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Investigating the Role of Stoichiometry as an Influence on Soil Phosphorus Content and Forms

A thesis submitted to The University of Adelaide in fulfilment of the
requirements for the degree of Doctor of Philosophy

Melinda R.S. Moata

BSc and MSc (Soil Science)

School of Agriculture, Food and Wine
The University of Adelaide, Australia

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To my husband, Butje A. Louk Fanggi
And my kids, Kayleen and Keanu Louk Fanggi

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FLOW DIAGRAM OF THESIS

Investigating the Role of Stoichiometry as an Influence on Soil Phosphorus Content and Forms

Background:

- Stoichiometry of C and N relative to P is important for nutrient dynamics and transformation.
- Organic P is important in controlling P but there are analytical challenges in determining its concentration and chemical nature.

Hypotheses:

- Modifying the ratio soil to solution during NaOH-EDTA extraction can improve the sensitivity of subsequent NMR analysis.
- Some organic P species may exhibit tighter correlations with C and N than others.
- Different physical fractions of soil may also exhibit different stoichiometry of C:N:P.
- The cellular P (lipid P, RNA P, diester P and pyrophosphate) component of soil organic matter (SOM) is closely related to traditional measures of soil microbial biomass (SMB).

Improving sensitivity of solution ³¹P NMR analysis in Australian Red Chromosols

Finding:

A 1:4 extraction ratio provided better quality NMR spectra without reducing extraction efficiency or affecting the distribution of P species compared to the traditional 1:20 extraction ratio.

Outcome:

Development of methodology to improve the sensitivity of NMR spectroscopy through modifying the ratio of soil to solution during NaOH-EDTA extraction for low P soils was successful.

Organic phosphorus speciation in Australian red Chromosols: stoichiometric control

Findings:

- Strong correlation between two inositol phosphate isomers and various combinations of lipid P, RNA P, diester P and pyrophosphate.
- The cellular P has strong correlations with C and N.

Outcome:

Development of a four pool model of soil P and the establishment of strong stoichiometry of C and N to one of these pools, i.e. the “cellular P” pool consisting of phospholipid, RNA, diester P and pyrophosphate.

Stoichiometry of carbon, nitrogen and phosphorus in soil physical fractions

Findings:

- Constrained and similar stoichiometry of C:N in whole soil, fine & coarse fractions.
- Soils organic matter stoichiometry is less constrained for P than it is for N, and is less constrained for humified organic matter than for particulate organic matter.

Outcome:

Establishing that C: organic P stoichiometry was more constrained in coarse than fine soil fractions in a set of twenty Australian Red Chromosol soils.

Comparison of microbial phosphorus determined by hexanol fumigation-extraction and NMR spectroscopic methods

Findings:

- Glucose addition increased microbial respiration.
- Underestimation of microbial biomass P (MBP) because some inorganic P taken up by microbes was transformed to organic P forms and was not detected by colorimetry following hexanol fumigation.
- No close relationship between the cellular P pool determined by NMR and the MBP pool determined by the hexanol fumigation-extraction method.

Outcome:

Establishing that the cellular P pool is not closely related to the traditionally determined soil MBP pool.

Future research 1:

Test the generality of the four pool model of soil P) and the finding that cellular P is under stronger stoichiometric control (to C and N) than other soil P pools by completing similar analysis for other soil types e.g. Calcarosol, Sodosol.

Future research 2:

Test the hypothesis that cellular P is primarily derived from plant material rather than soil microbes by completing incubation studies with plant residues and glucose treatments.

Future research 3:

Examine the effect of soil moisture, the rate of soluble carbon (glucose) addition and the incubation period on P composition.

Future research 4:

Gain a better understanding of the decomposition rate of inositol phosphate by conducting incubation studies by adding inositol phosphate to high organic P soils and directly measure the decomposition rate.

