The Isotopic Discrimination of Copper in Soil-Plant Systems: Examining Sources, Uptake and Translocation Pathways

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

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21st November 2014

CONTENTS

ABSTRACT

Copper (Cu) is an essential micronutrient for plants and many microorganisms, playing a key role in electron transport during photosynthesis, lignin formation and cell wall metabolism. However, when Cu is present at elevated concentrations it can cause toxicity with impacts on the growth, reproduction and survival of aquatic and terrestrial organisms. The biogeochemical cycle of Cu in aquatic and terrestrial environments can be influenced by numerous biological (e.g. root rhizosphere) and physicochemical (e.g. redox, pH) properties. A better understanding of Cu biogeochemical cycling is required to ensure optimal Cu supply to organisms. As such, there is an increasing need for the development of new analytical tools that can be used in complex environmental systems to examine this.

This thesis investigates the use of Cu stable isotopes to yield new information on the behaviour of Cu in soil-plant systems. Copper has two stable isotopes, ^{63}Cu and ^{65}Cu, and the different partitioning of these two isotopes between Cu pools (known as fractionation) can provide information on the reactions and mechanisms involved in Cu transport from one pool to another. Stable isotope data from plant growth studies were coupled with solid phase speciation and dialysis solution speciation to yield a better understanding of the isotopic signature of bioavailable Cu and the mechanisms by which Cu is absorbed into, and translocated throughout, plants.

The effect of Cu complexation by soluble organic matter was quantified to assess whether the isotopic signature of bioavailable 'free' Cu differed to that of the total soil solution Cu. This is important as fractionation between soil solution and plants cannot be accurately measured if the isotopic signature of

the available pool of Cu is not accurately known. Copper isotope fractionation was examined in solutions of both synthetic organic ligands and Suwannee River fulvic acid (SRFA) using Donnan dialysis to separate the free and complexed Cu pools. The results showed that Cu contained within the organic complex was enriched in the heavy isotope, with the magnitude of fractionation proportional to the strength of the Cu-ligand bond. These results highlight the importance of determining the isotopic signature of the bioavailable 'free' Cu when looking at plant uptake mechanisms, as the isotopic signature of the total solution Cu is different to that of free Cu if Cu is partly complexed with organic ligands, as is usually the case in environmental samples.

When using Cu isotope fractionation to assess root absorption mechanisms, it is important to consider the contribution of Cu adsorbed to the cell wall. In order to assess the isotope fractionation involved with Cu adsorption onto plant cell walls, four-week old plants and seedlings of Fe-acquisition Strategy I and Strategy II species were exposed to various concentrations of Cu for short periods of time. Adsorbed Cu was then desorbed from four-week old tomato and oat plants, using 3 different desorption techniques to determine which root washing technique quantitatively released adsorbed Cu while not extracting symplastic Cu. The results showed that the root wash procedure based on cation exchange using La and Ca was the best extractant to exclusively target the apoplastic Cu, while EDTA and HCl extractants showed signs of symplastic Cu removal. No significant isotope fractionation was found during adsorption onto the surface of monocotyledonous (monocot, Strategy II) plant roots, but adsorption onto the surface of dicotyledonous (dicot, Strategy II) plant roots yielded Cu isotope fractionations on the order of that seen during Cu complexation with fulvic acid ($\Delta^{65}Cu_{root\text{-}solution}$ = *ca.* 0.2‰). The results suggested that a difference in the type and/or strength of Cu binding sites on the cell walls exists for monocot and dicot species, and highlight the importance of root washing when assessing isotope fractionation due to root absorption.

The fractionation of Cu stable isotopes during uptake into plant roots and translocation to shoots was used to gain new information on Cu acquisition mechanisms by plants. Copper isotope fractionation values were coupled with intact tissue speciation techniques (X-ray absorption spectroscopy, XAS) to examine the uptake, translocation and speciation of Cu in a dicot (tomato) and monocot (oat) plant species. Plants were grown in solution culture where Cu was maintained as free Cu by regular replacement of the nutrient solution, so that complexation-induced isotope fractionation in the solution did not complicate the determination of fractionation due to plant uptake. The iron (Fe) conditions were varied to test whether the stimulation of Fe acquiring mechanisms can affect Cu uptake in plants. The results showed that isotopically light Cu was preferentially incorporated into tomatoes ($\Delta^{65}Cu_{whole\ plant-solution}$= *ca.* -1‰), whereas oats showed minimal isotopic fractionation, with no effect on isotope fractionation with changing Fe conditions in either species. The presence of isotopically light Cu in tomatoes was attributed to a reductive uptake mechanism. The heavier isotope was preferentially translocated to shoots in tomato, while oat plants showed no significant fractionation during translocation. The translocation fractionation observed for tomatoes was suggested to be linked to an oxidation and organic complexation with nicotianamine, as both of oxidation and complexation processes lead to heavy isotope enrichment. The majority of Cu in roots and leaves of both species existed as sulphur-coordinated Cu(I) species indicating glutathione/cysteine-rich proteins. The lack of isotopic discrimination in oat plants suggests that Cu uptake and translocation was not redox-selective and different translocation pathways exist between monocot and dicot plant species.

