



THE UNIVERSITY
of ADELAIDE

**Isotope studies of accumulation and cycling of phosphorus and
nitrogen below-ground in canola and lupin**

This thesis is submitted in fulfilment of the requirements
for the degree of Doctor of Philosophy

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July 2016

TABLE OF CONTENTS

Abstract	ix
Declaration	xv
Publication arising from this thesis	xvii
Acknowledgements	xix
Structure of the thesis	xxi
Chapter 1: General introduction and review of the literature	1
General introduction	3
Global P and N cropping systems budgets	5
<i>P in global cropping systems</i>	5
<i>N in global cropping systems</i>	8
<i>Conclusion</i>	10
Soil-plant P and N cycles - regulating fertility in agricultural systems	11
<i>P cycle</i>	11
<i>N cycle</i>	13
<i>Phosphorus and N in break crop residues AG and BG</i>	16
<i>Amounts and chemical speciation of P and N in AG break crop residues</i>	16
<i>Amounts of P and N in BG break crop residues</i>	19
<i>Difficulties in measuring total root mass and nutrient accumulation</i>	21
<i>Isotope studies to quantify BG N and P</i>	22
<i>Conclusions</i>	25
Phosphorus and N cycling of break crop residues	26
<i>Residue quality parameters</i>	26
<i>Proportion and amounts of N and P ‘released’ from break crop residues</i>	28

<i>Release of P by break crop residues and uptake by subsequent plants</i>	28
<i>Release of N by break crop residues and uptake by subsequent plants</i>	32
<i>Conclusions</i>	34
Overall conclusions and aims of the thesis	34
References	36
Chapter 2: Preliminary studies: towards accurate quantification of phosphorus accumulation in crop plant root systems	53
Introduction	55
Study 1: Comparison of two techniques to recover clean root samples from soil	55
Materials and Methods	57
<i>Field core collection and plant density counts</i>	57
<i>Extraction of roots using washing or freeze drying method</i>	58
<i>Dry matter (DM) and nutrient analysis of clean root samples</i>	59
<i>Statistical analysis of data</i>	59
Results and Discussion	61
Study 2: Effectiveness of stem wick-feeding ³³ P for labelling roots of lupin	65
Materials and Methods	66
<i>Details of experiment – design, set-up, nutrition and watering regime</i>	66
<i>³³P Stem wick-feeding</i>	67
<i>Sampling and analysis of plants</i>	68
Results and Discussion	69
Study 3: Specific activity of ³³ P in plants delivered through stem wick-fed ³³ P grown in soils with different application rates of fertiliser P	71
Materials and Methods	72
<i>Details of experiment</i>	72
<i>Sampling and analysis</i>	72

Results and Discussion	72
Overall conclusions	74
References	76
Chapter 3: <i>In situ</i> ³³P-labelling of canola and lupin to estimate total phosphorus accumulation in the root system	81
Abstract	85
Introduction	85
Materials and method	86
<i>Experimental set up</i>	86
³³ P feeding	87
<i>Sampling and processing</i>	87
<i>Measuring of microbial biomass P in bulk and rhizosphere soil</i>	87
<i>Statistical analysis</i>	88
Results	88
<i>Plant dry matter and P content</i>	88
<i>Recovery of ³³P and specific activity of shoot and root</i>	88
<i>Microbial P</i>	88
<i>Estimation of fine root P</i>	88
Discussion	89
Conclusion	91
Acknowledgments	91
References	91
Chapter 4: Quantifying total phosphorus accumulation below-ground by canola and lupin plants using ³³P-labelling	95
Abstract	101
Introduction	101

Materials and method	103
<i>Soil collection and potting</i>	103
<i>Details of the experiment</i>	103
<i>Harvesting, radioactive sample processing and analysis</i>	104
<i>Calculation of root-derived P in bulk and rhizosphere soil, and total below-ground P</i>	105
<i>Statistical analysis</i>	105
Results	105
<i>Plant dry weight, P content and shoot to root ratio</i>	105
<i>Shoot to recovered ratio</i>	105
<i>³³P distribution and recovery in soil-plant system and specific activity of shoot and recovered roots</i>	106
<i>Root-derived P in bulk and rhizosphere soil and total below-ground P</i>	106
<i>Estimates of unrecovered root dry weight</i>	107
Discussion	108
<i>Direct estimation of total BGP</i>	108
<i>Influence on BGP estimation of fed ³³P distribution</i>	108
<i>Roots as a sink for stem-fed ³³P</i>	109
<i>Shoot: root ratios</i>	110
Conclusion	110
Acknowledgments	110
References	110
Chapter 5: Use of ³³P to trace in situ the fate of canola below-ground phosphorus, including wheat uptake in two contrasting soils	113
Abstract	117
Introduction	118

