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

FOYJUNNESSA

School of Agriculture Food and Wine
The University of Adelaide

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

P in global cropping systems 5

N in global cropping systems 8

Conclusion 10

Soil-plant P and N cycles - regulating fertility in agricultural systems 11

P cycle 11

N cycle 13

Phosphorus and N in break crop residues AG and BG 16

Amounts and chemical speciation of P and N in AG break crop residues 16

Amounts of P and N in BG break crop residues 19

Difficulties in measuring total root mass and nutrient accumulation 21

Isotope studies to quantify BG N and P 22

Conclusions 25

Phosphorus and N cycling of break crop residues 26

Residue quality parameters 26

Proportion and amounts of N and P 'released' from break crop residues 28

Release of P by break crop residues and uptake by subsequent plants 28

Release of N by break crop residues and uptake by subsequent plants 32

Conclusions 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

Field core collection and plant density counts 57

Extraction of roots using washing or freeze drying method 58

Dry matter (DM) and nutrient analysis of clean root samples 59

Statistical analysis of data 59

Results and Discussion 61

Study 2: Effectiveness of stem wick-feeding ^{33}P for labelling roots of lupin 65

Materials and Methods 66

Details of experiment – design, set-up, nutrition and watering regime 66

^{33}P Stem wick-feeding 67

Sampling and analysis of plants 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

Details of experiment 72

Sampling and analysis 72

Results and Discussion 72

Overall conclusions 74

References 76

Chapter 3: *In situ* ^{33}P-labelling of canola and lupin to estimate total phosphorus accumulation in the root system 81

Abstract 85

Introduction 85

Materials and method 86

Experimental set up 86

^{33}P feeding 87

Sampling and processing 87

Measuring of microbial biomass P in bulk and rhizosphere soil 87

Statistical analysis 88

Results 88

Plant dry matter and P content 88

Recovery of ^{33}P and specific activity of shoot and root 88

Microbial P 88

Estimation of fine root P 88

Discussion 89

Conclusion 91

Acknowledgments 91

References 91

Chapter 4: Quantifying total phosphorus accumulation below-ground by canola and lupin plants using ^{33}P-labelling 95

Abstract 101

Introduction 101

Materials and method 103

Soil collection and potting 103

Details of the experiment 103

Harvesting, radioactive sample processing and analysis 104

Calculation of root-derived P in bulk and rhizosphere soil, and total below-ground P 105

Statistical analysis 105

Results 105

Plant dry weight, P content and shoot to root ratio 105

Shoot to recovered ratio 105

^{33}P distribution and recovery in soil-plant system and specific activity of shoot and recovered roots 106

Root-derived P in bulk and rhizosphere soil and total below-ground P 106

Estimates of unrecovered root dry weight 107

Discussion 108

Direct estimation of total BGP 108

Influence on BGP estimation of fed ^{33}P distribution 108

Roots as a sink for stem-fed ^{33}P 109

Shoot: root ratios 110

Conclusion 110

Acknowledgments 110

References 110

Chapter 5: Use of ^{33}P 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

Soil characteristics 120

Canola phase of the experiment 120

^{33}P labelling of canola 121

Fallow & wheat phase of experiment 122

Sample harvesting, processing and analysis for both phase of the experiment 123

Calculation, assumptions and statistical analysis 125

Results 126

Canola phase 126

Plant dry weight, P concentration and P content of mature canola plants 126

^{33}P activity below-ground and specific activity of recovered roots at two soil depths (0-10 cm and 10-35 cm) 126

Estimates of RDP and $DW_{unrecrt}$ for canola at maturity 129

Recovery and distribution of fed ^{33}P in the mature canola-soil system 131

Wheat phase 131

Plant dry weight, P concentration and P content of wheat after canola 131

Distribution and recovery of canola total $BG^{33}P$ in the subsequent wheat-soil system 132

Discussion 133

Below-ground P input by canola at maturity 133

Fate of mature canola BG P 136

Agronomic significance of canola BG P 140

Technical consideration of the isotope technique for estimating BG P 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 *in situ* below-ground residues 157

Abstract 161

Introduction 163

Materials and method 167

Soil preparation and pot set up 167

Break crop phase-details of the experiment 168

Dual-labelling (^{15}N and ^{33}P) of break crops using stem wick-feeding 169

Fallow and wheat phase 170

Sample processing and analysis 170

Estimation of canola and lupin RD P and RD N and calculation of total BG P and total BG N 172

Calculation of P and N in wheat derived from (df) BG P and BG N of the previous lupin or canola 173

Statistical analysis 174

Results 174

Canola and lupin total plant dry matter, N and P concentration and content 174

Recovery and distribution of fed ^{33}P and ^{15}N excess in canola or lupin, wick and soil 176

Amounts of total BG P and BG N and proportion as root-derived P (RD P) and root-derived N (RD N) 178

C: N and C: P ratio of recovered roots and N:P ratio for shoot, recovered root and root-derived fraction 179

Wheat plant dry matter, P and N concentration of shoot and recovered root 180

Recovery and distribution of lupin and canola BG^{33}P and BG^{15}N excess in subsequent wheat 180

Amounts of P and N in wheat derived from lupin or canola BG N and BG P 182

Discussion 183

The relative contribution of N and P to wheat from canola and lupin in situ BG residues 183

Is apparent mineralisation related to ratios between carbon, nitrogen and phosphorus? 186

Allocation of N and P BG in lupin and canola at maturity 189

Partitioning of labelled N and P above- and below-ground in lupin and canola 190

Potential errors associated with the use of isotope-labelling in this study 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

Corrigendum to Chapter 6 in PhD Thesis Foyjunnessa 2016

The data in this chapter has been substantially revised since publication of the thesis.

The reader is referred to the following publication for the updated presentation and interpretation of data from this chapter:

Foyjunnessa, McNeill, A., Doolette, A., Mason, S., 2018. Dual-labelling (^{15}N and ^{33}P) provides insights into stoichiometry and release of nitrogen and phosphorus from *in situ* mature lupin and canola below-ground residues. *Plant and Soil* **426**, 77-93.

November 2018

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.

CHAPTER 1

GENERAL INTRODUCTION

AND

REVIEW OF THE LITERATURE

1. General Introduction

It is commonly acknowledged that cycling of nutrients, including nitrogen (N) and phosphorus (P), 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 N and P as fertilisers or manures are limited. Furthermore, the per annum efficiency of applied fertiliser worldwide is reported to be less than about 50% for N (Raun and Johnson 1999) and 30% for P (McBeath et al. 2012) and therefore in many situations a large proportion of the N (as much as 80%) and P (as much as 95%) taken up by crops will be derived from other sources. The primary alternative source of plant-available nutrients is considered to be soil organic matter decomposition, including N and P mineralised from recently added plant residues, although the desorption of residual inorganic fertiliser is likely to be of importance for P but this isn't the case for N, and less likely is the release of any fixed ammonium for N. Hence, in crop rotation systems legume and oilseed break crops are valued not only for the cereal root disease break they generally provide (Cook et al. 2002; Kirkegaard et al. 2008a; Norton et al. 2013), but also for their potential contribution to soil organic matter cycling *via* unharvested plant components such as stems, leaves and roots (i.e. residues) that decompose and may benefit the nutrition of a following crop.

Optimal management of any N and P cycled from break crop residues requires a sound understanding of the quantity of each nutrient in residues and what proportion of it will potentially become available for a following cereal crop. These factors have been reasonably well-described for the above-ground (AG) residues of some break crop plants but are much less well understood for the below-ground (BG) residues, inclusive of both roots and rhizodeposits such as sloughed root cells and root exudates. One key reason for this gap in

the knowledge is the inherent problem that regardless of the technique adopted it is almost impossible to fully recover entire root systems from soil, which leads to inaccuracies in quantitative assessment of root mass and nutrient inputs from roots to soil. Isotope techniques have been used to address this issue and have provided improved estimates of BG N accumulation by break crops (Arcand et al. 2013; Mayer et al. 2003; Russell and Fillery 1996a; Yasmin et al. 2006), as well as quantitative information regarding the fate of break crop BG N in a following cereal crop phase (Arcand et al. 2014b; McNeill and Fillery 2008). However, similar information for BG P is lacking and hence estimates of the quantities of P input by break crop root systems are rudimentary (Damon et al. 2014), and furthermore the stoichiometry of BG N and BG P is not well described.

This review briefly considers global cropping system budgets for P and N with reference to the amounts of legume and oil crop residues generated and the potential importance for cycling of nutrients, especially in semi-arid cropping regions such as southern Australia. The key physico-chemical and biological components of soil-plant P and N cycles that regulate fertility in agricultural systems are also briefly described. The major focus of the review is an examination of current knowledge regarding the contribution of unharvested AG and BG residues of break crop species to maintenance of P and N fertility, particularly in low-input cropping systems. Data generated from studies using isotopes to assess N accumulation by crop species as well as N and P turnover from residues of these species is presented. The review highlights knowledge gaps regarding accurate quantification of P accumulation BG by plants and release of that P *via* decomposition *in situ* of undisturbed BG residues. It is concluded that stem-wick feeding ^{33}P to plants alone, or in conjunction with ^{15}N, may be a suitable approach to improve knowledge in this area.

Consideration of the information provided in the review supports a hypothesis that nutrients accumulated in break crop (canola and lupin) roots are a significant contributor to P and N cycling in soils, and hence to the P and N nutrition of a following cereal crop.

2. Global P and N cropping systems budgets

This section briefly presents information from a global perspective, and with reference to Australia, on the important pools of P and N in agricultural soils, uptake of P and N by crops, removal of P and N in produce, amounts of P and N returned as organic matter in crop AG residues, and amounts of N fixed by crop legumes (pulses). Information is presented to highlight the context of this PhD study: - that cycling of nutrients *via* crop residues is potentially an important source of available P and N in Australian semi-arid cropping systems that are characterised by relatively low inputs of fertiliser by world standards (FAO 2015; Liu and Chen 2008) and inherently low soil organic matter (McKenzie et al. 2004).

2.1 P in global cropping systems

Agriculture globally has been mining P from soils since it began and this is exacerbated as yield improvements are made and thus more P is taken off in grain, and is further exacerbated as the area of land under production has increased (Wong et al. 2012). In general, fertiliser P applications, globally, do not match P removed in produce (Hinsinger et al. 2011; Weaver and Wong 2011). Fertiliser P efficiency worldwide is less than adequate, partly due to P sorption reactions in soils – and in Australia many of the soils of the cropping zone are markedly P sorbing, especially where they are low in pH with presence of Fe/Al or high pH with carbonates. There are also some concerns about the length of time that P fertiliser resources will continue, and although this is debated (Cordell et al. 2011; Dawson and Hilton 2011; Van Vuuren et al. 2010) it is accepted that P fertiliser prices are intrinsically linked to

those of the energy (oil) used to produce them, and hence subject to price fluctuations. Furthermore, a large proportion of P reserves are located in countries where long term access for major manufacturers and users of fertiliser P may not be guaranteed due to political or socio-economic volatility (Kauwenbergh 2010). Additions of organic matter (OM) in the form of manures, biosolids, composts and other industrial and agricultural waste products have been shown to contribute to inorganic and organic P pools in soils, with the amount of P generated in animal wastes estimated globally to be in the order of 16-20 million metric tonnes per annum (Liu and Chen 2008). However, use of these organic amendments in cropping systems can be costly due to the transport costs involved if the sources (such as intensive chicken, cattle and pig enterprises) are not adjacent to the cropping enterprise. Increasing P through such OM inputs is not considered feasible for the majority of the semi-arid cropping belt of Australia (McNeill and Penfold 2009) since there are few centres of intensive animal production relative to the extensive areas growing crops. Furthermore mean crop yields for these Australian semi-arid cropping regions tend to be low (<2 t ha^{-1}), and therefore P fertiliser inputs tend to be at the lower end of the range 3-34 kg P ha^{-1} quoted for global applications (Liu and Chen 2008). So, given the constraints evident for P supply to crops in Australian rainfed cropping systems in semi-arid areas the retention of residues may be an important part of the management to increase the cycling of nutrients, including P, in the soil in order to increase overall P efficiency for the system. The adoption of no-till systems has increased, both worldwide and in Australia (Llewellyn et al. 2012). This, coupled with reductions in stubble burning due to concerns about influences on global climate, and an increase in the areas continuously cropped, has meant that returns as crop residues have become even greater.

An analysis of the allocation of P for the world harvest of agricultural products (Table 1) shows that about two-thirds of the P is present in harvested produce, chiefly grains, with the

remainder in AG residues of crops (e.g. straw, stems, chaff). The majority of crop P globally (70%) is present in cereals (Table 1) and apart from P in root crops which is included in the 'other' category in Table 1, there is no account taken of P accumulated by the root systems of crops with this BG portion of the residues of crops generally being ignored in net plant P budgets as it is likely considered as P in soil organic matter. Yet, recent root residues of cereal, oil and pulse crops will be subject to cycling in a similar manner to the AG residues, and in fact may be cycled more readily given they reside in the soil, whereas the AG residues may be incorporated, or can be left as standing stubbles for periods of time, particularly in no-till systems. Furthermore surface applications of fertiliser P have resulted in P becoming highly stratified (concentrated in the top few cm of soil) and this has been amplified by adoption of no till therefore essentially there is little P at depth (>20 cm). Indeed benefits have occurred from placing fertiliser P at depth in environments where top soil is prone to drying (Ma et al. 2009). Phosphorus in root residues may have the advantage of a more

Table 1 Dry matter (DM) and P allocation (Tg yr^{-1}) in world harvest 2005

Crop	Harvested portion of crop		Crop above-ground residues		Total P in harvest plus residues
	Dry matter	P	Dry matter	P	
Cereals	1,968	5.9	2,947	2.9	8.9
Pulses	58	0.3	61	0.1	0.4
Oil crops	102	0.1	92	0.1	0.2
Other *	1,363	1.9	1,063	1.4	3.2
Total	**3,491**	**8.2**	**4,163**	**4.5**	**12.7**

Sources: Data adapted from Liu and Chen (2008) and estimated from FAO Statistics

Tg = 10^{12} g * Other includes forage crops, sugar crops, roots and tubers, vegetables and fruits

diffuse and deeper distribution in the soil than P applied as fertiliser, and as these residues decompose they could provide an important source of P to the following crop at a time when the surface soil is dry, particularly in Australian dry land farming systems. It can be seen (Table 1) that the amount of P in AG residues of oil crops and pulses globally are much less than cereals, but since the phosphorus harvest index (PHI) calculated from this global data is similar for cereals and pulses (66% and 75%) and lower for oil crops (50%) there is an indication that oil crop AG residues may return more P to soil. Indeed, it is reported that P concentrations of cereal residues tends to be lower than those of both oil and pulse crops (Grant and Bailey 1993; Keerthisinghe et al. 1998; Soon and Arshad 2002), so the relative return of P to soil per tonne of residue should be greater from both these crops. Furthermore, some of the P in the residues of these crops, including canola and lupin, may have been accessed from a less or non-labile form in soil *via* excretion of acids (Gardner and Boundy 1983; Grant and Bailey 1993), which can be viewed as a further contribution to the P efficiency of the system by moving P from a non-available pool in the soil to a more dynamic pool in the recent residue. Thus, the residues of these crops may well be an important contributor to P cycling in regard to the rotational systems in southern Australia where diversity of cropping has expanded over the past few decades (Kirkegaard et al. 2011) such that 2.76 M tonnes of oilseeds and 1.78 M tonnes of pulses were sown in Australia in 2010-11 (ABARES 2011), compared to less than 0.5 M tonnes for each in the 1980's).

2.2 *N in global cropping systems*

N inputs to global agriculture croplands are increasing mainly as mineral fertilisers and manures and *via* legume biological nitrogen fixation (BNF), although there is also some due to atmospheric deposition (Conant et al. 2013). These fertiliser and BNF inputs are likely to remain high in order to increase global crop production to match the demand for food

(Tilman et al. 2002). Concurrent with increased production, and in the absence of a change in N HI, there should be an increase in the amount of N being returned to soil in plant residues for cycling. However, it is not always a certainty that yield will increase with additions of N (organic or inorganic) and in some cases yields have declined with increasing fertiliser N. Fertiliser efficiency is less than optimal (30-50%) due to gaseous and leaching losses (Cassman et al. 2002; Smil 1999). In fact Galloway et al. (2004) suggested that when fertiliser N is added to agroecosystems >75% is removed *via* a combination of harvested crop materials, gaseous losses or leaching. The authors also suggested that a smaller amount of N (<10%) is stored in the systems compared to a relatively higher amount of N (10-40%), with greater variability, being denitrified to N_2. Hence, effective N management is a central goal of sustainable agricultural crop production in order to reduce these N losses that have the potential for environmental (air and water) pollution. Optimum application of livestock and human wastes can contribute to increased N use efficiency (Tilman et al. 2002), but as commented previously in regard to P are less likely to be significant inputs for southern Australian dry land farming systems. Whereas, no-till or reduced tillage systems and rotational cropping, as well as the use of cover crops, which have all have been reported to reduce N losses from volatilisation, leaching or erosion (Cassman et al. 2002; Ladd et al. 1981; Smil 1999), should increase N use efficiency, and as mentioned already these practices have increased in recent years in Australia.

Estimates of N in the AG residues of major world crops for the mid 1990's (Table 2) show that although 70% of the N in crops is harvested, and 60% of N resides in cereal AG residues, the N in oil crop and legume residues together is about half that in cereal residues, and 28% of the N present in the residues of all world crops (Smil 1999). This is despite the fact that DM of cereal residues is much greater than that of oil and legume crop residues, and so the calculated average N concentration for cereal residues is 0.6% N, whereas it is 2.5% for

legume and 2% for oil crops. Thus the residues of oil and legume crops are N rich and so should contribute more N to the plant available pool on decomposition. Furthermore, it is important to note that the amount of N calculated for these crop residues does not include N accumulated BG, as mentioned previously in the section on P. Indeed, a review of global N fixation inputs to agricultural systems (Herridge et al. 2008) pointed out this issue in regard to estimates of BNF inputs by legumes based solely on shoot N data only. The authors calculated that if BG N accumulation was accounted for then estimates of the total amount of N_2 fixed globally by the major pulse and oilseed crop legumes would increase by about one-third, from 14.35 to 21.45 Tg N per annum.

Table 2 Estimated DM and N (Tg yr^{-1}) for the world harvest of major crops in the mid-1990s

Crop	Harvested portion of crops		Crop above-ground residues		N in harvest plus residues
	Dry weight	N	Dry weight	N	
Cereals	1,670	30	2,500	15	45
Legumes	190	10	200	5	15
Oil crops	110	4**	100	2**	6
Other*	1,280	16	950***	3***	19
Total	**3,250**	**60**	**3,750**	**25**	**85**

Source: Data adapted from (Smil 1999) and sourced from FAO statistics

* Other includes forage crops, sugar crops, roots and tubers, vegetables and fruits

** includes some other minor unspecified crops *** excludes forage residues

2.*3 Conclusion*

In conclusion to this section it is important to note that unharvested parts of a crop contain P and N that has been accessed from both labile and recalcitrant soil P and N pools, plus in the

case of legumes a portion may be derived from the atmospheric N_2 pool. These nutrients are stored as either organic or inorganic forms in the plant tissues and may become plant available following residue decomposition. Break crops, such as oilseed and legumes, that are known to mobilise P and/or fix N_2 may be particularly valuable for increasing P and N cycling. Global nutrient budgets focus on nutrients returned in AG residues only and ignore those sequestered in BG residues. Rotations where oilseed and legume crops are grown in alternate seasons with cereals are increasingly important components of sustainable agricultural systems (Kirkegaard et al. 2008a). The cycling of nutrients *via* both AG and BG residues of these crops may be especially significant for low input farming systems in southern semi-arid Australia.

3. Soil-plant P and N cycles - regulating fertility in agricultural systems

The following is a brief description of the key pools of P and N in the soil-plant P and N cycles with reference to how agricultural plants may influence those pools both during crop growth and *via* decomposition of residues. Information is presented regarding the amounts and speciation of P and N in AG and BG residues of break crop species, particularly lupin and canola. Difficulties in assessment of total root system biomass are highlighted and results from ^{15}N isotope studies to quantify BG N accumulation including rhizodeposition are discussed. The potential to use P isotopes to provide similar quantification of total BG P accumulation by plants is considered.

3.1 P cycle

There are two major P pools in soil- organic and inorganic (Fig. 1). In the relatively large organic pool there are three different types of P compounds- active organic P (Po) that can release P easily (labile), stabilized Po that can hold P tightly and resist decomposition, and P

contained in microbial biomass which is a very small pool but plays an important role in the organic pool as microbes are responsible for the biological process (mineralisation) where inorganic P is released from organic matter. This biological process is highly influenced by temperature, soil moisture, types of microorganism and the amount of C present in the soil. The inorganic P pool has four different types of P compounds- sorbed P, secondary P minerals, primary P minerals and soil solution P (Fig. 1). From these P compounds some of the P is very available for plant uptake as orthophosphate (PO_4) and very soluble in the soil solution which is a function of soil physico-chemical processes influenced by sorption, desorption and edaphic environment (Fig. 1).

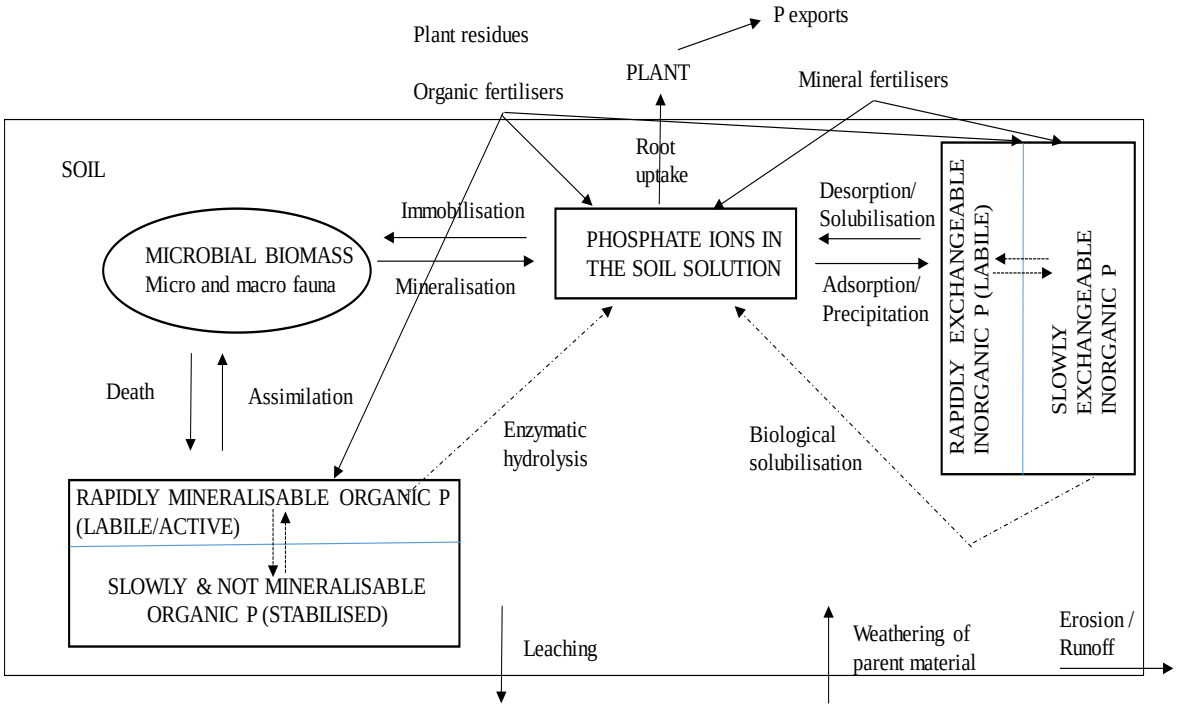


Figure 1. The P cycle in the soil-plant system- redrawn and modified from Frossard et al. (2011)

There are some loss pathways for P in the soil-plant system: soil erosion causes loss of P that is sorbed to minerals and OM, and there is potential for some leaching loss where soil solution P is high in a low P sorption soil.

Phosphorus uptake by plants *via* roots ultimately reduces total soil P when plant biomass (grain) is removed as occurs in broad acre cropping in agriculture, whereas the P cycle in natural ecosystems is considered closed, where fluxes are fast and P moves from one pool to another but rarely gets removed in harvested produce or lost *via* leaching or erosion. Active plant roots take up P from the soil solution but can influence soil P pools in several ways. For example some plants can excrete acids to solubilise less labile P or have specialised roots with greater surface area to access more solution P. Other plants take up P *via* vesicular-arbuscular mycorrhizal (VAM) that form a symbiotic relationship on their root systems, although not all agricultural plants do this e.g. canola and lupin plants are not mycorrhizal. The solution pool in agricultural systems is managed *via* fertiliser P addition largely, although organic sources like manures (Oberson et al. 2010) and plant residues (as will be further discussed later) have varying amounts of soluble P in them so they may also influence this pool, whereas the organic P pool is affected by inputs of OM from living roots, wastes imported from elsewhere or recycled *via* AG and BG residues.

3.2 N cycle

The major N pools in soil are organic and inorganic (Fig. 2). However N in soil is predominantly present in an organic form (>90%) in most environments, and highly influenced by biological processes, climatic conditions that affect soil moisture and temperature, and soil chemical and physical properties largely dictated by texture. Soil N availability is determined by the active soil N pools (Deng et al. 2000; Duxbury et al. 1991) and this mineralization-immobilization turnover (MIT) or short-term biological cycling of N is primarily controlled by microbial cycling (Fig. 2). There are three different active N pools in soil; inorganic N, mineralisable N, and microbial biomass N, and living soil microorganisms play an important role in each of these soil pools (Deng et al. 2000; McCarty

et al. 1995). Nitrogen present in the organic pool as crop residues, soil OM and manures can be converted to available inorganic N (NH_4^+) by soil microorganisms, and can be made readily accessible to plants through mineralization process followed by nitrification (NH_4^+ to NO_2^- to NO_3^-).

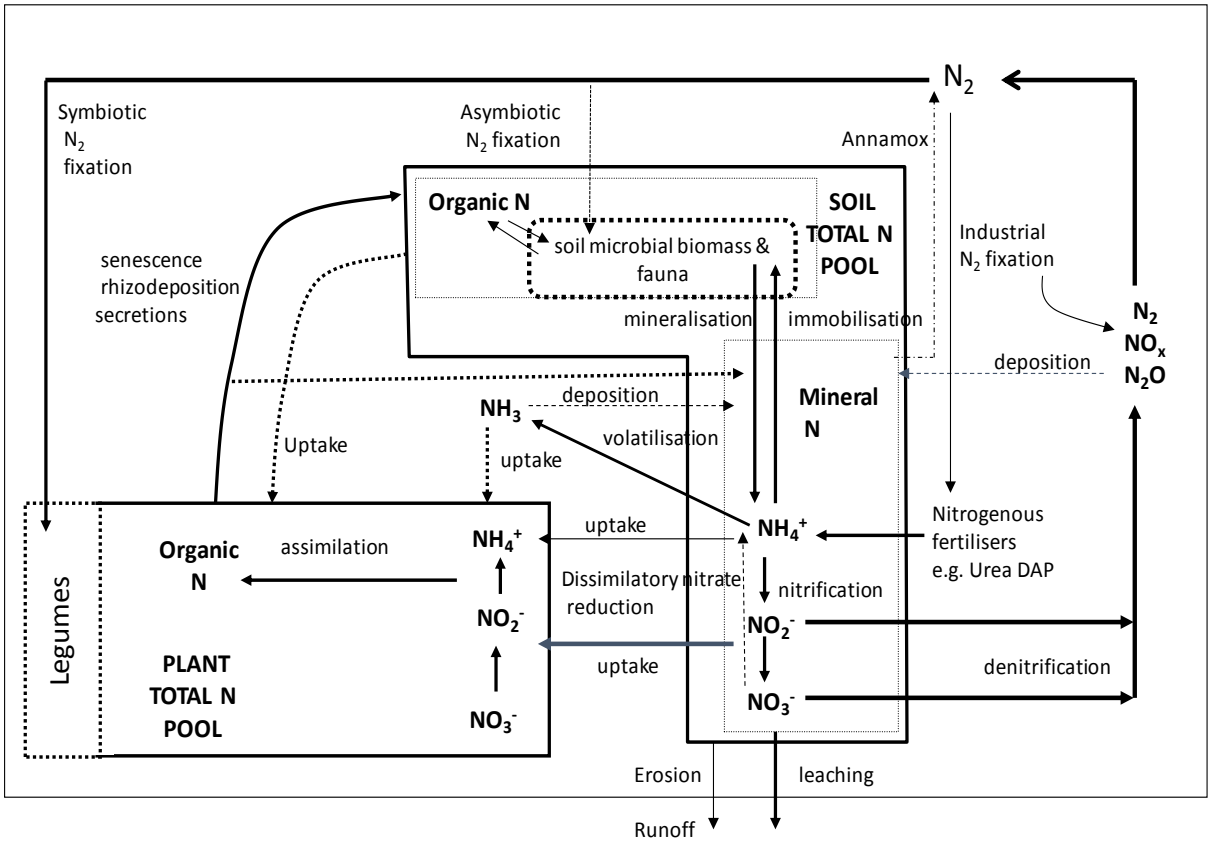


Figure 2. Important pools, processes and fluxes of N in the soil-plant system (redrawn and modified from McNeill and Unkovich (2007))

Although atmospheric N_2 is highly abundant, comprising 79% of the atmosphere and an almost infinite source of N, it is unavailable to most plants. However many crop and pasture legumes can biologically fix and convert atmospheric N_2 to a plant available form through a symbiotic relationship with rhizobia. These amounts may be substantial, with an average of 98.5 kg N ha^{-1} reported fixed by annual narrow-leaf lupin in the south-eastern region of

Australia (Evans et al. 1989), and 222 kg N ha^{-1} reported for the same crop in the western region of Australia when N in roots recovered by coring was included in the measurement (Unkovich et al. 1994). Hence many crop legume species have the potential to input additional N to the soil as unharvested crop residues including root residues. Atmospheric N_2 can be fixed *via* non-symbiotic fixation, by some soil microrganisms, particularly in tropical regions, but its significance in most cropping systems is minimal, estimated at less than 10kg/ha in low rainfall areas in Australia (Unkovich and Baldock 2008). In terms of physico-chemical process in the N cycle, some ammonium fixation may occur in certain heavy textured soils but overall it has far less influence on N availabiity in soils than sorption does for P availability in soils.

Loss pathways are more reactive for N than for P since NO_3^- is highly water soluble and can be leached easily particularly in sandy soils. There is evidence that nitrate derived from decomposition of N-rich AG and BG residues of lupins will leach on sandy soils (McNeill and Fillery 2008; Russell and Fillery 1999) and management to minimise this can be difficult due to a general disconnect for Australian systems in the demand for N by crops and the supply from residues *via* mineralisation (Angus 2001). Ammonia volatilization may be a significant pathway for fertliser N loss when ammonium-based fertilisers are applied on alkaline soils under warm wet conditions (Sommer et al. 2004), and again there is potential for similar loss pathways from plant residues although most reports are for green manures (immature material) not dessicated mature residues, or as loss from growing plants (Bouwman et al. 1997; Wetselaar and Farquhar 1980).

As mentioned in the preceding sections, break crops can directly affect soil P and N cycles during a growing period *via* root-associated activities and at maturity *via* return of AG and

BG residues to soil that contain N and P, and the latter will be explored further in the rest of this review.

3.3 Phosphorus and N in break crop residues AG and BG

Amounts of P and N in plant material can be calculated from measurements of dry matter and P or N concentration and next section present this information for AG and BG residues of break crops. The difficulty with quantifying total BG nutrient accumulation by plants due to incomplete root recovery when sampling is discussed as this relates to the development of isotope techniques for more accurate quantification of partitioning of N and P BG. The chemical speciation of N and P in plant residues is also considered in relation to the concept of residue quality parameters as drivers for cycling of nutrients.

3.3.1 Amounts and chemical speciation of P and N in AG break crop residues

Dry matter partitioning AG between the harvested crop materials (grains or seeds) and the residues is generally expressed as the harvest index (HI) and similarly the partitioning of P or N can be expressed as an N HI or a P HI. Amounts of N and P in mature AG residues of break crops are generally considered a relatively minor proportion of whole plant P and N at maturity due to the strong sink for nutrients in harvested grain. Reports for canola suggest a P HI range of 70-80% (Jackson 2000; Rose et al. 2007; 2008) with a similar P HI for lupin of 75%, as calculated from data in Bolland et al. (1989) and also a 75% N HI for pulses in the field in Canada (Gan et al. 2010). Reports for canola N HI are not dissimilar to P HI, ranging 70-80% (Gan et al. 2010; Hocking et al. 1997; Schjoerring et al. 1995; Svečnjak and Rengel 2006), although slightly lower in water-stressed canola (54%). Similarly N HI of 46% was calculated from a field N balance for lupin in semi-arid Western Australia (Unkovich et al. 1994). Partitioning of dry matter and nutrients in crops is a function of many things including the edaphic and climatic environment, stage of growth of the plant which influences the sink

for nutrients, and plant physiological, genetic and morphological attributes, particularly in relation to acquisition of nutrients by roots. Specific plant morphological features such as extensive root systems and root hairs as observed for canola (Grant and Bailey 1993; Liu et al. 2010) may facilitate greater uptake of nutrient, or plant may have physiological adaptations such as the excretion of organic acids by roots of various legume species including lupin that solubilise P (Keerthisinghe et al. 1998; Neumann and Römheld 1999). As mentioned earlier (in sections on P and N cycles) some plants may have symbiotic associations of roots with VAM fungi that facilitate P uptake or with rhizobia that fix atmospheric N_2. Hence it is not surprising that crop residues of different or even the same species may vary in P and N content.

Values for the concentration of P for AG residue materials from vegetative and mature break crop species from field and glasshouse trials range from 1.1 to 9.0 g P kg^{-1} dry matter (Gardner et al. 2002; Hocking 2001; Khan et al. 2002; Lupwayi et al. 2007; Mat Hassan et al. 2012; Noack et al. 2012; Nuruzzaman et al. 2005). Values for N content of grain legumes residues (stubbles, leaves, stems, pods and roots) are typically 0.5-1.5% N with roots tending to be lower than for stubbles (Farrington et al. 1977; Gladstones and Loneragan 1975; Russell and Fillery 1996a; Russell and Fillery 1999; Unkovich et al. 1994). Arcand et al. (2013) measured 0.32% N for mature canola AG residues in a glasshouse study and similarly Svečnjak and Rengel (2006) reported 0.35 to 0.59% N for stems and fallen leaves of mature canola depending on fertiliser N application. Field data is also in the same range with 0.59 - 0.7% N for canola straw reported by Soon and Arshad (2002).