ABSTRACT

The elemental composition (stoichiometry) of soil organic matter (SOM) plays an important role in the dynamics of nutrient transformations in terrestrial ecosystems. Many previous studies have reported several factors affect the stoichiometry of SOM (e.g. climate, land use, soil properties) and confirmed a relatively constrained stoichiometry of carbon (C) and nitrogen (N). On the other hand, correlations of C and N to phosphorus (P) in SOM are usually much weaker. This can partly be attributed to the fact that soils contain substantial quantities of inorganic as well as organic P. Some studies have reported stronger correlations of organic P than total P to the key elements of SOM (i.e. C and N), though are still not as strong as between C and N (mostly present as organic N). There are multiple possible reasons for this, including limitations in the way organic P is measured and the diversity of organic P forms that may be present in soils. Recent advances in solution ^{31}P NMR (nuclear magnetic resonance) analysis provide an opportunity to reassess the stoichiometry of P in SOM, as NMR provides a detailed and quantitative assessment of the various classes of organic P present in soils. Thus the implementation of solution ^{31}P NMR analysis facilitates investigation of whether the weak overall stoichiometric relationships of P in SOM may be masking stronger stoichiometric control of particular SOM components or chemical forms. These issues are agronomic and environmental importance because organic phosphorus represents a substantial pool of P in soils. Although organic P is not directly plant available, it can become available to plants through microbial mineralisation. Understanding the processes involved would be of potential benefit to agricultural producers in assessing P fertility of soils and better estimating P fertiliser requirements and also to environmental managers in assessing the risk of P transfer from soils to water ways where excess P is a major cause of eutrophication. This thesis describes a range of activities aimed at improving understanding the role of stoichiometry of P in SOM.

The main limitation of solution ^{31}P NMR spectroscopy as a method for analysis of soil P is the inherently low sensitivity of NMR. This results in long acquisition times (typically one day per sample) and hence low sample throughput. Furthermore, low sensitivity limits detection and quantification of species present in low concentrations. The first part of this thesis reports on efforts to improve sensitivity by tightening the ratio of soil to solution in the extraction step preceding NMR analysis. The most commonly used procedure involves extraction with a mixture of NaOH and EDTA at a ratio of 1:20. A range of tighter extraction ratios down to 1:4 were tested for a set of four Red Chromosol topsoils with low P contents, and it was shown that at lower extraction ratios the signal to noise ratio of spectra was improved with little or no loss in extraction efficiency, there was no loss in signal resolution and no difference in the distribution of P species detected. Thus it can be confidently claimed that, at least for soils similar to those tested, employing an extraction ratio of 1:4 provides a sensitivity benefit with no detrimental effects on resolution or quantitation.

Using the improved NMR methodology, a wider set of twenty Red Chromosol soils were analysed and a range of P forms quantified in the extracts. A novel P pool structure was proposed based on strong correlations among sets of P species present. A set of four pools was proposed: (i) orthophosphate, (ii) humic P, (iii) cellular organic P (the sum of lipid P, RNA P, diester P and pyrophosphate) and (iv) inositol phosphate P (the sum of *myo*- and *scyllo*-inositol hexakisphosphate). In terms of stoichiometry, it was shown that the cellular organic P pool had closer relationships than other P pools with C and N. These findings support the overall hypothesis that some SOM pools would exhibit stronger P stoichiometry than other pools. These findings also raise the question of whether the cellular P pool is closely related to the microbial biomass P pool or a plant residue P pool.

In part, to address the possible correspondence of the cellular P pool with plant residues, physical fractionation (fine fraction < 50 µm and coarse fraction > 50 µm) of the twenty Red Chromosol soils was carried out and the elemental composition (C, N, P) of the fractions determined. Carbon concentrations were found to correlate strongly with N in both size fractions. In contrast, C concentrations correlated moderately ($r^2=0.37$, $p < 0.01$) with P in the coarse but not in the fine fractions. Again, these results support the overall hypothesis that some SOM pools would exhibit stronger P stoichiometry than other pools. In particular, since the organic matter in the coarse fraction isolated in this manner, which is often referred to as particulate organic carbon (POC), is usually presumed to be dominated by plant residues, these results suggest stronger stoichiometric constraint of the plant-dominated fraction of soil organic matter. Solution ^{31}P NMR analysis was carried out on a small selection of fractionated soils and this indicated consistent differences in organic P composition between the fractions, with the fine fractions containing a larger proportion of humic P and the coarse fraction containing relatively more cellular P. Unfortunately, practical constraints relating to the amount of soil available and the low sensitivity of NMR analysis restricted the number of soil fractions that could be analysed in this way.