Materials and method	120
<i>Soil characteristics</i>	120
<i>Canola phase of the experiment</i>	120
³³ P labelling of canola	121
<i>Fallow & wheat phase of experiment</i>	122
<i>Sample harvesting, processing and analysis for both phase of the experiment</i>	123
<i>Calculation, assumptions and statistical analysis</i>	125
Results	126
Canola phase	126
<i>Plant dry weight, P concentration and P content of mature canola plants</i>	126
³³ P activity below-ground and specific activity of recovered roots at two soil depths (0-10 cm and 10-35 cm)	126
<i>Estimates of RDP and DW_{unrecr} for canola at maturity</i>	129
<i>Recovery and distribution of fed ³³P in the mature canola-soil system</i>	131
Wheat phase	131
<i>Plant dry weight, P concentration and P content of wheat after canola</i>	131
<i>Distribution and recovery of canola total BG³³P in the subsequent wheat-soil system</i>	132
Discussion	133
<i>Below-ground P input by canola at maturity</i>	133
<i>Fate of mature canola BG P</i>	136
<i>Agronomic significance of canola BG P</i>	140
<i>Technical consideration of the isotope technique for estimating BG P</i>	143
Conclusion	146
Acknowledgments	146
References	146

Chapter 6: Dual-labelling (^{15}N and ^{33}P) quantifies relative contributions to nitrogen and phosphorus uptake by wheat from lupin and canola <i>in situ</i> below-ground residues	157
Abstract	161
Introduction	163
Materials and method	167
<i>Soil preparation and pot set up</i>	167
<i>Break crop phase-details of the experiment</i>	168
<i>Dual-labelling (^{15}N and ^{33}P) of break crops using stem wick-feeding</i>	169
<i>Fallow and wheat phase</i>	170
<i>Sample processing and analysis</i>	170
<i>Estimation of canola and lupin RD P and RD N and calculation of total BG P and total BG N</i>	172
<i>Calculation of P and N in wheat derived from (df) BG P and BG N of the previous lupin or canola</i>	173
<i>Statistical analysis</i>	174
Results	174
<i>Canola and lupin total plant dry matter, N and P concentration and content</i>	174
<i>Recovery and distribution of fed ^{33}P and ^{15}N excess in canola or lupin, wick and soil</i>	176
<i>Amounts of total BG P and BG N and proportion as root-derived P (RD P) and root-derived N (RD N)</i>	178
<i>C: N and C: P ratio of recovered roots and N:P ratio for shoot, recovered root and root-derived fraction</i>	179
<i>Wheat plant dry matter, P and N concentration of shoot and recovered root</i>	180
<i>Recovery and distribution of lupin and canola BG^{33}P and BG^{15}N excess in subsequent wheat</i>	180
<i>Amounts of P and N in wheat derived from lupin or canola BG N and BG P</i>	182

Discussion	183
<i>The relative contribution of N and P to wheat from canola and lupin in situ BG residues</i>	183
<i>Is apparent mineralisation related to ratios between carbon, nitrogen and phosphorus?</i>	186
<i>Allocation of N and P BG in lupin and canola at maturity</i>	189
<i>Partitioning of labelled N and P above- and below-ground in lupin and canola</i>	190
<i>Potential errors associated with the use of isotope-labelling in this study</i>	193
Conclusions	194
Acknowledgments	194
References	195
Chapter 7: General discussions and future research directions	207
Overview	209
Reliability of isotope-based assessments of P accumulation below-ground by break crop species	211
The fate of P and N from break crop BG residues including uptake by wheat	214
What is the agronomic significance of the findings?	217
Future research directions	218
Conclusion	220
References	221
Appendix	225

ABSTRACT

It is commonly acknowledged that the cycling of nutrients, including phosphorus (P) and nitrogen (N), from plant residues in crop rotations is important for the sustainability of agricultural systems. This is especially the case for Australian low input rain-fed cropping systems, where, due to economic, climatic and edaphic factors, additions of P and N as fertilizers or manures are limited. Optimal management of P and N cycled from break crop residues requires a sound understanding of the quantity of each nutrient in residues and what proportion potentially becomes available for a following cereal crop. A review of the literature (Thesis Chapter 1) highlighted that whilst there is information concerning quantities of N, and to a lesser extent P, contained in mature above-ground crop residues, much less has been reported concerning quantities of P or N of below-ground (BG) residues from various crop species. This is partly because root studies are time consuming and hence expensive to undertake, but also quantification is hampered by the certainty that not all roots can be recovered from soils, especially in fine textured soils. As a result root turnover and nutrient release have largely been investigated under somewhat ‘artificial’ or ‘unrealistic’ conditions - using roots that have been extracted from soil, dried, often chopped or finely ground and finally incorporated back into soil to decompose.