Reports of field measures for amounts of P and N physically retained in AG residues after harvest of canola and lupin break crops have increased in the last few years but are still not all that common, compared for example to field data for tropical crops. The scant field data

for canola suggests returns of P at harvest from 2.4-12 kg P ha^{-1} in residue dry matter ranging from 2.1-12.7 t ha^{-1}(Bünemann et al. 2006; Jackson 2000; Lupwayi et al. 2007), and 2.2-3.1 kg P ha^{-1} for lupin (Bolland et al. 1989). Whilst Unkovich et al. (1994) reported 80 kg N ha^{-1} in the unharvested stubble and fallen leaves of narrow-leaved lupin in Western Australia, and Gan et al. (2010) a mean of 30.6 and 28.2 kg N ha^{-1} respectively in pulse and oilseed straw at maturity. It is not always easy to obtain representative samples of mature plant residues as they can be fragile and easily disintegrate and disperse, especially in the field and hence accurate DM assessment is often an issue. Nevertheless, there is definitely a requirement for more empirical field measures of these variables for these crops in temperate agriculture to facilitate increased assessment of their significance and a greater understanding of how they affect P and N cycling in agricultural soils.

Measurements of the chemical nature of residues tend to have focussed on AG rather than root residues, and are more commonly found for immature rather than mature plant materials. However, a recent study of plant stem residues for mature wheat, pea, lupin and canola, demonstrated that a large proportion (25 to 75 %) of the P in these residues is in an inorganic form (orthophosphate), potentially readily available to plants and soil microbes (Noack et al. 2014a). Whereas, solid-state ^{15}N NMR of 76 days old shoot and root residues for a range of species including wheat and pea (Smernik and Baldock 2005) suggested that the majority of the N was in a fairly recalcitrant form since only 2-17% was measured as amide and very little (<5%) was as nitrate. Differences between the forms of P and N in residues may be attributed to P being more associated with labile cell components (e.g. ATP, lipid membranes) whereas N may be more equally distributed between soluble and less soluble fractions as shown by Smernik and Baldock (2005). Iqbal (2009) also using NMR, reported for residues of a number of break crop species including lupin and canola, that the chemical

and biochemical properties varied depending on stage of maturity of crop residue and also differed between plant parts.

3.3.2 Amounts of P and N in BG break crop residues

Phosphorus concentrations of oilseed and grain legume crop plant roots have been mainly reported from controlled environment studies (Föhse et al. 1991; Iqbal 2009; Liu et al. 2013; Nuruzzaman et al. 2005) with scant field data for P in mature break crop roots (Nebiyu et al. 2014; Soon and Arshad 2002), and the number of break crop studies that report both N and P are relatively rare (Soon and Arshad 2002). Some reports suggest a higher P concentration for roots of grain legumes than canola; for example 1.3-2.0 g P kg^{-1} for lupin (Mat Hassan et al. 2012), 1.7 g P kg^{-1} for faba bean (Alamgir et al. 2012) grown in the glasshouse, and 1.54-1.58 g P kg^{-1} for field grown pea (Soon and Arshad 2002), compared to P concentrations for canola roots from glasshouse grown plants of 0.057-1.42 g P kg^{-1} depending on P fertiliser application (Noack et al. 2014a), and 1.22-1.44 g P kg^{-1} for canola roots over a two year field study (Soon and Arshad 2002).

Generally roots have lower concentrations and amounts of nutrients than AG residues as reported for N in lupin roots compared to stubbles (Unkovich et al. 1997) and for N in pea roots compared to straw (Soon and Arshad 2002); although this is not always the case, e.g. P concentration of mature pea stem and leaf (AG) residues collected from an agricultural crop in South Australia was similar (0.75 g kg^{-1}) to that of canola roots from the same region (Noack et al. 2014a; Noack et al. 2014b). Indeed, in the field study of Soon and Arshad (2002), mature canola roots actually had higher P (1.22-1.44 g P kg^{-1}) and N (8.57-8.84 g N kg^{-1}) concentration than canola straw (0.81-1.29 g P kg^{-1} and 5.86-7.04 g N kg^{-1}) but there was an order of magnitude difference in the DM of the AG residues compared to that for the recovered roots which resulted in much smaller calculated amounts of N and P in BG

compared to AG residues. More accurate data is clearly required for root biomass in order to clearly quantify how much P and N there is in roots of crops in the field, and hence if these amounts may be significant for P and N cycling.

It is clear from some glasshouse studies that roots can potentially comprise a reasonably high proportion of total plant P and N (Table 3). Nearly one-third (28 %) of the total plant P was measured in the roots of mature chickpea, with a similar proportion (26%) but lower amounts for roots of white lupin (Mat Hassan et al. 2012). Roots of mature faba bean and narrow-leafed lupin also contained reasonably large proportions (19 and 17 % respectively) of total plant P in roots (Table 3). Similar unpublished data for N (Table 3) also shows that the proportion of N present in roots of mature faba bean, chick pea and white lupin plants can be reasonable (14 to 24 %). Whilst a study by Iqbal (2009) reported no significant differences in

Table 3. Total P and N content (mg pot^{-1}) for roots of mature grain legumes and root N as a proportion (%) of total plant N

Legume species	Root N (mg pot^{-1})	Root N (% of total plant N)	Root P (mg pot^{-1})	Root P (% of total plant N)
Faba bean	78	24	15	19
Chick pea	22	14	21	28
White lupin	31	18	9	26
Narrow-leafed lupin	-	-	7	17

P data from Mat Hassan et al. (2012), and N data from McNeill and Unkovich (unpublished)

mature shoot and root P in wheat and lupin in terms of concentrations of total and water soluble P, it was found that total P concentration in canola roots were almost 5 times higher (2.3 g kg^{-1} DM) than wheat residues (0.6 g kg^{-1} DM) and lupin (0.8 g kg^{-1} DM).

3.3.3 Difficulties in measuring total root mass and nutrient accumulation

One of the major impediments to accurately quantifying the total accumulation of nutrients by plants belowground, especially for field situations, is the difficulty in isolating total root systems from soil in order to quantify biomass and to obtain a representative sample to determine nutrient concentrations. Although many root studies have relied on soil cores and minirhizotron observations (Cheng et al. 1991; Zobel 2008), root data from these studies does not represent total root systems of the crops as fine roots are often unrecovered during extraction from cores and minirhizotrons tend to record a limited portion of the root system. It is time consuming and expensive to recover roots in a realistic and non-destructive manner and be certain that all roots are recovered (Wichern et al. 2008) even when a very fine sieve is used (Gasser et al. 2015; Liu et al. 2010; Mayer et al. 2003). Furthermore, by maturity it is harder to extract intact root systems due to the age and fragility of the roots, the fact that they have started to decompose, and also often the dry soil conditions.

Measuring BG biomass and nutrient concentration (P and N) are subject to relatively larger errors compared to AG biomass (Hoad et al. 2001). These inaccuracies and the general paucity of information on root dry matter and nutrient content occur because fine roots are hard to quantify, and root P is relatively harder to trace compared to N due to the short half-life of P radioisotopes (^{32}P/ ^{33}P). P radio tracer (^{32}P) has been used but to look at root distribution rather than to determine root biomass *in situ* (Abbott and Fraley Jr 1991; Rennie and Halstead 1965).

3.3.4 Isotope studies to quantify BG N and P

During the last two decades there has been an increase in studies labelling plants '*in situ*' with ^{15}N stable isotope using a stem wick-feeding technique. The aim being to improve estimates of BG N accumulation in root systems of crop legumes and oilseeds by accounting for N in fine roots that are not recovered using standard physical root recovery methods (Arcand et al. 2013; 2014a; Arcand et al. 2014b; Khan et al. 2002; Mahieu et al. 2007; McNeill and Fillery 2008; Russell and Fillery 1996a; Wichern et al. 2007; Yasmin et al. 2006). There do not appear to have been any similar studies for estimating BG P accumulation. These ^{15}N studies of BG N use the specific enrichment of recovered roots, usually obtained by sieving the soil through a 1-2mm mesh sieve, in order to estimate unrecovered root N from the amount of enriched N in the bulk soil from which the labelled roots have been removed. Unrecovered root N and total BG N are calculated as shown in the following equations taken from McNeill (2001):

Specific enrichment of coarse root

= ^{15}N Enrichment of coarse root/N content of coarse root

Estimated amount of root N in bulk soil

= total ^{15}N excess in bulk soil / Specific enrichment of coarse root

Total below-ground N = N content of coarse root + estimated root N in bulk soil

It is clear from discussion in the literature cited above that assumptions underlying this ^{15}N approach for estimating BG N are debated. The major assumption, common to all studies using isotopes, is that uniform labelling of the discrete pool of interest is obtained. Clearly, it is simplistic to describe a root system as a discrete pool since it is a spatially and temporally non-homogeneous dynamic entity, with processes such as exudation, sloughing of root cells, mucilage production and loss of root hairs all contributing in varying degrees to movement of

N from the intact root system into the rhizosphere. Production of these root-derived materials has been collectively termed rhizodeposition (Schenck zu Schweinsberg-Mickan et al. 2010) and has been shown to occur over weeks to months with estimates that plants can annually release 7-27% of the total plant mass annually as rhizodeposition (Lynch and Panting 1980). Many of the ^{15}N studies quantifying BG N accumulation include rhizodeposition of N in estimates of total plant BG N accumulation. Whilst some studies do investigate the proportion of root-derived N in labile soil N pools (microbial and mineral) there appears to be only one study (McNeill and Fillery 2008) that not only differentiated the ^{15}N in the bulk soil after recovery of ^{15}N-labelled roots in the microbial and inorganic N pools, but also partitioned the organic N pool into soluble and 'insoluble' fractions, and reported that 36-60% % of the ^{15}N in the bulk soil (depending on soil type) was associated with this more insoluble or recalcitrant fraction. It was suggested this was evidence that a large proportion of the plant-derived N in soil was therefore likely to be residing in fine root material, a suggestion supported by a much earlier study which demonstrated the rapid and permanent accumulation of stem-fed ^{15}N in the insoluble N fraction of pea roots (Oghoghorie and Pate 1972). Other evidence that suggests assimilation and partitioning of fed ^{15}N to non-labile N components in roots is given in a more recent study where there was no apparent redistribution of stem-fed ^{15}N from the roots of white lupin and pea, at least in the short term (Wichern et al. 2011). Further evidence is suggested by data from a study of several grain legumes, pea, lupin and faba bean (Mayer et al. 2003) which showed that the sum of ^{15}N in three N pools in soil - micro-roots, microbial biomass and mineral N, only accounted for 28-52% of the total ^{15}N measured BG.

Another assumption underlying ^{15}N feeding of plants to label roots is that no immediate leakage of highly enriched N from the root occurs. Opinion is clearly divided on this issue with a recent study suggesting it may occur and cause substantial errors in estimation of root-

derived N (Gasser et al. 2015), whilst others consider it is not highly likely to occur (Gardner et al. 2012; McNeill 2001). The potential for leakage may be related to the frequency of feeding or the N concentration of the fed solution (Mahieu et al. 2009), and these clearly require careful consideration to avoid supplying disruptive amounts of isotope or nutrient to plants.

Nevertheless, despite a potential for errors, the isotope approach for estimating N in roots that are unrecovered from soil represents some advance over not accounting for this fraction at all. Estimates for the proportion of N unrecovered by standard root recovery for a single particular break crop species can be in broad agreement across studies; for example, lupin roots recovered using a 2mm mesh sieve contained 21-45% of the total estimated BG N in one study depending on soil type (McNeill and Fillery 2008) and 35% in another study using the same recovery method (Russell and Fillery 1996b). A study that used a 2mm sieve for the bulk soil plus a 0.5mm sieve for the small amount of rhizosphere soil reported slightly lower values for other break crop species - 23% of estimated total BG N for canola as recovered roots, 19% for field pea (Arcand et al. 2013), and less (15%) for lentil (Arcand et al. 2014b). Mayer et al. (2003) reported lower estimates of the % of total BG N as roots recovered by hand for lupin (7%) than previous studies, but similar proportions to grain legumes in other studies for faba bean (16%) and pea (31%), although they also measured a further 20% for each species in the mineral and microbial pools together, as has been similarly reported for lupin (McNeill and Fillery 2008).

Methods for root recovery do vary across studies, most specifically the mesh size used for sieving bulk and/or rhizosphere soils, and furthermore there are not enough studies reported for any one species to undertake robust comparisons. Nevertheless, some confidence in the validity of these estimates of total BG N accumulation by legumes generated using ^{15}N is

suggested given their application in adjustments to global N_2 fixation budgets (Herridge et al. 2008; Peoples et al. 2009) and in simple linear models for estimation of N_2 fixation (Unkovich et al. 2010).

An important question arises as to whether P radio-isotopes could be used for estimation of total BG P accumulation by plants root systems in the same way that ^{15}N has been used. An earlier review (Abbott and Fraley Jr 1991) of radiotracer methods used in root studies suggested that they could be used to provide qualitative information on the distribution of living roots but not on root quantitative measures due to non-uniform labelling of root systems in some species. In this review it was also highlighted that non-uniform labelling for different parts of the root system may be a potential problem in the use of ^{32}P as a radiotracer for quantifying roots. Indeed, another study using ^{32}P to label wheat (using stem injection method) supports the above mentioned summary (Racz et al. 1964) as ^{33}P movement across the root systems varied with plant age. However, other studies using ^{32}P stem injection reported that there was rapid movement of ^{32}P into the root system and was uniformly distributed (Halstead and Rennie 1965; Rennie and Halstead 1965). McLaughlin et al. (1987) also reported a similar rapid translocation to roots for wheat that was stem-injected with ^{33}P. Overall, there seems to be a potential to use P radio-isotope to investigate P accumulation in root systems although the feasibility requires investigation.

3.4 Conclusions

Whilst nutrients in AG residues can be easily quantified and chemically characterised the amount of information for mature AG residues is still relatively limited and very scarce for mature BG plant residues. There is less known about the accumulation of P BG by break crops relative to N, although the accuracy of estimation for BG N is still debated. Nevertheless, current data for BG P accumulation in roots of crop species is likely to be an

underestimate of the true value due to difficulties in recovering all roots from soil. Isotopes offer a way forward for investigating accumulation of P in roots and to gain insight into root P dynamics and P associated with rhizodeposition. However, feasibility studies need to consider limitations to the use of radio-isotope tracers such as half-life, safety and suitability for field application. Improved quantitation of the amounts of P sequestered BG by plants should facilitate more robust models of P partitioning in break crops and could improve inputs to models of P cycling from BG residues. Ultimately this may assist in defining the value of these break crops for maintenance of soil fertility in rotation systems.

4. Phosphorus and N cycling of break crop residues

This section discusses residue quality parameters available for predicting P and N release from plant residues, and briefly summarises the body of information that is available regarding the proportion of P and N released during the decomposition and mineralisation of break crop residues.

4.1 Residue quality parameters

It is well known that the release of nutrients from crop residues is dependent on a number of edaphic factors, such as texture, tenperature, moisture, biological activity and organic matter content (Smith and Paul 1990) but also nutrient release is particularly influenced by the quality of the residue inputs (Palm and Rowland 1997). Residue quality is a function of two key parameters; (i) the biochemical nature of the residues (Bertrand et al. 2006; Jensen et al. 2005), including the relative proportion of nutrients such as N and P in forms that are more readily accessible compared with N and P associated with reclcitrant compounds such as lignin, hemi-cellulose, polyphenols, pyrophosphates and phytate (Lupwayi et al. 2007; Palm and Sanchez 1991), and (ii) the quality of these nutrients (N and P) in relation to carbon (C),

expressed as a mass based C:N or C:P ratio (Heal et al. 1997; Kwabiah et al. 2003a; Lupwayi et al. 2006; Nicolardot et al. 2001; Swift et al. 1979). There are also a few reports of N:P ratios as an indicator of nutrient release (Kwabiah et al. 2003b; Vogt et al. 1986).

There is wide variation in values reported for the critical concentration of crop residues for N or P release since studies of residue decomposition have investigated vastly different materials. Critical values for P concentration range from 2.0-3.0 g P kg^{-1} (Fuller et al. 1956; Kwabiah et al. 2003b; Singh and Jones 1976), and for N concentration from 15-20 g N kg^{-1} (Campbell 1978). There is similar wide variation in reported critical C:P ratios from 100:1 to 300:1 (Blair and Bolland 1978; Cheshire and Chapman 1996; Fuller et al. 1956; Iqbal 2009; Kwabiah et al. 2003b; Lousier and Parkinson 1978; White and Ayoub 1983), although less variation for critical C:N ratio ranging from 20-30:1 (Campbell 1978; Reinertsen et al. 1984).

Nutrient release in the early stages of residue decomposition may be more related to a water soluble fraction or to the critical total nutrient concentration than to a C:nutrient ratio. As mentioned earlier, there can be a substantial proportion of soluble P in plant residues (Jones and Bromfield 1969) including canola (Noack et al. 2012) which can be rapidly released, as has been shown for residues of a range of crop and pasture species including roots (Blair and Bolland 1978; Martin and Cunningham 1973). Kwabiah et al. (2003b) examined tropical AG residues and found total P and water soluble P contents were best predictors of P release as measured by P availability (resin). Similarly, Jensen et al. (2005) investigated a wide range of plant residues and plant parts, although not including roots, and found that N mineralisation up to 22 days correlated well with water soluble N in residues.

It has been suggested that roots may decompose slower than shoots (Balesdent and Balabane 1996) and Abiven et al. (2005) found that wheat root C:N ratio was not well related to N release as shoot C:N ratio, suggesting this may be due to biochemical differences in

constituents. However, there are a number of studies where net release of N (Till et al. 1982) and P (Alamgir et al. 2012; Dalal 1979; Till et al. 1982) from root residues has been shown to be similar or greater than from shoot residues, although in some of these studies temporal pattern of release differed but ultimately the proportion of residue P and N apparently mineralised was the same. The limited studies on N:P ratio report critical ratios in the range 7:1-14:1 (Kwabiah et al. 2003b; Vogt et al. 1986). Bell et al. (2014) state that N:P ratios will depend upon where in the plant tissue the nutrient is located since N:P in metabolic tissue is around 20:1 but may be 50:1 in woody structural tissue (Reiners 1986) and this will have implications with regard to the maturity of the residue when incorporated to soil. Indeed, in most of the studies referred to above the residue materials were not mature.

4.2 Proportion and amounts of N and P 'released' from break crop residues

There are many reports from incubation studies where the residues are added to soil cores, either with or without plants, and measures have been made of changes in labile nutrient pools in soil (microbial, mineral, exchangeable) and/or plant uptake, generally to produce net measures of apparent mineralisation or immobilisation. Direct measure of N or P release from residues in such experiments requires the use of isotope-labelled residues. Other studies measure the nutrient loss from residues as they decompose encased in mesh bags either placed on the soil surface or buried. The following discussion examines P and N release from break crop residues, particularly lupin and canola residues.

4.2.1 Release of P by break crop residues and uptake by subsequent plants

Information regarding P mineralisation of plant residues, mostly from incubation studies and relatively limited for canola and lupin, 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); and that rates of P mineralisation for canola residues are faster than field pea (Soon and Arshad 2002). Around 20% of P was released from mature canola above-ground residues during an 8 week period following burial of litter bags in the field (Lupwayi et al. 2007).

Isotope P studies indicate wide variation in the amount of P released from crop residues for uptake by plants, with proportions ranging from 5-40% of the P input. Many of these studies focused on AG shoot residues of crops (Nachimuthu et al. 2009; Noack et al. 2014b) or pastures (McLaughlin and Alston 1986) or used whole plants (Blair and Bolland 1978). Most studies that have assessed the fate of P in AG residues have incorporated the residues into the system (Nachimuthu et al. 2009; Noack et al. 2014b; Soon and Arshad 2002) which may not reflect the effect of standing stubbles in modern no-till systems, unless the soil has been cultivated (Nebiyu et al. 2014). Therefore the P benefits from the AG residues inferred from these studies could, in practical terms, be overestimates. Although, a recent study (Noack et al. 2014b) reported that the proportion of P accessed by wheat from surface placed large pieces of mature pea residue (13%) was similar to that from incorporated large pieces (8%), but significantly less than that from incorporated finely ground pea residues (27%).

Information for root residues has been generated for roots that have been extracted from soil or produced in solution culture prior to incorporation (Blair and Bolland 1978; Dalal 1979; Friesen and Blair 1988; Martin and Cunningham 1973; Nachimuthu et al. 2009). Some data suggest shoot and root residues may release a similar or greater proportion of their P. 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 quantitatively roots are considered a smaller P pool as mentioned before. 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). Such magnitude of P release, as pointed out by the authors, is likely to have occurred because the residues 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^{-1}. Nevertheless, a litter bag study in the field also reported a greater proportion of N and P turned over from mature roots of canola and pea than from straw of the same species: 22-64% of root N and 53-76% of root P compared to 4-42% of straw N and 18-38% of straw P. 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. As yet it appears there are no studies that have investigated P release from labelled *in situ* BG root residues.

There is also a wide range in reported P benefits to wheat from release of P by break crop residues, represented in terms of the proportion of wheat plant P that is derived from the residues. The proportion of P in 60 day old wheat derived from incorporated or surface mature shoot residues of field pea ranged from 5-29% and increased by 80 days to 9-44% (Noack et al. 2014b). Some measures of P benefit from residues are very low, e.g. the 4-5% contribution to P uptake of 35 day old corn from faba bean and field pea root residues in a glasshouse study (Nachimuthu et al. 2009). Field results from litter bag decomposition studies of Soon and Arshad (2002) demonstrate net P mineralization (equivalent to 0.1 to 0.3 kg P ha^{-1}) from root residues of wheat, canola and pea, although little variation between species.

Phosphorus availability from decomposing residues has been described as occurring in three phases (Kwabiah et al. 2003c) 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 has been suggested that sorption of P by soil minerals is a principal determinant of plant availability of P mineralised from plant residues in the same way as it is for fertiliser (Guppy and McLaughlin 2009). Since, as already mentioned, a relatively large proportion of the P in most plant residues is soluble (orthophosphate) it is highly likely to be released quickly and subject to sorption, depending on the soil characteristics, as shown by Friesen and Blair (1988). However, fate of this rapidly released P from residues is also likely to depend on the activity of other P 'sinks' like microbial biomass, or the demands of a plant if one is present. Indeed, substantial uptake by microbial biomass of P released from medic residues in the field and glasshouse has been measured (McLaughlin and Alston 1986; McLaughlin et al. 1988). Also, a recent 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). The significance of understanding the role of the microbial biomass was highlighted in a recent review (Damon et al. 2014) where sensitivity analysis of an empirical model for P release from residues indicated that uptake of P by the microbial biomass and residue P concentration and speciation were the most sensitive parameters in terms of altering model output.

Several studies highlight that a large proportion of the P in plant residues remains in the soil following a period of incubation or after a subsequent crop (Friesen and Blair 1988; McLaughlin et al. 1988). Thus, up to 80% of the P in a crop not receiving P fertiliser will be 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 two soil P sources have not been clearly separated.

4.2.2 Release of N by break crop residues and uptake by subsequent plants

There is a substantial body of empirical data from incubation experiments worldwide on N release from residues, especially for pasture and crop legumes, although data from field studies is less common (Kumar and Goh 1999; Martens 2001; Murphy et al. 2003). Field data for temperate conditions in Australia suggested about 30% of the N in stubbles of grain legumes and 20% of the N in the root material may mineralise during the year following the grain legume crop (Evans et al. 2001; Fillery and Recous 2001; Unkovich et al. 1997). Direct measures of N release from canola residues in Australia appear difficult to find, although enhanced accumulation of mineral N following canola has been observed in a field study but could not be linked to increased populations of soil organisms associated with mineralisation (Kirkegaard et al. 1999). An incubation study in Europe found from 14-27% of the N in canola residues (pods, stems and roots) mineralised (Trinsoutrot et al. 2000).

Use of the previously mentioned *in situ* ^{15}N isotope-labelling technique in studies to quantify BG N accumulation has also allowed in some cases for direct tracing of the decomposition of intact BG residues of break crop species. The proportion of BG N released on decomposition, measured in some studies as subsequent plant uptake of labelled ^{15}N, ranged from 12-27% for lupin and was 6.5% for canola, which contributed 7-27% of the N uptake of a subsequent cereal (Arcand et al. 2014b; McNeill and Fillery 2008). Similar values for this apparent mineralisation (inferred from plant uptake only) of break crop BG N from other glasshouse and field studies using ^{15}N-labelling of BG residues of oilseeds (Arcand et al. 2014b) and grain legumes including lupin (Arcand et al. 2014a; Mayer et al. 2003; McNeill and Fillery 2008; Wichern et al. 2011) range from 8.6-20%. The range accords with an earlier field study

using isotopically labelled residues re-introduced to soil (Ladd et al. 1981) which demonstrated an apparent net N mineralisation of 10.9 to 17.3 % of legume residues during the following wheat phase. A field study of N loss from residues in mesh bags buried in soil in the field demonstrated a higher net N mineralisation (22 to 38%) of canola roots (Soon and Arshad 2002), but perhaps this is not surprising given the technique incorporates N from residues that will have been incorporated into microbial biomass and mineral N pools. Studies of the fate of BG N residues have measured from 2-11% of the residue-derived N in the microbial pool (Mayer et al. 2003; McNeill and Fillery 2008; Wichern et al. 2011) with some variation in the proportion of residue-derived N reported in the mineral N pool from negligible amounts (Arcand et al. 2014a) up to 10% (McNeill and Fillery 2008) with additional loss of 8-14% of N derived from lupin BG residues as nitrate leached to 1m soil depth (McNeill and Fillery 2008). Thus, apparent mineralisation rates for BG N based on following plant N uptake only are likely to be underestimates.

Benefits to following crops of N released from AG canola and legume residues appear relatively small with N derived from residues comprising up to only 15% of following wheat N uptake (Arcand et al. 2014a; Jensen 1996a; Ladd et al. 1981). The N benefit from BG residues is reportedly similar to that from AG residues ranging from a 15% contribution by lentil BG residues and 3-10% by lupin BG to wheat N uptake in field studies (Arcand et al. 2014b; McNeill and Fillery 2008). Values for contribution from faba bean, pea and lupin BG residues to plant N uptake obtained in a glasshouse study (Mayer et al. 2003) are slightly higher (42, 18 and 31% respectively) than these field data. As noted for residue-derived P, a large proportion of residue-derived N remains in soil in a non-labile form for the longer term (Amato et al. 1984; Arcand et al. 2014a; Glasener et al. 2002; Jensen 1996b; McNeill and Fillery 2008)

4.3 Conclusions

Clearly BG plant residues have the potential to release N and P at a similar rate to AG residues and have been shown to benefit following crops to varying extents. However, it is also clear that a large proportion of N and P in the residue remains unavailable to a following plant and is likely to contribute in the longer term to maintenance of soil fertility. Overall, data from field studies is much less common than data from controlled environment and glasshouse studies. Information regarding cycling of nutrients from roots is largely available from studies where roots have been excavated from soil, dried and re-introduced which may not reflect the cycling of these roots if left undisturbed *in situ*. ^{15}N isotope has been used to trace the fate of *in situ* BG N for a number of pulses, canola and wheat. $^{32}P/^{33}P$ isotope-labelling has been used to trace the fate of P from crop residues.

5. Overall conclusions and aims of the thesis

Available P and N derived from the decomposition of plant residues will contribute to the nutrition of following crops to varying degrees depending on edaphic, climatic and management factors. However, this review highlighted that there is a preponderance of data concerning P and N release from shoot rather than root residues and clarified that very little is known about the total accumulation of P BG by break crops relative to that for total BG N accumulation and turnover. Hence, the importance of root residues to nutrient cycling, particularly P cycling cannot be easily quantified. This is particularly so since roots are extremely difficult to quantify in a realistic and non-destructive manner. Hence accurate quantitative information for dry matter and P contents of mature root systems is limited, especially relative to that for N. Standard root recovery methods often underestimate the total P accumulation in the BG root residue since P in unrecovered fine roots and rhizodeposits in the soil is not taken into account. Whilst the ^{15}N stem wick-feeding technique has

successfully been used to quantify total BG N in some break crop species including legumes and oilseeds, the potential to adapt this technique for assessing total BG P has not been tested. Development of an *in situ* ^{33}P method may allow for more accurate assessment of BG P accumulation and turnover by break crop root systems Furthermore, ^{33}P and ^{15}N dual-labelling could provide some unreported simultaneous measures of the *in situ* contribution of undisturbed break crop roots to P and N uptake by subsequent wheat, and allow for a stoichiometric analysis of N and P during root decomposition. Such knowledge will advance quantitative understanding of P and N cycling by BG residues of break crop species and may assist in defining the P fertility benefits from break crops in rotation systems.

The broad aim of this PhD study is to move beyond the limitations inherent in current studies of root dry matter quantification and nutrient cycling from BG residues where plant roots have been excavated, dried, weighed, ground and re-introduced to soil, in order to:

i) test the efficacy of the ^{33}P stem wick-feeding technique for labelling roots *in situ*

ii) estimate total below-ground (BG) P accumulation *in situ* for canola and lupin

iii) assess soil texture effects on the accumulation of BG P *in situ* by these break crop species and on the proportion of recovered roots to root-derived P (RDP)

iv) directly trace and quantify *in situ* the cycling of mature canola BG P in soil and the contribution to P uptake by subsequently grown wheat; and

v) simultaneously trace and quantify *in situ* the contribution of labelled BG P relative to BG N of the break crops to following wheat by using ^{33}P and ^{15}N dual-labelling.

References

ABARES (2011) Agricultural statistics commodity 2011. Department of Agriculture, Fisheries and Forestry, Canberra, Australia.

Abbott M L and Fraley Jr L (1991) A review: Radiotracer methods to determine root distribution. Environmental and Experimental Botany 31, 1-10.

Abiven S, Recous S, Reyes V and Oliver R (2005) Mineralisation of C and N from root, stem and leaf residues in soil and role of their biochemical quality. Biology and Fertility of Soils 42, 119-128.

Alamgir M, McNeill A, Tang C and Marschner P (2012) Changes in soil P pools during legume residue decomposition. Soil Biology and Biochemistry 49, 70-77.

Amato M, Jackson R, Butler J and Ladd J (1984) Decomposition of plant material in Australian soils. II. Residual organic ^{14}C and ^{15}N from legume plant parts decomposing under field and laboratory conditions. Soil Research 22, 331-341.

Angus J F (2001) Nitrogen supply and demand in Australian agriculture. Australian Journal of Experimental Agriculture 41, 277-288.

Arcand M, Knight J D and Farrell R (2013) Estimating belowground nitrogen inputs of pea and canola and their contribution to soil inorganic N pools using ^{15}N labeling. Plant and Soil 371, 67-80.

Arcand M, Knight J D and Farrell R (2014a) Differentiating between the supply of N to wheat from above and belowground residues of preceding crops of pea and canola. Biology and Fertility of Soils 50, 563-570.

Arcand M, Lemke R, Farrell R and Knight J D (2014b) Nitrogen supply from belowground residues of lentil and wheat to a subsequent wheat crop. Biology and Fertility of Soils 50, 507-515.

Balesdent J and Balabane M (1996) Major contribution of roots to soil carbon storage inferred from maize cultivated soils. Soil biology & biochemistry 28, 1261-1263.

Bell C, Carrillo Y, Boot C M, Rocca J D, Pendall E and Wallenstein M D (2014) Rhizosphere stoichiometry: are C : N : P ratios of plants, soils, and enzymes conserved at the plant species-level? New Phytologist 201, 505-517.

Bertrand I, Chabbert B, Kurek B and Recous S (2006) Can the Biochemical Features and Histology of Wheat Residues Explain their Decomposition in Soil? Plant and Soil 281, 291-307.

Blair G J and Bolland O W (1978) The release of phosphorus from plant material added to soil. Australian Journal of Soil Research 16, 101-111.

Bolland M, Paynter B and Baker M (1989) Increasing phosphorus concentration in lupin seed increases grain yield on phosphorus deficient soil. Australian Journal of Experimental Agriculture 29, 797-801.

Bouwman A, Lee D, Asman W, Dentener F, Van Der Hoek K and Olivier J (1997) A global high-resolution emission inventory for ammonia. Global Biogeochemical Cycles 11, 561-587.

Bünemann E K, Heenan D P, Marschner P and McNeill A M (2006) Long-term effects of crop rotation, stubble management and tillage on soil phosphorus dynamics. Soil Research 44, 611-618.

Campbell C A (1978) Soil organic carbon, nitrogen and fertility. Elsevier Scientific Publishing Co., Amsterdam, The Netherlands.

Cassman K G, Dobermann A and Walters D T (2002) Agroecosystems, Nitrogen-use Efficiency, and Nitrogen Management. AMBIO: A Journal of the Human Environment 31, 132-140.

Chauhan B S, Stewart J W B and Paul E A (1979) Effect of carbon additions on soil labile inorganic, organic and microbially held phosphate. Canadian Journal of Soil Science 59, 387-396.

Cheng W, Coleman D C and Box Jr J E (1991) Measuring root turnover using the minirhizotron technique. Agriculture, Ecosystems & Environment 34, 261-267.

Cheshire M V and Chapman S J (1996) Influence of the N and P status of plant material and of added N and P on the mineralization of C from 14C-labelled ryegrass in soil. Biology and Fertility of Soils 21, 166-170.

Conant R T, Berdanier A B and Grace P R (2013) Patterns and trends in nitrogen use and nitrogen recovery efficiency in world agriculture. Global Biogeochemical Cycles 27, 558-566.

Cook R J, Schillinger W F and Christensen N W (2002) Rhizoctonia root rot and take-all of wheat in diverse direct-seed spring cropping systems. Canadian Journal of Plant Pathology 24, 349-358.

Cordell D, Rosemarin A, Schröder J J and Smit A L (2011) Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options. Chemosphere 84, 747-758.

Dalal R C (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, 913-916.

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

Dawson C J and Hilton J (2011) Fertiliser availability in a resource-limited world: Production and recycling of nitrogen and phosphorus. Food Policy 36, S14-S22.

Deng S P, Moore J M and Tabatabai M A (2000) Characterization of active nitrogen pools in soils under different cropping systems. Biology and Fertility of Soils 32, 302-309.

Duxbury J M, Lauren J G and Fruci J R (1991) Measurement of the biologically active soil nitrogen fraction by a 15N technique. Agriculture, Ecosystems & Environment 34, 121-129.

Evans J, McNeill A M, Unkovich M J, Fettell N A and Heenan D P (2001) Net nitrogen balances for cool-season grain legume crops and contributions to wheat nitrogen uptake: a review. Australian Journal of Experimental Agriculture 41, 347-359.

Evans J, O'Connor G, Turner G, Coventry D, Fettell N, Mahoney J, Armstrong E and Walsgott D (1989) N_2 fixation and its value to soil N increase in lupin, field pea and other legumes in south-eastern Australia. Australian Journal of Agricultural Research 40, 791-805.

FAO (2015) World fertilizer trends and outlook to 2018. Rome.

Farrington P, Greenwood E, Titmanis Z, Trinick M and Smith D W (1977) Fixation, accumulation, and distribution of nitrogen in a crop of Lupinus angustifolius cv. Unicrop. Crop and Pasture Science 28, 237-248.

Fillery I R and Recous S (2001) Use of enriched ^{15}N sources to study soil N transformations. In Stable Isotope Techniques in the Study of Biological Processes and Functioning of Ecosystems. pp 167-194. Springer.