The final set of experiments described in this thesis addressed the potential connection between the cellular organic P pool and the microbial biomass P (MBP) pool. These experiments involved incubation of a pasture soil rich in organic matter with an easily assimilated source of carbon (glucose). Glucose addition resulted in the expected increase in soil respiration, but the effects on measured soil P pools were unexpected. The traditional measure of the MBP pool – the difference in resin P between fumigated and unfumigated soils was only slightly higher for the glucose amended soil, although closer inspection of the components of this measurement indicated a complex response in which resin P was strongly

depleted on glucose amendment but fumigation failed to release all of the extra P that had been taken up. Solution ^{31}P NMR analysis clearly showed that glucose addition did not significantly increase the concentration of cellular organic P. The implication is that the cellular P pool detected by NMR is not closely related to established methods of determining MBP.

The results presented in this thesis provide insight into the role of stoichiometry as a control on the P content of SOM. Overall, the hypothesis that weak overall stoichiometric control of P in SOM masks stronger stoichiometric control of particular components or chemical forms was sustained. In particular, amongst chemical pools, P stoichiometry was strongest for a pool identified as cellular organic P and amongst physical pools, P stoichiometry was stronger for the coarse fraction than the fine fraction. The use of combinations of techniques (e.g. NMR and size separation) and comparison of techniques (e.g. NMR and traditional measures of MBP) was important in providing this new insight and this general approach should be pursued further to better understand the complex transformations that control the fate of P in soils.

DECLARATION

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

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Melinda R.S. Moata _____

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PUBLICATIONS ARISING FROM THIS THESIS

Journal papers

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Conferences

Melinda R.S. Moata, Ann M. McNeill, Ronald J. Smernik, Lynne M. Macdonald, Ashlea L. Doolette (2014). Understanding organic phosphorus speciation in agricultural soils: Correlation between P types in relation to carbon (C), nitrogen (N), and organic phosphorus (Po) compounds. *The 20th World Congress of Soil Science (20WCSS)*. Jeju-South Korea, 8-13 June 2014, (Oral presentation).

Melinda R.S. Moata, Ann M. McNeill, Ronald J. Smernik, Lynne M. Macdonald, Ashlea L. Doolette (2014). A three-pool model of soil organic phosphorus. *Australia National Soil Science Conference*. Victoria-Melbourne, 23-27 November 2014, (Oral presentation).

STRUCTURE OF THIS THESIS

This thesis is presented as a combination of papers that have been published and chapters that may be submitted for publication.

Chapter 1 presents an overview of the literature on the concept of stoichiometry in terrestrial ecosystems and soil organic matter pools, especially organic phosphorus pools. This chapter also includes the aim of this research.

Chapter 2 comprises a paper published in *Communications in Soil Science and Plant Analysis*. It describes how solution ^{31}P NMR spectroscopic analysis of soil organic phosphorus can be improved for some soils by tightening the ratio of soil to solution during NaOH-EDTA extraction.

Chapter 3 comprises a paper that has been accepted for publication in *Soil Research*. It describes an investigation into organic P speciation in Australian Red Chromosol soils, with a focus on stoichiometric control of C, N and P in the organic matter of these soils.

Chapter 4 describes an investigation of the stoichiometry of C, N, and P in physical fractions (fine and coarse fractions) of Australian Red Chromosol soils. These results have not been submitted for publication.

Chapter 5 describes an incubation experiment aimed at examining the effect of adding glucose to soil on the rate of soil respiration and the size of the microbial biomass P pool. These results have not been submitted for publication.

Chapter 6 provides a synthesis of the findings contained in this thesis and includes recommendations for future study.