilizer on the yield and uptake of phosphorus by wheat. *Australian Journal of Experimental Agriculture* **5**(17), 152-157.

Soon Y, Arshad M (2002) Comparison of the decomposition and N and P mineralization of canola, pea and wheat residues. *Biology and Fertility of Soils* **36**(1), 10-17.

Tamburini F, Pfahler V, von Sperber C, Frossard E, Bernasconi SM (2014) Oxygen isotopes for unraveling phosphorus transformations in the soil–plant system: A review. *Soil Science Society of America Journal* **78**(1), 38-46.

Thibaud M-C, Morel C, Fardeau J-C (1988) Contribution of phosphorus issued from crop residues to plant nutrition. *Soil Science and Plant Nutrition* **34**(4), 481-491.

Unkovich M, Baldock J, Peoples M (2010) Prospects and problems of simple linear models for estimating symbiotic N2 fixation by crop and pasture legumes. *Plant Soil* **329**(1-2), 75-89.

Weaver DM, Wong MTF (2011) Scope to improve phosphorus (P) management and balance effeciency of crop and pasture soils with contrasting P status and buffering indices. *Plant Soil* **349**, 37-54.

White RE, Ayoub AT (1983) Decomposition of plant residues of variable C/P ratio and the effect on soil phosphate availability. *Plant Soil* **74**(2), 163-173.

Wilhelm NS Warm season cropping in the southern cropping zone of Australia. In 'Proceedings of 10th Australian Agronomy Conference', 2001, Hobart, Australia,

Zadoks JC, Chang TT, Konzak CF (1974) Decimal code for growth stages of cereals. *A Weed Research* **14**, 415-421.

Zarcinas BA, Cartwright B (1983) Analysis of soil and plant material by inductively coupled plasma-optical emission spectrometry. CSIRO, Australia. Division, Soils, Technical Paper No. 45: 1-35.

Zarcinas BA, McLaughlin MJ, Smart MK (1996) The effect of acid digestion technique on the performance of nebulization systems used in inductively coupled plasma spectrometry. *Communications in Soil Science and Plant Analysis* **27**(5-8), 1331-1354.

Fig.1 Total root P calculated as the sum of measured recovered root P of mature canola grown in sand and loam and estimated root-derived P (a) and total root dry weight represented as measured recovered root dry weight and estimated unrecovered root dry weight in soil (b) (^{33}P was fed using a cotton-wick stem feeding technique at 45 days and plants were harvested at 105 days after sowing). Error bars represent standard errors of $n=4$ and different letter within same shade and pattern indicate significant differences ($p \le 0.005$).
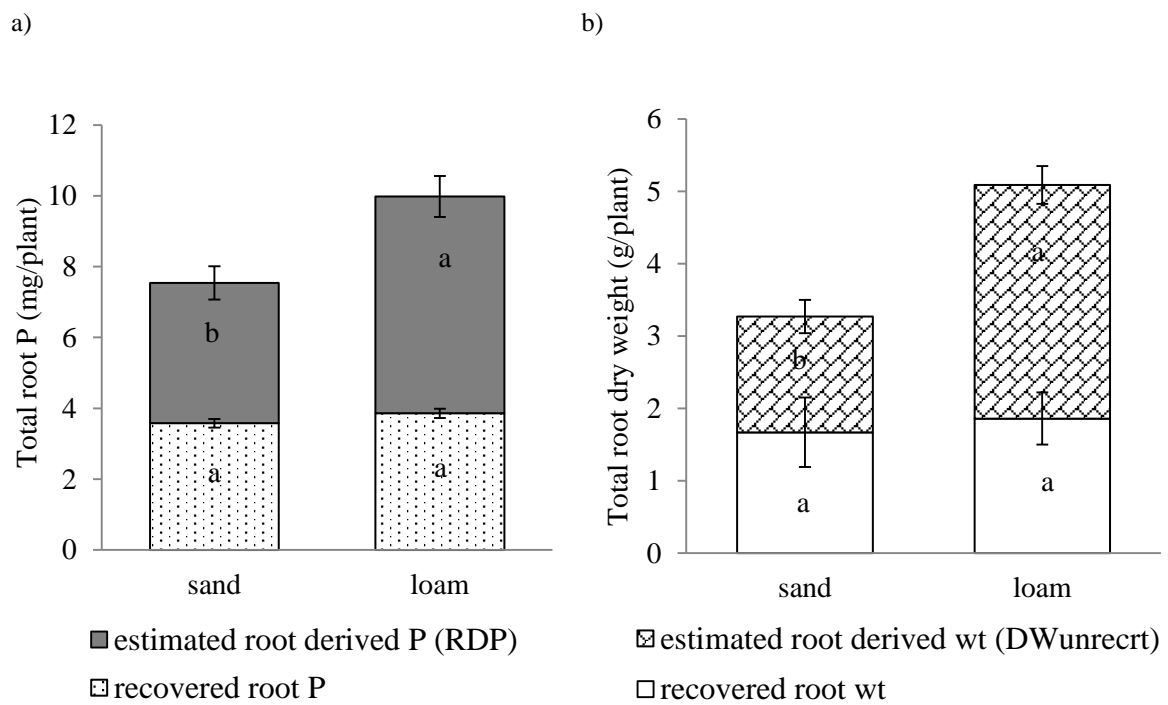
a)   b)

estimated root derived P (RDP)   estimated root derived wt (DWunrecrt)
recovered root P   recovered root wt