Föhse D, Claassen N and Jungk A (1991) Phosphorus efficiency of plants. Plant and Soil 132, 261-272.

Friesen D and 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, 355-366.

Frossard E, Achat D L, Bernasconi S M, Bünemann E K, Fardeau J-C, Jansa J, Morel C, Rabeharisoa L, Randriamanantsoa L, Sinaj S, Tamburini F and Oberson A (2011) The use of tracers to investigate phosphate cycling in soil–plant systems. In Phosphorus in Action. Eds. E Bünemann, A Oberson and E Frossard. pp 59-91. Springer Berlin Heidelberg.

Fuller W H, Nielsen D R and Miller R W (1956) Some Factors Influencing the Utilization of Phosphorus from Crop Residues1. Soil Science Society of America Journal 20, 218-224.

Galloway J N, Dentener F J, Capone D G, Boyer E W, Howarth R W, Seitzinger S P, Asner G P, Cleveland C C, Green P A, Holland E A, Karl D M, Michaels A F, Porter J H, Townsend A R and Vöosmarty C J (2004) Nitrogen Cycles: Past, Present, and Future. Biogeochemistry 70, 153-226.

Gan Y, Campbell C, Janzen H, Lemke R, Basnyat P and McDonald C (2010) Nitrogen accumulation in plant tissues and roots and N mineralization under oilseeds, pulses, and spring wheat. Plant and Soil 332, 451-461.

Gardner C M K, Cooper D M and Hughes S (2002) Phosphorus in soils and field drainage water in the Thame catchment, UK. Science of The Total Environment 282–283, 253-262.

Gardner M, Peoples M, Condon J, Li G, Conyers M and Dear B (2012) 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, Armidale, Australia.

Gardner W K and Boundy K A (1983) The acquisition of phosphorus byLupinus albus L. Plant and Soil 70, 391-402.

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

Gladstones J and Loneragan J (1975) Nitrogen in temperate crop and pasture plants. Crop and Pasture Science 26, 103-112.

Glasener K M, Wagger M G, MacKown C T and Volk R J (2002) Contributions of shoot and root nitrogen-15 labeled legume nitrogen sources to a sequence of three cereal crops. Soil Science Society of America Journal 66, 523-530.

Grant C A and Bailey L D (1993) Fertility management in canola production. Canadian Journal of Plant Science 73, 651-670.

Guppy C N a and McLaughlin M J (2009) Options for increasing the biological cycling of phosphorus in low-input and organic agricultural systems. Crop and Pasture Science 60, 116-123.

Halstead E and Rennie D (1965) The movement of injected P^{32} throughout the wheat plant. Canadian Journal of Botany 43, 1359-1366.

Heal O W, Anderson J M and Swift M J (1997) Plant litter quality and decomposition: an historical overview. In Driven by Nature: Plant Litter Quality and Decomposition. Eds. G Cadisch and K E Gill. pp 3-30. CAB International, Wallingford, UK.

Herridge D F, Peoples M B and Boddey R M (2008) Global inputs of biological nitrogen fixation in agricultural systems. Plant and Soil 311, 1-18.

Hinsinger P, Betencourt E, Bernard L, Brauman A, Plassard C, Shen J, Tang X and Zhang F (2011) P for Two, Sharing a Scarce Resource: Soil Phosphorus Acquisition in the Rhizosphere of Intercropped Species. Plant Physiology 156, 1078-1086.

Hoad S P, Russell G, Lucas M E and Bingham I J (2001) The management of wheat, barley, and oat root systems. In Advances in Agronomy. pp 193-246. Academic Press.

Hocking P J (2001) Organic acids exuded from roots in phosphorus uptake and aluminum tolerance of plants in acid soils. In Advances in Agronomy. pp 63-97. Academic Press.

Hocking P J, Randall P J and DeMarco D (1997) The response of dryland canola to nitrogen fertilizer: partitioning and mobilization of dry matter and nitrogen, and nitrogen effects on yield components. Field Crops Research 54, 201-220.

Iqbal S M (2009) Effect of crop residue qualities on decomposition rates, soil phosphorus dynamics and plant phosphorus uptake. In Soil and Land Systems. pp 1-220. The University of Adelaide, Adelaide.

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

Jensen E (1996a) Compared cycling in a soil-plant system of pea and barley residue nitrogen. Plant and Soil 182, 13-23.

Jensen E S (1996b) Rhizodeposition of N by pea and barley and its effect on soil N dynamics. Soil Biology and Biochemistry 28, 65-71.

Jensen L S, Salo T, Palmason F, Breland T, Henriksen T, Stenberg B, Pedersen A, Lundström C and Esala M (2005) Influence of biochemical quality on C and N mineralisation from a broad variety of plant materials in soil. Plant and Soil 273, 307-326.

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

Kauwenbergh (2010) World P Phosphate Rock Reserve. http://firt.org/sites/default/files/SteveVanKauwenbergh_World_Phosphate_Rock_Reserve.pdf.

Keerthisinghe G, Hocking P J, Ryan P R and Delhaize E (1998) Effect of phosphorus supply on the formation and function of proteoid roots of white lupin (Lupinus albus L.). Plant, Cell & Environment 21, 467-478.

Khan D F, Peoples M B, Chalk P M and Herridge D F (2002) Quantifying below-ground nitrogen of legumes. 2. A comparison of ^{15}N and non isotopic methods. Plant and Soil 239, 277-289.

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

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

Kirkegaard J A, Mele P M and Howe G N (1999) Enhanced accumulation of mineral-N following canola. Australian Journal of Experimental Agriculture 39, 587-593.

Kirkegaard J A, Peoples M B, Angus J F and Unkovich M J (2011) Diversity and Evolution of Rainfed Farming Systems in Southern Australia Rainfed Farming Systems. Eds. P Tow, I Cooper, I Partridge and C Birch. pp 715-754. Springer Netherlands.

Kumar K and Goh K M (1999) Crop Residues and Management Practices: Effects on Soil Quality, Soil Nitrogen Dynamics, Crop Yield, and Nitrogen Recovery. In Advances in Agronomy. Ed. L S Donald. pp 197-319. Academic Press.

Kwabiah A B, Palm C A, Stoskopf N C and Voroney R P (2003a) Response of soil microbial biomass dynamics to quality of plant materials with emphasis on P availability. Soil Biology and Biochemistry 35, 207-216.

Kwabiah A B, Stoskopf N C, Palm C A and Voroney R P (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, 53-61.

Kwabiah A B, Stoskopf N C, Palm C A, Voroney R P, Rao M R and Gacheru E (2003c) Phosphorus availability and maize response to organic and inorganic fertilizer inputs in a short term study in western Kenya. Agriculture, Ecosystems & Environment 95, 49-59.

Ladd J N, Oades J M and Amato M (1981) Distribution and recovery of nitrogen from legume residues decomposing in soils sown to wheat in the field. Soil Biology and Biochemistry 13, 251-256.

Liu J, Khalaf R, Ulén B and Bergkvist G (2013) Potential phosphorus release from catch crop shoots and roots after freezing-thawing. Plant and Soil 371, 543-557.

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

Liu Y and Chen J (2008) Phosphorus Cycle. In Encyclopedia of Ecology. Eds. J Sven Erik and F Brian. pp 2715-2724. Academic Press, Oxford.

Llewellyn R S, D'Emden F H and Kuehne G (2012) Extensive use of no-tillage in grain growing regions of Australia. Field Crops Research 132, 204-212.

Lousier J D and Parkinson D (1978) Chemical element dynamics in decomposing leaf litter. Canadian Journal of Botany 56, 2795-2812.

Lupwayi N Z, Clayton G W, O'Donovan J T, Harker K N, Turkington T K and Soon Y K (2006) Nitrogen release during decomposition of crop residues under conventional and zero tillage. Canadian Journal of Soil Science 86, 11-19.

Lupwayi N Z, Clayton G W, O'Donovan J T, Harker K N, Turkington T K and Soon Y K (2007) Phosphorus release during decomposition of crop residues under conventional and zero tillage. Soil and Tillage Research 95, 231-239.

Lynch J M and Panting L M (1980) Cultivation and the soil biomass. Soil Biology and Biochemistry 12, 29-33.

Ma Q, Rengel Z and Rose T (2009) The effectiveness of deep placement of fertilisers is determined by crop species and edaphic conditions in Mediterranean-type environments: a review. Soil Research 47, 19-32.

Mahieu S, Fustec J, Faure M-L, Corre-Hellou G and Crozat Y (2007) Comparison of two ^{15}N labelling methods for assessing nitrogen rhizodeposition of pea. Plant and Soil 295, 193-205.

Mahieu S, Fustec J, Jensen E S and Crozat Y (2009) Does labelling frequency affect N rhizodeposition assessment using the cotton-wick method? Soil Biology and Biochemistry 41, 2236-2243.

Martens D A (2001) Nitrogen cycling under different soil management systems. Advances in Agronomy 70, 143-192.

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

Mat Hassan H, Marschner P, McNeill A and Tang C (2012) Growth, P uptake in grain legumes and changes in rhizosphere soil P pools. Biology and Fertility of Soils 48, 151-159.

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

McBeath T M, McLaughlin M J, Kirby J K and Armstrong R D (2012) The effect of soil water status on fertiliser, topsoil and subsoil phosphorus utilisation by wheat. Plant and Soil 358, 337-348.

McCarty G W, Meisinger J J and Jenniskens F M M (1995) Relationships between total-N, biomass-N and active-N in soil under different tillage and N fertilizer treatments. Soil Biology and Biochemistry 27, 1245-1250.

McKenzie N, Jacquier D, Isbell R and Brown K (2004) Australian soils and landscapes: an illustrated compendium. CSIRO publishing.

McLaughlin M and 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, 517-526.

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

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

McNeill A (2001) Stable isotope techniques using enriched “N and" 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. Eds. M Unkovich, J Pate, A McNeill and D J Gibbs. pp 195-218. Springer Netherlands.

McNeill A and 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 and Soil 302, 297-316.

McNeill A and Unkovich M (2007) The Nitrogen Cycle in Terrestrial Ecosystems. In Nutrient Cycling in Terrestrial Ecosystems. Eds. P Marschner and R Z. pp 37-64. Springer-Verlag, Berlin.

McNeill A M and Penfold C M (2009) Agronomic management options for phosphorus in Australian dryland organic and low-input cropping systems. Crop and Pasture Science 60, 163-182.

Murphy D, Recous S, Stockdale E, Fillery I, Jensen L, Hatch D and Goulding K (2003) Gross nitrogen fluxes in soil: theory, measurement and application of 15 N pool dilution techniques. Advances in Agronomy 79, 69-118.

Nachimuthu G, Guppy C, Kristiansen P and 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 and Soil 314, 303-310.

Nebiyu A, Vandorpe A, Diels J and Boeckx P (2014) Nitrogen and phosphorus benefits from faba bean (Vicia faba L.) residues to subsequent wheat crop in the humid highlands of Ethiopia. Nutrient Cycling in Agroecosystems 98, 253-266.

Neumann G and Römheld V (1999) Root excretion of carboxylic acids and protons in phosphorus-deficient plants. Plant and Soil 211, 121-130.

Nicolardot B, Recous S and Mary B (2001) Simulation of C and N mineralisation during crop residue decomposition: A simple dynamic model based on the C:N ratio of the residues. Plant and Soil 228, 83-103.

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

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

Noack S R, McBeath T M, McLaughlin M J, Smernik R J and Armstrong R D (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, 31-39.

Norton A, Kirkegaard J, Angus J and T P (2013) Canola in Rotations 7. The Regional Institute, http://www.regional.org.au/au/gcirc/canola/p-06.htm.

Nuruzzaman M, Lambers H, Bolland M D A and Veneklaas E J (2005) Phosphorus benefits of different legume crops to subsequent wheat grown in different soils of Western Australia. Plant and Soil 271, 175-187.

Oberson A, Tagmann H, Langmeier M, Dubois D, Mäder P and Frossard E (2010) Fresh and residual phosphorus uptake by ryegrass from soils with different fertilization histories. Plant and Soil 334, 391-407.

Oghoghorie C G and Pate J S (1972) Exploration of the nitrogen transport system of a nodulated legume using ^{15}N. Planta 104, 35-49.

Palm C A and Rowland A P (1997) A minimum dataset for characterization of plant quality for decomposition. In Driven by nature plant litter quality and decomposition. Eds. G Cadisch and K E Giller. CAB International, Wallingford, UK.

Palm C A and Sanchez P A (1991) Nitrogen release from the leaves of some tropical legumes as affected by their lignin and polyphenolic contents. Soil Biology and Biochemistry 23, 83-88.

Peoples M B, Brockwell J, Herridge D F, Rochester I J, Alves B J R, Urquiaga S, Boddey R M, Dakora F D, Bhattarai S, Maskey S L, Sampet C, Rerkasem B, Khan D F, Hauggaard-Nielsen H and Jensen E S (2009) The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. Symbiosis 48, 1-17.

Racz G J, Rennie D A and Hutcheon W L (1964) The ^{32}P injection method for studying the root system of wheat. Canadian Journal of Soil Science 44, 100-108.

Raun R W and Johnson V G (1999) Improving nitrogen use effeciency for cereal production. Agronomy Journal 91, 357-363.

Reiners W A (1986) Complementary models for ecosystems. American Naturalist, 59-73.

Reinertsen S A, Elliott L F, Cochran V L and Campbell G S (1984) Role of available carbon and nitrogen in determining the rate of wheat straw decomposition. Soil Biology and Biochemistry 16, 459-464.

Rennie D and Halstead E (1965) A ^{32}P injection method for quantitative estimation of the distribution and extent of cereal grain roots. Proc. Isotopes and Radiations in soil-plant nutrition studies. FAO/IAEA Ankara 489.

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

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

Russell C and Fillery I (1996a) Estimates of lupin below-ground biomass nitrogen, dry matter, and nitrogen turnover to wheat. Australian Journal of Agricultural Research 47, 1047-1059.

Russell C and Fillery I (1996b) *In situ* ^{15}N labelling of lupin below-ground biomass. Australian Journal of Agricultural Research 47, 1035-1046.

Russell C A and Fillery I R P (1999) Turnover of nitrogen from components of lupin stubble to wheat in sandy soil. Soil Research 37, 575-592.

Schenck zu Schweinsberg-Mickan M, Joergensen R G and Müller T (2010) Fate of 13C- and 15N-labelled rhizodeposition of Lolium perenne as function of the distance to the root surface. Soil Biology and Biochemistry 42, 910-918.

Schjoerring J K, Bock J G H, Gammelvind L, Jensen C R and Mogensen V O (1995) Nitrogen incorporation and remobilization in different shoot components of field-

grown winter oilseed rape (Brassica napus L.) as affected by rate of nitrogen application and irrigation. Plant and Soil 177, 255-264.

Singh B B and Jones J P (1976) Phosphorous sorption and desorption characteristics of soil as affected by organic residues. Soil Science Society of America Journal 40, 389-394.

Smernik R and Baldock J (2005) Solid-state ^{15}N NMR analysis of highly ^{15}N-enriched plant materials. Plant and Soil 275, 271-283.

Smil V (1999) Nitrogen in crop production: An account of global flows. Global Biogeochemical Cycles 13, 647-662.

Smith J L and Paul E A (1990) The significance of soil microbial biomass estimations. Soil biochemistry 6, 357-396.

Sommer S G, Schjoerring J K and Denmead O (2004) Ammonia emission from mineral fertilizers and fertilized crops. Advances in Agronomy 82, 557-622.

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

Svečnjak Z and Rengel Z (2006) Nitrogen utilization efficiency in canola cultivars at grain harvest. Plant and Soil 283, 299-307.

Swift M J, Heal O W and Anderson J M (1979) Decomposition in Terrestrial Ecosystems. Blackwell Scientific Publications, Oxford.

Till A R, Blair G J, Dalal R C, Freney J R and Galbally I E (1982) Isotope studies of the recycling of carbon, nitrogen, sulfur and phosphorus from plant material. In The cycling of carbon, nitrogen, sulfur and phosphorus in terrestrial and aqautic ecosystems. pp 51-59. Springer-Verlag.

Tilman D, Cassman K G, Matson P A, Naylor R and Polasky S (2002) Agricultural sustainability and intensive production practices. Nature 418, 671-677.

Trinsoutrot I, Recous S, Mary B and Nicolardot B (2000) C and N fluxes of decomposing 13C and 15N Brassica napus L.: effects of residue composition and N content. Soil Biology and Biochemistry 32, 1717-1730.

Unkovich M and Baldock J (2008) Measurement of asymbiotic N2 fixation in Australian agriculture. Soil Biology and Biochemistry 40, 2915-2921.

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

Unkovich M, Pate J and Hamblin J (1994) The nitrogen economy of broadacre lupin in southwest Australia. Australian Journal of Agricultural Research 45, 149-164.

Unkovich M J, Pate J S and Sanford P (1997) Nitrogen fixation by annual legumes in Australian Mediterranean agriculture. Australian Journal of Agricultural Research 48, 267-293.

Van Vuuren D P, Bouwman A F and Beusen A H W (2010) Phosphorus demand for the 1970-2100 period: A scenario analysis of resource depletion. Global Environmental Change 20, 428-439.

Vogt K A, Grier C C and Vogt D (1986) Production, turnover, and nutrient dynamics of above-and belowground detritus of world forests. Advances in Ecological Research 15, 3-377.

Weaver D and Wong M F (2011) Scope to improve phosphorus (P) management and balance efficiency of crop and pasture soils with contrasting P status and buffering indices. Plant and Soil 349, 37-54.

Wetselaar R and Farquhar G D (1980) Nitrogen losses from tops of plants. Advances in Agronomy 33, 263-302.

White R E and Ayoub A T (1983) Decomposition of plant residues of variable C/P ratio and the effect on soil phosphate availability. Plant and Soil 74, 163-173.

Wichern F, Andreeva D, Joergensen Rainer G and Kuzyakov Y (2011) Stem labeling results in different patterns of 14C rhizorespiration and 15N distribution in plants compared to natural assimilation pathways. Journal of Plant Nutrition and Soil Science 174, 732-741.

Wichern F, Eberhardt E, Mayer J, Joergensen R G and Müller T (2008) Nitrogen rhizodeposition in agricultural crops: Methods, estimates and future prospects. Soil Biology and Biochemistry 40, 30-48.

Wichern F, Mayer J, Joergensen R G and Müller T (2007) Release of C and N from roots of peas and oats and their availability to soil microorganisms. Soil Biology and Biochemistry 39, 2829-2839.

Wong M, Grundy M, Barson M and Walcott J (2012) A strategic framework to improve phosphorus management in the Australian grains industry. CSIRO, Australia.

Yasmin K, Cadisch G and Baggs E M (2006) Comparing ^{15}N-labelling techniques for enriching above- and below-ground components of the plant-soil system. Soil Biology and Biochemistry 38, 397-400.

Zobel R W (2008) Hardware and software efficacy in assessment of fine root diameter distributions. Computers and Electronics in Agriculture 60, 178-189.

CHAPTER 2

PRELIMINARY STUDIES: TOWARDS ACCURATE QUANTIFICATION OF PHOSPHORUS ACCUMULATION IN CROP PLANT ROOT SYSTEMS

Chapter 2

Preliminary studies: towards accurate quantification of phosphorus accumulation in crop plant root systems

2.1 Introduction

As highlighted in the literature review (Chapter 1) there is no technique available that will completely recover all root material from soil, so in order to quantify total below-ground (BG) accumulation of P for break crop plants, an isotope approach is required similar to that used to successfully quantify BG N for some crop species. One study was undertaken to determine a suitable procedure to recover roots from soils to use in the proposed isotope studies. Two subsequent studies assessed the efficacy of stem-feeding ^{33}P for labelling plant roots and how this may be influenced by (a) the growth stage of the plant at time of feeding the isotope and (b) available P in soils as manipulated by P fertiliser application.

2.2 Study 1: Comparison of two techniques to recover clean root samples from soil

The most common method to recover roots, from soil in studies of nutrient accumulation and turnover BG, is washing accompanied by sieving (Arcand et al. 2013; Gasser et al. 2015; Khan et al. 2002; Mayer et al. 2003; Parker et al. 1989; Wichern et al. 2011). Although in some cases freeze drying of intact root-soil entities followed by separation into rhizosphere soil and cleaning roots using a soft brush has been used (McNeill and Fillery 2008; Yasmin et al. 2006). The key advantage of washing is that clean roots can be obtained that are relatively free of any soil contamination and hence accurate nutrient concentrations can be measured. However in relation to root nutrient studies there are some limitations of using a root washing method – loss of soluble P from the roots could potentially be a problem as quite a high proportion of P in roots is present as soluble/water extractable orthophosphate

(Noack et al. 2014). Indeed, Mayer et al. (2003) dried and analysed the soil-water slurry obtained after washing ^{15}N-labelled roots from soil and detected ^{15}N in that sample, indicating extraction of soluble root-derived N by washing. Loss of fine roots and root hairs will also occur during extraction and washing of roots from soil, to a greater or lesser extent depending on the mesh size of the sieve used, and the degree of care taken in removing roots from the sieve. Freeze drying followed by cleaning of dried roots is a technique that has been far less commonly used for root studies with the main potential advantage being reduced likelihood of soluble nutrients in roots being leached. However, a disadvantage of obtaining clean roots by freeze drying and fine brushing is the increased potential for root contamination with soil since, without the thorough washing action of water, the cleanliness of the roots may be compromised. This could interfere with accurate nutrient content measurements, particularly for trace nutrients which are often measured using ICP after digestion with acid (Wheal et al. 2011) and also macro-nutrients like P that have relatively low concentrations in the plant compared to other macro-nutrients such as N. Any method for extraction of roots from soil is time consuming and laborious and there is always a trade-off between effort invested and accuracy of the result. All of this needs to be considered when designing a suitable method to improve the quantification of root dry matter and obtaining more accurate measures of root nutrient content.

The primary aim of the first study was to measure root dry matter (DM) and P concentration of clean roots recovered using washing or freeze drying approaches, and to assess any potential for soil contamination. The study used root samples collected from the field and presented an opportunity to generate field estimates for DM and P content of roots from some break crops in a semi-arid environment which, as mentioned in the literature review (Chapter 1), are scarce.

2.2.1 Materials and Methods

2.2.1.1 Field core collection and plant density counts

Intact soil-root samples of canola (*Brassica napus*), lupin (*Lupinus angustifolius*) and rye (*Secale cereale*) were collected from an agricultural field experiment on a sandy soil (table 1, chapter 4) at Karoonda (36°04′S, 140°05′E) South Australia. Samples were collected at two times during the growing season; one when the crops were flowering and considered close to peak biomass (103 days after sowing) and the other when the crops were at physiological maturity and ready for grain harvest (184 days after sowing). At both sampling times 0-15 cm PVC cores (diameter 8 cm) were used to collect intact soil-root samples from the 0-10 cm soil depth immediately underneath individual plants (Plate 1). Two paired cores were collected at three randomly selected locations within each of the three field replicate plots of the above listed crops. After sampling, cores were transported in a cool box to the laboratory and stored at 4^0C for a maximum of 2 days until further processing. The mean value of the three sampled cores within the field replicates (three) of each crop species was used for the statistical analysis.

Plant density counts were also undertaken at peak biomass in three randomly selected $1m^2$ areas in the same field replicates (n=3) where the cores were sampled and then the mean value of the three sampled areas per replicate were used for the plant density.

Plate 1. Collection of intact soil-root sample from field using 0-15 cm core

2.2.1.2 Extraction of roots using washing or freeze drying methods

The details of the steps involved in recovery of clean roots from intact soil-root cores using washing or freeze drying approaches are shown in Figure 2.1. Briefly, paired collected cores with intact root systems plus soil from the 0-10cm depth were sieved (2 mm mesh sieve) in the field moist state. A clean root sample (>1mm) was then extracted from this >2mm fraction containing coarse roots plus adhering rhizosphere soil of one of the paired cores by wet sieving (1 mm mesh) plus additional recovery of clean fine roots (>1mm) from the root washing water containing rhizosphere soil by sieving this fraction after oven drying it at 60°C. A clean root sample (>1mm) from the same >2mm fraction of the other paired core was extracted after the fraction was frozen (-18°C) and freeze dried (-45°C), the freeze-dried

sample was then dry sieved (1 mm mesh), with roots in this >1mm fraction being manually cleaned using a paint brush. It is clear that recovering clean roots (>1 mm) using the freeze drying method was less time consuming with fewer steps than the washing method (Figure 2.1).

2.2.1.3 Dry matter (DM) and nutrient analysis of clean root samples

Clean root samples obtained by both methods were weighed, finely ground; acid digested and analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICPOES) for P concentration plus other trace elements (Ti, Fe and Al) which was used as indicators of soil contamination. The recovered root dry matter and root P contents, measured on a per plant basis were converted into kg ha^{-1} using a mean plant density of 52, 27 and 61 plants m^{-2} respectively for canola, lupin and rye. This was done to allow for comparison with agronomic data that is available on fertilizer P applications and above-ground crop residue P content.

2.2.1.4. Statistical analysis of data

Analysis of Variance (ANOVA) of the data was undertaken using the GENSTAT version 13 statistical package (VSN International, Rothamsted, UK). Least significance of difference (l.s.d) between methods was determined at <5 % significance using Fisher's protected l.s.d.

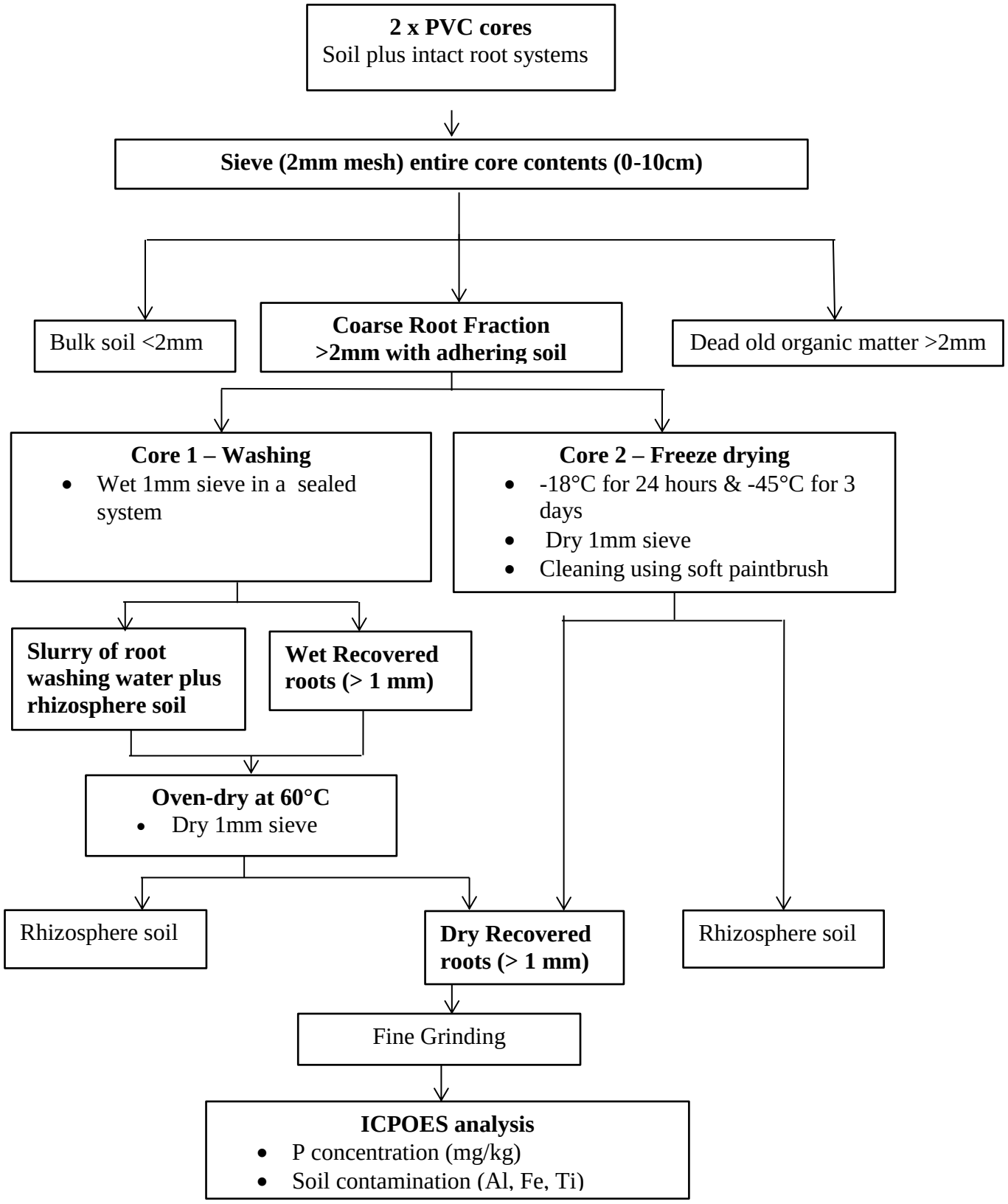


Figure 2.1 Flow diagram showing the steps to recover clean roots (>1mm) from intact soil cores using two different root recovery approaches (washing and freeze drying).

2.2.2 Results and Discussion

Results from this experiment clearly demonstrate that the freeze drying approach is as effective for obtaining a clean root sample to analyse P as the washing approach, at least for this sandy soil. The primary evidence is that there are no significant differences (p>0.05) in the measured P concentration of clean roots obtained by the two approaches for each species at either of the sampling times (Table 2.1). There also appear to be no differences in the quantity of root DM recovered by the two methods at either growth stage (Table 2.1). Indeed, the freeze drying approach is simpler and quicker in terms of processing and labour required (Figure 2.1) provided one has access to a freeze drying machine. Further evidence that the freeze drying approach can produce recovered roots that are as clean as those obtained by washing is seen in the ICP analysis for trace elements associated with potential soil contamination of plant samples (Table 2.2). Data indicate that Ti is present in the clean root samples at concentrations ranging from 0.58 to 8.27 mg kg^{-1} depending on species. Values above 1ppm Ti are considered by Wheal et al. (2011) to indicate potential for trace soil contamination, and indeed the ICP data show that the concentrations of Fe and Al, two other trace elements often closely associated with soil contamination tend to increase as the concentration of Ti increases (Table 2.2). However, overall it seems potential contamination is greatest for clean roots of rye and negligible at least for canola but importantly, within any one species it does not differ between the two approaches for clean root recovery. Furthermore, the fact that the values of Ti are all <10 ppm suggests that soil contamination is relatively low (Fleming 1963).

A decrease in clean root P content between flowering and physiological maturity was only seen in canola and not in lupin and rye. This could possibly be attributable to root senescence which is more likely than translocation to other plant P sinks since canola root P

concentration also declined between the two growth stages (Table 2.1). There were anticipated differences in recovered clean root P concentration between species which are likely to reflect inherent species differences in the efficiency of plant P acquisition (Wissuwa 2003). For example, the different efficiency with which plant species acquire P refers to the fact that canola may produce more root hairs (Misra et al. 1988) and can excrete more organic acids that can solubilize more P from the soil (Grant and Bailey 1993) than rye and lupin.

Field based estimates of recovered root DM and P concentration at maturity and flowering were greatest for canola and smallest for lupin (Table 3). Phosphorus in recovered roots was close to 4.0 kg ha^{-1} for canola, an amount that is not dissimilar to the maintenance of P fertilizer replacement rates, e.g. 2 t ha^{-1} wheat grain yield = P replacement rate of 6 kg ha^{-1} (Norton 2012). Recovered root DM was close to 1.0 t ha^{-1} for canola, < 0.7 t ha^{-1} for rye and <0.5 t ha^{-1} for lupin; a much smaller amount than the average standing root biomass for croplands of 0.15 kg m^{-2} reported by Jackson et al. (1996). This is particularly so since roots are notoriously difficult to quantify, and accurate information on root DM and P content, especially for field situations, is limited (Fageria and Moreira 2011; Gregory and Reddy 1982; Hoad et al. 2001).

Table 2.1 Recovered root dry matter of collected root samples using washing and freeze drying technique at flowering and maturity

Method	Species	Dry matter (kg ha^{-1})		P content (kg ha^{-1})		P concentration (mg kg^{-1})	
		Flowering	Maturity	Flowering	Maturity	Flowering	Maturity
Washing	Canola	704±151	655±82	3.51±0.60	2.75±0.37	4900±227	3500±207
Freeze drying		915±151	724±82	4.07±0.60	2.77±0.37	4500±227	3733±207
Washing	Lupin	235±87	226±45	0.70±0.26	0.54±0.07	3300±185	2433±180
Freeze drying		240±87	237±45	0.58±0.26	0.43±0.07	2570±185	2570±180
Washing	Rye	667±107	566±84	1.21±0.37	0.93±0.16	1853±172	1833±179
Freeze drying		719±107	510±84	1.55±0.37	1.30±0.16	2180±172	2165±179
P value							
Method		0.486	0.491	0.613	0.97	0.328	0.230
Species		0.006	0.003	<0.001	<0.001	<0.001	<0.001
Species x method		0.777	0.958	0.857	0.678	0.281	0.918

Data represent means (n=3) following means (± standard errors) between root recovery method

Table 2.2 Iron (Fe), aluminium (Al) and titanium (Ti) concentration (mg kg^{-1}) of canola, lupin and rye roots recovered from intact soil cores by washing or freeze drying sampled at two times (flowering and maturity) during the growing season.

Method	Species	Fe (mg kg^{-1})		Al (mg kg^{-1})		Ti (mg kg^{-1})	
		Flowering	Maturity	Flowering	Maturity	Flowering	Maturity
Washing	Canola	118±71	104±42	103±51	73±43	0.88±0.31	0.58±0.25
Freeze drying		243±71	173±51	186±59	98±54	1.70±0.38	1.02±0.30
Washing	Lupin	570±96	437±59	403±66	257±63	3.90±0.44	1.91±0.35
Freeze drying		660±96	587±72	453±77	260±44	4.03±0.52	2.37±0.43
Washing	Rye	2007±174	1773±102	1147±132	860±76	8.27±0.76	4.47±0.47
Freeze drying		1977±174	1697±96	1217±94	903±107	8.17±0.54	5.40±0.61
P value							
Method		0.549	0.435	0.392	0.705	0.529	0.111
Species		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Species x method		0.806	0.317	0.984	0.966	0.682	0.820

Data represent means (n=3) following means (± standard errors) between root recovery method ($p>0.05$)

2.3 Study 2: Effectiveness of stem wick-feeding ^{33}P for labelling roots of lupin

As highlighted in the literature review (Chapter 1) the efficacy of different methods for feeding ^{33}P to plant shoots in order to effectively label roots has been highly variable (McLaughlin et al. 1987; Rennie and Halstead 1965; Vijayalakshmi and Dakshinamurti 1977). Stem-wick-feeding ^{15}N to lupin appears quite efficient at labelling root N (Russell and Fillery 1996, McNeill and Fillery 2008) and so it seems appropriate to assess stem wick-feeding of ^{33}P using that same species. However, a radio-isotope such as ^{33}P requires some different considerations to those that apply when using a stable isotope such as ^{15}N including safe handling issue (Appendix 1). A key difference is the half-life of the ^{33}P radio-isotope (25 days) which effectively limits the length of time it can be traced before it decays below detection limits. This means a relatively high initial activity of radio-isotope needs to be used in studies where a significant length of time will elapse before measurements are to be taken. Another factor that decreases detection of the ^{33}P isotope is potential dilution with ^{31}P which will already be present when the material is fed ^{33}P plus additional ^{31}P will be taken up by the labelled plant from soil available P pools during any growth period subsequent to feeding. Furthermore there may be translocation of ^{31}P from one plant organ to another as plants mature, such as from root to shoot since developing grain is generally regarded as a sink for P (Batten and Khan 1987). Any ^{31}P uptake or translocation will affect the specific activity (SA) of the plant parts involved. The SA (as stated in the literature review, chapter 1) is defined as the ratio of the amount of the major isotope (in this case ^{31}P) to total activity of the minor isotope (kBq ^{33}P) in the measured pool/plant part. SA is important in isotope studies for quantifying total BG P since the SA of clean recovered roots (^{31}P mg plant^{-1}/^{33}P kBq plant^{-1}) is applied to estimate amounts of P in unrecovered roots and root derived entities such as sloughed root material, root hairs and root exudates.