More recent innovative studies, summarised in the review (Chapter 1), have used a stem wick-feeding technique to label crop root systems *in situ* with the ^{15}N isotope. These studies demonstrated that total BG N accumulation for these crop species was larger than quantified from recovered roots alone. The labelling technique allowed for direct *in situ* quantitative tracing of the N from legume and oilseed root residues into subsequent wheat plants. It was demonstrated that up to 20% of wheat N uptake may be derived from the BG N input by root systems of a previous break crop. The review (Chapter 1) further highlighted that quantitative assessment of the amounts of P accumulated by crop root systems were extremely scarce and

there did not appear to be any *in situ* isotope studies related to P accumulation BG. Hence the work described in this thesis broadly explored the potential to adapt the approaches used for ^{15}N isotope studies in order to quantitatively assess *in situ* P accumulation BG by break crop species in soils differing in texture, and the uptake of P derived from those BG break crop residues by a following wheat plant. The specific aims of the work were: i) to adapt the stem wick-feeding technique for use with ^{33}P to allow *in situ* quantification of total BG P accumulation by plants, ii) to quantify and compare BG P in two break crops species (an oilseed and a legume) important in Australian rain-fed cropping systems, iii) to assess and measure whether soil texture influences BG P accumulation in canola (oilseed) and lupin (legume), and iv) to trace the fate of break crop BG P relative to BG N in a following cereal (wheat).

Preliminary assessment of methodologies used in estimation of BG N in crop plants and their suitability for ^{33}P studies for BG P were undertaken (Thesis Chapter 2). It was found that the ‘dry’ method frequently used to recover roots for isotope studies (*viz*: freeze dry manually picked roots with adhering soil, brush roots clean) was comparable to the conventional ‘wet’ root recovery method (*viz*: washing soil from roots over a sieve), in that similar amounts of root were recovered, which did not differ in P concentration and were not contaminated by soil. Recovery and measurement of roots from field soil cores suggested the amount of P in canola roots in the topsoil (to 0.1m) could be as much as 4 kg ha^{-1} compared to 1.5 kg ha^{-1} for rye and less than 1 kg ha^{-1} for lupin. Other preliminary studies identified that in stem wick-fed plants, ^{33}P isotope activity was lower where soil P availability (manipulated by P fertiliser addition) was greater. However, the feeding technique could be used to effectively label root systems of lupin with ^{33}P even at a late vegetative stage of plant growth when it might be considered that the shoot would be the primary sink for P redistributed within the plant.

A further study (Chapter 3; Paper 1) confirmed that a substantial proportion (26-51%) of wick-fed ^{33}P was allocated to recoverable roots of canola and lupin grown in sand. Since this first main study did not detect any ^{33}P in soil, a mass balance approach was used to determine the amount of unrecovered ^{33}P , which was suggested to be largely present in unrecovered fine roots, designated as root-derived (RD) P. Using this indirect approach it was estimated that RD P represented 15% of total BG P for canola and 32% for lupin. A subsequent study in deeper pots (Chapter 4; Paper 2) fed a larger amount of ^{33}P and extended scintillation counting time for samples to improve the method detection limit. This facilitated the direct estimation of unrecovered RD P for canola and lupin at late vegetative stage in two contrasting soil textures, sand and loam. Estimated total BG P accumulation by both crop species was at least twice that of recovered root P and was a greater proportion of total plant P for lupin than canola. There was more unrecovered RD P in the loam than the sand within each species. No ^{33}P was detected in labile P pools (resin-P or hexanol released-microbial P) at this late vegetative stage of sampling which suggested that there had been no active efflux of ^{33}P -labelled orthophosphate from labelled roots or any root turnover. However, from a subsequent study (Chapter 5; Paper 3) where ^{33}P labelled canola plants were sampled at maturity it was evident that after the late vegetative stage root turnover may occur, with 3-5% of fed ^{33}P detected in the hexanol-released pool and 6-10% in the resin P pool– the higher values being for a loam textured soil which contained a higher proportioned of the fed ^{33}P than the sand. There appeared to be no translocation of P from roots to shoot between late vegetative stage and maturity since the proportion of fed ^{33}P recovered BG was the same (70%) at both times. The proportion and amount of canola BG ^{33}P that was recovered in subsequently grown wheat was higher in the loam (26%; 2.6 mg P) than sand (22%; 1.5 mg P) reflecting the larger pool of BG P in the loam and the faster turnover rate of BG residues. However, this P derived from the previous crop BG residues represented an equal proportion

(20%) of the total wheat P uptake in both soils (Chapter 5, Paper 3) since wheat dry matter production was less in the sand. Hence the P benefit from the previous plant BG residues was the same for wheat on both soils.