Fig.2 Distribution of fed ^{33}P in a mature canola-soil system grown in sand and loam (a and b: Pie chart) and in resin P and microbial P pools within the soil (a and b: Bar chart) (^{33}P was fed using a cotton-wick stem feeding technique at 45 days and plants were harvested at 105 days after sowing)

a) Canola sand
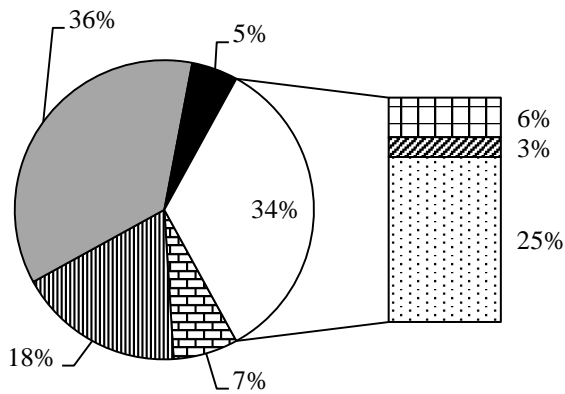
b) Canola loam
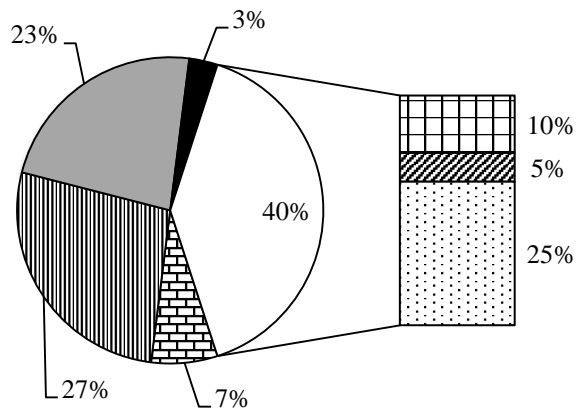
Legend:

Pie chart ⊞ wick ⦚ shoot ▢ recovered roots ■ lost

▢ Soil (bulk plus rhizosphere)

Bar chart ⊟ resin ▨ microbial ⊡ Other form*

*Other forms of P present in soil = Unrecovered fine roots <2 mm plus root-derived P not in microbial or resin P pools

Fig.3 Distribution of canola total BG ^{33}P in the following wheat-soil system grown in sand and loam. The proportional distribution and recovery of canola BG ^{33}P in the wheat shoot, recovered roots and soil are significantly different ($p \leq 0.05$) between the soil textures. Data represent means (n=4).
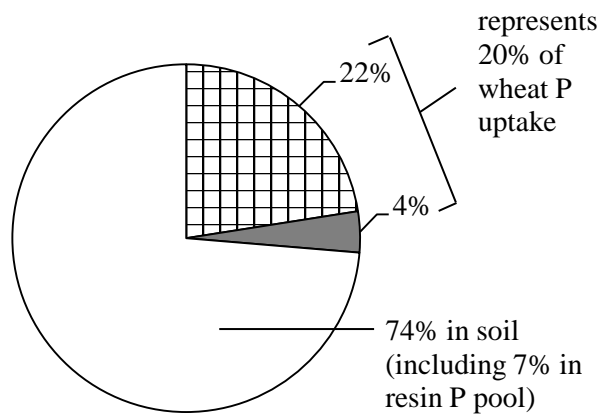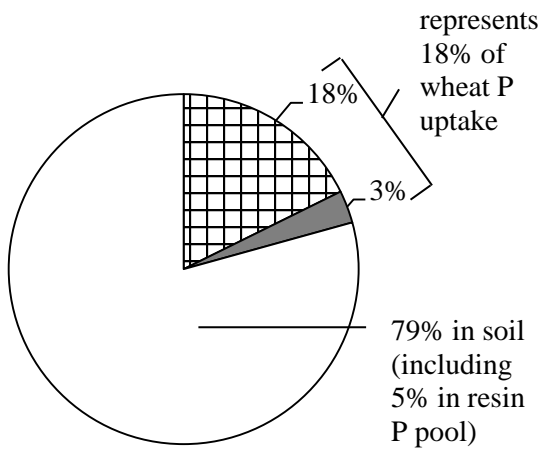
a) Wheat sand

b) Wheat loam

Table 1 Plant dry weight, P concentration and P content of (a) mature canola grown in sand and loam soil and fed with ^{33}P using a ^{33}P stem-wick feeding technique at 45 days and (b) wheat after canola grown for 5 weeks

a)