The aim of the second pilot study was therefore to assess the proportion of stem wick-fed ^{33}P that could be partitioned to lupin roots and how this was influenced by the age of the plant when it was fed and hence the length of time that elapsed after the feed before the plant was sampled.

2.3.1 Materials and Methods

2.3.1.1 Details of experiment – design, set-up, nutrition and watering regime

This glasshouse experiment was carried out for 12 weeks. Six pre-germinated seeds of narrow-leaf lupin (*Lupinus angustifolius*) were sown in a single large pot (30 cm diameter) containing 5 kg of acid washed sand (Plate 2). A single nutrient solution containing required macro and micro nutrients were added to the soil at the beginning of the experiment as a basal application. The rate of the nutrients based on soil weight were: nitrogen (NH_4NO_3) 75 mg kg^{-1} soil, phosphorus (KH_2PO_4) 5 mg kg^{-1} soil, potassium (KCl) 100 mg kg^{-1} soil, magnesium ($MgSO_4.7H_2O$) 25 mg kg^{-1} soil, sulfate mg kg^{-1} soil, manganese ($MnSO_4.H_2O$) 2 mg kg^{-1} soil, copper ($CuSO_4.5H_2O$) 10 mg kg^{-1} soil and zinc ($ZnSO_4.7H_2O$) 15 mg kg^{-1} soil. Briefly, 50 ml of nutrient solution plus 350 ml of deionized water was applied to the dry soil and mixed thoroughly before potting up. Further once off top up application of N (25 ml) was performed at three weeks after sowing. The pot was watered with deionized water 3-4 times a week (depending on temperature) in order to maintain 60% field capacity. During the growing period glasshouse temperature ranged from 20°C to 31°C with a day length of 12 h and humidity 35-85% (RH) in natural day-light in a Mediterranean climate environment.

Plate 2. Six narrow-leaf lupin (*Lupinas angustifolius*) grown in a single pot

2.3.1.2 ^{33}P Stem wick-feeding

Carrier-free ^{33}P solution ($H_3{}^{33}PO_4$) with 99% isotopic purity was made using deionized water on the day of feeding to achieve a 250 kBq ml^{-1} of solution. One ml of ^{33}P solution with an activity of 250 kBq ml^{-1} was stem fed to each of three plants in the container at five weeks after sowing and to each of the remaining three plants at nine weeks after sowing using a stem wick-feeding technique previously described for ^{15}N feeding (McNeill et al. 1997). Briefly, using a micro pipette the solution was placed into a small vial (5ml) that was attached by a cotton wick to the stem of a plant. The initial radioactivity of the fed ^{33}P was chosen to minimize any potential radiation effects on the plants whilst at the same time aiming to

achieve an activity of 250 kBq per plant at harvesting. After the solution was taken up by the plants, generally within 24 hours after feeding, 1 ml of deionized water was added to the vial to rinse any adhering ^{33}P solution off the wall of the vial. The stem feed was removed after this water had been taken up by the plant. The three lupin plants that were chosen for feeding at five weeks were not in good health at that time due to some unknown reason, did not thrive and subsequently dropped leaves. However, the plants fed later at nine weeks which were from larger seeds were much healthier. In addition, there could have been some N stress since the seeds were not inoculated and the plants were not nodulated.

2.3.1.3 Sampling and analysis of plants

All six plants were harvested at 11 weeks after sowing. As mentioned earlier, the three early fed plants were unhealthy and there was some losses of plant shoot as fallen leaves. Since these could not be allocated a specific source plant they were collected but not analysed. At harvesting shoots were cut off directly above the soil surface (1 cm) and the roots were extracted from the coarse sand using the freeze-drying approach described in the previous pilot study in this chapter. Freeze dried shoots and recovered roots were finely ground and digested (0.5 g) using 5 ml concentrated HNO_3 (Zarcinas and Cartwright 1983). Total P concentration of the digests was determined using inductively-coupled plasma atomic emission spectroscopy (ICP-AES) at 214.97 nm and ^{33}P activity determined (8 ml scintillate to a 2 ml digested aliquot) using Rack Beta II Liquid Scintillation Counter. Recorded ^{33}P activity in each sample was then back corrected for decay and expressed as kBq kg^{-1} using the equation: $N= N_0(1/2)^t$ where, 'N' is the radioactivity at counting day, 'N_0' is the initial radioactivity of ^{33}P (dpm mL^{-1}), and 't' is time days elapsed/ 25.34 (half-life of ^{33}P).

Since the experiment was not replicated the data cannot be subject to rigorous statistical testing, therefore the results are simply presented as the mean of the three plants fed ^{33}P at

either 5 or 9 weeks with a standard deviation term that indicates the variability between the three plants.

2.3.2 Results and Discussion

The SA of plants fed ^{33}P at nine weeks after sowing was greater than those fed at five weeks (Figure 2.2) which is not simply due to the later time of feeding since the activity is back-corrected for decay. Whilst this result implies that the roots were a greater sink for the fed P at the later stage than at the earlier growth stage since the plants were not thriving when fed at 5 weeks, it could also be associated with the loss of P (and ^{33}P) in fallen leaves and later redistribution of P (and ^{33}P) from roots. Indeed, it seems that the plants which were not healthy at five weeks and lost leaves had 'recovered' by the sampling time at 11 weeks since both dry matter and P concentration of the two sub-sets of plants did not markedly differ (Table 2.3). The overall aim of using ^{33}P stem wick-feeding is to enable estimation of any unrecovered plant P BG and to do this a measurable SA of clean recovered roots is required, which can then be applied to a measured amount of ^{33}P activity in the soil taken to be representative of unrecovered BG P of the plant. Total recovery of fed ^{33}P at sampling was 40% in shoots and recovered roots of plants fed at 5 weeks whereas it was 99% for those fed ^{33}P at 9 weeks (Figure 2.2). The soil was not analysed for ^{33}P in this study and it could be that some of the unaccounted for ^{33}P in the earlier fed plants was present in unrecovered root or root derived products in the soil, although this seems unlikely given the potential for loss of ^{33}P in fallen leaves coupled with the virtually complete recovery of fed ^{33}P for the plants fed later.

Table 2.3 Plant dry weight and phosphorus (P) content of lupin grown in acid wash sand in a glasshouse, fed with ^{33}P using a ^{33}P stem wick-feeding technique either 5 weeks or 9 weeks after sowing, and harvested at 11 weeks.

Treatments	Dry weight (g plant^{-1})		P content (mg plant^{-1})		P concentration (mg kg^{-1})	
	Shoot	Recovered roots	Shoot	Recovered roots	Shoot	Recovered roots
Early fed (5 weeks)	0.45±0.02	0.42±0.07	0.29±0.05	0.24±0.03	644±70	581±69
Late fed (9 weeks)	0.35±0.06	0.67±0.13	0.23±0.04	0.35±0.04	641±17	533±71

Data represent means of three plants grown in a single pot ± standard deviation.

The high recovery of ^{33}P for the later-fed set of plants in this study suggests that there has been minor, if any, loss of fed ^{33}P via immediate exudation from roots or sorption to the wick, loss pathways which have been reported to occur for stem-fed ^{15}N (Mayer et al. 2003; Wichern et al. 2007). It also suggests that there is little or no ^{33}P remaining in the soil as unrecovered root or root-derived material which means that the objective of applying the SA of recovered roots for estimation of unrecovered root materials in soil cannot be achieved. The apparent complete recovery of roots in this study may be the result of using coarse acid-washed sand and the technique needs to be tested using soils differing in texture as well as for other species with potentially finer root systems, such as canola.

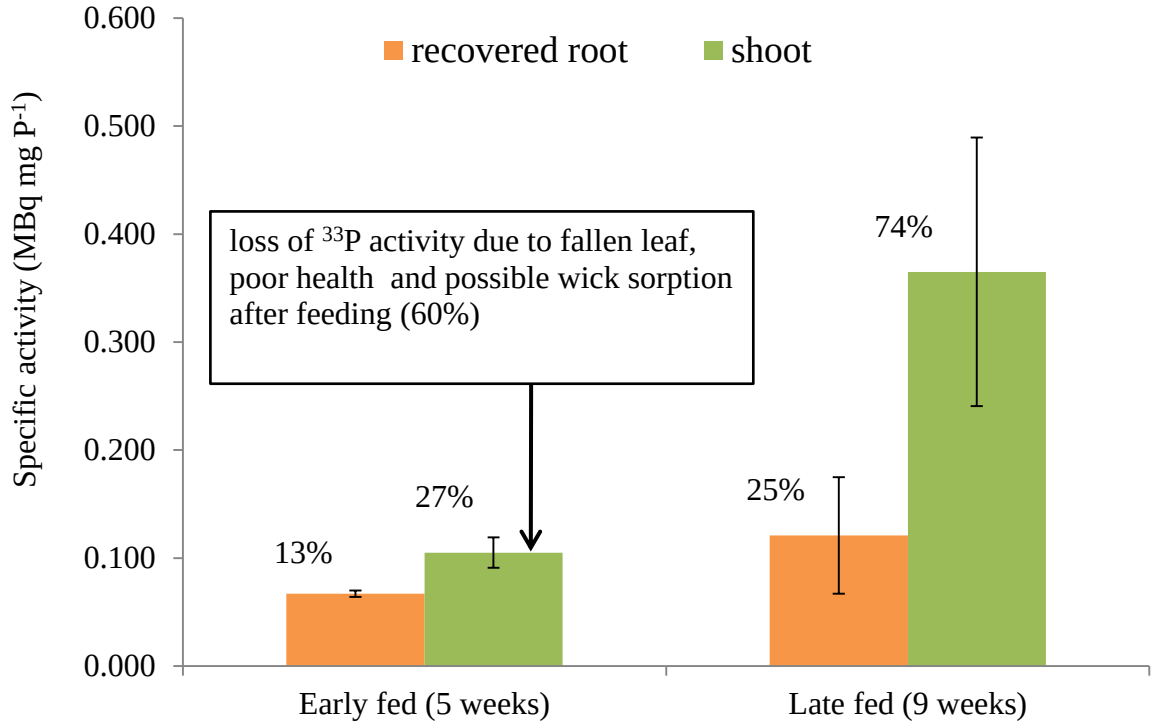


Figure 2.2 Specific activity of fed ^{33}P of recovered root and shoot over time of lupin grown in sand (^{33}P was fed using a stem wick-feeding technique at 5 weeks and 9 weeks after sowing). Numbers on top of bars represent distribution of recovered label of fed ^{33}P (%). Error bars represent variability (standard deviation) between three plants grown in a single pot.

2.4 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

As mentioned previously any pool of ^{31}P accessed by plants fed ^{33}P will dilute the SA of the radio-isotope in the plant organs and so fed plants grown in soils with greater P availability may have reduced SA of ^{33}P which may decrease the utility of the technique for estimating and tracing the fate of BG P. The aim of this third pilot study was therefore to determine how the ^{33}P SA of recovered roots of plants fed ^{33}P may be influenced by the available P status of soil as manipulated by fertiliser P application.

2.4.1 Materials and Methods

2.4.1.1 Details of experiment

This experiment was carried out for 6 weeks in under controlled glasshouse conditions. Three pre-germinated seeds of narrow-leaf lupin (*Lupinus angustifolius*) were sown in pots containing 2 kg acid washed sand per fertiliser rate treatment (one pot per six treatments) (Plate 3). The nutrients used in this experiments and water management were the same as for the previous study except for P. In this experiment six rates of P fertiliser: 5, 10, 15, 20, 25, 30 mg kg^{-1} were added as KH_2PO_4 solution by pipette to the sand as basal application after adding other nutrients. Plants were fed with carrier-free ^{33}P solution as $H_3{}^{33}PO_4$ solution as described for the previous study (2.3.1.2) at five weeks after sowing. All pots were harvested at six weeks after sowing (one week after feeding). During the growing period the glasshouse temperature, day length and relative humidity were as reported for study 2. The stem feeding vials were removed one day before harvesting.

2.4.1.2 Sampling and analysis

All plants were harvested at 6 weeks after sowing, processed and analysed as reported for study 2 in the previous section.

2.4.2 Results and Discussion

Specific activity generally decreased as the amount of P applied to the soil increased (Fig 2.3). There was a marked decline in SA of shoots and recovered roots as the rate of P applied increased from 5 to 10 mg P kg^{-1} soil, a more gradual decline as P applied increased to 15 mg P kg^{-1} soil, and little further decline up to the maximum rate (30 mg P kg^{-1} soil). The ultimate goal for feeding ^{33}P to this break crop species (lupin) is to be able to trace that ^{33}P activity

Plate 3. Three narrow-leaf lupin (*Lupinas angustifolius*) grown in a single pot per six P fertilizer rate treatment

into a subsequent crop, and hence the higher the SA of the plant roots, the more likely it will be that the P released as these roots decay will be above detection limits. However, as the amount of P applied to the soil increases there appears to be less variability in the SA of the plant shoot and roots, therefore labelling plants under low P conditions to obtain a relatively higher SA for ^{33}P in roots may cause greater variability in the SA obtained which might be a limitation of this technique. Furthermore, to gain a measure of BG P accumulation that is relevant to agricultural crops it should be undertaken on plants that are not P deficient. Since the critical value of shoot P concentration of young lupin plants is 2 mg P g^{-1} (Bolland and Brennan 2008) the data in this study indicate that the lupins grown with 5 and 10 mg P kg^{-1} fertiliser additions are P deficient (Figure 2.4). The SA of the recovered roots of P sufficient lupin plants is less than 0.02 MBq mg^{-1} which may not be sufficient to ensure detection in

longer term studies of root decomposition and hence the feeding of higher doses of ^{33}P needs to be explored.

The recovery of ^{33}P in the plants in this study was virtually complete (97-99%) which, as in the previous study particularly in the late fed, indicates little or no unrecovered BG plant material in an acid washed sand and highlights the need to undertake these ^{33}P feeding studies in field soils and in soils that have heavier/finer textures where not all roots will be recovered.

2.5 Overall conclusions

It can be concluded from these studies that recovering plant roots from soil using a freeze drying approach is as effective as a washing approach in terms of the mass of roots recovered and the integrity of the roots for measuring nutrient concentration. Within a species there was no difference between the two approaches in terms of the likelihood of soil contamination of cleaned roots. It is suggested that measured amounts of BG P in roots of some field grown crop species are potentially significant in relation to average amounts of fertilizer P added per annum to semi-arid agricultural systems in Australia. Furthermore it was demonstrated that the stem wick-feeding technique was effective for introducing measurable amounts of ^{33}P into roots of lupin plants, although the radio-isotope dose may need to be increased above that used in these studies if BG^{33}P is to be traceable in the medium to longer term in soils with P fertility typical of agricultural regions in Australia. Also, soils with a range of textures rather than the coarse acid washed sand used here need to be investigated to assess the fraction of unrecovered plant P BG for relevant crop species using *in situ* ^{33}P-labelling of roots. Finally, it can be concluded that the techniques investigated here are potentially valuable tools for studies that improve the accuracy and understanding of accumulation of P in plant root systems and the subsequent contribution to P cycling.

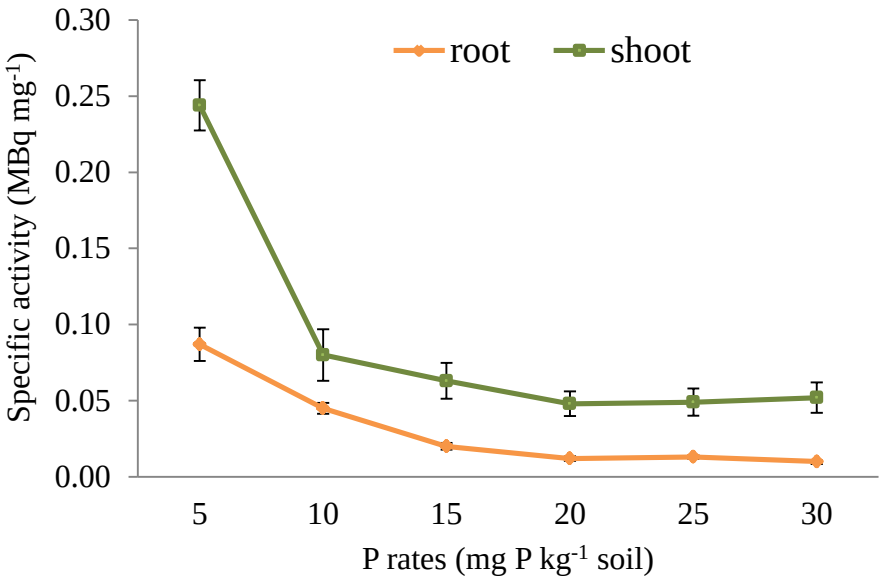


Figure 2.3: Shoot and root specific activity for plants stem fed ^{33}P at five weeks and harvested at six weeks after sowing. Error bars (standard deviation) represent within plant variability in one pot per treatment.

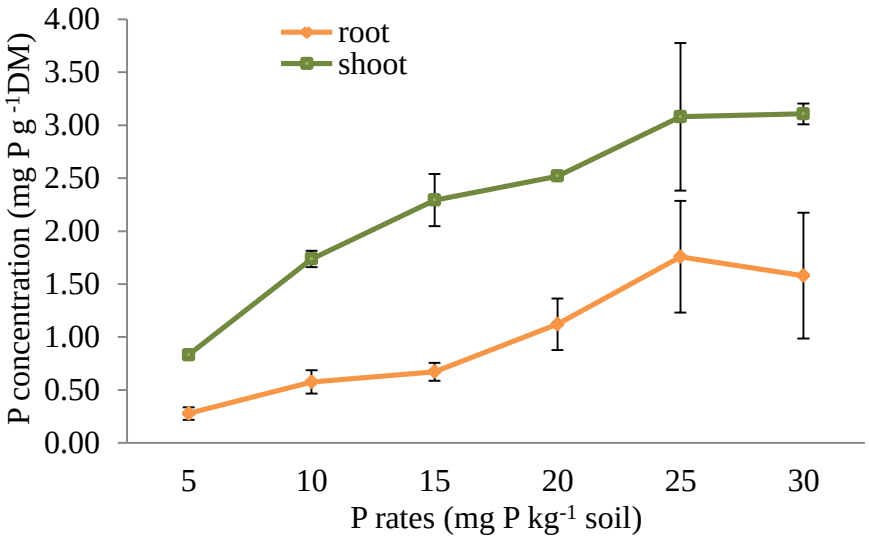


Figure 2.4: Shoot and root P concentration (mg P g^{-1}DM) for plants stem fed ^{33}P at five weeks and harvested at six weeks after sowing. DM = dry matter and error bars (standard deviation) represent within plant variability in one pot per treatment.

References

Arcand M, Knight J D and Farrell R (2013) Estimating belowground nitrogen inputs of pea and canola and their contribution to soil inorganic N pools using ^{15}N labeling. Plant and Soil 371, 67-80.

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

Bolland M D A and Brennan R F (2008) Comparing the phosphorus requirements of wheat, lupin, and canola. Australian Journal of Agricultural Research 59, 983-998.

Fageria N K and Moreira A (2011) Chapter four - The role of mineral nutrition on root growth of crop plants. In Advances in Agronomy. Ed. L S Donald. pp 251-331. Academic Press.

Fleming G A (1963) Distribution of major and trace elements in some common pasture species. Journal of the Science of Food and Agriculture 14, 203-208.

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

Grant C A and Bailey L D (1993) Fertility management in canola production. Canadian Journal of Plant Science 73, 651-670.

Gregory P J and Reddy M S (1982) Root growth in an intercrop of pearl millet/groundnut. Field Crops Research 5, 241-252.

Hoad S P, Russell G, Lucas M E and Bingham I J (2001) The management of wheat, barley, and oat root systems. In Advances in Agronomy. pp 193-246. Academic Press.

Jackson R B, Canadell J, Ehleringer J R, Mooney H A, Sala O E and Schulze E D (1996) A global analysis of root distributions for terrestrial biomes. Oecologia 108, 389-411.

Khan D F, Peoples M B, Chalk P M and Herridge D F (2002) Quantifying below-ground nitrogen of legumes. 2. A comparison of ^{15}N and non isotopic methods. Plant and Soil 239, 277-289.

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

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

McNeill A and 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 and Soil 302, 297-316.

McNeill A M, Zhu C and Fillery I R P (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, 295-304.

Misra R K, Alston A M and Dexter A R (1988) Role of root hairs in phosphorus depletion from a macrostructured soil. Plant and Soil 107, 11-18.

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

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

Parker C J, Carr M K V, Jarvis N J, Evans M T B and Lee V H (1989) Effects of subsoil loosening and irrigation on soil physical properties, root distribution and water uptake of potatoes (Solanum tuberosum). Soil and Tillage Research 13, 267-285.

Rennie D and Halstead E (1965) A ^{32}P injection method for quantitative estimation of the distribution and extent of cereal grain roots. Proc. Isotopes and Radiations in soil-plant nutrition studies. FAO/IAEA Ankara 489.

Vijayalakshmi K and Dakshinamurti C (1977) Limitations of the ^{32}P isotope injection technique for the study of the root systems of wheat, mung and cowpeas. Plant and Soil 46, 113-125.

Wheal M S, Fowles T O and Palmer L T (2011) A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis of plant essential elements. Analytical Methods 3, 2854-2863.

Wichern F, Andreeva D, Joergensen Rainer G and Kuzyakov Y (2011) Stem labeling results in different patterns of 14C rhizorespiration and 15N distribution in plants compared to natural assimilation pathways. Journal of Plant Nutrition and Soil Science 174, 732-741.

Wichern F, Mayer J, Joergensen R G and Müller T (2007) Rhizodeposition of C and N in peas and oats after 13C–15N double labelling under field conditions. Soil Biology and Biochemistry 39, 2527-2537.

Wissuwa M (2003) How Do Plants Achieve Tolerance to Phosphorus Deficiency? Small Causes with Big Effects. Plant Physiology 133, 1947-1958.

Yasmin K, Cadisch G and Baggs E M (2006) Comparing ^{15}N-labelling techniques for enriching above- and below-ground components of the plant-soil system. Soil Biology and Biochemistry 38, 397-400.

Zarcinas B A and Cartwright B (1983) Analysis of soil and plant material by inductively coupled plasma-optical emission spectrometry. In Division of soils technical paper /

Commonwealth Scientific and Industrial Research Organization. pp 1-36. CSIRO, Australia. Division, Soils, Technical Paper No. 45: 1-35.

CHAPTER 3

IN SITU ^{33}P-LABELLING OF CANOLA AND LUPIN TO ESTIMATE TOTAL PHOSPHORUS ACCUMULATION IN THE ROOT SYSTEM

The work contained in this chapter has been published in *Plant and Soil*

Foyjunnessa ., McNeill, A., Doolette, A., Mason, S., Mike J. 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.

Statement of Authorship

Title of Paper	*In situ* ^{33}P-labelling of canola and lupin to estimate total phosphorus accumulation in the root system
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	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.

Principal Author

Name of Principal Author (Candidate)	Foyjunnessa		
Contribution to the Paper	Assisted in experimental design, performed experiment and analysis on all samples, interpreted results, wrote the manuscript, illustrated all figures and tables, and acted as corresponding author.		
Overall percentage (%)	85%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Ann McNeill		
Contribution to the Paper	Supervised experimental design and development of the work, help in soil collection and interpretation of results, and manuscript evaluation		
Signature		Date	3/11/2015

Name of Co-Author	Ashlea Doolette		
Contribution to the Paper	Supervised development of the work, help in soil collection and sample analysis, interpretation of results, and manuscript evaluation		
Signature		Date	

Name of Co-Author	Sean Mason		
Contribution to the Paper	Supervised development of the work, help in soil collection and sample analysis, interpretation of results, and manuscript evaluation		
Signature		Date	

Name of Co-Author	Mike J. McLaughlin		
Contribution to the Paper	Help in experimental design, interpretation of results, and manuscript evaluation		
Signature		Date	24/10/15

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(1), 291-299.

CHAPTER 4

QUANTIFYING TOTAL PHOSPHORUS ACCUMULATION BELOW-GROUND BY CANOLA AND LUPIN PLANTS USING ^{33}P-LABELLING

The work contained in this chapter has been accepted by *Plant and Soil*

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

Statement of Authorship

Title of Paper	Quantifying total phosphorus accumulation below-ground by canola and lupin plants using ^{33}P-labelling
Publication Status	☐ Published ☑ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	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*. DIO: 10.1007/s11104-015-2545-y

Principal Author

Name of Principal Author (Candidate)	Foyjunnessa		
Contribution to the Paper	Assisted in experimental design, performed experiment and analysis on all samples, interpreted results, wrote the manuscript, illustrated all figures and tables, and acted as corresponding author.		
Overall percentage (%)	85%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Ann McNeill		
Contribution to the Paper	Supervised experimental design and development of the work, help in soil collection and interpretation of results, and manuscript evaluation		
Signature		Date	03/11/2015

Name of Co-Author	Ashlea Doolette		
Contribution to the Paper	Supervised development of the work, help in soil collection and sample analysis, interpretation of results, and manuscript evaluation		
Signature		Date	

Name of Co-Author	Sean Mason		
Contribution to the Paper	Supervised development of the work, help in soil collection and sample analysis, interpretation of results, and manuscript evaluation		
Signature		Date	

Name of Co-Author	Mike J. McLaughlin		
Contribution to the Paper	Help in experimental design, interpretation of results, and manuscript evaluation		
Signature		Date	29/10/15

Foyjunnessa, McNeill, A., Doolette, A., Mason, S. & McLaughlin, M.J., (2016). Quantifying total phosphorus accumulation below-ground by canola and lupin plants using ^{33}P labelling.
Plant and Soil, 401(1), 39-50.

CHAPTER 5

USING ^{33}P TO TRACE *IN SITU* THE FATE OF CANOLA BELOW-GROUND PHOSPHORUS INCLUDING WHEAT UPTAKE IN TWO CONTRASTING SOILS

The work contained in this chapter has been accepted by the journal *Crop and Pasture Science*.

Statement of Authorship

Title of Paper	Using ^{33}P to trace the fate of canola below-ground phosphorus including wheat uptake in two contrasting soils
Publication Status	☐ Published ☐ Accepted for Publication ☑ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	The manuscript has been submitted to *Crop and Pasture Science*.

Principal Author

Name of Principal Author (Candidate)	Foyjunnessa
Contribution to the Paper	Performed experiment and analysis on all samples, interpreted results, wrote the manuscript, illustrated all figures and tables, and acted as corresponding author.
Overall percentage (%)	80%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 27/6/2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Ann McNeill
Contribution to the Paper	Supervised experimental design and development of the work, interpretation of results, and manuscript evaluation
Signature	Date 28/6/2016

Name of Co-Author	Ashlea Doolette
Contribution to the Paper	Supervised development of the work, help in sample analysis, interpretation of results, and manuscript evaluation
Signature	Date 27/6/2016

Name of Co-Author	Sean Mason		
Contribution to the Paper	Supervised development of the work, help in sample analysis, interpretation of results, and manuscript evaluation		
Signature		Date	27/6/2016

Name of Co-Author	Mike J. McLaughlin		
Contribution to the Paper	Help in experimental design, interpretation of results, and manuscript evaluation		
Signature		Date	

Abstract. Our understanding of the contribution of crop root residues to phosphorus (P) cycling is mainly derived from studies using excavated roots re-introduced to soil. This study aims to quantify total below-ground P (BGP) of mature canola *in situ* and to estimate directly the proportion accessed by subsequent wheat. ^{33}P-Labelled phosphoric acid was fed by stem wick 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 5 weeks in the remaining undisturbed pots. At canola maturity, 23–36% of the ^{33}P was partitioned in recovered roots and 34–40% in the soil. More ^{33}P was recovered in the loam than the sand. 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 of shoot P : BGP (8 : 1 in sand and 15 : 1 in loam) were much narrower than those of shoot P : recovered root P (17 : 1 in sand and 39 : 1 in 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. Input of P below-ground by mature canola and subsequent P benefit to wheat was greater in loam than sand. The P from canola below-ground residues contributed up to 20% of P uptake in wheat during the first 5 weeks of growth. Longer term benefits of P from below-ground residues require investigation.

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

5.1 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, the United Kingdom, Australia and the United States (Rahman and McClean 2013). 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 because it provides a disease break for cereals (Norton et al. 2013). It is also considered a phosphorus (P)- efficient crop compared with wheat (Bolland and Brennan 2008), 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 with cereals (Norton et al. 2013). Indeed, Jackson (2000) reported that 30% of P accumulated aboveground by a spring canola crop remained in the residues after harvest. Release of some of this P as the residues mineralise is a potential benefit to subsequently grown crops such as cereals.

Information about P mineralisation of plant residues is mostly from incubation studies and is relatively limited for canola. Published information generally 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 than more mature material (Iqbal 2009; Kirkegaard et al. 1994; Soon and Arshad 2002), that rates of P mineralisation for canola residues are faster than for some other species, (Soon and Arshad 2002), that P release is highly correlated with total P content of residues in general (Baggie et al. 2005; Kwabiah et al. 2003c) 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, studies with P isotopes indicate wide variation in the measured amounts of P taken up from crop residues, ranging from 5% to 40% of the P input (Blair and Bolland 1978; Nachimuthu et al. 2009; Noack et al. 2014b) and that buried residues decompose faster than surface residues (Franzluebbers et al. 1996).

No-till systems being widely adopted in Australia (Llewellyn et al. 2012). In these systems, a greater proportion of aboveground residue tends to remain on the soil surface, so the contribution of nutrients 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 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 or exudates). A recent isotope study showed that the total below-ground P (BGP) 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 BGP may be re-translocated to the shoot as the plants matured, because the developing grain is a sink for P (Batten and Khan 1987; Batten et al. 1986; Smith 1965). Hence, it is necessary to quantify BGP at maturity to compare with previous estimates at peak growth.

Recovery of roots from soils is also widely believed to be more difficult with a greater proportion of clay, and so unrecovered BGP in finer textured soils is expected to be greater than in coarse-textured sandy soils. However, this needs verification. Furthermore, as plants progress to maturity, some leaves and roots senesce. Therefore, 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 BGP input

from the crop. This study uses a ^{33}P cotton-wick stem feeding technique (Foyjunnessa et al. 2014) to label root systems of canola *in situ* to determine: (i) quantities of root and root-derived BGP for canola grown in two different soil textures (sand and loam) at maturity, and (ii) the proportion of canola BGP accessed during early growth by subsequently grown wheat.

5.2 Materials and methods

5.2.1 Soil characteristics

Topsoil (0–10 cm) of two agricultural soils was collected in South Australia from Karoonda (36°04′S, 140°05′E) and Roseworthy (34°32′S, 138°45′E) (Foyjunnessa et al. 2015). The soils were classified as Kandosol (Karoonda) and Chromosol (Roseworthy) (Isbell 1996). The physiochemical properties of the soils from Karoonda and Roseworthy, respectively, were: soil texture sand (<5% clay) and loam (25% clay); pH_w (1 : 5) 6.4 and 7.6; P-buffering index (Moody 2007) 4.2 and 61; total P concentration 114 and 827 mg/kg; total carbon (C) content (Matejovic 1997) 0.33% and 1.78%; resin P (Kouno et al. 1995) 29 and 137 mg/kg; Colwell P (Rayment and Higginson 1992) 32 and 163 mg/kg; DGT-P (Mason et al. 2010)330 and 466 µg/L; and available sulfur (S) using mono-calcium-phosphate (MCP) solution (Rayment and Lyons 2011) 3.7 and 18 mg/kg .

5.2.2 Canola phase of the experiment

A complete nutrient solution without P was prepared. Over the canola phase, each pot received a total of nitrogen (N) as a combination of $Ca(NO_3)_2.4H_2O$, NH_4NO_3, and $Mg(NO_3)_2.6H_2O$ at 400 mg N/pot; potassium (K) and S as K_2SO_4 at 350 mg K and 200 mg S/pot; zinc (Zn) as $ZnSO_4.7H_2O$ at 50 mg Zn/pot; copper (Cu) as $CuCl_2.2H_2O$ at 40 mg

Cu/pot; and manganese (Mn) as $MnCl_2.4H_2O$ at 10 mg Mn/pot. Nutrient solution was added to the dry soil and adjusted to 70% of maximum water-holding capacity (WHC) with deionised water (respective WHC for the sand and loam 12% and 22%). Then the soil was packed sequentially (15 cm by 10 cm by 10 cm) into a PVC cylinder (height 40 cm, inner diameter 15 cm) capped at one end (referred to as 'pots' herein), to a bulk density of 1.2 g/cm^3 (sand) and 1.1 g/cm^3 (loam). The total soil weight for sand was 8.5 kg/pot and loam 8 kg/pot.

Under controlled glasshouse conditions with an average of 12 h daylight, canola was grown in pots filled with either sand or loam with eight replicates per soil type. Each pot was sown with four pre-germinated seeds of canola (cv. Tanami) and then thinned to 1 plant/pot after the first week. A complete randomised design was followed and the pots were re-randomised every week. During the first 2 weeks of canola growth, the pots were watered daily with deionised water to maintain 70% of the maximum WHC; after 2 weeks, the pots were watered every second day. Watering was terminated at 1 week before 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 during the day. Average relative humidity was 73%.

5.2.3 ^{33}P-labelling of canola

Canola was labelled by 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 cotton-wick stem feeding technique (Foyjunnessa et al. (2014). Briefly, a 5-mL vial was attached with a 100% cotton wick to the stem of the plant at ~3 cm above the surface via a drilled hole (1 mm diameter). A single dose of ^{33}P radioactive solution (1 mL $H_3{}^{33}PO_4$ containing 0.0000746 µg P) was placed into the vial via a micropipette. 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 minimise evaporation loss of the ^{33}P solution on the way from the vial to the stem. The ^{33}P radioactive solution (1 mL) was then introduced to the vial by a third pre-drilled hole in the cap, via a micropipette. The uptake of ^{33}P solution by the plants took 2–3 days and 1 mL deionised water was then added to ensure that 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 plants were then grown to maturity (105 DAS, 60 days after feeding commenced) and half of the replicates (four per soil type) were destructively sampled.

