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

FOYJUNNESSA

School of Agriculture Food and Wine
The University of Adelaide

July 2016

TABLE OF CONTENTS

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 mg N plant^{-1}) than canola 68 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* ^{33}P-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.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that the copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

FOYJUNNESSA . (Candidate)

Signed Date

Publications arising from this thesis

Journal articles

Foyjunnessa, McNeill, A., Doolette, A., Mason, S., McLaughlin, M.J., 2014. *In situ* ^{33}P-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 ^{33}P-labelling. *Plant and Soil* (online first).

Foyjunnessa, McNeill, A., Doolette, A., Mason, S., McLaughlin, M.J., 2015. Use of ^{33}P *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 (^{15}N and ^{33}P) 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 ^{33}P 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 Mobaroqul 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 Mobaroqul 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.