Treatments	Dry weight (g /pot)		P concentration (mg /kg)		P content (mg /pot)	
	Shoot	Recovered roots	Shoot	Recovered roots	Shoot	Recovered roots
Canola sand	14.1 b	1.67 a	4236 b	2211 b	60 b	3.58 a
Canola loam	31.7 a	1.86 a	4702 a	2683 a	149 a	3.85 a
P value	<0.001	0.051	0.015	0.021	<0.001	0.249

Data represent means (n=4); different letters within column indicate significant difference ($p \leq 0.05$) between treatments

b)

Treatments	Dry weight (g /pot)		P concentration (mg /kg)		P content (mg /pot)	
	Shoot	Recovered roots	Shoot	Recovered roots	Shoot	Recovered roots
Wheat sand	1.82 b	0.93 a	1698 b	809 b	6.19 b	1.50 b
Wheat loam	2.51 a	0.96 a	2141 a	1515 a	10.76 a	4.12 a
P value	<0.001	0.644	0.006	<0.001	<0.001	<0.001

Data represent means (n=4); different letters within column indicate significant difference ($p \leq 0.05$) between treatments

Table 2 Activity of ^{33}P in recovered roots and soil (bulk soil plus rhizosphere soil) of canola grown in sand and loam soil in a glasshouse and fed with ^{33}P using a ^{33}P cotton-wick stem feeding technique at 45 days and harvested 105 days after sowing at maturity.

Treatments	Fed ^{33}P (kBq ^{33}P /pot)	^{33}P activity (kBq ^{33}P /pot)		
		Recovered roots	Bulk soil	Rhizosphere soil
Canola sand	459.25	163 a	129 b	26 a
Canola loam	459.25	107 b	161 a	25 a
P value		<0.001	<0.001	0.210

Data represent means (n=4); same letters within columns indicate no significant difference ($p \geq 0.05$) between treatments

Table 3 Activity of ^{33}P (kBq plant^{-1}), amounts of ^{31}P (mg plant^{-1}) and specific activities (SA, kBq ^{33}P mg^{-1} ^{31}P) of recovered roots from two soil depths for mature canola grown in sand and loam. Plants were fed with ^{33}P using a ^{33}P cotton-wick stem feeding technique at 45 days and harvested 105 days after sowing at maturity. Data represent means (n=4); ns following means within a column means non-significant and different letters following means within a column indicate that values are significantly different according to the LSD$_{0.05}$.

Treatments	Sand			Loam		
Depths	^{33}P (kBq plant^{-1})	^{31}P (mg plant^{-1})	SA (kBq mg^{-1})	^{33}P (kBq plant^{-1})	^{31}P (mg plant^{-1})	SA (kBq mg^{-1})
0-10 cm	141	2.98	48 a	88	3.27	27 ns
10-35 cm	22	0.60	38 b	19	0.60	33 ns
LSD$_{0.05}$ (SA)			Treatment	8.1		
			Depth	8.1		
			Treatment * Depth	11.5		

Table 4 Bulk soil resin available P, microbial P (hexanol released) and ^{33}P activity where canola was grown to maturity in a sand or loam in a glasshouse and fed with [33]P (459.25 kBq [33]P /plant) using a ^{33}P stem-wick feeding technique at 45 days after sowing

Treatments	Resin available P (mg /kg)		Microbial P (mg /kg)		[33]P activity at maturity (kBq [33]P /pot)	
	Initial	Maturity	Initial	Maturity	Resin P	Microbial P
Canola sand	29 b	6 b	1.1 b	2.8 b	29.1 b	13.4 b
Canola loam	137 a	68 a	7.1 a	17.1 a	46.4 a	22.5 a
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Data represent means (n=4); different letters within columns indicate significant difference ($p \leq 0.05$) between treatments

Table 5 Bulk soil resin available P, microbial P (hexanol-released) and recovery of ^{33}P activity in bulk soil after wheat was grown in sand or loam in a glasshouse following canola that had been fed with ^{33}P (459.25 kBq ^{33}P /plant) using a ^{33}P cotton-wick stem feeding technique at 45 days after sowing.

Treatments	Resin P (mg /kg)	Microbial P (mg /kg)	^{33}P activity after wheat (kBq ^{33}P /pot)	
			Resin P	Microbial P
Wheat sand	6.1 b	2.0 b	23.2 b	Below detection
Wheat loam	68.1 a	16.1 a	30.1 a	Below detection
P value	<0.001	<0.001	<0.001	

Data represent means (n=4); different letters within columns indicate significant difference ($p \leq 0.05$) between treatments