Dual feeding with ^{33}P and ^{15}N was used in the final study reported in this thesis (Chapter 6; Paper 4) to simultaneously assess *in situ* (i) BG N and BG P accumulation by mature lupin and canola, and (ii) the relative contribution from the decomposition of these BG residues to the N and P nutrition of following wheat. The hypothesis tested was that P release from canola BG residues would be relatively greater than from lupin BG residues whereas N release would be relatively smaller. Partitioning of fed ^{15}N differed from ^{33}P with the majority of fed ^{15}N recovered in shoots while a larger proportion of fed ^{33}P was allocated BG. The amount of total BG P was greater for canola than lupin although lupin had a higher amount of total BG N ($75 \text{ mg N plant}^{-1}$) than canola ($68 \text{ mg N plant}^{-1}$). C:P ratio of lupin roots was 708:1 and 188:1 for canola. Root C:N ratio was 39:1 for canola and 24:1 for lupin. The N:P ratio for lupin roots was wider (29:1) than canola (5:1), but the N:P ratio of the RD fractions was similar (6:1 canola; 7:1 lupin). Proportion of BG P taken up by wheat was significantly, but only slightly greater after canola (21%) than after lupin (19%), and since BG P was greater for canola this represented 20% of total wheat P uptake and 12% for wheat after lupin. Despite larger lupin BG N, a lower proportion (~8%) was taken up by wheat than from canola BG N (~12%) and so contribution to wheat total N uptake by lupin BG residues (~10%) was surprisingly less than from canola (12.5%). It was concluded from this final study that P uptake by wheat from residues was related to total BG P of the residues but not total BG N. The proportion of P and N from BG residues of mature canola and lupin taken up by wheat did not appear driven by C:P or C:N ratio of recovered roots, but by P concentration of roots, and possibly N:P ratio of BG residues.

Research presented in this thesis demonstrates significantly greater amounts of P in BG residues compared to those previously estimated using root recovery methods alone, and that about one-third of total plant P may be partitioned BG. Thus potential P and N benefits to wheat from cycling of break crop root residues are likely to be more substantial than currently thought, and potentially comparable to contributions from an annual P fertilizer addition in low input rain-fed systems. Results further suggest an interaction between release of N and P from BG residues, with an apparent P limitation to the release of N by lupin BG residues; hence C to nutrient ratio of roots was not a good predictor of nutrient release. Lastly, this research also highlights the contribution by root residues of break crops to the longer term fertility of soils, since a large proportion of the BG P and N remains in soil after wheat.

In summary, this work develops greater quantitative understanding of the direct contribution of the BG P and BG N of canola and lupin to wheat in terms of P and N supply, and a greater understanding of P and N accumulation in break crop roots. The adaptation of the stem wick-feeding technique for *in situ* ³³P-labelling of plants opens up exciting future research opportunities in determining the accumulation, fate and interactions of break crop BG P and BG N under undisturbed conditions in following cereals.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Date

PUBLICATIONS ARISING FROM THIS THESIS

Journal articles

- Foyjunnessa**, McNeill, A., Doolette, A., Mason, S., McLaughlin, M.J., 2014. *In situ* ³³P-labelling of canola and lupin to estimate total phosphorus accumulation in the root system. *Plant and Soil* 382, 291-299.
- Foyjunnessa**, McNeill, A., Doolette, A., Mason, S., McLaughlin, M.J., 2015. Quantifying total phosphorus accumulation below-ground by canola and lupin plants using ³³P-labelling. *Plant and Soil* (online first).
- Foyjunnessa**, McNeill, A., Doolette, A., Mason, S., McLaughlin, M.J., 2015. Use of ³³P *in situ* the fate of canola below-ground phosphorus, including wheat uptake in two contrasting soils. *Crop and Pasture Science* (accepted).
- Foyjunnessa**, McNeill, A., Mason, S., Doolette, A., McLaughlin, M.J., 2015. Dual-labelling (¹⁵N and ³³P) quantifies relative contributions to nitrogen and phosphorus uptake by wheat from lupin and canola *in situ* below-ground residues. Journal targeted *Plant and Soil* (in preparation).