5.2.4 Fallow & wheat phase of experiment

The aboveground dry matter (DM) (canola shoots) was removed (1 cm above soil level) from the other half of the replicates (four), and the ^{33}P-labelled below-ground systems (roots plus root-derived (RD) P) was left undisturbed in the PVC pots of soil for 3 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 and relative humidity from 25% to 82%, and daylength was 11 h. 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 (mg/pot) of K 25, S 20, Ca 5, magnesium (Mg) 5, iron (Fe) 0.3, Mn 0.5, Cu 1, Zn 1, N 75 and P nil. Pots were allowed to dry naturally for 2 weeks during the fallow period, which resulted in the WHC decreasing to 30%. The pots were rewet to 60% WHC at 1 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 (Z43) (Zadoks et al. 1974). Wheat roots were separated from canola roots based on visual

judgment, which was considered accurate given the difference in morphological features of canola and wheat roots.

*5.2.5 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 no input of aboveground 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 added to the relevant aboveground shoot DM 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 and 10–35 cm, by 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 h and sieved with a 2-mm sieve. All root materials and fine roots >2 mm from each of the two soil depths 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 a 100-g subsample of bulk soil) were frozen (–18°C) and subsequently freeze-dried before processing and analysis. An additional 100-g fresh subsample of bulk soil was stored immediately after harvesting in a cool room (4°C) for ≤24 h until microbial biomass P was determined. Following freeze-drying, clean roots

(recovered) were obtained by brushing off rhizosphere soil with a soft paint brush. Fresh and dry weights of shoots, dry weight of recovered roots, and soil fractions (rhizosphere and bulk soil) were recorded and digested for chemical analysis (e.g. P concentration and ^{33}P activity) as described below. Subsequently harvested wheat was processed and analysed similarly to canola.

All freeze-dried plant samples (shoots and recovered roots) were finely ground and digested (1.0 g) by using 5 mL concentrated HNO_3 (Zarcinas and Cartwright 1983). Bulk soil and rhizosphere soil was digested in 5 mL aqua regia (HNO_3 : HCL 1 : 3) by the method of Zarcinas et al. (1996). The resulting digest solution was then used to determine total P concentration by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) at 214.97 nm, and ^{33}P activity (8 mL scintillate to 2 mL digested aliquot) by using a Rack Beta II liquid scintillation counter. Each cotton wick used to feed ^{33}P (16 wicks) 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 determined by the hexanol fumigation method (McLaughlin and Alston 1986) were measured on fresh bulk soil. Briefly, duplicate soil samples (one treated with 1 mL hexanol, the other without) were extracted by using distilled H_2O (soil : water ratio 1 : 15) containing one resin strip (BDH#55164), by simultaneous liquid fumigation and extraction for 16 h (Kouno et al. 1995). The resin strips were then eluted with 0.1 M NaCl–HCl for at least 2 h, and orthophosphate in the elution solution was measured colourimetrically (Murphy and Riley 1962). Plant-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. No correction was made for sorption because >90% of a P spike (10 mg P/kg) was recovered from non-fumigated samples.

5.2.6 Calculations, assumptions and statistical analysis

Root-derived P (RDP) and total BGP (BGP_{total}) for canola were calculated by a modified approach of that used in ^{15}N studies to estimate total BGN for legumes (McNeill 2001). RDP (Eqn I) was calculated as:

$$RDP\ (mg) = {}^{33}P \text{ activity in soil } (kBq\ {}^{33}P\ pot^{-1})/SA \text{ of clean roots } (kBq\ {}^{33}P\ mg^{-1}P) \quad (1)$$

where SA is the specific activity of clean roots (^{33}P in recovered roots (kBq) / ^{31}P content of recovered roots (mg)).

The 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 that all had the same specific activity.

Total BGP (Eqn 2) 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:

$$BGP_{total} = P_{recrt} + RDP_{bulk} + RDP_{rh} \quad (2)$$

An estimate of the dry mass of unrecovered root materials ($DW_{unrecrt}$) represented by the measured ^{33}P present in the soil (assuming that all ^{33}P in bulk soil was in unrecovered roots apart from that measured in pools of microbial and resin P) was also derived as follows (Eqn 3):

$$DW_{unrecrt}\ (g) =$$

$$^{33}P \text{ activity in soil (kBq)}/(^{33}P \text{ activity in recovered roots (kBq)/Recovered roots wt (g)}) \quad (3)$$

The distribution of canola BGP to subsequently grown wheat was calculated by using Eqn 4 because any ^{33}P activity detected in wheat after canola will have been derived from canola $BG^{33}P$:

Contribution 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) ×100 (4)

Data were tested for normality and homogeneity of variance from each treatment. A one-way analysis of variance (ANOVA) was undertaken by using the GENSTAT version 15 statistical package (VSN International, Hemel Hempstead, UK), except for specific activity data, where two-way ANOVA was performed. Least significance of variances (l.s.d.) between treatments was determined at $P = 0.05$ level of significance using Fisher's protected l.s.d.

5.3 Results:

5.3.1 Canola phase

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

Shoot dry weight of mature canola (Table 1) 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. Because the recovered root dry weights were similar, there was no significant difference in the P content (mg/plant) of recovered roots (Table 1).

5.3.1.2 ^{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,

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
Sand	14.1 b	1.67 a	4236 b	2211 b	60 b	3.58 a
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
Sand	1.82 b	0.93 a	3396 b	1617 b	6.19 b	1.50 b
Loam	2.51 a	0.96 a	4281 a	4029 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.

owing 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 specific activities of recovered roots at the two depths (0–10 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 specific activity of canola roots grown in the sand was greater at 0-10 cm than10-35 cm depth.

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	163 a	129 b	26 a
Canola loam	459	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.

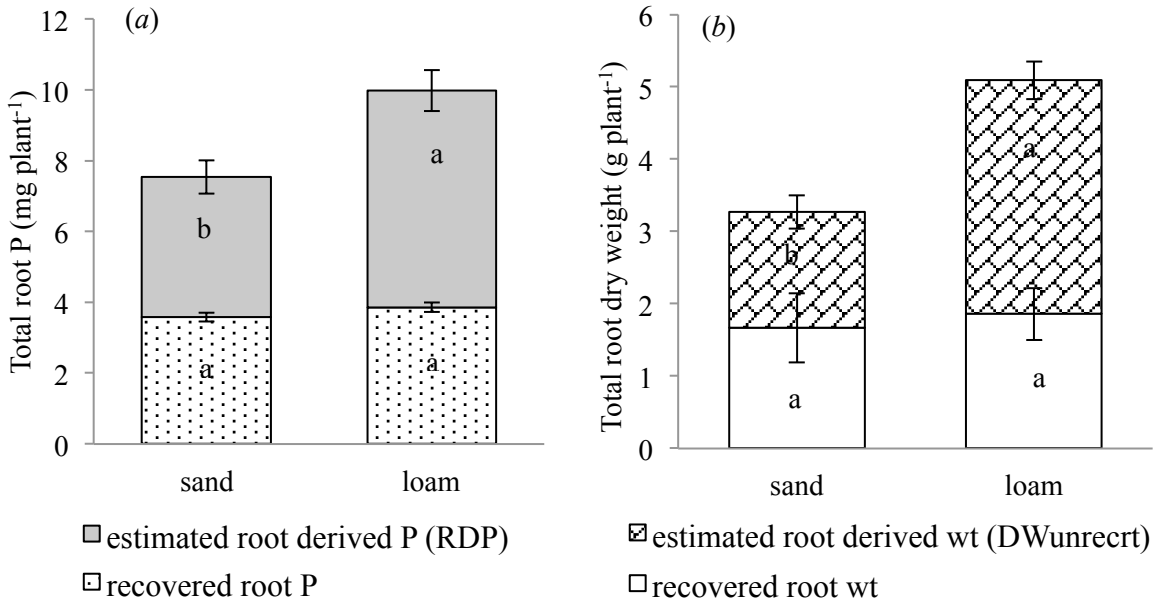
Table 3. Activity of ^{33}P (kBq plant^{-1}), amounts of ^{31}P (mg plant^{-1}) and specific activities (SA, kBq ^{33}P mg^{-1} ^{31}P) of recovered roots from two soil depths for mature canola grown in sand and loam. Plants were fed with ^{33}P using a ^{33}P cotton-wick stem feeding technique at 45 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^{-}
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		

*5.3.1.3 Estimates of RDP and $DW_{unrecrt}$ for canola at matur*ity

The RDP in the soil of pots containing canola at maturity (Fig. 1) was significantly higher in the loam (6.12 mg/plant) than in the sand (3.96 mg/plant). The amount of RDP in soil was 1.5 times greater than recovered root P when canola was grown in the loam, whereas RDP in soil was similar to recovered root P when canola was grown in sand. Estimates of $DW_{unrecrt}$ followed a pattern similar 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

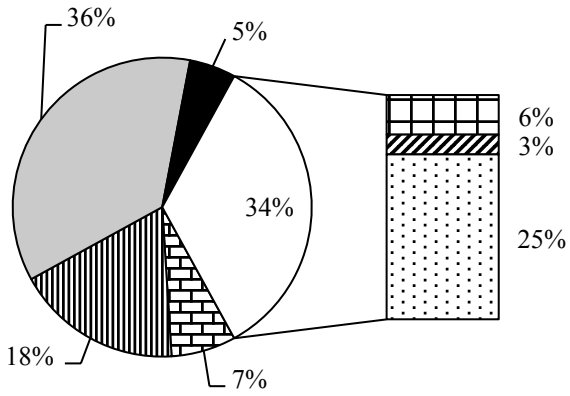
Fig. 1. Total root P calculated as the sum of measured recovered root P of mature canola grown in sand and loam and estimated root-derived P (*a*) and total root dry weight represented as measured recovered root dry weight and estimated unrecovered root dry weight in soil (*b*) (^{33}P was fed using 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 ($p \leq 0.005$).



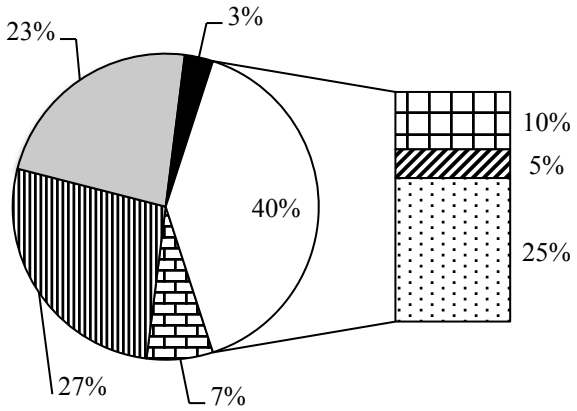
mature canola results in substantially narrower ratios than if recovered root P or DM were used. Hence, in sand, the shoot P content : recovered root P content ratio was 17 : 1 and the shoot P : BGP_{total} ratio was 8 : 1. For the loam, the respective ratios were 39 : 1 and 15 : 1.

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

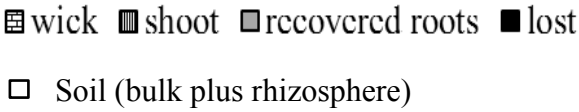


b) Canola loam

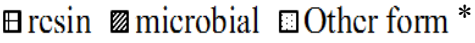


Legend:

Pie chart



Bar chart



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

5.3.1.4 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% (Fig. 2)with sorption by the wick being only 7%. Distribution of ^{33}P in aboveground (shoot) and below-ground 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 (Fig. 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 pool of resin P and3–5% was in the pool of microbial (hexanol-released) P (Fig. 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 associated with unrecovered fine roots and products of root turnover, was the same (25%) for both soil textures (Fig. 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) could not 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 aboveground plant material (shoot), regardless of soil types (Fig. 2).

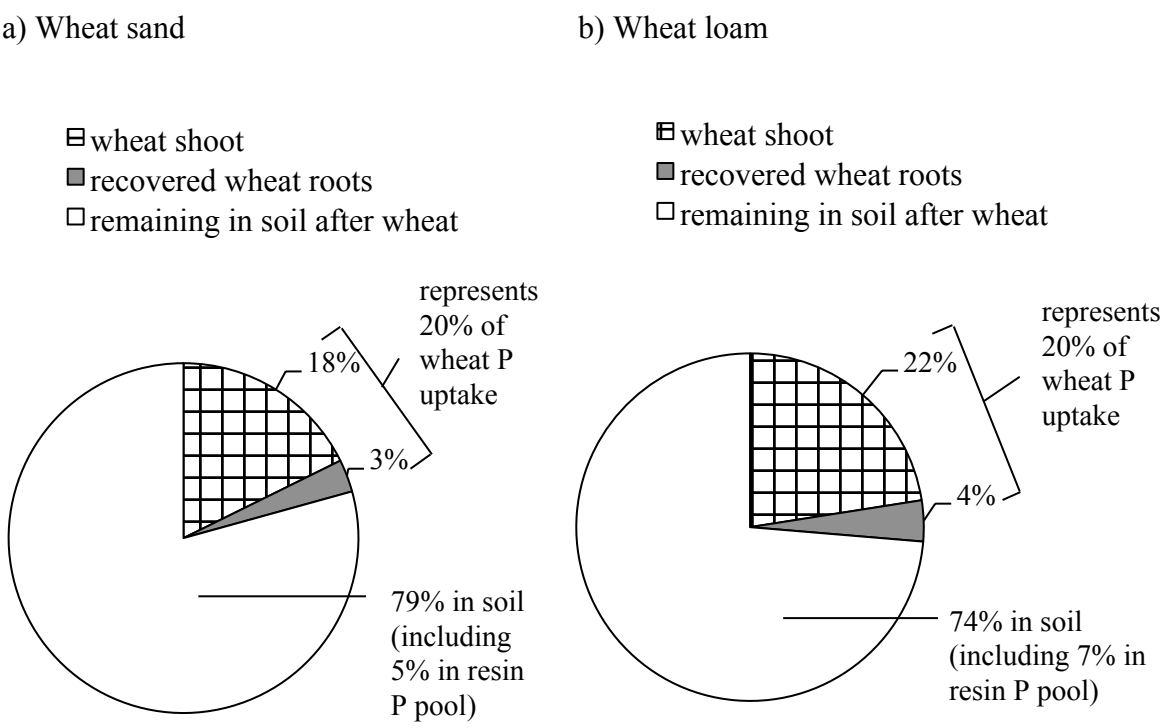
5.3.2 Wheat phase

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

Dry weights of shoot and recovered roots of wheat after canola followed similar trends to those of mature canola grown in sand and loam (Table 1). Similarly, P concentrations of shoot and recovered roots of wheat after canola were also affected by soil texture and were significantly higher in the loam than in the sand (Table 1). The bigger differences in wheat than canola shoot and root P concentrations between the two soil textures meant that

recovered root P content (mg/plant) of wheat after canola (Table 1) was also significantly greater in the loam (4.12 mg/plant) than in the sand (1.50 mg/plant).

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).



5.3.2.2 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 ($P < 0.001$), with the proportion of canola BG^{33}P recovered in the shoot and roots of wheat significantly higher in the loam than in the sand (Fig. 3). Although this represented an equal proportion (20%) of the total wheat P uptake in both soils (Fig. 3), 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 remained in soil after the wheat was harvested (Fig.

3) and constituted a significantly ($P < 0.001$) greater proportion of the BG^{33}P in the sand (79%) than in the loam (74%).

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 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.

Available P in the bulk soil of wheat at harvest was affected by soil texture, whereby it was higher ($P < 0.001$) in the loam than in the sand, probably due to the higher fertility of the loam, as evidenced by the large difference in initial resin-P status of these soils (Tables 4 and 5). ^{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 was also significantly higher in the loam than the sand after wheat (Table 4), but no ^{33}P activity was detected in the microbial P pool in bulk soil at wheat harvest (Table 5).

5.4 Discussion

5.4.1 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

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 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.

influence of soil texture on the amount of root and root-derived BGP. Irrespective of soil texture, the amount of P allocated below-ground 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 BGP is similar to that recently reported for canola at late flowering stage (Foyjunnessa et al. 2015). However, in the present study of canola at maturity, less canola BGP is accounted for as recovered roots and more as root-derived P, including some ^{33}P detected in pools of resin and microbial P 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 below-ground, which was greater in loam than sand, probably as a result of the overall higher plant DM and higher P concentration for plants grown in the greater yield potential loam.

Our results highlight that the quantitative contribution of the BGP component of crop plants to soil P pools is likely to be higher than inferred from studies to date, where a proportion of the plant's roots have not been assessed because of the sampling techniques used, which 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 BGP from more readily assessed shoot P contents by using the ratios generated in this present study, as has been done for estimating BGN of legumes (Peoples et al. 2001; Unkovich et al. 2010). Applying the ratios of shoot P content : total BGP 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. If estimates were based only on recovered roots, these values would be 0.47–0.54 kg P/ha, which fall within the range 0.35–0.84 kg P/ha derived from DM and P concentration data reported for recovered roots of field-grown canola (Soon and Arshad 2002). Estimated BGP 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 0–0.15 m soil depth sampled at maturity under a dryland canola crop, grown at the same site in South Australia from which the sand in this present study was collected, were in the order of 2.75 kg P/ha (Foyjunnessa, unpublished data). Assuming that 25% of the BGP was not recovered (with a mesh size of 1 mm used in the field study) compared with 52% of

unrecovered BGP (with mesh size <2 mm), this translates to an estimated total BGP input of ~3.5 kg P/ha. Such quantities of P as BGP inputs may be important for long-term sustainability of organic matter and nutrient supply, especially in low-fertility, semi-arid farming systems.

As discussed in detail in 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 below-ground, 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 below-ground at maturity was similar to that measured at peak growth of canola (Foyjunnessa et al. 2015), suggesting 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; 2008). However, 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), because the P concentration of the shoots in this study was above that considered adequate for canola (Reuter and Robinson 1997).

5.4.2 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 trace directly the fate of undisturbed BGP 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 below-ground residues was assessed in the presence of young wheat plants. 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 2-month period following removal of the shoots of mature canola, up to one-third of the canola residue BGP 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 BGP decomposition, with net release of P being a greater proportion of the canola BGP in the higher fertility loam than in the sand, and even though wheat DM was greater for the loam, the proportion of P in the wheat derived from the canola BGP was also greater. This greater P benefit from BGP in a loam soil is likely due to a combination of factors including: greater BGP 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 BGP and for wheat roots to access any available P derived from decomposition of canola BGP. 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; indeed, the wheat in the loam had a higher shoot P concentration than wheat in the sand.

Phosphorus availability from decomposing residues has been described as occurring in three phases (Kwabiah et al. 2003c): (i) an initial rapid P release from sparingly soluble inorganic plant materials; (ii) a phase when P in solution comes from both soluble P and mineralisation of plant materials; and (iii) a final phase when P in solution is influenced by its equilibrium with P sorption processes. It is highly likely that much of the ^{33}P release from the decomposing roots in the present study was directly as soluble P, because 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, because it occurred from sterile roots, was attributed to autolytic degradation of organic P in the roots. Rapid loss of P from clover root residues encased in mesh bags in the field has been observed, especially where residues were buried rather than left on the surface (Buchanan and King 1993), and because initial losses of P were not correlated with 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, much 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 below-ground 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 the more soluble organic forms (Friesen and Blair 1988). Although ^{33}P was present in the microbial biomass at canola maturity in this study, indicating that some BGP from roots had already been released and utilised as the plants senesced, no ^{33}P was measurable 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 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 below-ground input. Indeed, a longer term study of the decomposition of ^{32}P-labelled residues of mature field pea shoot 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 the 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); 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. The lignin content of canola shoot residues has been reported to be higher than of some other residues (Lupwayi et al. 2007), so this may also be the case for canola roots.

A 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 P release (per unit biomass) comparable to that of the shoot residue component. Indeed, the proportion of P (26–33%) released from the mature canola root BGP residues in this present study was slightly greater than that reported from mature canola aboveground 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 of roots added to soils. For example, Dalal (1979) reported greater apparent mineralisation of P from clover root residues than from shoots, with 42% of root P used by subsequent oats during 10 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 study, >40% of P from oat root residues was measured as inorganic P forms in soil only 11 days after incorporation, and 30–40% of P from oat root residues was taken up by the succeeding plants after 50 days (Friesen and Blair 1988). However, the growth period of the subsequent crop in that study was 2

weeks longer than in our study. Such a 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 while potentially altering the forms of P in the residues. However, it may also in part reflect that the roots were from young plants (only 3 weeks old), with a relatively high P concentration (1.6 mg/g). The threshold for P concentration of residues above which net P mineralisation occurs is wide-ranging (1–3 mg/g). Investigations in both tropical and temperate soils have largely focused 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 relatively few studies considering roots (Fuller et al. 1956; Soon and Arshad 2002). Net mineralisation of canola and clover roots with total P concentrations of 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, is not surprising. Clearly, as mentioned earlier, factors other than just P concentration of residues are governing the rate of release of P.

5.4.3 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 whether P fertility benefits over a longer period are considered. 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 were considered ‘not agronomically significant’ for Australian systems. Modelling suggested, however, 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. We agree that this could be the case in the context of dryland cropping systems in southern Australia assuming a median P fertiliser rate of 12 kg P/ha.year (Weaver and Wong 2011), a maximum (year 1) 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 for grain yields ranging from 1 to 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 ~0.8 kg/ha in the year after incorporation, which, according to our estimation, would provide only 3–4% of the P requirement of the following cereal crop in that environment. A radiotracer study using young (23-day-old) legume root residues produced in solution culture concluded that they contributed <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 that up to 20% of the P nutrition for a developing wheat crop can be contributed from decomposition of canola root residues. Given the importance of P availability during early growth of a wheat crop in Australia(Bolland 1997), this amount of P may be considered valuable because P uptake from fertiliser can be similar to or less than that from plant residues (McBeath et al. 2012; Thibaud et al. 1988).

The results of the present 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 inputs have not been clearly identified. Longer term soil P-

fertility benefits from the contribution of P-rich, below-ground plant residues, such as those of canola, may indirectly accrue as a result of the concomitant increase in soil P status. It has been suggested that increasing the 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). Another longer term potential value of BGP in root systems is that it will naturally be distributed to some depth through the soil profile (Read and Campbell 1981). This contrasts with 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) where, in dryland cropping systems, the topsoil can be periodically too dry for nutrient uptake to occur (Armstrong et al. 2015). Although this study indicates that a large proportion of BGP is concentrated in the top 0.1 m of soil depth, this is for well-watered soil columns with a defined diameter. A different distribution may be found in crops grown under field conditions where lateral and vertical root growth is influenced by soil profile volume and rates of drying. Nevertheless, these studies on the contribution of root residues *in situ* are highly relevant to conservation farming systems, especially in Australia where there is a high rate of adoption of no-till (Llewellyn et al. 2012). In no-till systems, the contribution from surface retention of aboveground residues to P nutrition of crops may be less immediate and longer term (Noack et al. 2014b), so that, by inference, the relative importance of the below-ground residues for immediate P supply may increase.

In this study, the wheat, which was sown just 3 weeks after harvest of the canola, was able 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 inputs (cycling) of P

from roots are also highly relevant to the intercropping or pasture cropping systems that have been suggested as economically viable and environmentally sound options for cropping regions across Australia (Bengough et al. 2001; Craig et al. 2013). These inputs would be important in dual-purpose systems where canola is utilised for both grazing and grain (Kirkegaard et al. 2008b). Where some time may elapse between harvest of canola and sowing of the subsequent crop (e.g. 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 be reduced by edaphic factors such as wetting and drying cycles that increase P availability in the short term (Butterly et al. 2009) but can also enhance soil P-sorption reactions (Olsen and Court 1982). It is not possible to trace the release of P from the canola below-ground residues over the long term by using the technique described here because of the relative short (25 days) half-life of the ^{33}P isotope used. Recent developments, however, using stable oxygen isotopes (Tamburini et al. 2014), may allow P cycling in soils to be traced over longer periods in the future.

Opinion on the agronomic significance of P inputs from crop residues in Australian cropping systems is divergent, with field data especially for below-ground residues scarce, and the longer term fate of P from crop residues unknown but clearly requiring further investigation. The present study of the potential of canola below-ground 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 whether there are management options for manipulation of fertiliser P applications in cereals following canola.

5.4.4 Technical consideration of the isotope technique for estimating BG P

Although this study using ^{33}P isotope purports to provide improved estimation of BGP compared with 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. (Gasser et al. 2015) suggested that substantial errors may occur in the estimation of root-derived N when using ^{15}N isotope because there may be immediate leakage of small amounts (0.5–1.0%) of highly enriched, fed ^{15}N isotope from the roots, which then are not representative of root or root-derived N. Although 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 by 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, in another study, Gardner et al. (2012) detected excess ^{15}N in soil 24 h after feeding ^{15}N to the plant via the leaves, and suggested that this could be attributed to ^{15}N in unrecovered very fine roots in soil and hence concluded that feeding did not induce substantial exudation. Whether the fed P in our 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 being 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 compared with the P present in other 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 these authors suggest 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 such as the sand in this present study. Alternatively, assimilation by microbial biomass in the rhizosphere may have occurred, as has been reported for exudates from wheat fed ^{32}P (McLaughlin et al. 1987). In a previous study complementary to the present one, in which plants fed ^{33}P at 45 DAS were sampled at peak vegetative stage (57 DAS) (Foyjunnessa et al. 2015), no ^{33}P was detected in labile soil P pools such as microbial biomass or resin P. This, in our opinion, supports the suggestion that rapid leakage of any fed P is unlikely to have occurred. However, we do agree that because these measures were taken 12 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 test rigorously 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 before feeding the isotope could remain unlabelled, although given the inter-connectivity of the vascular system in plants this seems unlikely. This study provides some evidence of spatial homogeneity in label distribution within the root system of canola grown in the loam, as suggested by our specific activity data; however, this was not the case in the sand (Table 3). Furthermore, we did not investigate different orders of roots. There is some evidence that different root cohorts may not be labelled uniformly, at least by leaf feeding of the isotope (Gasser et al. 2015), and any implications of this with regard to the estimates of BGP generated by this study need to be considered. Thus, more work is warranted to test the specific activity 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 clarify further the uniformity of ^{33}P labelling across root systems.

5.5 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 BGP input and the subsequent P benefit to young wheat plants was greater in a loam of high P fertility than a sandy soil of lower P fertility. Some cycling of root P occurs between flowering and break crop senescence. Phosphorus from canola below-ground residues contributed up to 20% of the P uptake of seedling wheat, and further work is required to clarify the longer term P fertility benefits from below-ground residues.

Acknowledgments

The authors thank the Grains Research and Development Corporation for providing top up funding (GRS10026) to support this research as this work contributes to outputs in GRDC project UA00119 and the University of Adelaide for an Australian Postgraduate Scholarship. We also thank CSIRO for use of their radioisotope laboratory facilities and access to a field site for soil collection.

References

ABS (2008) Agricultural Commodities: Small Area Data, Australia, 2005-06 (Reissue). https://www.rirdc.infoservices.com.au/downloads/10-113.

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

Armstrong R D, Dunsford K, McLaughlin M J, McBeath T, Mason S and Dunbabin V M (2015) Phosphorus and nitrogen fertiliser use efficiency of wheat seedlings grown in soils from contrasting tillage systems. Plant and Soil, 1-13.

Baggie I, Rowell D L, Robinson J S and Warren G P (2005) Decomposition and phosphorus release from organic residues as affected by residue quality and added inorganic phosphorus. Agroforestry Systems 63, 125-131.

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

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

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

Bengough A G, Lijima M and Barlow P W (2001) Image Analysis of Maize Root Caps—Estimating Cell Numbers from 2-D Longitudinal Sections. Annals of Botany 87, 693-698.

Birch C and 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 G J and Bolland O W (1978) The release of phosphorus from plant material added to soil. Australian Journal of Soil Research 16, 101-111.

Bolland M D A (1997) Comparative phosphorus requirement of canola and wheat. Journal of Plant Nutrition 20, 813-829.

Bolland M D A and Brennan R F (2008) Comparing the phosphorus requirements of wheat, lupin, and canola. Australian Journal of Agricultural Research 59, 983-998.

Brewster J L, Bhat K K S and Nye P H (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 and Soil 44, 279-293.

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

Bünemann E K, Bossio D A, Smithson P C, Frossard E and 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, 889-901.

Butterly C R, Bünemann E K, McNeill A M, Baldock J A and 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, 1406-1416.

Chalk P M, Peoples M B, McNeill A M, Boddey R M, Unkovich M J, Gardener M J, Silva C F and 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, 10-21.

Chauhan B S, Stewart J W B and Paul E A (1979) Effect of carbon additions on soil labile inorganic, organic and microbially held phosphate. Canadian Journal of Soil Science 59, 387-396.

Craig P R, Coventry D and Edwards J H (2013) Productivity advantage of crop–perennial pasture intercropping in Southeastern Australia. Agronomy Journal 105, 1588-1596.

Dalal R C (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, 913-916.

Damon P M, Bowden B, Rose T and 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 and 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 M J S (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, 173-185.

Foyjunnessa, McNeill A, Doolette A, Mason S and McLaughlin M (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 and McLaughlin M (2015) Quantifying total phosphorus accumulation below-ground by canola and lupin plants using ^{33}P-labelling. Plant and Soil, 10.1007/s11104-11015-12545-y.

Franzluebbers A J, Arshad M A and Ripmeester J A (1996) Alterations in canola residue composition during decomposition. Soil Biology and Biochemistry 28, 1289-1295.

Friesen D and 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, 355-366.

Fuller W H, Nielsen D R and Miller R W (1956) Some Factors Influencing the Utilization of Phosphorus from Crop Residues1. Soil Science Society of America Journal 20, 218-224.

Gardner M, Peoples M, Condon J, Li G, Conyers M and Dear B (2012) 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, Armidale, Australia.

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

Grant C A and Bailey L D (1993) Fertility management in canola production. Canadian Journal of Plant Science 73, 651-670.

Ha K V, Marschner P and Bünemann E K (2008) Dynamics of C, N, P and microbial community composition in particulate soil organic matter during residue decomposition. Plant and Soil 303, 253-264.

Helal H M and Sauerbeck D R (1984) Influence of plant roots on C and P metabolism in soil. Plant and Soil 76, 175-182.

Iqbal S M (2009) Effect of crop residue qualities on decomposition rates, soil phosphorus dynamics and plant phosphorus uptake. In Soil and Land Systems. pp 1-220. The University of Adelaide, Adelaide.

Isbell R (1996) The Australian soil classification. Australian soil and landsurvey handbook (4).

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

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

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

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

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

Kirkegaard J A, Sprague S J, Dove H, Kelman W M, Marcroft S J, Lieschke A, Howe G N and Graham J M (2008b) Dual-purpose canola—a new opportunity in mixed farming systems. Australian Journal of Agricultural Research 59, 291-302.

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

Kwabiah A B, Palm C A, Stoskopf N C and Voroney R P (2003a) Response of soil microbial biomass dynamics to quality of plant materials with emphasis on P availability. Soil Biology and Biochemistry 35, 207-216.

Kwabiah A B, Stoskopf N C, Palm C A and Voroney R P (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, 53-61.

Kwabiah A B, Stoskopf N C, Palm C A, Voroney R P, Rao M R and Gacheru E (2003c) Phosphorus availability and maize response to organic and inorganic fertilizer inputs in a short term study in western Kenya. Agriculture, Ecosystems & Environment 95, 49-59.

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

Llewellyn R S, D'Emden F H and Kuehne G (2012) Extensive use of no-tillage in grain growing regions of Australia. Field Crops Research 132, 204-212.

Lupwayi N Z, Clayton G W, O'Donovan J T, Harker K N, Turkington T K and Soon Y K (2007) Phosphorus release during decomposition of crop residues under conventional and zero tillage. Soil and Tillage Research 95, 231-239.

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

Mason S, McNeill A, McLaughlin M and Zhang H (2010) Prediction of wheat response to an application of phosphorus under field conditions using diffusive gradients in thin-films (DGT) and extraction methods. Plant and Soil 337, 243-258.

Matejovic I (1997) Determination of carbon and nitrogen in samples of various soils by the dry combustion. Communications in Soil Science and Plant Analysis 28, 1499-1511.

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

McBeath T M, McLaughlin M J, Kirby J K and Armstrong R D (2012) The effect of soil water status on fertiliser, topsoil and subsoil phosphorus utilisation by wheat. Plant and Soil 358, 337-348.

McLaughlin M and 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, 517-526.

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

McLaughlin M, Alston A and Martin J (1988) Phosphorus cycling in wheat pasture rotations .I. The source of phosphorus taken up by wheat. Soil Research 26, 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. Eds. M Unkovich, J Pate, A McNeill and D J Gibbs. pp 195-218. Springer Netherlands.

McNeill A and 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 and Soil 302, 297-316.

McNeill A M, Zhu C and Fillery I R P (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, 295-304.

Misra R K, Alston A M and Dexter A R (1988) Role of root hairs in phosphorus depletion from a macrostructured soil. Plant and Soil 107, 11-18.

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

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

Nachimuthu G, Guppy C, Kristiansen P and 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 and Soil 314, 303-310.

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

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

Noack S R, McBeath T M, McLaughlin M J, Smernik R J and Armstrong R D (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, 31-39.

Norton A, Kirkegaard J, Angus J and T P (2013) Canola in Rotations 7. The Regional Institute, http://www.regional.org.au/au/gcirc/canola/p-06.htm.

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

Olsen R G and Court M N (1982) Effect of wetting and drying of soils on phosphate adsorption and resin extraction of soil phosphate. Journal of Soil Science 33, 709-717.

Peoples M B, Bowman A M, Gault R R, Herridge D F, McCallum M H, McCormick K M, Norton R M, Rochester I J, Scammell G J and Schwenke G D (2001) Factors regulating the contributions of fixed nitrogen by pasture and crop legumes to different farming systems of eastern Australia. Plant and Soil 228, 29-41.

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

Rayment G and Higginson F R (1992) Australian laboratory handbook of soil and water chemical methods. Inkata Press Pty Ltd, Melborne.

Rayment G E and Lyons D J (2011) Soil chemical methods: Australasia. CSIRO publishing.

Read D W L and Campbell C A (1981) Bio-cycling of phosphorus in soil by plant roots. Canadian Journal of Soil Science 61, 587-589.

Reuter D J and Robinson B J (1997) Plant Analysis: an Interpretation Manual. CSIRO, Collingwood, Australia.

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

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

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

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

Rovira A D and Bowen G D (1970) Translocation and loss of phosphate along roots of wheat seedlings. Planta 93, 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, 152-157.

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

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

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

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

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

White R E and Ayoub A T (1983) Decomposition of plant residues of variable C/P ratio and the effect on soil phosphate availability. Plant and Soil 74, 163-173.

Wilhelm N S (2001) Warm season cropping in the southern cropping zone of Australia. In Proceedings of 10th Australian Agronomy Conference, Hobart, Australia.

Zadoks J C, Chang T T and Konzak C F (1974) Decimal code for growth stages of cereals. A Weed Research 14, 415-421.