The results presented in this thesis provide significant new information on the behaviour of Cu isotopes in soil-plant systems. For the first time it has been shown that Cu complexation with soluble organic matter and adsorption onto plant roots can cause notable isotopic fractionation, with the organic complex or root surface enriched in the heavy isotope. The most significant findings of this research relate to differences observed between Cu uptake and translocation mechanisms between monocot and dicot species, elucidated from Cu isotope fractionations and XAS analysis. These data open the door to future research into Cu source tracing using isotopic signatures, further investigations into Cu behaviour in soil solutions in-situ, as well as field studies looking at Cu uptake and translocation mechanisms in plants grown in soil environments.

DECLARATION

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ACKNOWLEDGEMENTS

First and foremost I would like to sincerely thank my supervisors Mike McLaughlin, Jason Kirby, Fien Degryse and Kate Scheiderich. Their continual support, positivity and encouragement has enabled the completion of this work despite the challenges and hurdles it has thrown my way. I am extremely grateful for their willingness to share their vast knowledge, experience and time with me to help this thesis come together. Secondly, I would like to thank Hugh Harris and Claire Weekley for their invaluable assistance with XAS beamline operation and data analysis. Having their expert knowledge and assistance added immense value to the quality of work that I was able to produce within this thesis.

To my incredibly understanding and supportive employer, Jim Kelly, I have nothing but praise and thanks for you. Your understanding and flexibility over the past 12 months as I have endeavoured to complete the write up of this thesis has been invaluable. Thanks must also be passed onto Bruno and Chad for their fantastic artistic contributions to this thesis. I could not have finished this work in a timely manner without the assistance I have received from the entire Arris team and I thank them for all they have done.

I would like to acknowledge two of the best housemates one could ask for while completing a PhD; Claire and Stacey. They have provided me with unlimited support, encouragement and bribes to meet my deadlines to completion and for that I thank them from the bottom of my heart. Furthermore, they never once complained about my requests to proof read whatever I put in front of them, no matter how boring or poorly written. To that end I have been privileged to have an incredible support network to see me through the challenges of the past few years, with amazing people on hand who knew when to leave me alone to get my work done and when to drag me out and distract me so I could de-stress. Thanks must go to Mr Ben Murphy, Sarah, Emma, Casey, Shanagh, Frances, E-Mac, Heather, Tehlia, and Shane Koimans for adequately performing these tasks. A special thanks to Nick Skewes for his expert steak preparation efforts; it was greatly appreciated.

Finally, to Maurice, Faye and Kate, who will be as excited as I am to see the end of this PhD, I offer nothing but gratitude for your never ending support. Also, a special thanks to Maurice for his assistance with equipment building and design that facilitated many of my experimental set ups and to Faye for all her assistance with formatting and proof reading.

PUBLICATIONS ARISING FROM THIS THESIS

Ryan, B.M., Kirby, J.K., Degryse, F., Harris, H., McLaughlin, M.J., Scheiderich, K., 2013. Copper speciation and isotopic fractionation in plants: uptake and translocation mechanisms. New Phytologist, 199: 367-378.

Ryan, B.M., Kirby, J.K., Degryse, F., Scheiderich, K., McLaughlin, M.J., 2014. Copper isotope fractionation during equilibration with natural and synthetic ligands. Environmental Science and Technology, **DOI:** 10.1021/es500764x.

STATEMENT OF AUTHORSHIP

Components of the research described in this thesis have been published or have been submitted for publication (as listed below). The contribution of each author to these works is described below.

Chapter 3: Environmental Science and Technology; **DOI:** 10.1021/es500764x

Chapter 4: Environmental Science and Technology; submitted

Chapter 5: New Phytologist; 2013, 199: 367-378

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STRUCTURE OF THESIS

This thesis is presented as a combination of papers that have been published, accepted or submitted for publication.

Chapter 1 provides an overview of the literature on Cu in soil-plant systems, as well as stable isotope geochemistry and its application to tracing element biogeochemical cycling. This chapter also includes the proposed objectives of the research presented throughout this thesis.

Chapter 2 provides an overview of the sample preparation methods developed for three key sample types analysed throughout this thesis: plant tissues, solutions exposed to plant roots, and dialysis solutions of Cu in a strontium nitrate background electrolyte. This chapter outlines the procedures that were trialled in order to obtain an optimised digestion, purification and isotope analysis procedure for Cu.

Chapter 3 describes how Donnan dialysis was used to separate free and complexed soluble Cu to determine the isotopic fractionation resulting from Cu complexation with soluble organic matter. This work has been accepted for publication in Environmental Science and Technology.

Chapter 4 details an investigation into the Cu isotope fractionation arising from root adsorption in the apoplast in various Strategy I and Strategy II plant species. This work also highlights the importance of selecting an appropriate root washing technique to avoid symplastic nutrient release. This chapter has been prepared as a manuscript and submitted to Environmental Science and Technology for publication.

Chapter 5 describes a comprehensive study using Cu isotope ratios and X-ray Absorption Spectroscopy to examine Cu uptake and translocation mechanisms in hydroponically grown plants. This work has been published in New Phytologist.

Chapter 6 provides a summary of the findings and implications of the research presented in this thesis and includes some key recommendations and future research arising from this work.