Conference abstracts

- Foyjunnessa**, McNeill A, Doolette A, Mason S, McLaughlin M (2014). Direct tracing of the phosphorus contribution to wheat from intact root residues of lupin. National Soil science Conference, MCG, Melbourne. November 2014.
- Foyjunnessa**, McNeill A, Doolette A, Mason S, McLaughlin M (2014). Using ³³P to quantify phosphorus accumulation below-ground by canola and the contribution to following wheat. Phosphorus in Soils and Plants Symposium, Montpellier, France. August 2014.

ACKNOWLEDGEMENTS

This PhD thesis would not have been possible without the guidance and continuous encouragement of Ann McNeill, my principal supervisor. I am equally thankful to all my supervisors, Ann McNeill, Ashlea Doolette, Sean Mason and Mike McLaughlin for the time and effort they have devoted to supervising me as a PhD student. I am very fortunate and grateful for the opportunity to undertake my PhD journey under their positive, professional guidance and to be a part of wonderful soils group.

I would not have been able to carry out my experiments and analysis without the help of Md Mobarouq Ahsan Chowdhury, Ashleigh Broadbent, Colin Rivers, Bogumila Tomczak, Caroline Johnston and Philippa Tansing. I would also like to thank my friendly fellow students of the soils group, in particular Yulin Zhang, Melinda Moata, Daniela Montalvo, Courtney Peirce, Cuicui Zhao and Sarah Noack for the great times shared and their support through my candidature.

I greatly appreciate the support of Ron Smernik for his independent advice on my Core Component of the Structured Program (CCSP) and his scientific writing class that help me to write scientific papers. Many thanks must go to Murray Unkovich for his support and help with my EndNote issues, Margaret Cargill for her advice and patience in my writing and Cam Grant for his fantastic support as the Postgraduate Coordinator.

I would like to thank CSIRO for the use of their radioisotope facilities and allowing me access to sample their field trial sites. Thanks to Waite Analytical Services and The University of New England, Armidale for sample analysis.

I would like to acknowledge the Grains Research and Development Corporation (GRDC) for their generous project funding through the project GRS10026, travel award funding from the

Crop Nutrition Trust, Soil Science Australia and the Young Scientist Workshop (YSW) Grant
– SPS 2014 (France).

My greatest thanks are reserved for my loving husband Mobarouqul Ahsan Chowdhury and my super patience son Faiyaj Abrar Chowdhury who have been there through all the ups and downs. I truly believe I could not have completed my PhD without the support and encouragement of my family.

This thesis is dedicated to the memory of my Father, Mohiuddin Ahmed, who served his whole career life working in the area of plant pathology, and encouraged me to take the opportunity to study agricultural science to become a scientist. Finally, I always remember his last speech, *“No matter how many awards you receive in your life, being a good human with honesty is the most rewarding thing of your life even though the pathway is often cruel and painful”*.

FOYJUNNESSA . (Candidate)

Signed

Date

STRUCTURE OF THE THESIS

This thesis is presented as a combination of chapters that have been published, are in press, have been submitted for publication or are soon to be submitted for publication.

Chapter 1 provides an overview of the literature highlighting the importance of organic sources including above- and below-ground crop residues. More specifically it discusses P and N release from root residues and the subsequent benefit to cereal with a focus on break crops in rotation. This chapter also includes the proposed objectives of this study along with the research hypotheses.

Chapter 2 provides an estimation of the magnitude of root P from crop species collected in the field. This chapter also examines the effects of stem-wick ^{33}P feeding at different plant growth stages on the recovery of the isotopes in the shoots and roots of lupin grown under glasshouse conditions.

Chapter 3 comprises a paper that has been published in *Plant and Soil*. This paper describes a technique that was developed from ^{15}N studies to label break crop root P *in situ* using ^{33}P stem wick-feeding.

Chapter 4 comprises a paper that has been published in *Plant and Soil*. It describes the differences in root recovery between, two crop species and in soils with contrasting textures and ultimately provides an estimation of total below ground P.

Chapter 5 comprises a paper that has been submitted to *Crop and Pasture Science*. It describes the fate of below-ground P including root-derived P from mature canola into the following wheat phase and differences between soil textures.

Chapter 6 comprises a paper that will be submitted to *Plant and Soil*. It describes the dual-labelling of ^{33}P and ^{15}N in canola and lupin *in situ* and provides an insight to the uptake of the below-ground P relative to below-ground N by the following wheat.

Chapter 7 provides a synthesis of the findings contained in this thesis and includes recommendations for future research.