Zarcinas B A and Cartwright B (1983) Analysis of soil and plant material by inductively coupled plasma-optical emission spectrometry. In Division of soils technical paper / Commonwealth Scientific and Industrial Research Organization. pp 1-36. CSIRO, Australia. Division, Soils, Technical Paper No. 45: 1-35.

Zarcinas B A, McLaughlin M J and Smart M K (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, 1331-1354.

CHAPTER 6

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

The work contained in this chapter has been prepared with an intention to submit to *Plant and Soil*.

Corrigendum to Chapter 6 in PhD Thesis Foyjunnessa 2016

The data in this chapter has been substantially revised since publication of the thesis.

The reader is referred to the following publication for the updated presentation and interpretation of data from this chapter:

Foyjunnessa, McNeill, A., Doolette, A., Mason, S., 2018. Dual-labelling (^{15}N and ^{33}P) provides insights into stoichiometry and release of nitrogen and phosphorus from *in situ* mature lupin and canola below-ground residues. *Plant and Soil* **426**, 77-93.

November 2018

Statement of Authorship

Title of Paper	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
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	This chapter is being prepared for publication and will be submitted to *Plant and Soil*.

Principal Author

Name of Principal Author (Candidate)	Foyjunnessa		
Contribution to the Paper	Performed experiment and analysis on all samples, interpreted results, wrote the manuscript, illustrated all figures and tables, and acted as corresponding author.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Ann McNeill		
Contribution to the Paper	Supervised experimental design and development of the work, interpretation of results, and manuscript evaluation		
Signature		Date	23/1/2015

Name of Co-Author	Sean Mason		
Contribution to the Paper	Supervised development of the work, help in sample analysis, interpretation of results, and manuscript evaluation		
Signature		Date	

Name of Co-Author	Ashlea Doolette		
Contribution to the Paper	Supervised development of the work, help in sample analysis, interpretation of results, and manuscript evaluation		
Signature		Date	

Name of Co-Author	Mike J. McLaughlin		
Contribution to the Paper	Help in experimental design, interpretation of results, and manuscript evaluation		
Signature		Date	29/10/15

Abstract

Background and aims

Belowground (BG) residues may contribute significant amounts of N and P *via* nutrient cycling to following crops, particularly in low fertiliser input systems or where all the above-ground residues are removed. Reports of simultaneous measures of nitrogen (N) and phosphorus (P) contributions from mature crop residues to wheat, especially from roots, are rare. Dual-feeding with ^{33}P and ^{15}N was used to simultaneously assess *in situ* (i) BG N and BG P accumulation by lupin and canola, and (ii) the contribution of N and P from the decomposition of these BG residues to N and P uptake by following wheat.

Methods

One set of pots were destructively sampled at lupin and canola maturity to quantify total accumulation of BG P and BG N, including estimated N and P in unrecovered roots plus root-derived materials (RD N and RD P). Shoots were removed from a second set of pots into which wheat was sown after a three week fallow. Uptake of P and N from the decomposing *in situ* $^{33}P/^{15}N$ labelled lupin and canola BG residues was measured in five week old wheat.

Results

65% of fed ^{33}P and 10% of fed ^{15}N was partitioned BG. 20-30% fed ^{15}N was not recovered. Canola root had higher P and lower N concentrations than lupin. Canola total BG P was greater than lupin with a higher proportion as estimated RD P. Estimated RD N was similar in both species but lupin had more N in roots and so higher total BG N. 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. N:P ratio for lupin roots was wider (29:1) than canola (5:1), but N:P ratio of RD fractions was similar (6:1 canola; 7:1 lupin). Proportion of BG P taken up by wheat was 21% after canola and 19%

after lupin, 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 less than from canola (12.5%)

Conclusion

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 in the short term did not appear to be 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.

Keywords: Simultaneous assessment, Dual-labelling *in situ***,** BG residues N:P ratio, N and P benefits of BG residues

1 Introduction

Break crops such as oilseeds and legumes in rotations have many benefits for the growth of cereal crops (Karlen et al. 1994), but in low input systems the contribution from decomposition of break crop residue inputs to nutrient cycling are considered particularly important (Schomberg et al. 1994). It is well known that the release of nutrients from crop residues is dependent on a number of edaphic factors, such as soil texture, temperature, moisture, biological activity and organic matter content (Smith and Paul 1990) but also nutrient release is particularly influenced by the quality of the residue inputs (Palm and Rowland 1997). It seems generally accepted that this release is broadly a function of two key parameters; (i) the biochemical nature of the residue (Bertrand et al. 2006; Jensen et al. 2005), including the relative proportion of nutrients such as N and P in forms that are more readily accessible (often soluble) such as proteins, orthophosphates, phospholipids and nucleic acids, compared with N and P associated with recalcitrant compounds such as lignin, hemi-cellulose, polyphenols , pyrophosphates and phytate (Lupwayi et al. 2007; Palm and Sanchez 1991), and (ii) the quantity of these nutrients (N and P) in relation to carbon, expressed as a mass based C:N or C:P ratio (Heal et al. 1997; Kwabiah et al. 2003a; Lupwayi et al. 2006; Nicolardot et al. 2001; Swift et al. 1979). There are also a few reports of N:P ratio as an indicator of nutrient release (Kwabiah et al. 2003b; Vogt et al. 1986). However, there appears to be no single quality parameter that can be universally applied for prediction of N or P release from plant residue decomposition (Abiven et al. 2005; Bertrand et al. 2006), and this is not that surprising given that residue quality parameters will vary considerably depending on species, age of plant at harvest and type of residue e.g. seed, stem, leaf or root (Gan et al. 2011).

The many reported studies of residue decomposition have investigated vastly different materials and so there is wide variation in values reported for the critical concentration of crop

residues for N or P release. Critical values for P concentration range from 2 to 3.0 g P kg^{-1} (Fuller et al. 1956; Kwabiah et al. 2003b; Singh and Jones 1976), and for N concentration from 15-20 g N kg^{-1} (Campbell 1978). There is also extremely wide variation in reported critical C:P ratios from 100:1 to 300:1 (Blair and Bolland 1978; Cheshire and Chapman 1996; Fuller et al. 1956; Iqbal 2009; Kwabiah et al. 2003b; Lousier and Parkinson 1978; White and Ayoub 1983), although somewhat less variation for critical C:N ratio ranging from 20-30:1 (Campbell 1978; Reinertsen et al. 1984). Despite these wide ranges of critical values for quality parameters it is often stated that residues of oilseed and legumes input more P and N to the soil than cereal residues because the P and N concentrations of these break crop species are higher with narrower C:P and C:N ratios than those of cereals (Armstrong et al. 1994; Nebiyu et al. 2014; Strong et al. 1986).

Assessment of nutrient release in the early stages of decomposition may correlate more closely to a water soluble fraction or to the critical total nutrient concentration than to a C:nutrient ratio since nutrient in a more labile or soluble form in the residues is likely to be more accessible for assimilation by microbial biomass or plant uptake *via* release directly into the soil solution. There can be a substantial proportion of soluble P in plant residues (Jones and Bromfield 1969) including canola (Noack et al. 2012) which can be rapidly released, as has been shown for residues of a range of crop and pasture species including roots (Blair and Bolland 1978; Martin and Cunningham 1973). Indeed, Kwabiah et al. (2003b) examining tropical above-ground residues found total P and water soluble P contents were best predictors of P release as measured by P availability (resin). Similarly, Jensen et al. (2005) investigated a wide range of plant residues and plant parts, although again this did not include roots, and found that N mineralisation up to 22 days correlated well with water soluble N in residues. They also reported that thereafter N mineralisation most closely correlated with total plant N and neutral detergent soluble fraction, and unlike many other studies they did not find a relationship of N

mineralised to C:N or lignin:N. It has been suggested that roots may decompose slower than shoots (Balesdent and Balabane 1996) and hence it could be expected they would release nutrients slower. Indeed, Abiven et al. (2005) found that wheat root C:N ratio was not as well related to N release as shoot C:N ratio and indicated this may be due to biochemical differences in constituents of shoots versus roots. However there are a number of studies where net release of N (Till et al. 1982) and P (Alamgir et al. 2012; Dalal 1979; Till et al. 1982) from root residues has been shown to be similar or greater than from shoot residues, although in some of these studies temporal patterns of release differed but ultimately the proportion of residue P and N apparently mineralised was the same. The limited studies on N:P ratio report critical ratios in the range 7:1 - 14:1 (Kwabiah et al. 2003b; Vogt et al. 1986). Bell et al. (2014) state that N:P ratios will depend upon where in the plant tissue the nutrient is located since N:P in metabolic tissue is around 20:1 but may be 50:1 in woody structural tissue (Reiners 1986) and this will have implications with regard to the maturity of the residue when incorporated to soil. Indeed, in most of the studies referred to above the residue materials were often not mature.

It is evident that information on N and P release is much more widely available for above-ground plant residues than roots (Arcand et al. 2014a) even though roots may be a reasonable proportion of plant dry matter production (Gan et al. 2009; Gregory 2008) and generally remain *in situ* as an organic matter input to soil after harvest. Where there is information for roots it is for roots that have been extracted from soil prior to re-incorporation or produced in solution culture (Friesen and Blair 1988; Martin and Cunningham 1973; Nachimuthu et al. 2009). The contribution to soil nutrient cycling *via* root residues may be especially significant in agricultural systems where all above-ground residues are harvested, in low fertiliser input systems common to semi-arid environments, and in no-till systems where above-ground residues accumulate on the soil surface or as standing stubbles and are not necessarily in immediate contact with soil. The quantity of nutrients in residues, calculated as a product of

the dry mass and the concentration, can be determined easily for above-ground plant residues such as stubbles, stems and leaves. However, quantifying N and P in the same manner using such data for recovered roots (Gan et al. 2011; Soon and Arshad 2002) may underestimate the total quantity of nutrient that the plant inputs BG since nutrients in fine roots and root-derived materials in soil are not recovered, as has been demonstrated for lupin BG N (McNeill and Fillery 2008; Wichern et al. 2011), canola BG N (Arcand et al. 2013) and canola BG P (Foyjunnessa et al. 2014; Foyjunnessa et al. 2015). The *in situ* isotope-labelling used in such studies also allowed in some cases for direct tracing of the decomposition of intact BG residues of these crop plant species. The proportion of BG N released on decomposition ranged from 12-27% for lupin and was 6.5% for canola which contributed 7-27% of the N uptake of a subsequent cereal (Arcand et al. 2014b; McNeill and Fillery 2008), whereas in a recent study P release (assessed as plant P uptake plus P in resin and microbial pools) from BG decomposition of canola in two different soils ranged from 26-34% and contributed 20% of the P uptake of a subsequently planted cereal (Chapter 5, submitted to *Crop and Pasture Science*). Dual-labelling with N and P isotopes would allow for simultaneous study of N and P release from BG plant residues *in situ* which we believe has not yet been reported, and could enable direct examination of any relationship with residue C:N, C:P and N:P ratio. A seminal study employing multiple-isotope labelled plant residues (shoots and roots) added to soil, including ^{15}N and ^{32}P, demonstrated that a larger proportion of P (42%) than N (30%) from clover residues was accessed by oats grown for 70 days (Till et al. 1982). However, the residues were immature and so the relative release of these nutrients may not represent that from the decomposition of *in situ* mature roots.

Information is certainly scarce for the combined accumulation *in situ* of N and P BG by break crops such as canola and lupin, and the relative release of these nutrients as the BG residues decompose. Hence, we proposed to use dual isotope-labelling ($^{15}N/^{33}P$) in plants to provide

simultaneous insights into the fate of P and N from mature canola and lupin BG residues. We tested the hypothesis that P release from canola BG residues would be relatively greater than from lupin BG residues whereas N release would be relatively smaller. The specific aims of this study were to i) estimate total amounts of N and P accumulated *in situ* BG by mature canola and lupin plants, ii) directly trace and quantify simultaneously the uptake of that N and P by subsequently planted wheat as these *in situ* BG residues decompose following shoot removal.

2 Materials and methods

2.1 Soil preparation and pot set up

As previously described (Foyjunnessa et al. 2015) an agricultural soil (top 10 cm) was collected from South Australia; at Karoonda (36°04′S, 140°05′E). The soil was air-dried at room temperature, sieved (2 mm mesh) to remove any large stones and organic materials. The initial chemical properties of the soil are described in detail elsewhere (Foyjunnessa et al. 2014; Foyjunnessa et al. 2015). Briefly, the soil was 95% sand with < 5% clay, had a low PBI of 4.2 (Burkitt et al. 2002) and contained total P of 114 mg kg^{-1} including a resin P of 29 mg kg^{-1} (Kouno et al. 1995), total N of 0.3 g kg^{-1} and total C of 3.3 g kg^{-1}. No P fertilizer was added to the soils.

Preparation of the nutrient solutions for canola (with N) and for lupin (without N) are described in detail in Foyjunnessa et al. (2015). A cement mixer was used to mix batches of 35 kg dry soil plus nutrient solution and additional water to maintain 70% of maximum water holding capacity. After mixing the soil was packed sequentially, layer by layer, into a PVC pot (height 40 cm, inner diameter 15 cm) that had been capped at one end, to a bulk density of 1.2 g cm^{-3}.

2.2 Break crop phase- details of the experiment

Canola (*Brassica napus* L. cv. Tanami) and narrow-leaf lupin (*Lupinus angustifolius* L. cv. Mandelup) seeds were pre-germinated on moist filter paper at room temperature. Germinated canola or lupin seeds were sown (4 seeds pot^{-1}) into the prepared PVC pots. Lupin was inoculated at sowing with a peat-water suspension (2 ml pot^{-1}) containing legume specific Rhizobium inoculant (Nodulated ^{TM} Legume Peat Inoculant Group G). All pots were thinned to one plant per pot in the first week. The pots were watered daily with deionised water for the first 14 days after sowing, then on alternate days for the remainder of the experiment, and maintained at 70% water holding capacity throughout the experimental period. The pots were arranged on a bench in the glasshouse using a randomized complete block design with eight replicates per treatment (two treatments; canola and lupin) plus two replicate control plants (non-labelled) per treatment for determining the natural abundance (atom% ^{15}N) of soil and plants. Over the duration of this phase of the study (105 days) each pot received a total (mg) of K (300), Mg and Ca (60), Zn and Cu (45), S (150), Mn (7) and N (400 – canola, 195 – lupin) from the initial application described above plus three top up applications of nutrient solution to each canola and lupin pot on 21, 27 and 34 days after sowing. Throughout the growing period of the break crop phase (105 days) plants received an average day length of 12 hours, temperature ranged from 16^0 to 28^0 C and an average relative humidity of 73 (% RH).

2.3 Dual-labelling (^{15}N and ^{33}P) of break crops using stem wick-feeding

Five weeks (35 days) following sowing, when canola was at early flowering stage and lupin at pre-flowering stage, each plant was fed with ^{15}N-enriched urea (0.4 % w/v, 98.61 atom% ^{15}N) using the stem wick-feeding technique for ^{15}N (McNeill 2001). Briefly, a single dose (1 ml) of ^{15}N-enriched urea (containing 1.83 mg ^{15}N excess ml^{-1}) was placed into a 5 ml vial that was attached by a cotton wick fed through the stem of the plant approximately 3 cm above the soil

surface *via* a pre-drilled hole (0.5 mm diameter). Canola and lupin plants were supplied with less than 2 mg N $plant^{-1}$ with the aim of minimising the likelihood of any necrotic effects on the plants (leaf burn) as previously reported by Palta et al. (1991) or stem scar tissue development as reported by (Arcand et al. 2013). Uptake of the ^{15}N-enriched urea solution was completed within three days and was followed by two sequential flushing feeds each using 1 ml of deionised water. Ten days after the introduction of the ^{15}N-enriched urea solution to the plants (45 days after sowing), a single dose (1 ml) of ^{33}P radioactive solution as $H_3{}^{33}PO_4$ solution (99% isotopic purity) with an activity of 459 kBq $plant^{-1}$ was placed into the same vial. The uptake of the radioactive solution was slower than the uptake of the ^{15}N-enriched urea solution and was completed within five days, and was followed by the two sequential flushing feeds using deionised water (1 ml). The vials were removed after this and the wicks were stored for further analysis. Following feeding of isotope and up until harvest any leaves, flowers, or pods that matured and detached from the canola and lupin plants were collected, stored in a paper bag and if the source plant was known, were added to the relevant above-ground shoot dry matter at harvesting. Where source plant could not be identified these fallen plant materials were discarded. All canola and lupin plants including controls were grown to maturity and 50% of the replicates (four labelled plants plus one unlabelled control per treatment and species) were destructively sampled at 105 DAS, processed and analysed as described later.

2.4 Fallow and wheat phase

The above-ground dry matter (shoot) of canola and lupin plants were removed (1 cm above soil surface) from the other 50% of the replicates (four labelled plus one control per treatment and species). The dual-labelled (^{15}N and ^{33}P) below-ground systems (entire root system plus soil) were left undisturbed in the PVC pots 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 temperature ranged from 15^0 to 24^0 C, relative humidity ranged from 25 to 82% and day length was 11 h. Over the duration of the wheat phase (5 weeks) each PVC pot was given a single application of nutrient solution (including N) at 21 DAS and received (mg) of K (25), S (20), Ca (5), Mg (5), Fe (0.3), Mn (0.5), Cu (1), Zn (1), N (75) and zero P. Soil moisture was maintained at 60% of the maximum water holding capacity throughout the experiment with frequent small additions of deionised water. All wheat plants were harvested 35 days after sowing when the boot was just visibly swollen (GS43) (Zadoks et al. 1974).

2.5 Sample processing and analysis

At the destructive harvest of the mature canola and lupin plants the shoots were cut directly above the soil surface (1 cm) and removed whilst ensuring that there was no input of above-ground plant materials into the soil. Sealed caps were removed from the base of the pots which were then placed in a plastic tray (60 cm length). A special piston shaped tool with marginally smaller diameter (14.5 cm) than the PVC pot (15 cm) was used to extrude each intact root-soil column by pushing from the base of the pots. All visible roots and root fragments with adhering rhizosphere soil were recovered by hand and stored in plastic bag. The remaining entire bulk soil was then air dried in room temperature (glasshouse) for 24 hours, sieved using 2 mm sieve and then subsampled (100 g) for further analysis. Root materials >2 mm on the sieve were added to the hand recovered root sample. All sample fractions (shoot, recovered roots plus rhizosphere soil and 100 g sub-sample of bulk soil) were frozen (-18°C) and subsequently freeze dried before further processing and chemical analysis. Following freeze drying, clean roots (recovered) were obtained by brushing off rhizosphere soil using a soft paint brush. Fresh and dry weight of shoot, dry weight of recovered roots and soil fractions (rhizosphere and bulk soil) were recorded. Subsequently harvested wheat was processed similarly.

All freeze dried plant samples (shoots and recovered roots) and soil samples were finely ground in a ball mill and subsamples were weighed into tin capsules and analysed for N concentration (%) and atom% ^{15}N using a continuous flow isotope ratio mass spectrometer (Sercon 20-22) connected to an elemental analyser sample preparation unit (ANCA-GSL) (Todd and Paul 2001). Atom% ^{15}N excess in plant and soil samples were determined by deducting the ^{15}N atom% natural abundance values for control (non-labelled) plants from the ^{15}N atom% values of the ^{15}N-enriched urea labelled plants (Schmidtke 2005). Other subsamples of the finely ground plant and soil samples were digested (1.0 g), and total P concentration, ^{33}P activity in the digest solution, and minimum detection limit (kBq kg soil^{-1}) were determined as described previously (Foyjunnessa et al. 2015), using the mean of the background isotope activity (23.5) for un-spiked soils plus 10*standard deviation (2 replicates) of the background activity (Thompson et al. 1987). Each cotton-wick used to feed the ^{15}N and ^{33}P was also digested (four replicates) and analysed similarly for ^{33}P sorption to the wick. Recorded ^{33}P activity in each sample was then back corrected for decay and expressed as kBq.

2.6 Estimation of canola and lupin RD P and RD N and calculation of total BG P and total BG N

Root-derived P (RD P) and N (RD N) in bulk and rhizosphere soil fractions were estimated as described previously (Foyjunnessa et al. 2015; McNeill 2001), using the ^{33}P specific activity (SA) or ^{15}N excess specific enrichment (SE) of clean recovered roots as follows:

RD P = ^{33}P activity in soil (kBq pot^{-1})/ ^{33}P SA of clean roots (kBq mg P^{-1})……(I)

RD N = ^{15}N excess in soil (µg ^{15}N excess pot^{-1})/ SE of clean roots (µg ^{15}N excess mg N^{-1}) .(II)

^{33}P SA of clean recovered roots was calculated as ^{33}P in recovered roots (kBq)/ ^{31}P content of recovered roots (mg). Total ^{33}P activity for the 8 kg soil was calculated from the kBq detected in a digested sub-sample (1.0 g).

^{15}N excess SE was calculated as ^{15}N excess in a clean recovered root sample (µg pot^{-1}) divided by the total N of the clean recovered root sample (mg pot^{-1}).

Final units of mg N or P pot^{-1} equate to mg N or P $plant^{-1}$ since there was one plant per pot.

Total BGP (mg P $plant^{-1}$) was calculated as the sum of P measured in recovered roots (P_{recrt}) plus the estimated amounts of root-derived P in the bulk (RD P_{bulk}) and rhizosphere (RD P_{rh}) soils as calculated using equation I.

RD P as a percentage of total BG P was then calculated as:

RD P as % of total BGP =

$$(RD\ P\ mg\ P\ plant^{-1} / Total\ BGP\ mg\ P\ plant^{-1}) * 100 \ldots\ldots\ldots\ldots\ldots\ldots (III)$$

Total BG N (mg N $plant^{-1}$) was calculated as the sum of N measured in recovered roots (N_{recrt}) plus the estimated amounts of root-derived N in the bulk (RD N_{bulk}) and rhizosphere (RD N_{rh}) soils as calculated using equation II.

RD N as a percentage of total BG N (equation IV) was then calculated as:

RD N as % of total BG N =

$$(RD\ N\ mg\ N\ plant^{-1} / Total\ BG\ N\ mg\ N\ plant^{-1}) * 100 \ldots\ldots\ldots\ldots\ldots (IV)$$

RD P and RD N were considered to be mainly unrecovered roots plus any P or N-containing derivatives from roots such as exudates, sloughed material or products of any root decomposition including microbial biomass (Foyjunnessa et al. 2015; McNeill 2001). Estimates of BG P and BG N were based on the assumption inherent to all isotope studies of homogeneity of labelling for the pool of interest (in this case the roots), and also that there was no root senescence or nutrient exudation prior to labelling, and no immediate loss of label after feeding *via* excretion from roots (Chapter 5; submitted to *Crop and Pasture Science*).

2.7 Calculation of P and N in wheat derived from (df) BG P and BG N of the previous lupin or canola

The amount of P and N in the wheat derived from (df) the lupin or canola BG P and BG N was calculated on a mass balance basis as follows:

P_{df} BG P of lupin or canola (mg plant^{-1}) =

(^{33}P activity wheat (kBq plant^{-1})/ ^{33}P activity total BG P of lupin or canola (kBq plant^{-1})) total BG P of lupin or canola (mg plant^{-1})……..(V)*

N_{df} BG N of lupin or canola (mg N plant^{-1}) =

(^{15}N excess of wheat (µg ^{15}N excess plant^{-1}) /^{15}N excess in BG N of lupin or canola (µg ^{15}N excess plant^{-1})) estimated BG N of lupin or canola (mg N plant^{-1})…….(VI)*

The proportion (%) of wheat shoot and root P and N that was derived from (df) lupin or canola BG P or BG N was calculated as:

% wheat shoot or root P_{df} BG P of lupin or canola =

*(Amount wheat shoot or root P_{df} BG P of lupin or canola (mg P plant^{-1}) / wheat shoot or root P (mg plant^{-1}))*100……..(VII)*

% wheat shoot or root N_{df} BG N of lupin or canola =

*(Amount wheat shoot or root N_{df} BG N of lupin or canola (mg N plant^{-1}/ wheat shoot or root N (mg plant^{-1})*100……..(VIII)*

2.8 Statistical analysis

The experiment design consisted of two treatments (species) with four replicates. Data from each treatment were tested for normality and homogeneity of variance. A one-way Analysis of

Variance (ANOVA) was undertaken using the GENSTAT statistical package (Version 15; 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.

3 Results

3.1 Canola and lupin total plant dry matter, N and P concentration and content

Total plant dry matter (i.e shoot plus recovered root >2 mm sieve mesh), shoot and root P concentrations were all significantly higher ($p<0.05$) for canola than lupin, and N concentrations significantly lower (Table 1). Hence total plant P content (Fig. 1a) for canola (67.1 mg P plant^{-1}) was slightly more than twice that for lupin (32.3 mg P plant^{-1}) whilst total plant N content of canola (273.4 mg N plant^{-1}) was significantly ($p<0.05$) less than for lupin (424.5 mg N plant^{-1}) (Fig. 1b).

Table 1 Total plant dry matter, shoot and root (>2mm mesh) phosphorus and nitrogen concentrations, and estimated root carbon:nitrogen and carbon phosphorus ratios at 105 days after sowing (maturity) for isotope-labelled glasshouse-grown lupin and canola.

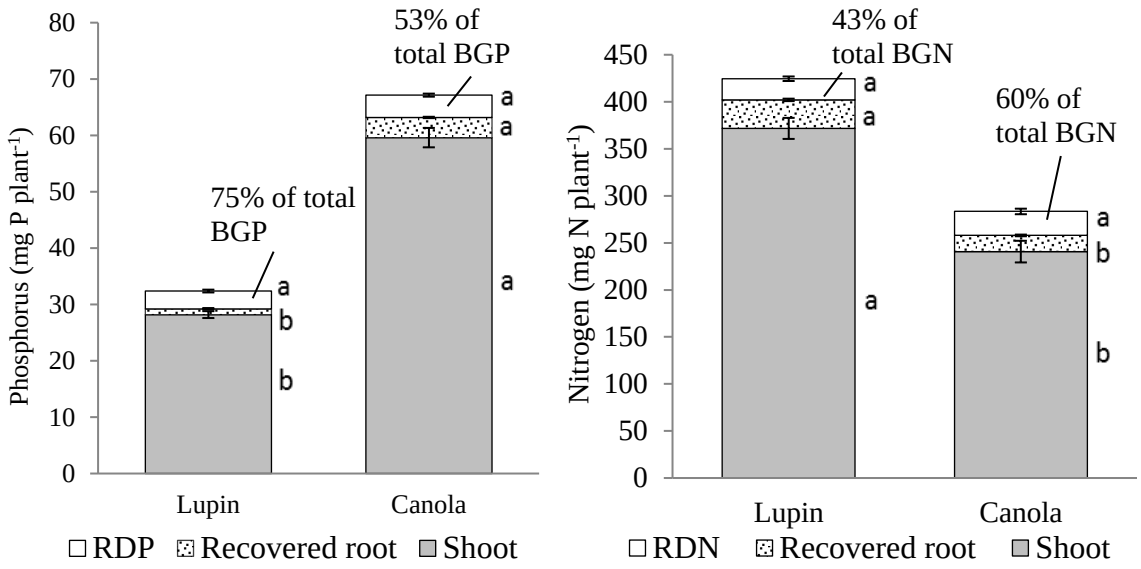
Species	Dry matter	P concentration (g kg^{-1})		N concentration (g kg^{-1})		C**: P	C**: N
	Total (g $plant^{-1}$)	Shoot	Root	Shoot	Root	Root	Root
Lupin	14.29 b	2.26 b	0.64 b	29.79 a	16.91 a	708 a	24 b
Canola	15.78 a	4.23 a*	2.21 a*	17.05 b	10.24 b	188 b	39 a
$lsd_{0.05}$	1.3	0.2	0.3	1.8	0.7	131	2.3

Data are means (n=4); different letters following means within a column indicate significant differences between species at P<0.05

* data for canola P concentration reported previously (Foyjunnessa *et al*; submitted to *Crop and Pasture Science*)

Figure 1 (a) Phosphorus (mg P plant^{-1}) and (b) nitrogen content (mg N plant^{-1}) of lupin and canola shoot and recovered root (2mm mesh sieve), and estimated amounts of (a) root derived P (RDP) or (b) root derived N (RDN) calculated from isotope activity in the soil (<2 mm fraction). Plants were dual-labelled with ^{15}N (35 DAS)/ ^{33}P (45 DAS) using a stem wick-feeding technique and harvested at 105 DAS. Data are means (n=4) and different letters within same pattern indicate significant differences (lsd0.05). Canola plant P previously reported (Chapter 5, submitted to *Crop and Pasture Science*)

a) b)



3.2 Recovery and distribution of fed ^{33}P and ^{15}N excess in canola or lupin, wick and soil

Total recovery of fed ^{33}P was 95-97% which included a measured constant 6.9% sorption to the feeding wick (Table 2). In contrast, total recovery of fed ^{15}N excess was lower (70-80%) and although any wick sorption of excess ^{15}N was not assessed because all the wicks were utilised for ^{33}P measurement the extent of the 'loss' indicates other loss pathways from the soil or plant. A greater proportion of the fed ^{33}P was distributed below-ground, with 24-35% of fed

^{33}P in recovered roots and 34-47% in soil, compared to a mean of 18.5% of fed ^{33}P in the mature shoot including grain (Table 2). Conversely, the distribution of fed ^{15}N excess was greater to the shoots (60-69%) compared to 2.5-4.5% in recovered roots and 6.6-7.5% in soil (Table 2). There was no significant difference between the two species for recovery of ^{33}P in shoots but below-ground there was proportionately more ^{33}P recovered in canola roots and less in the soil (estimated root-derived fraction) compared with lupin (Table 2). Significantly more ^{15}N excess was partitioned in lupin shoots and recovered roots than in the same plant components for canola, whereas more ^{15}N excess was recovered in the soil for canola than for lupin (Table 2).

Table 2 Recovery (%) of fed ^{33}P and ^{15}N (459.25 kBq plant^{-1}) at 105 days after sowing (DAS) for lupin and canola plants dual-labelled with ^{15}N (1831 µg ^{15}N excess plant^{-1}) at 35 DAS and ^{33}P (459.25 kBq plant^{-1}) at 45 DAS using a stem-wick feeding technique.

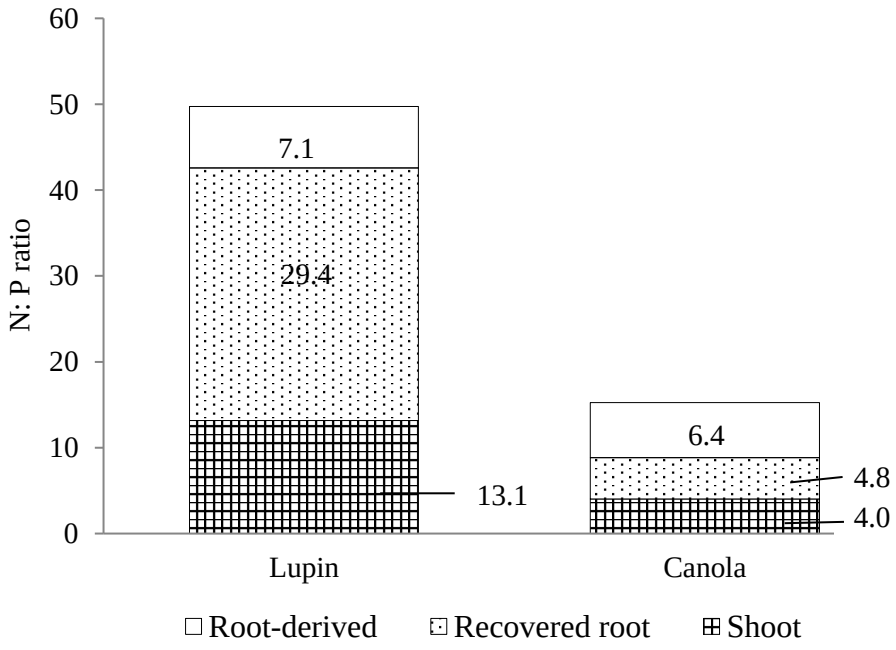
	Recovered ^{33}P (% of fed)			Recovered ^{15}N excess (% of fed)		
	Lupin	Canola *	$lsd_{0.05}$	Lupin	Canola	$lsd_{0.05}$
Shoot	19.0 ns	18.3 ns	2.2	69.2 a	60.01 b	4.7
Roots (>2mm sieve)	23.9 b	35.5 a	2.5	4.5 a	2.5 b	0.4
Soil	47.2 a	33.9 b	2.1	6.6 b	7.5 a	0.4
Wick	6.9	6.9		nd	nd	
Total	97.1	94.6		80.2	70.0	

Data are means (n=4); different letters following means within rows for each isotope (^{33}P or ^{15}N) indicate significant difference between species ($lsd_{0.05}$); ns indicates no significant difference within a row; nd indicates value not determined; * ^{33}P recovery for canola previously reported (Foyjunnessa *et al*; submitted to *Crop and Pasture Science*)

3.3 Amounts of total BG P and BG N and proportion as root-derived P (RD P) and root-derived N (RD N)

Canola had significantly more total BG P than lupin but less total BG N, however a higher proportion (75%) of the total BG P was present as estimated RD P (Fig. 1a) in the lupin (3.15 mg P plant^{-1}) compared to 53% (3.96 mg P plant^{-1}) for canola. There was much less recovered root P for lupin (1.03 mg P plant^{-1}) compared to 3.58 mg P plant^{-1} for canola (Fig 1a). Total BG N was significantly greater for lupin (52.8 mg N plant^{-1}) than canola (42.7 mg N plant^{-1}) but both species had similar amounts of estimated RD N (Fig. 1b), 22.5 mg N plant^{-1} for lupin and 25.5 mg N plant^{-1} for canola. Hence they differed significantly in amounts of recovered root N (Fig. 1b); 30.3 mg N plant^{-1} for lupin compared to 17.2 mg N plant^{-1} for canola.

Figure 2 N: P ratio (mass basis) of shoot, recovered root and estimated root derived fraction for lupin and canola harvested at 105 days after sowing (DAS). Data are means (n=4)



3.4 C:N and C:P ratio of recovered roots and N:P ratio for shoot, recovered root and root-derived fraction

Estimated C:N ratio of recovered roots was wider for canola than lupin (Table 1) whereas estimated C:P ratio for the recovered lupin roots was much wider than for canola roots, due primarily to the very low P concentration of lupin roots (Table 1). N:P ratio of shoot and recovered root were significantly ($p<0.01$) wider for lupin than canola (Fig. 2), again especially for the recovered roots where P concentration for lupin was markedly lower than for canola (Table 1). However, the estimated root-derived fraction N:P ratio was similar for both species (Fig 2).

Table 3 Plant dry matter, phosphorus and nitrogen concentrations (shoot and root >2mm mesh) of wheat plants grown for 5 weeks in a glasshouse (no P fertilizer applied) in undisturbed soil cores where previously ^{15}N-^{33}P labelled lupin or canola had been grown and the shoots removed at 105 DAS. The labelled below-ground system (intact root plus soil) was left undisturbed for three weeks before wheat was grown.

