Nano-scale Secondary Ion Mass Spectrometry – Application to soil organic matter research

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Key Words
Image techniques, isotopes, carbon, nitrogen, ^{13}C, ^{15}N.

Introduction

Many microbial-mediated processes exhibit high spatial variability across a wide range of scales (nm to cm) and at this scale very little is known about the spatial organization of soil particles, soil organic matter, plant roots and microorganisms and their interactions. Understanding the link between the heterogeneity of the soil's physical/chemical environment and its impact on biological processes is a major challenge in soil science. Nano-scale secondary ion mass spectrometry (NanoSIMS) links a high resolution ion probe with isotopic analysis, which allows precise, spatially-explicit, elemental and isotopic analyses to be image mapped at the micro-scale (*ca*. 100 nm) (Herrmann *et al.* 2007a; 2007b). The power of NanoSIMS lies in the ability of the instrument to distinguish stable isotopes of elements with a high sensitivity, i.e. concentrations of sub parts per million can be detected. Here we illustrate the potential of NanoSIMS to examine plant root-bacterial and ectomycorrhizal competition for ^{15}N- and ^{13}C-labeled low molecular weight organic molecules. Amino acids are an important source of organic N for plants and C and N for microorganisms and as such these organic molecules are a major factor regulating ecosystem productivity. ^{15}N- and ^{13}C-labelled amino acids are often used to determine the relative competition between plants and microorganisms for dissolved organic matter. However, this has traditionally required bulk sample analysis (e.g. ground plant root material) which does not enable spatial resolution of the isotopes at a scale relevant to organic matter utilisation and competition by individual microbial and plant root cells.

As examples, we present data of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ NanoSIMS imaging to investigate (i) the competition between wheat root cells and bacteria for ^{15}N in the rhizosphere of an agricultural soil (Clode *et al.* 2009) and (ii) the flow of ^{15}N and ^{13}C across the ectomycorrhizal roots (mantle and hartig net) of the herbaceous plant *Polygonum viviparum* L.which has a widespread distribution in polar arctic and alpine regions. Enriched ^{15}N- and ^{13}C-labelled solutions of amino acid were injected into the soil surrounding the root zone of these plant species. Plant roots were sampled from individual plants over an uptake period of between 1 to 1000 minutes. Subsamples allowed the traditional bulk determination of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios for roots, soluble N pools and residual soil. In addition, samples were rapidly fixed and subsequently resin embedded so that $^{15}N/^{14}N$ and $^{13}C/^{12}C$ isotopic ratio image maps (10-30 μm^2) of cross-sections of bacterial-wheat root cell interactions or fungal-*Polygonum* root cell interactions could be obtained by NanoSIMS. Data will be presented to illustrate differential enrichment of root cells and microbes and show clear spatial patterns between the soil physical matrix (assessed as ^{28}Si), soil organic matter (assessed as ^{12}C), bacterial cells (^{15}N), fungal cells (^{15}N and ^{13}C) and plant roots (^{15}N and ^{13}C).

Conclusions

We conclude that NanoSIMS enables visualisation and isotopic ratio quantification of organic matter resource capture between competing plant and microbial cells. The ability to measure ^{15}N and ^{13}C enrichment within the rhizosphere at the sub-micron scale provides great opportunity to simultaneously quantify and image nutrient flow pathways in complex biological systems at a scale appropriate to the size of the competing organisms.

Acknowledgements

The authors acknowledge the facilities, scientific, and technical assistance of the Australian Microscopy and Microanalysis Research Facility at the Centre for Microscopy, Characterisation and Analysis, University of Western Australia, a facility funded by the university and state and commonwealth governments. DVM receives funding for microbial research from the Australian Research Council (DP0985832) and for soil

organic matter research from the Grains Research and Development Corporation (GRDC) and the Australian Department of Agriculture, Fisheries and Forestry (DAFF). Funding for the polar work was provided by the UK Natural Environment Research Council Antarctic Funding Initiative.

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