Treatments	Dry matter (g plant^{-1})	P concentration (g kg^{-1})		N concentration (g kg^{-1})	
	Total	Shoot	Root	Shoot	Root
Wheat after lupin	2.70 ns	2.88 b	1.77 ns	32.41 a	12.33 b
Wheat after canola	2.75 ns	3.39* a	1.61* ns	28.30 b	14.85 a
$lsd_{0.05}$	0.13	0.39	0.66	2.74	0.85

Data are means (n=4); different letters within a column indicate significant difference between treatments; ns indicates non-significance within a column; * P concentration for wheat after canola previously reported (Foyjunnessa *et al*; submitted to *Crop and Pasture Science*)

3.5 Wheat plant dry matter, P and N concentration of shoot and recovered root

There were no differences in plant (shoot plus recovered root) dry matter of wheat grown after canola or after lupin (Table 3). However, shoot P concentration was significantly higher for wheat after canola and was borderline deficient (<0.3% P) for the GS42 growth stage (Reuter and Robinson 1997) of the wheat after lupin, whereas shoot N concentration was sufficient for wheat after both species, although higher for wheat after lupin (Table 3). Overall the nutrient concentrations of recovered roots were lower than shoots. P concentration of recovered roots was similar for wheat after canola and lupin, with N concentration of recovered roots greater for wheat after canola than after lupin (Table 3).

3.6 Recovery and distribution of lupin and canola $BG^{33}P$ and $BG^{15}N$excess in subsequent wheat

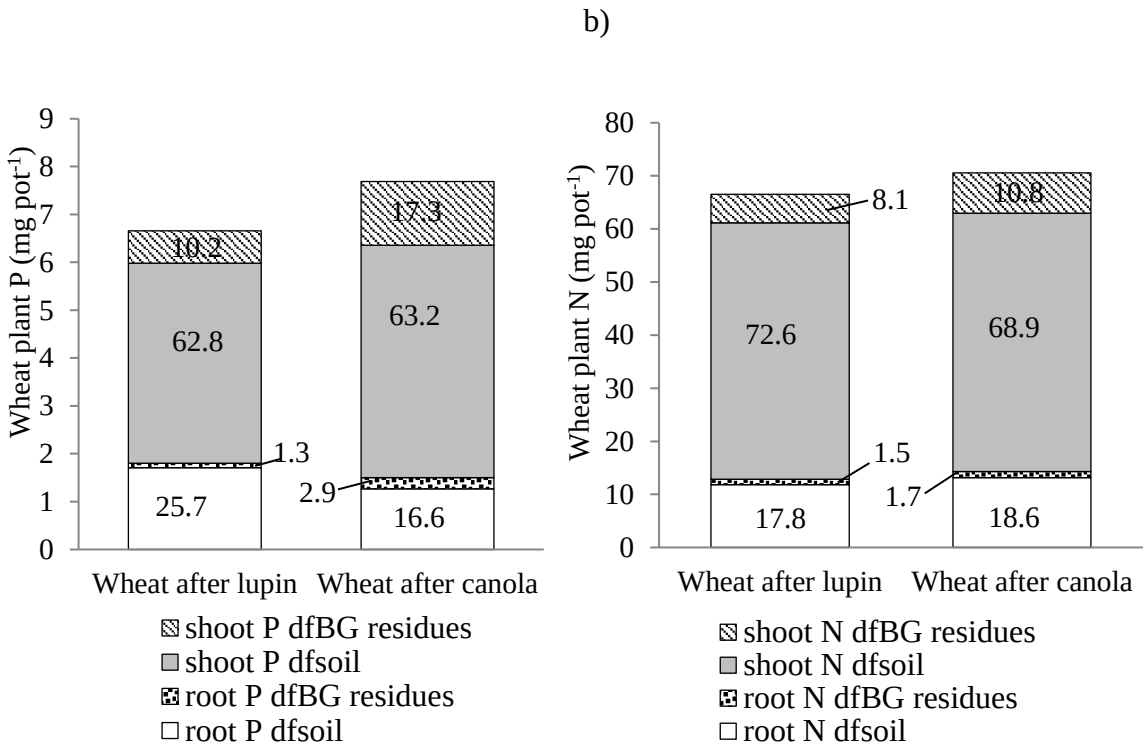
The recovery of $BG^{33}P$ and $BG^{15}N$ excess was greater in wheat after canola than after lupin, being significantly greater for N in both wheat shoot and recovered root, and for P in recovered root only (Table 4). A substantial proportion of the $BG^{33}P$ (19-21%) and somewhat less of the $BG^{15}N$ excess (8-12%) was accessed by the five week old wheat plants (Table 4). However the majority of the labelled $BG^{33}P$ (79-81%) and $BG^{15}N$ excess (63-64%) remained in the soil following the three week fallow and five week wheat growth period, although incomplete recovery of $BG^{15}N$ excess indicated a 'loss' of 24-29% (Table 4).

Table 4 Recovery (%) of ^{33}P and ^{15}Nexcess in plant-soil systems of wheat grown for 5 weeks (no fertilizer applied) in intact soil cores containing dual-labelled ($^{33}P/^{15}N$) canola or lupin BG residues.

	Distribution of BG^{33}P (%)			Distribution of BG ^{15}Nexcess (%)		
	Wheat after lupin	Wheat after canola*	$LSD_{0.05}$	Wheat after lupin	Wheat after canola	$LSD_{0.05}$
Shoot	16.7 ns	17.7 ns	1.1	6.9 b	10.1 a	0.8
Recovered roots	2.3 b	3.0 a	0.2	1.2 b	1.7 a	0.2
Soil	80.9 ns	79.3 ns	2.1	62.9 ns	64.1 ns	6.8
Total	99.9	100		71	75.9	

Data are means (n=4); different letters within rows and within isotope (^{33}P or ^{15}N) indicate significant difference ($lsd_{0.05}$); ns indicates no significance; * distribution of recovered BG^{33}P in wheat after canola has been reported previously (Chapter 5; submitted to *Crop and Pasture Science*)

Figure 3 Amounts of P and N (mg pot^{-1}) derived from (df) BG residues of lupin or canola and from unlabelled soil sources and relative proportion (%, numbers shown on histograms) in shoot and recovered roots (>2mm mesh sieve) of wheat (4 plant pot^{-1}). Data are means (n=4). P in wheat after canola previously reported (Foyjunnessa *et al*; submitted to *Crop and Pasture Science*).



3.7 Amounts of P and N in wheat derived from lupin or canola BG N and BG P

Due to the significantly larger amount of total BG P in canola (Fig. 1) and the slightly larger proportion of labelled BG P accessed by wheat after canola (Table 4), the uptake of P from canola total BG P by subsequent wheat was 1.56 mg P pot^{-1} compared to an uptake from lupin total BG P of 0.77 mg P pot^{-1} (Fig. 3a). This represented about 12% of the total P uptake by wheat after lupin and about 20% for wheat after canola (Fig. 3a). The proportion of BG^{15}N recovered by wheat was significantly greater after canola (Table 4) and the amount was 8.79 mg N pot^{-1} compared to 6.37 mg N pot^{-1} for wheat after lupin (Fig. 3b). These amounts

represented about 10% of the total N uptake by wheat after lupin and about 12% for wheat after canola (Fig. 3b). However, 80-88% of wheat P uptake (Fig. 3a) and 88-90% of wheat N uptake (Fig. 3b), in the absence of shoot residues or additional P fertiliser, was sourced from residual soil sources (plus added fertiliser N) that were not associated with BG P/N of previously grown lupin or canola.

4. Discussion

4.1 The relative contribution of N and P to wheat from canola and lupin in situ BG residues

The hypothesis tested in this study was that P release from canola BG residues would be relatively greater than from lupin BG residues whereas N release would be relatively smaller. The hypothesis proved correct for P since a significant, although only slightly greater, proportion of the P present in canola (21%) than lupin (19%) BG residues was apparently mineralised (as inferred from P uptake by the wheat), and the quantity of P benefit for wheat from BG residues was almost twice after canola (1.56 mg P pot^{-1}) than lupin (0.77 mg P pot^{-1}) due to the larger BG P input by canola. However, the hypothesis for N proved incorrect since a lower proportion of N from BG residues of lupin (`8%) than canola (~12%) was apparently mineralised; and furthermore the N benefit from BG residues of canola was greater despite the larger BG N input by lupin. We acknowledge that apparent net mineralisation, as inferred from wheat uptake of P derived from BG residues, will be an underestimate of total net mineralisation since N and P derived from BG residues resident in labile pools in soil (mineral/extractable and microbial) have not been taken into account. An indication of the likely magnitude of any underestimation for P release from the BG residues in this study can be gained from previously reported data for the wheat after canola (Chapter 5; *submitted to Crop and Pasture Science*) and for the wheat after lupin (data not shown; see supplementary table for this chapter) where, in both cases, no BG residue-derived ^{33}P was detected in the

microbial (hexanol-released) P pool and 3-5% was measured in the resin extractable P pool. However, our values for proportion of P in lupin and canola BG residues accessed by the subsequent plant broadly agree with those from other isotope studies using crop or pasture plant residues (both roots and shoots), although there is a large amount of variation in the reported proportion of the residue P that is accessed by the plant, ranging from 8-42% in glasshouse studies (Blair and Bolland 1978; Dalal 1979; Noack et al. 2014b; Till et al. 1982) to 5.4% in a field study of medic residues (McLaughlin et al. 1988).

It is clearly difficult to compare across studies, in terms of the proportion of subsequent plant P uptake that P from residue represents, since the time period for growth of the subsequent plant often differs, as well as the species used. This is in part unavoidable due to the half-life of P isotopes dictating to some extent how long plants can be grown. Nevertheless, our data of 12-20% of P uptake by 35 day old wheat derived from P in canola and lupin BG residues is in the middle of the range (5-29%) reported for proportion of P in 60 day old wheat derived from incorporated or surface mature shoot residues of field pea (Noack et al. 2014b) and corresponds well also with the results obtained by McLaughlin and Alston (1986) in a growth chamber experiment, where 13.7% of P uptake by 34 day old wheat plants was derived from medic residues. Our results are slightly greater than the value of 7% of wheat P uptake in the field from medic, where most of this uptake occurred within the first 3 weeks after residue addition and sowing of wheat (McLaughlin et al. 1988), and this difference may partly be a reflection of the more favourable growing conditions for the wheat under well-watered glasshouse conditions. Although, this does not explain why our results for P contribution from BG residues are also higher than the 4-5% contribution to P uptake of 35 day old corn from faba bean and field pea root residues in a glasshouse study (Nachimuthu et al. 2009).

The values for proportion of N in lupin and canola BG residues accessed by the following plant (8-12%) in this study, generally accord with those derived from other glasshouse and field studies using ^{15}N-labelling of BG residues of oilseeds (Arcand et al. 2014b) and grain legumes including lupin (Mayer et al. 2003; McNeill and Fillery 2008; Wichern et al. 2011); Arcand et al 2014a) which range from 8.6-20%. An earlier field study using isotope (Ladd et al. 1981) demonstrated an apparent net N mineralisation of 10.9 to 17.3 % of legume residues during the following wheat phase which is also not dissimilar to our data, although it was over a longer period. A field study of N loss from residues in mesh bags buried in soil in the field demonstrated a much higher net N mineralisation (22 to 38%) of canola roots (Soon and Arshad 2002), even though N concentration of canola roots in that study was lower (8.5 to 8.8 mg g^{-1}) than in this present study (10 mg g^{-1}), but perhaps this is not surprising given the field study was over a much longer time period (10-11months) than ours, and also would account for N lost from residues that will have been incorporated into microbial biomass and mineral N pools. Studies of the fate of BG N residues have measured from 2-11% of the residue-derived N in the microbial pool (Mayer et al. 2003; McNeill and Fillery 2008; Wichern et al. 2011) with some variation in the proportion of residue-derived N reported in the mineral N pool from negligible amounts (Arcand et al. 2014a) up to 10% (McNeill and Fillery 2008) with additional loss of 8-14% of N derived from lupin BG residues as nitrate leached to 1m soil depth (McNeill and Fillery 2008). Thus, apparent mineralisation rates for BG N based on following plant N uptake only are likely to be underestimates by at least 50% which, if applied to the values in our study, would bring them close to those values obtained from Soon and Arshad (2002) for N lost from canola roots over a season.

Finally it needs to be noted that the addition of N fertiliser during the wheat phase, which was done because the plants appeared deficient, may have positively influenced the rate of BG residue decomposition and the release of P and N (Cheshire and Chapman 1996). More

importantly, the addition of unlabelled fertiliser N at 21 days will have exacerbated the pool dilution effect on the enrichment of the mineral N pool that is already inherent in such studies Murphy et al. (2003) and Kirkham and Bartholomew (1954). Hence, wheat will have accessed a mineral N pool with lower ^{15}N enrichment between 21-35 days compared to that before N fertiliser addition, and so measures of apparent N mineralisation of residues based on recovery of labelled residue N by wheat will be underestimates of what may have occurred in the absence of that fertiliser application.

Nevertheless, overall we can conclude with some degree of confidence that canola BG residues were more readily mineralised in the short term than lupin BG residues, contributed a greater proportion of the N and P uptake by following wheat, and supplied a similar proportion of their N and P to wheat as that reported for above-ground residues.

4.2 Is apparent mineralisation related to ratios between carbon, nitrogen and phosphorus?

The rationale underlying the hypothesis for this study was that since canola reportedly has relatively high plant tissue P concentrations it would have a narrower C:P ratio than lupin which as a legume that fixes N_2 has high plant tissue N concentration and thus narrower C:N than canola, and indeed the root residues obtained in this study did follow these anticipated trends. However, apparent net P mineralisation was observed for the BG residues of both species despite the large differences in C:P ratio of recovered roots. This accords with results of Soon and Arshad (2002) demonstrating net P mineralization (equivalent to 0.1 to 0.3 kg P ha^{-1}) from root residues of wheat, canola and pea, although little variation between species. Whilst C:P for canola recovered root was within the reported range of critical C:P ratios of 156: 1 to 252: 1, the C:P ratio for lupin recovered roots was greater than 500:1 indicating potential for P immobilisation (Kwabiah et al. 2003b). Admittedly the ratios are based on the use of a mean value of 40% of root DW to estimate canola and lupin root C content (Gan et al. 2011) but the

difference between the two ratios would still be substantial even if the carbon concentration differed between the species, as may be the case given the ranges for C concentration of mature canola (47-51%) and legume/pea (40-42%) roots reported by Soon and Arshad (2002). A likely reason why there was net P release from both BG residues in this present study is that a similar proportion of the P derived from the BG residues may exist in a soluble form. This suggestion is supported by data from Noack et al. (2012) showing the same proportion (~55%) of orthophosphate in lupin and canola chaff and also a high proportions in both canola (75%) and lupin (50%) stems. This highly soluble P form is likely to be rapidly released from decomposing roots, not necessarily undergoing biological mineralisation, so not closely linked to C, but directly entering the labile P pool in soil solution and being readily accessed by wheat and/or microbes immediately. Thus a C:P ratio may be less useful in predicting P release over the time period of this study in particular (8 weeks total - 3 weeks fallow and five weeks wheat) as was discussed in the introduction.

Apparent N mineralisation from BG residues was greater for canola (12%) than for lupin (8%) which again did not correspond with the hypothesis of this study given that the wider C:N ratio for canola recovered root of 39:1 is considered (Kwabiah et al. 2003b) to indicate likely net immobilisation compared to the lupin recovered root ratio of 24:1. However, it is possible that biological mineralisation of N from lupin BG residues may have been P limited due to the extremely low concentration of P in the lupin recovered roots compared to those of canola, and the fact that no fertiliser P was applied prior to the wheat phase. In fact the wheat after lupin was marginally P deficient suggesting the P fertility of the soil during this phase of the study was low whereas P concentration in the wheat after canola was adequate under the same soil conditions, suggesting the canola BG residues had provided sufficient P not only for the plant's needs but also for greater biological N mineralisation. The lower N mineralisation by lupin does relate to the difference seen in the N:P ratio of lupin and canola recovered roots with lupin

being 29:1 and canola 5:1 – the latter within the critical N:P ratio range of 7:1 to 14:1 observed by Kwabiah et al. (2003b). It was interesting that the N:P ratio for unrecovered BG residues of canola (6:1) was similar to the canola recovered roots suggesting an overall similarity in biochemical composition of these two fractions of canola BG residues. Furthermore N:P ratio of unrecovered lupin BG residues was also similar to that for canola and it could be that this fraction of the BG residues may have been the more labile fraction in terms of supplying P for wheat. Another possibility is that there was more structural N in lupin roots associated with recalcitrant C (lignin, cellulose, hemi-cellulose) than in canola roots, since narrow-leaved lupin used in this study has been described as having a large taproot and primary roots but not many secondaries (Clements et al. 1993) in contrast to canola which has a large proportion of fine roots (Liu et al. 2010).

The P concentration of the recovered mature lupin roots in this study (0.64 g kg^{-1}) was extremely low compared to reports for mature roots of other grain legumes such as field pea of 1.54 – 1.58 g P kg^{-1} (Soon and Arshad 2002) and faba bean of 1.7 g P kg^{-1} (Alamgir et al. 2012), although higher than the reported P concentrations (0.057 and 0.073 g kg^{-1}) for mature roots of canola and wheat that had been grown on a P deficient calcareous soil with a low P fertiliser application (Noack et al. 2014a), and very similar to the P concentration (0.75 g kg^{-1}) of mature pea stem and leaf residues collected from an agricultural crop in South Australia (Noack et al. 2014b). Cheshire and Chapman (1996) conclude that decomposition may be slowed when decomposer organisms are limited by a nutrient lack in the plant residue, and this effect may have been amplified since the soil in this present study was also relatively low P status with no fertiliser P added in the wheat phase. Given no ^{33}P detection in microbial biomass after the wheat phase of this study, but only in the resin pool (data not shown; see supplementary table for this chapter), supports the suggestion that there may have been a P limitation to microbial breakdown and N release by the lupin residue. Indeed, as mentioned

earlier, other isotope studies of residue decomposition have reported residue-derived P and N in microbial biomass at harvest of the following plant which suggests that in soils where fertility may have been greater the microbial biomass was less limited. Furthermore, it has been reported (Nguluu et al. 1997) that N mineralisation from cowpea residues with low P concentration (1.1 g kg^{-1}) was consistently less than from those with higher P concentrations (1.2-2.0 g kg^{-1}) which supports the observation in this study of lower apparent N mineralisation from lupin than canola residues despite a higher N concentration. This highlights that the residues of a crop that has grown with only a single nutrient limitation may then be constrained in terms of the release of other nutrients.

In summary, it appears that the proportion of P and N from BG residues of mature canola and lupin taken up by wheat in the short term did not appear to be driven by C:P or C:N ratio of recovered roots but by P concentration, and possibly by N:P ratio in BG residues.

4.3 Allocation of N and P BG in lupin and canola at maturity

The *in situ* dual-labelling (^{33}P and ^{15}N) technique used in this study provide unique data for simultaneous estimation of total BG P and BG N for these species at maturity, as well as the proportion of P and N BG that was unrecoverable by simple standard root recovery using a 2mm mesh sieve. Total BG P was greater for canola (7.5 mg P) than lupin (4.1 mg P) largely due to the much higher P concentration for canola roots compared to lupin which had a relatively low P concentration as discussed in the previous section. Canola is reputedly a highly P efficient species (Grant and Bailey 1993; Norton et al. 2013) and so the root P concentration is not unexpected although it is higher than some other reports as discussed earlier.

A similar proportion of total BG P (53-75%) and total BG N (60-75%) was in the unrecoverable RD P and RD N fractions for mature canola and lupin. The unrecoverable RD P portion had increased compared to that measured for the same species at late flowering (Foyjunnessa et al.

2015) although total BG P remained the same. As discussed elsewhere (Chapter 5; submitted to Crop and Pasture Science) the larger unrecovered proportion of RD P is associated with root senescence and turnover between plant peak biomass (flowering) and maturity with some RD P being measured in microbial (hexanol-released) and resin P pools. The measures in this study of RD N as a proportion of total BG N in canola and lupin accord with observation from a ^{15}N stem feeding study (glasshouse) in lentil and wheat that RD N can comprise 85-89% of total BG N (Arcand et al. 2014b). A field study by McNeill and Fillery (2008) using the same technique observed estimates of 80-87% of total BG N as RD N in deep sand soil and 63-71% of total BG N as RD N in sand over clay soil, including measured labile pools (microbial biomass, inorganic N and soluble organic N), further substantiating our data. The implications of these unrecoverable fractions of plant BG N and P in terms of accurate assessment of inputs to organic matter cycling by plant roots have been acknowledged previously ((Arcand et al. 2014a; Arcand et al. 2014b; Soon and Arshad 2002); Foyjunnessa et al 2015; Chapter 5).

4.4 Partitioning of labelled N and P above- and below-ground in lupin and canola

This study clearly showed that partitioning of stem-fed ^{33}P differed completely from that of stem-fed ^{15}N, with more of the fed ^{33}P recovered BG (64-71%) whilst a larger proportion of fed ^{15}N (60-69%) was recovered in shoots. This difference between the two may be due to the fact that a large proportion of the P required for shoot growth was taken up prior to the ^{33}P feeding stage at early flowering (45 DAS), which is likely given the importance of P supply for early growth, as shown for canola (Rose et al. 2007). Alternatively, it may be that transfer of stem-fed ^{33}P from the phloem to xylem in the roots may not occur readily, as discussed elsewhere (Foyjunnessa et al. 2015). However, not all studies have obtained such high partitioning of fed P isotope to roots with reported recoveries ranging from as little as 0.5-3% (Rennie and Halstead 1965) in wheat to 22% (McLaughlin et al. 1987) in medic although these

studies did not use stem wick-feeding of ^{33}P. Similar above- and below-ground allocation of stem-fed ^{15}N to that obtained in this study has been reported by others using stem feeding of isotopes in grain legumes including lupin (Mahieu et al. 2007; Mayer et al. 2003; McNeill and Fillery 2008) and in canola (Arcand et al. 2014a), and it is indicative of a strong shoot (and grain) sink for N. Nevertheless, the proportion of fed ^{15}N that was allocated BG (10-11%) in our study resulted in mean ^{15}N enrichments of 0.0077 and 0.0086 ^{15}N atom % excess in bulk soil, and 0.2689 and 0.2710 ^{15}N atom % excess in recovered roots, which have been shown to be sufficient for estimating BG N accumulation in grain legumes and oilseeds (Arcand et al. 2013; Mahieu et al. 2009; Mayer et al. 2003; Yasmin et al. 2010). The lower total recovery of fed ^{15}N than ^{33}P in the break crop phase highlights that N is often more susceptible to losses from plant-soil systems than P. The recoveries of fed ^{15}N in lupin and canola (70-80%) however are very similar to those obtained in several other studies including Gasser et al. (2015) for red clover leaf-labelled with a single pulse of ^{15}N-urea and (Arcand et al. 2013) for stem wick-fed canola and field pea. Some ^{15}N (2-29%) may be unaccounted for due to wick sorption (Wichern et al. 2007) which was not determined in this present study as the wicks were all utilised for ^{33}P analysis to enable replicated measures. Wick sorption for ^{15}N was not anticipated as there are many reports of virtually complete (>90%) recoveries of ^{15}N when labelling using stem wick-feeding (Khan et al. 2002; McNeill and Fillery 2008; McNeill et al. 1997; Russell and Fillery 1996), however we cannot definitively say it did not occur. It may be that sorption is determined by the type of material used as the wick and this could be investigated. We do know that an unknown proportion of fed ^{15}N was unaccounted for in material (leaves and flowers) that senesced, was shed from the canola and lupin plants, could not be assigned to a specific pot and so was not measured. Further loss of fed ^{15}N, in the order of 1-4% may have occurred directly due to N volatilisation from the canola and lupin plants, particularly at times of rapid

uptake of N by roots or during leaf senescence as plants matured (Sommer et al. 2004; Wetselaar and Farquhar 1980).

What was more unexpected was the apparent large loss (21-29%) of ^{15}N from the labelled total BG residues during the fallow and wheat phase, although a similar loss was reported in a study of wheat after labelled BG residues of pea (Arcand et al. 2014a). There are apparent losses of ^{15}N that can be attributed to experimental errors in the mass balance between the calculated ^{15}N fed and/or present in the BG residue and the ^{15}N determined from analysis of the various plant and soil fractions that differ in pool size and N content. However we believe the loss was greater than could be attributed to the ~10% total error associated with the assumption that the mean value for amount of excess ^{15}N obtained for the BG residues from destructively sampled cores was representative of the amount of excess ^{15}N in the undisturbed BG residues in the cores where the shoots were removed. The ^{15}N loss is difficult to completely explain since the study was undertaken in sealed pots and thus the most plausible explanation would be denitrification, which was not anticipated as the soil was sandy and not saturated – although there is some possibility that if WHC exceeded the target of 60% this could have created microsites for nitrification to occur. Loss *via* denitrification has been proposed to account for a loss of 50% of the ^{15}N in labelled lupin BG residues in the field during a wheat season when the sandy top horizon of a texture-contrast soil was periodically waterlogged (McNeill and Fillery 2008). Any unexplained loss of isotope, although by no means unique to this study, does reduce confidence in the use of these isotope techniques for both quantifying total nutrient accumulation BG and assessing the subsequent release of those nutrients, and indeed potential errors associated with the technique are discussed in the next section.

4.5 Potential errors associated with the use of isotope-labelling in this study

One key assumption inherent in isotope studies is that the 'discrete' pool of interest is uniformly labelled (Frossard et al. 2011; Vijayalakshmi and Dakshinamurti 1975). This assumption has been discussed in great detail with regard to estimation of total BG P (Rennie and Halstead 1965) and BG N (Khan et al. 2003) where ^{33}P specific activity (SA) or ^{15}N excess specific enrichment (SE) of recovered roots (>2 mm) are used for estimation of root-derived P (RD P) or RD N in soil, and it is clear that there is debate as to whether the assumption holds. A previous study (Foyjunnessa et al. 2015) did show a positive linear relationship ($R^2 = 0.91$) between recovered root dry weight and ^{33}P activity in recovered root but this is still not unequivocal evidence that ^{33}P is uniformly distributed, as there may be more ^{33}P in fine roots and less in coarse roots, or more in younger roots and less in older roots. Furthermore this same study showed that ^{33}P SA may be the same or different for recovered roots from different soil depths depending on soil type (Chapter 5; submitted to Crop and Pasture Science). Similarly, some ^{15}N studies provide evidence that recovered root SE remains constant at different depths in some species but not others (McNeill and Fillery 2008; McNeill et al. 1997). Clearly this is an area that requires further investigation to give confidence in estimates of amounts of P and N in unrecovered roots and root-derived materials in soil.

Another assumption is that stem wick-fed nutrients are not immediately exuded by roots as suggested for leaf-fed ^{15}N by Gasser et al. (2015), who observed ^{15}N leakage (0.5±0.2% of the fed ^{15}N) within twenty four hours after labelling and thus indicated a potential for overestimation of N in unrecovered root materials. Detailed time-course measurements not undertaken in this study are required to rigorously test for ^{15}N and ^{33}P leakage. The likelihood that there was no ^{33}P leakage immediately post-feed for this study has been discussed in detail in a previous paper (Chapter 5; *submitted to Crop and Pasture Science*). However, to ensure

that all assumptions underlying the quantitative estimation of total BG N and P using isotopes are not causing large errors in the estimations obtained using these techniques, more experiments aimed at assessing uniformity of labelling of the discrete pool or pools of interest are required. Nevertheless, dual-isotope labelling offers great opportunities to compare N and P accumulation BG and to trace the relative benefit to following crop species. Current estimates of fine root P and N are not included in nutrient cycling budgets for rotational farming systems, in particular for P (Oberson et al. 2011), and robust quantitative information regarding the direct contribution of P and N from the BG residues of crop species to subsequent wheat is required.

6. Conclusions

The amount of P taken up by wheat derived from residues was related to total BG P of the residues but not total BG N; hence canola accumulated more BG P and contributed twice as much P as lupin BG residues to wheat P uptake whereas a greater amount of lupin BG N did not result in a greater N contribution than canola. The proportion of P and N from BG residues of mature canola and lupin taken up by wheat in the short term did not appear to be driven by C:P or C:N ratio of recovered roots but by P concentration, and possibly by N:P ratio of BG residues.

Acknowledgments

The University of Adelaide provided an Australian Postgraduate Scholarship and the Grains Research and Development Corporation provided top up funding (GRS10026) to support this research. This work contributes to outputs in GRDC project UA00119. CSIRO provided access to radioisotope laboratory facilities and a field site for soil collection Leanne Lisle at University of New England undertook the ^{15}N analysis.

References

Abiven S, Recous S, Reyes V and Oliver R (2005) Mineralisation of C and N from root, stem and leaf residues in soil and role of their biochemical quality. Biology and Fertility of Soils 42, 119-128.

Alamgir M, McNeill A, Tang C and Marschner P (2012) Changes in soil P pools during legume residue decomposition. Soil Biology and Biochemistry 49, 70-77.

Arcand M, Knight J D and Farrell R (2013) Estimating belowground nitrogen inputs of pea and canola and their contribution to soil inorganic N pools using ^{15}N labeling. Plant and Soil 371, 67-80.

Arcand M, Knight J D and Farrell R (2014a) Differentiating between the supply of N to wheat from above and belowground residues of preceding crops of pea and canola. Biology and Fertility of Soils 50, 563-570.

Arcand M, Lemke R, Farrell R and Knight J D (2014b) Nitrogen supply from belowground residues of lentil and wheat to a subsequent wheat crop. Biology and Fertility of Soils 50, 507-515.

Armstrong E, Pate J and Tennant D (1994) The field pea crop in south western Australia - patterns of water use and root growth in genotypes of contrasting morphology and growth habit. Functional Plant Biology 21, 517-532.

Balesdent J and Balabane M (1996) Major contribution of roots to soil carbon storage inferred from maize cultivated soils. Soil biology & biochemistry 28, 1261-1263.

Bell C, Carrillo Y, Boot C M, Rocca J D, Pendall E and Wallenstein M D (2014) Rhizosphere stoichiometry: are C : N : P ratios of plants, soils, and enzymes conserved at the plant species-level? New Phytologist 201, 505-517.

Bertrand I, Chabbert B, Kurek B and Recous S (2006) Can the Biochemical Features and Histology of Wheat Residues Explain their Decomposition in Soil? Plant and Soil 281, 291-307.

Blair G J and Bolland O W (1978) The release of phosphorus from plant material added to soil. Australian Journal of Soil Research 16, 101-111.

Burkitt L L, Moody P W, Gourley C J P and Hannah M C (2002) A simple phosphorus buffering index for Australian soils. Soil Research 40, 497-513.

Campbell C A (1978) Soil organic carbon, nitrogen and fertility. Elsevier Scientific Publishing Co., Amsterdam, The Netherlands.

Cheshire M V and Chapman S J (1996) Influence of the N and P status of plant material and of added N and P on the mineralization of C from 14C-labelled ryegrass in soil. Biology and Fertility of Soils 21, 166-170.

Clements J, White P and Buirchell B (1993) The root morphology of Lupinus angustifolius in relation to other Lupinus species. Crop and Pasture Science 44, 1367-1375.

Dalal R C (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, 913-916.

Foyjunnessa, McNeill A, Doolette A, Mason S and McLaughlin M (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 and McLaughlin M (2015) Quantifying total phosphorus accumulation below-ground by canola and lupin plants using ^{33}P-labelling. Plant and Soil, 10.1007/s11104-11015-12545-y.

Friesen D and 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, 355-366.

Frossard E, Achat D, Bernasconi S, Bünemann E, Fardeau J-C, Jansa J, Morel C, Rabeharisoa L, Randriamanantsoa L, Sinaj S, Tamburini F and Oberson A (2011) The use of tracers to investigate phosphate cycling in soil–plant systems. In Phosphorus in Action. Eds. E Bünemann, A Oberson and E Frossard. pp 59-91. Springer Berlin Heidelberg.

Fuller W H, Nielsen D R and Miller R W (1956) Some Factors Influencing the Utilization of Phosphorus from Crop Residues1. Soil Science Society of America Journal 20, 218-224.

Gan Y T, Campbell C A, Janzen H H, Lemke R, Liu L P, Basnyat P and McDonald C L (2009) Root mass for oilseed and pulse crops: Growth and distribution in the soil profile. Canadian Journal of Plant Science 89, 883-893.

Gan Y T, Liang B C, Liu L P, Wang X Y and McDonald C L (2011) C : N ratios and carbon distribution profile across rooting zones in oilseed and pulse crops. Crop and Pasture Science 62, 496-503.

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

Grant C A and Bailey L D (1993) Fertility management in canola production. Canadian Journal of Plant Science 73, 651-670.

Gregory P J (2008) Development and growth of root systems. In Plant Roots: Growth, Activity and Interactions with the Soil. Ed. G Peter J. Blackwell Publishing, Oxford UK.

Heal O W, Anderson J M and Swift M J (1997) Plant litter quality and decomposition: an historical overview. In Driven by Nature: Plant Litter Quality and Decomposition. Eds. G Cadisch and K E Gill. pp 3-30. CAB International, Wallingford, UK.

Iqbal S M (2009) Effect of crop residue qualities on decomposition rates, soil phosphorus dynamics and plant phosphorus uptake. In Soil and Land Systems. pp 1-220. The University of Adelaide, Adelaide.

Jensen L S, Salo T, Palmason F, Breland T, Henriksen T, Stenberg B, Pedersen A, Lundström C and Esala M (2005) Influence of biochemical quality on C and N mineralisation from a broad variety of plant materials in soil. Plant and Soil 273, 307-326.

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

Karlen D, Varvel G, Bullock D G and Cruse R (1994) Crop rotations for the 21st century. Advances in agronomy 53.

Khan D F, Peoples M B, Chalk P M and Herridge D F (2002) Quantifying below-ground nitrogen of legumes. 2. A comparison of ^{15}N and non isotopic methods. Plant and Soil 239, 277-289.

Khan D F, Peoples M B, Schwenke G D, Felton W L, Chen D and Herridge D F (2003) Effects of below-ground nitrogen on N balances of field-grown fababean, chickpea, and barley. Australian Journal of Agricultural Research 54, 333-340.

Kirkham D O N and Bartholomew W V (1954) Equations for following nutrient transformations in soil, utilizing tracer data. Soil Science Society of America Journal 18, 33-34.

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

Kwabiah A B, Palm C A, Stoskopf N C and Voroney R P (2003a) Response of soil microbial biomass dynamics to quality of plant materials with emphasis on P availability. Soil Biology and Biochemistry 35, 207-216.

Kwabiah A B, Stoskopf N C, Palm C A and Voroney R P (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, 53-61.

Ladd J N, Oades J M and Amato M (1981) Distribution and recovery of nitrogen from legume residues decomposing in soils sown to wheat in the field. Soil Biology and Biochemistry 13, 251-256.

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

Lousier J D and Parkinson D (1978) Chemical element dynamics in decomposing leaf litter. Canadian Journal of Botany 56, 2795-2812.

Lupwayi N Z, Clayton G W, O'Donovan J T, Harker K N, Turkington T K and Soon Y K (2006) Nitrogen release during decomposition of crop residues under conventional and zero tillage. Canadian Journal of Soil Science 86, 11-19.

Lupwayi N Z, Clayton G W, O'Donovan J T, Harker K N, Turkington T K and Soon Y K (2007) Phosphorus release during decomposition of crop residues under conventional and zero tillage. Soil and Tillage Research 95, 231-239.

Mahieu S, Fustec J, Faure M-L, Corre-Hellou G and Crozat Y (2007) Comparison of two ^{15}N labelling methods for assessing nitrogen rhizodeposition of pea. Plant and Soil 295, 193-205.

Mahieu S, Fustec J, Jensen E S and Crozat Y (2009) Does labelling frequency affect N rhizodeposition assessment using the cotton-wick method? Soil Biology and Biochemistry 41, 2236-2243.

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

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

McLaughlin M and 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, 517-526.

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

McLaughlin M, Alston A and Martin J (1988) Phosphorus cycling in wheat pasture rotations .I. The source of phosphorus taken up by wheat. Soil Research 26, 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. Eds. M Unkovich, J Pate, A McNeill and D J Gibbs. pp 195-218. Springer Netherlands.

McNeill A and 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 and Soil 302, 297-316.

McNeill A M, Zhu C and Fillery I R P (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, 295-304.

Murphy D, Recous S, Stockdale E, Fillery I, Jensen L, Hatch D and Goulding K (2003) Gross nitrogen fluxes in soil: theory, measurement and application of 15 N pool dilution techniques. Advances in Agronomy 79, 69-118.

Nachimuthu G, Guppy C, Kristiansen P and 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 and Soil 314, 303-310.

Nebiyu A, Vandorpe A, Diels J and Boeckx P (2014) Nitrogen and phosphorus benefits from faba bean (Vicia faba L.) residues to subsequent wheat crop in the humid highlands of Ethiopia. Nutrient Cycling in Agroecosystems 98, 253-266.

Nguluu S N, Probert M, Myers R and Waring S (1997) Effect of tissue phosphorus concentration on the mineralisation of nitrogen from stylo and cowpea residues. Plant and Soil 191, 139-146.

Nicolardot B, Recous S and Mary B (2001) Simulation of C and N mineralisation during crop residue decomposition: A simple dynamic model based on the C:N ratio of the residues. Plant and Soil 228, 83-103.

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

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

Noack S R, McBeath T M, McLaughlin M J, Smernik R J and Armstrong R D (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, 31-39.

Norton A, Kirkegaard J, Angus J and T P (2013) Canola in Rotations 7. The Regional Institute, http://www.regional.org.au/au/gcirc/canola/p-06.htm.

Oberson A, Pypers P, Bünemann E and Frossard E (2011) Management impacts on biological phosphorus cycling in cropped soils. In Phosphorus in Action. Eds. E Bünemann, A Oberson and E Frossard. pp 431-458. Springer Berlin Heidelberg.

Palm C A and Sanchez P A (1991) Nitrogen release from the leaves of some tropical legumes as affected by their lignin and polyphenolic contents. Soil Biology and Biochemistry 23, 83-88.

Palta J, Fillery I, Mathews E and Turner N (1991) Leaf feeding of (^{15}N) urea for labelling wheat with nitrogen. Functional Plant Biology 18, 627-636.

Reiners W A (1986) Complementary models for ecosystems. American Naturalist, 59-73.

Reinertsen S A, Elliott L F, Cochran V L and Campbell G S (1984) Role of available carbon and nitrogen in determining the rate of wheat straw decomposition. Soil Biology and Biochemistry 16, 459-464.

Rennie D and Halstead E (1965) A ^{32}P injection method for quantitative estimation of the distribution and extent of cereal grain roots. Proc. Isotopes and Radiations in soil-plant nutrition studies. FAO/IAEA Ankara 489.

Reuter D J and Robinson B J (1997) Plant Analysis: an Interpretation Manual. CSIRO, Collingwood, Australia.

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

Russell C and Fillery I (1996) Estimates of lupin below-ground biomass nitrogen, dry matter, and nitrogen turnover to wheat. Australian Journal of Agricultural Research 47, 1047-1059.

Schmidtke K (2005) How to calculate nitrogen rhizodeposition: a case study in estimating N rhizodeposition in the pea (Pisum sativum L.) and grasspea (Lathyrus sativus L.) using

a continuous 15N labelling split-root technique. Soil Biology and Biochemistry 37, 1893-1897.

Schomberg H H, Ford P B and Hargrove W L (1994) Influence of crop residues on nutrient cycling and soil chemical properties. In Managing Agricultural Residues. Ed. P W Unger. pp 99-121. Lewis Publisher of CRC Press, United States of America.

Singh B B and Jones J P (1976) Phosphorous sorption and desorption characteristics of soil as affected by organic residues. Soil Science Society of America Journal 40, 389-394.

Smith J L and Paul E A (1990) The significance of soil microbial biomass estimations. Soil biochemistry 6, 357-396.

Sommer S G, Schjoerring J K and Denmead O (2004) Ammonia emission from mineral fertilizers and fertilized crops. Advances in agronomy 82, 557-622.

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

Strong W, Harbison J, Nielsen R, Hall B and Best E (1986) Nitrogen availability in a Darling Downs soil following cereal, oilseed and grain legume crops. 2. Effects of residual soil nitrogen and fertiliser nitrogen on subsequent wheat crops. Australian Journal of Experimental Agriculture 26, 353-359.

Swift M J, Heal O W and Anderson J M (1979) Decomposition in Terrestrial Ecosystems. Blackwell Scientific Publications, Oxford.

Thompson M, Bee H M, Cheeseeman R V, Evans W H, Lord D W, Ripley B D and Wood R (1987) Recommendations for the definition, estimation and use of the detection limit. Analyst 112, 199-204.

Till A R, Blair G J, Dalal R C, Freney J R and Galbally I E (1982) Isotope studies of the recycling of carbon, nitrogen, sulfur and phosphorus from plant material. In The cycling

of carbon, nitrogen, sulfur and phosphorus in terrestrial and aqautic ecosystems. pp 51-59. Springer-Verlag.

Todd E D and Paul D B (2001) Fundamental of Stable Isotope Chemistry and Measurement. In Stable Isotope Techniques in the Study of Biological processes and Functioning of Ecosystems. Eds. U Murray, P John, A McNeill and D J Gibbs. pp 1-18. Kluwer Academic Publishers, Dordrecht/ Boston/ London.

Vijayalakshmi K and Dakshinamurti C (1975) Quantitative estimation of root weight using ^{32}P plant injection technique. Journal of Nuclear Agriculture and Biology 4, 98-100.

Vogt K A, Grier C C and Vogt D (1986) Production, turnover, and nutrient dynamics of above- and belowground detritus of world forests. Advances in Ecological Research 15, 3-377.

Wetselaar R and Farquhar G D (1980) Nitrogen losses from tops of plants. Advances in Agronomy 33, 263-302.

White R E and Ayoub A T (1983) Decomposition of plant residues of variable C/P ratio and the effect on soil phosphate availability. Plant and Soil 74, 163-173.

Wichern F, Andreeva D, Joergensen Rainer G and Kuzyakov Y (2011) Stem labeling results in different patterns of 14C rhizorespiration and 15N distribution in plants compared to natural assimilation pathways. Journal of Plant Nutrition and Soil Science 174, 732-741.

Wichern F, Mayer J, Joergensen R G and Müller T (2007) Release of C and N from roots of peas and oats and their availability to soil microorganisms. Soil Biology and Biochemistry 39, 2829-2839.

Yasmin K, Cadisch G and Baggs E M (2010) The significance of below-ground fractions when considering N and C partitioning within chickpea (Cicer arietinum L.). Plant and Soil 327, 247-259.

Zadoks J C, Chang T T and Konzak C F (1974) Decimal code for growth stages of cereals. A Weed Research 14, 415-421.

Supplementary table

Table 1. Bulk soil resin available P, microbial P (hexanol-released) and recovery of ^{33}P activity in mature lupin and canola and wheat phase grown in sand in a glasshouse following that had been fed with ^{33}P (459 kBq ^{33}P /plant) using a ^{33}P cotton-wick stem feeding technique at 45 days after sowing.

Treatments	Canola and lupin maturity phase (105 days after sowing)				Wheat phase			
	Available P (mg kg^{-1})		^{33}P activity (kBq pot^{-1})		Available P (mg kg^{-1})		^{33}P activity (kBq pot^{-1})	
	Resin available	Microbial	Resin available	Microbial	Resin available	Microbial	Resin available	Microbial
Lupin	6.9 ns	2.6 ns	34.7 a	14.8 ns	6.6 ns	2.0 ns	28.1 a	Below detection
Canola	6.0 ns	2.8 ns	29.1 b	13.4 ns	6.1 ns	1.9 ns	23.2 b	Below detection
lsd	0.96	0.83	4.3	2.1	0.92	0.76	4.1	

Data are means (n=4), ns= non-significant and different letters within column indicate significant differences (lsd 0.05).

CHAPTER 7

GENERAL DISCUSSION

AND

FUTURE RESEARCH DIRECTIONS

1. Overview

The overarching hypothesis of this study was that nutrient recycling by break crop roots may be a significant contributor to P and N cycling in soils, and hence to the P and N nutrition of a following cereal crop (Chapter 1). The research described by this thesis contributes novel information concerning P and N accumulation below-ground (BG) by two important break crop species for southern Australian rain-fed farming systems, and the turnover of those BG residues in relation to supply of P and N to a following crop species. The research demonstrated that the ^{33}P stem-wick feeding technique could be used to effectively label plant BG P *in situ* (Chapters 2 to 5) in a similar manner to that used for ^{15}N-labelling of BG N. The amount of P accumulated BG by lupin and canola was at least twice that measured by a standard root recovery method i.e. 2 mm mesh sieve commonly used in root studies (Chapters 3, 4 and 5; Fig 7.1), and it was mainly (>80%) distributed in the top 0-0.1m depth of a 0.35m diameter soil core (Chapter 5). Both total BG P and the unrecovered portions of P (termed root-derived P - RD P) were greater for plants grown in a loam than a sandy soil (Chapter 5). Lupin and canola BG P comprised at least one-third of total plant P, at both peak vegetative stage (flowering) and at maturity (Chapters 4 and 5; Fig 7.1). There was considerable cycling of BG P between flowering and senescence for canola with 4-8% of BG ^{33}P (3-5% of the fed ^{33}P) detected in the microbial (hexanol-released) P pool and 9-16% (6-10% of the fed ^{33}P) in the resin P pool at maturity (Chapters 5; Fig 7.1). During a period of 8 weeks following removal of mature shoots the cycling of canola BG P was greater in a loam than a sandy soil and provided more P for the following wheat crop, although the P benefit expressed as a proportion of wheat P uptake was the same on both soils since wheat DM production was lower on the sand (Chapter 5; Fig 7.2). A ^{15}N-^{33}P dual-labelling study of lupin and canola in the sandy soil suggested that the proportion of P and N from BG residues of mature lupin and canola taken up by wheat in the short term did not appear to be driven by

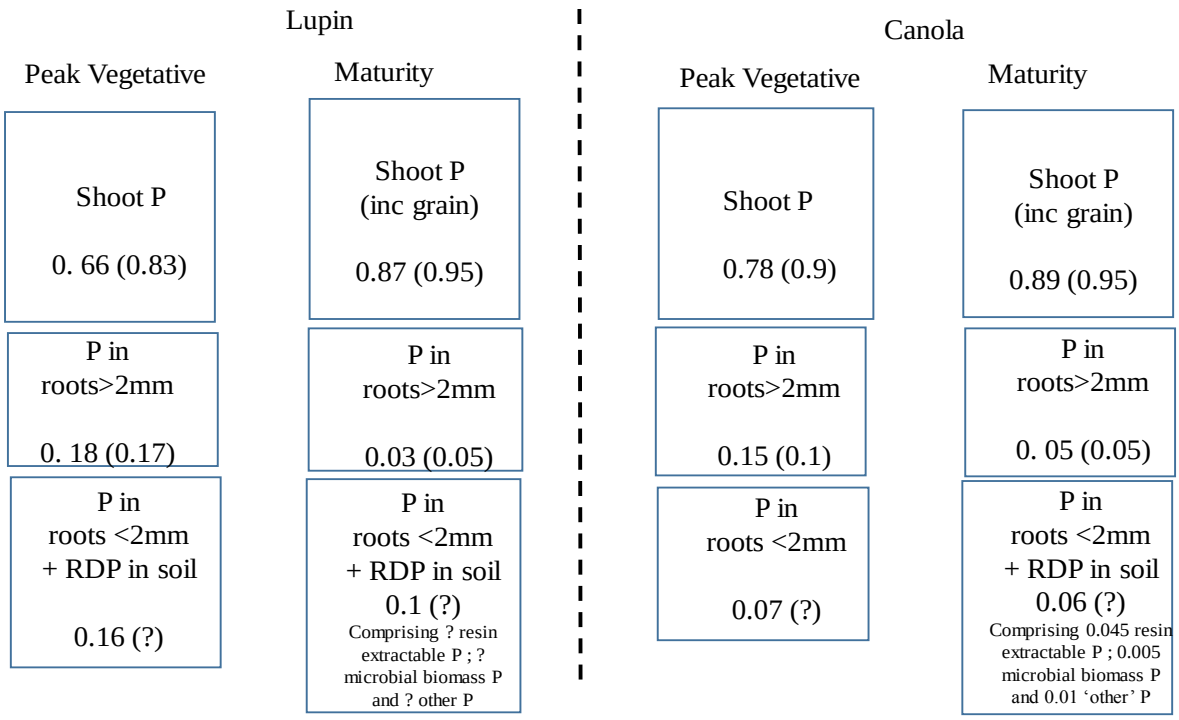


Fig 7.1 Suggested proportional distribution of P in lupin and canola above and below-ground based on the work in this thesis - with values in parentheses being best estimates from published information prior to thesis (box sizes not to scale)

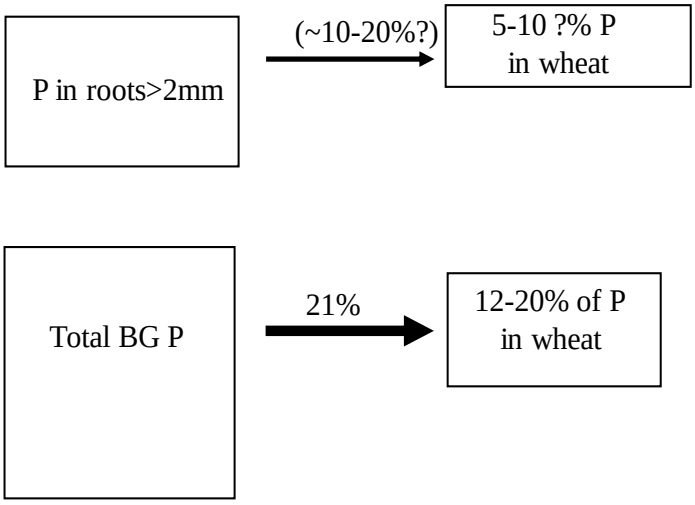


Fig 7.2 Conceptual diagram indicating an increased magnitude of P benefit to wheat if the estimates of total BG P accumulation of canola and lupin break crops generated by this thesis are incorporated into P cycling models currently based on estimated values for recoverable roots only (box sizes not to scale)

the C:P or C:N ratios of recovered roots but by P concentration, and possibly by N:P ratio of BG residues (Chapter 6). Canola BG residues appeared to be more readily mineralised in the short term than lupin BG residues, contributed a greater proportion of the P and N uptake to the following wheat crop, and supplied a similar proportion of their P and N to wheat as that reported for above-ground residues (Chapter 6).

The following sections are a critical reflection on the findings of this research in regard to scientific interpretation and any agronomic significance, and discussion on the limitations of the approach adopted and the methods used. Some future research directions that arise from the results are suggested and a concluding statement made regarding the broad hypothesis of the thesis.

2. Reliability of isotope-based assessments of P accumulation below-ground by break crop species

As mentioned in the discussion sections of earlier chapters a fundamental principal underlying the use of isotopes is that the 'discrete' pool of interest is labelled uniformly. All studies of the accumulation of P and N BG by plants are subject to the same issue in terms of defining the BG pool of a plant as 'discrete' since the intimate connect between the plant and the soil is a diffuse and incredibly dynamic rhizosphere (Richardson et al. 2009). Isotope approaches for estimating BG P and N in roots tend to be simplified by defining the BG pool as recovered roots plus unrecovered root or root-derived material (rhizodeposition) and any 'fine' flows of nutrients within the rhizosphere are integrated within these two pools. How much these fine nutrient dynamics influence estimates of BG P and N based on isotope methods is not clear. Since there were only two sampling time points post-feed during the life cycle of the break crop species, one at late-vegetative stage and one at maturity, it was not possible to examine in more detail the dynamic of the fed ^{33}P that was allocated BG, although

it was clear from the limited measures of labile P pools in this research that plant-derived P was cycled between those two times. The implication of such cycling for the nutrition of the growing plant, and in terms of dynamics of root-derived nutrients in soil, was not examined. Clearly, there is a great opportunity to utilise this stem wick-feeding technique for a more detailed understanding of rhizosphere P dynamics, which may be particularly valuable for quantifying the nutrient exchanges that occur in systems with multiple species such as inter-cropping or pasture.

The preliminary assessment of the stem wick-feeding technique for ^{33}P uptake and distribution (Chapter 3) was limited to testing the effects of a single feed of isotope, at different growth stages and for plants grown in soils differing in P fertility. Furthermore, the major studies in the thesis all used a single feed at late-vegetative stage. This approach was adopted based on reports for wick stem-feeding ^{15}N in the literature that indicated a single pulse labelling during vegetative growth appeared to adequately label pasture and grain legumes BG for determining total BG N accumulation (McNeill and Fillery 2008; McNeill et al. 1997). Furthermore, since the research in this thesis also aimed to examine the fate of the BG P post maturity of the break crop species including uptake by a subsequent plant, late-vegetative stage was preferred as the time for feeding since the relatively short half-life of the ^{33}P isotope limits the time period it can be traced following feeding. Uniformity of labelling of the root systems in those studies previously referred to was assessed using comparison of the specific enrichment of recovered roots from different depths in the soil profile. Indeed the work in this thesis showed a similar uniformity for ^{33}P in recovered roots of canola, although not for both soil types (Chapter 5). Results presented in this thesis also show that partitioning of ^{33}P to roots was positively correlated to recovered root dry matter suggesting a potential for uniform labelling. However, since in all these studies no measures were made of different root cohorts/ages or different orders of recovered roots, there could be no assessment of

whether the label was preferentially allocated to more active roots, which would lead to under-estimation of total BG P using the specific enrichment of those roots if they were recovered, or overestimation if they were the unrecovered roots, which is perhaps more likely. There are a number of studies quantifying BG N that have employed ^{15}N pulse-labelling (i.e. several feeds during the life cycle of the plant) with the stated aim of ensuring more uniform labelling of the root system (Arcand et al. 2013; Mayer et al. 2003; Russell and Fillery 1996; Yasmin et al. 2006). However, there is also some research suggesting that a large number of pulses of N isotope, or indeed continuous feeding, may lead to some 'leakage' of fed ^{15}N and overestimation of unrecovered roots or root-derived material (rhizodeposits) (Gasser et al. 2015). The spatial and temporal aspects of both ^{33}P and ^{15}N feeding clearly require further research to give greater confidence in the robustness of results regarding estimates of BG P and N allocation. Nevertheless, it is interesting that the proportion of total plant N estimated to be BG N for canola and lupin in those studies using pulse feeding was similar to that obtained in this thesis using a single feed of ^{15}N, and indeed to estimates obtained in other studies for lupin using a single feed. This suggests that if there is non-uniformity in isotope labelling, it is occurring despite attempts to overcome or reduce it and that the results of all these studies are subject to a similar error in terms of over- or under-estimation of plant BG N. Nevertheless, the labelling approach does at least allow for estimation of that unrecovered portion of roots that will always remain in soil despite best attempts to fully recover roots, and results highlight that current soil organic matter nutrient cycling models should be adjusted to more accurately reflect the true quantities of P (and N as suggested by this work and others) accumulated by root systems. Whether the adjustment factor is as much as suggested by this research needs further investigation.

Another aspect of quantifying total BG accumulation of nutrient by plants using isotopes is the necessity to obtain a clean root sample to generate a specific activity or enrichment. It

was found that the freeze drying technique used in these studies to separate a sample of clean recovered roots from rhizosphere soil was less laborious and time consuming than the washing technique used by many other studies where the slurry of root washing water has to be collected and processed to capture any labelled materials from the rhizosphere. It was also thought that the dry method avoided any leaching of soluble materials from the roots, and hence an incorrect allocation to the rhizosphere rather than to 'root' material. This may be more of a potential problem for P than N, since it is well known that there can be high amounts of water-soluble P in roots. However, it is interesting that the dry method for BG fractionation following isotope feeding does not appear to be an approach that other studies of BG accumulation and turnover have adopted. This could possibly be due to lack of access to a suitable freeze dryer or perhaps some concerns relating to the maintenance of integrity of plant material when being freeze-dried, or simply that others do not find the wet separation technique too arduous.

3. The fate of P and N from break crop BG residues including uptake by wheat

Although there have been dual-labelling isotope studies of the fate of C and N, or C and P, from residues (Blair et al. 2005; Dalal 1979; McNeill 2001), the research in this thesis provides a unique assessment of the relative fate of N and P from root residues of canola and lupin. The work highlighted that there can be interactions between N and P, independently of C, that will influence net release. Canola, being a more P efficient species than lupin, accumulated and released more BG P, but even though lupin, as an N_2 fixing plant, accumulated more BG N and had a higher root N concentration than canola, it appeared limited in N release from the BG residues by the very low P concentration of the roots.

It is very clear from the studies reported in this thesis (Chapter 5 and 6) that in the short term, and indeed from other studies of BG decomposition in the longer term (Glasener et al. 2002;

McNeill and Fillery 2008), that a large proportion of nutrients in the residues (>80%) are not immediately accessed by subsequent plants, but remains in soil for periods of time. Hence, the contribution to soil organic matter cycling by nutrients contained in plant roots is both short and long term. Indeed a recent report for ^{15}N labelled BG N of tropical legumes in the field indicated that 28-46% of the labelled BG N was recovered by a sequence of three cereal crops over two years (Glasener et al. 2002), much higher recovery than the 8-10% they recovered from ^{15}N labelled shoots for the same period. Exploration of the biochemical nature of root and root-derived nutrients, and the biological and chemical pools where they are found, will require studies that employ chemical speciation approaches such as NMR, combined with chemical or physical fractionation methods. It would be extremely useful if the phosphoric acid that is fed to the plants to label P in roots could also be associated with a stable oxygen isotope (^{18}O) so that longer term experiments could be undertaken. However this may not be possible due to decoupling of the P from the associated oxygen during biological reactions in the plants and soils (Larsen et al. 1989; Pfahler et al. 2013; Tamburini et al. 2012; Weiner et al. 2011).

A key limitation of the study, as discussed in Chapter 6, was that assessment of P and N release from BG residues of canola and lupin was largely based on P and N uptake over a five week period by wheat, planted three weeks after shoots had been removed from the break crop species. Measurements of labile pools were constrained to P only and further were only undertaken at a single point at the end of the eight week study period. This study is not unique in using plant uptake as an assessment of apparent net mineralisation, but it is acknowledged that estimates derived from this approach are clearly under-estimates of the gross cycling of nutrients in the BG residues. More measures of the labile P (hexanol-released and resin P) and N (microbial and mineral N) in soil would give some insight into true rates of nutrient cycling and may assist in determining by how much the values

generated for nutrient cycling using plant uptake as a proxy indicator of mineralisation are under-estimates. There is some indication for P from the results of this study that the underestimation of mineralisation is at least by 5%, whereas other studies suggest it may be as much as 50% for N (McNeill and Fillery 2008; Russell and Fillery 1999). Nevertheless, capture by wheat of nutrients released by the canola and lupin BG residues is a key aspect of understanding the effect of these residues in the plant-soil system, and it was clear that more P than N was accessed from BG residues of both species. This may reflect a greater proportion of soluble P than N in the residues with rapid release and ready access by wheat grown on a sandy soil with a low phosphorus buffering index. The interaction of soil type with availability of P released from these BG residues is an aspect that could be studied in much more detail than was possible in this PhD research, where a single study of canola suggested that P accumulation BG by canola and rate of release to subsequent wheat were greater for a loam than a sand (Chapter 5). For example, ^{33}P *in situ* labelling of root systems could be used to trace the fate of soluble P released from those residues in soils differing in P sorption capacities to determine if the enhanced P benefit to wheat from greater P accumulation BG in finer textured soils is mitigated to some extent by greater sorption of RD P.

Whilst the dual-labelling study was not specifically designed as a detailed assessment of the influence of BG residue quality parameters on nutrient release, it was possible from the results to draw some simple conclusions regarding the influence of residue quality measures such as C:P, C:N, critical P and N concentration on nutrient release from the canola and lupin BG residues. These measures are readily acknowledged in the literature as being somewhat variable with regard to their ability to predict nutrient release (Chapter 6) and the results of this PhD research support that statement. Clearly, as has been recently highlighted in the literature (Bertrand et al. 2006), there may be other biochemical indices that are more suitable

quality parameters for predicting nutrient release from residues, and so detailed speciation of BG residues using solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy (Noack et al. 2014a) and solid state ^{15}N NMR (Smernik and Baldock 2005a; b) in conjunction with the studies of isotope uptake from labelled residues undertaken in this thesis may provide useful information in this regard.

4. What is the agronomic significance of the findings?

It is generally difficult to extrapolate the results from glasshouse studies to the field. Nevertheless, the studies reported in this thesis (Chapter 5 and 6) demonstrate that about 20% of the needs of a young wheat plant may be provided from P that resided in the mature BG residues of a previous lupin or canola plant (Fig 7.2). Given that this is not dissimilar to the likely proportion contributed by the immediate application of fertiliser P or previous crop shoot residues (Blair and Bolland 1978; McBeath et al. 2012; Noack et al. 2014b), it could be deemed of agronomic significance. However, it needs to be remembered that the accumulation of P in those break crop root residues will inherently be linked to the soil P fertility including previous history of P fertiliser inputs, and further the cycling of P *via* decomposition of these residues will be affected by soil P sorption characteristics governing availability of P. So there is much work to be done to untangle the relative benefits of P derived from break crop BG P accumulation versus those from historical inputs of fertiliser P that may have been sorbed, and indeed from native P in soil organic matter which is partly a function of rotation history.

Whilst this research reports reasonable success in using the ^{33}P isotope to label *in situ* BG P in order to obtain a direct assessment of the accumulation of P in root systems and the effects on nutrient cycling, it may prove difficult to extend into the field. It may be applicable for field estimates of *in situ* accumulation of P BG by various crops but it is unlikely to be

feasible to study seasonal dynamics of the labelled BG residues in southern Australian farming systems where there is a summer fallow period between crops around six months, far beyond the likely detection period for the ^{33}P isotope with a half-life of around 26 days. Longer term studies may be feasible if the previously mentioned problem with the development of ^{18}O techniques is resolved. However, the ^{33}P-labelling technique for roots as used for the research in this thesis may be suited to field measures of the transfer of BG P between species in inter-cropping systems. The technique is attractive because it more closely reflects what is likely to be happening in terms of root residue decomposition in an undisturbed situation than previously reported approaches of incorporating labelled roots produced *ex situ*.

5. Future research directions

To address some of the points raised regarding the accurate quantification of BG P and N accumulation by this PhD research study, and in order to further develop an understanding of the interaction of P and N during cycling of BG residues and the fate of nutrients from BG residues, further research in the following areas is recommended.

1. Detailed investigation of the uniformity of labelling achieved in the BG pool and the potential for immediate leakage of fed ^{33}P and ^{15}N using a single feed and comparing with pulse feeding. Data on isotope distribution in different root cohorts (older and younger roots) and for different orders of roots (primary, first order and second order laterals) should be obtained employing a sequential sampling technique from immediately after the feed and thereafter at intervals. Immediate leakage of highly enriched isotope could be tested using a mesh enclosed root system on fed plants (McLaughlin et al. 1987) to measure rhizosphere soil and labile pools. More importantly, clarification of any effect of immediate leakage or non-uniform isotope

distribution on quantifying total BG P and N should contribute to an increased confidence that estimates of BG P and N are robust.

2. Chemical characterisation of the unrecovered 'root-derived' P fraction in soil where plant have been labelled, using fractionation techniques with bulk and/or rhizosphere soils, in conjunction with isotope analysis and NMR speciation studies. This will provide some detailed insight into the nature of what structures or entities the isotope is associated with in the BG pools, is it actually within intact fine roots or root-derived materials such as sloughed cells, exudates etc. – where it may differ of course depending on the plant growth stage.
3. Research needs to address an understanding of the contribution of the BG residues to nutrient cycling in the longer term. Given previously mentioned difficulty with phosphate (δ^{18}O-P) the other approaches mentioned earlier of chemical sequential fractionation combined with NMR of physical fractions of bulk soil after ^{33}P-labelled root systems attain maturity may be the best way forward to determine what forms of root P contribute to long term fertility.
4. Estimation of the BG P and BG N of break crops and their contribution to subsequent wheat in this PhD study was only undertaken for two crop species (canola and lupin) and two broad soil types (sand and loam). It would be valuable to investigate the accumulation of BG P and N of other important crop species (e.g. wheat, canola, field pea) grown in southern Australia, particularly to compare BG P accumulation by those genotypes, cultivars or species with different P acquisition abilities. Also knowledge regarding the influence of a wider range of soil types differing in P sorbing capacity on the availability of P and N from the previous crop root residues to the next crop would be of great value, especially in relation to start of season soil P test values for soils of the low input semi-arid agricultural region of southern

Australia. Finally this thesis suggested >80% of the BG P accumulated in the top 0-0.1m soil depth but this requires further investigation since one valuable role for break crops could be redistribution of P deeper in the profile which would be useful in rain-fed systems where the topsoil can be dry for much of the season.

5. Conclusion

The overarching hypothesis of this study was that nutrient inputs by break crop roots may be a significant contributor to P and N cycling in soils, and hence to the P and N nutrition of a following cereal crop. The study has clearly demonstrated that break crops accumulate more P and N BG than is accounted for by nutrient analysis of roots recovered using a standard 2 mm mesh sieve. These BG residues contributed up to 20% of the P (Chapter 5 and 6) and up to 12% of the N requirements of 5 week old wheat (Chapter 6). Below-ground residues of mature lupin and canola decomposed at comparable rates to those reported for above ground residues, releasing up to 12% of their N and 21% of their P for uptake by the following wheat crop. This work has developed a 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.

References

Arcand M, Knight J D and Farrell R (2013) Estimating belowground nitrogen inputs of pea and canola and their contribution to soil inorganic N pools using ^{15}N labeling. Plant and Soil 371, 67-80.

Bertrand I, Chabbert B, Kurek B and Recous S (2006) Can the Biochemical Features and Histology of Wheat Residues Explain their Decomposition in Soil? Plant Soil 281, 291-307.

Blair G J and Bolland O W (1978) The release of phosphorus from plant material added to soil. Australian Journal of Soil Research 16, 101-111.

Blair N, Faulkner R D, Till A R and Sanchez P (2005) Decomposition of 13C and 15N labelled plant residue materials in two different soil types and its impact on soil carbon, nitrogen, aggregate stability, and aggregate formation. Soil Research 43, 873-886.

Dalal R C (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, 913-916.

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

Glasener K M, Wagger M G, MacKown C T and Volk R J (2002) Contributions of shoot and root nitrogen-15 labeled legume nitrogen sources to a sequence of three cereal crops. Soil Science Society of America Journal 66, 523-530.

Larsen S, Middelboe V and Johansen H (1989) The fate of ^{18}O labelled phosphate in soil/plant systems. Plant and Soil 117, 143-145.

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

McBeath T M, McLaughlin M J, Kirby J K and Armstrong R D (2012) The effect of soil water status on fertiliser, topsoil and subsoil phosphorus utilisation by wheat. Plant and Soil 358, 337-348.

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

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. Eds. M Unkovich, J Pate, A McNeill and D J Gibbs. pp 195-218. Springer Netherlands.

McNeill A and 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 and Soil 302, 297-316.

McNeill A M, Zhu C and Fillery I R P (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, 295-304.

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

Noack S R, McBeath T M, McLaughlin M J, Smernik R J and Armstrong R D (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, 31-39.

Pfahler V, Dürr-Auster T, Tamburini F, M Bernasconi S and Frossard E (2013) 18O enrichment in phosphorus pools extracted from soybean leaves. New Phytologist 197, 186-193.

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

Russell C and Fillery I (1996) Estimates of lupin below-ground biomass nitrogen, dry matter, and nitrogen turnover to wheat. Australian Journal of Agricultural Research 47, 1047-1059.

Russell C A and Fillery I R P (1999) Turnover of nitrogen from components of lupin stubble to wheat in sandy soil. Soil Research 37, 575-592.

Smernik R and Baldock J (2005a) Does solid-state ^{15}N NMR spectroscopy detect all soil organic nitrogen? Biogeochemistry 75, 507-528.

Smernik R and Baldock J (2005b) Solid-state ^{15}N NMR analysis of highly ^{15}N-enriched plant materials. Plant and Soil 275, 271-283.

Tamburini F, Pfahler V, Bünemann E K, Guelland K, Bernasconi S M and Frossard E (2012) Oxygen isotopes unravel the role of microorganisms in phosphate cycling in soils. Environmental science & technology 46, 5956-5962.

Weiner T, Mazeh S, Tamburini F, Frossard E, Bernasconi S M, Chiti T and Angert A (2011) A method for analyzing the delta18O of resin-extractable soil inorganic phosphate. Rapid communications in mass spectrometry : RCM 25, 624-628.

Yasmin K, Cadisch G and Baggs E M (2006) Comparing ^{15}N-labelling techniques for enriching above- and below-ground components of the plant-soil system. Soil Biology and Biochemistry 38, 397-400.

APPENDIX

Safe handling of radioisotope (^{33}P), a beta radiation emitter

It is imperative that the following precautions are taken when handling ^{33}P radioisotope in the glasshouse and laboratory:

- Ensure that you are familiar with the radiation nature and handling procedure of the radioisotope.
- Wear suitable protective clothes (gloves, specialised lab coat, safety glasses, solid shoes).
- Have a suitable radiation monitor (beta counter) turned on and within reach.
- Ensure that working area is not contaminated from previous experiments, neat and tidy.
- Always check radioisotope container for contamination before use.
- Minimise exposure using distance (minimum 0.5 m), time and shielding (perspex).
- Label and seal radioactive container.
- Return to storage any left over radioactive liquid that is not used.
- Change gloves and monitor for contamination frequently.
- Dispose of solid and liquid waste in a designated labelled container.
- Ensure that spill kit is within reach.
- Always monitor working area and self after use of radioisotope and wash hands.
- Wear Thermo Luminescent Dosimeters (TLD) badge.