Applications of solid-state 15N NMR spectroscopy to the study of nitrogen cycling in sub-tropical forest plantations

Author

George, LL, Kannangara, GSK, Xu, Zhihong, Wilson, MA, Dennis, G, Reddy, N

Published

2007

Conference Title

Linking Local Management to Global Change Challenges: The Proceedings of the International Symposium on Forest Soils and Ecosystem Health

Applications of Solid-State ^{15}N NMR Spectroscopy to the Study of Nitrogen Cycling in Sub-Tropical Forest Plantations

LL George[1*], GSK Kannangara[1], ZH Xu[2], MA Wilson[1], G Dennis[1] & N Reddy[1].
[1] School of Natural Sciences, University of Western Sydney, Locked Bag 1797, Penrith South DC 1797, Australia,
[2] School of Science, Griffith University, Nathan, Qld , Australia,
*Telephone No: +61 2 96859956 E-mail: l.george@uws.edu.au
Key words Solid-state ^{15}N and ^{13}C NMR; nitrogen cycling; plant uptake; decomposition

Abstract A comprehensive study has been undertaken to further understand what forms nitrogen takes as it is cycled within forest plantations found in subtropical Queensland, Australia The uptake of nitrogen by *Eucalyptus Pilularis* and *Pinus Elliotti* was first studied in an experiment using hydroponically grown plants, with either 98%+ ^{15}N enriched calcium nitrate, ammonium sulfate, ammonium nitrate or urea as the only nitrogen source. ^{15}N enriched plant material was then used in a decomposition experiment with soil from a southeast Queensland plantation, to observe how the organic forms of nitrogen in the plant material were cycled back into the soil. Solid-state ^{15}N nuclear magnetic resonance (NMR) spectroscopy was used in both experiments to analyse samples.

Introduction

Forest industries are faced with the growing global demand for timber and forest based products This, however, is combined with increasing public awareness of the need for conservation of natural forests. This has led to the rapid expansion of tropical forest plantations in many developing countries, and more intensive management of existing subtropical forest plantations in developed countries such as Australia and the USA Development of effective soil management practices is therefore necessary for the sustainable production of intensively managed forest plantations. However, improved methods for assessing soil quality and identifying useful soil quality indicators are required for developing these effective soil management practices in forest plantation ecosystems.

Soil organic matter (SOM) is the principle source of nitrogen (N) in most forest ecosystems Therefore an understanding of nitrogen cycling processes in forest soils is needed to develop effective site management, enhance forest productivity, and to provide environmental benefits. The development of sound strategies for the management of forest plantations therefore require an understanding of the forms of N, and the influence of management practices on the forms and transformation rates of N in SOM. Improved methods for assessing and identifying soil quality indicators are also needed (Blumfield *et al* 2004).

The objective of this project was to test, develop and apply solid-state ^{15}N nuclear magnetic resonance (NMR) spectroscopy, techniques to advance our understanding of both the nitrogen cycle and nitrogen composition in subtropical forest plantations. NMR has been used, as it is a non-destructive form of analysis, which does not form any bi-products which may affect results, as other forms of analysis can do (Smernik and Baldock 2005) Previous ^{15}N NMR studies on forest soils have predominantly focused on SOM characterisation (Kogel Knabner 2000, Sjoberg *et al* 2004, Knicker and Hatcher 2001, Knicker 2000, Knicker *et al* 2000). This project will, however, use ^{15}N NMR to characterise fertilizers, plant and soil material, therefore observing the full nitrogen cycle As the natural abundance of the ^{15}N isotope is very low (0 37%), and therefore quite insensitive to NMR spectroscopy (Wilson 1987), all materials used in this project were ^{15}N enriched.

Materials and Methods

The compounds and samples used in this project were analysed at UWS Parramatta Campus, Australia, by solid-state ^{15}N and ^{13}C NMR. The ^{15}N spectra were obtained with a 200-MHz Bruker NMR spectrometer at a frequency of 20 28 MHz, using 4-mm diameter zirconium oxide rotors spun at a frequency of 7 kHz. Chemical shift values were referenced to glycine at –347 ppm NMR parameters for different nitrogen environments were first optimised using cross polarisation magic angle spinning (CPMAS), dipolar dephasing (DD-CPMAS) and Bloch decay ^{15}N NMR experiments on commercially available ^{15}N enriched and unenriched model compounds, before proceeding to study highly ^{15}N enriched organic material

To study the uptake of nitrogen, *Eucalyptus Pilularis* (Blackbutt) and *Pinus Elliotti* (Slash Pine) were chosen, as they are a hardwood and softwood grown by the Queensland Forestry Industry The *Eucalyptus Pilularis* plants were purchased from the Cumberland State Nursery NSW and were approximately 30cm in height. Roots were removed of all soil and washed with de-ionised water, then placed in plastic pots and filled with perlite *Pinus Elliotti* was grown from seeds in rockwool and then transferred to perlite pots with the same setup as *Eucalyptus Pilularis* For both species, the pots sat in a blackened container with holes cut out in the lid for the pots to sit in. Plants were separated into 6 groups Each group was given a different nutrient solution Nutrient solutions were made using Hoagland's Solution as the basic recipe All macro and micro nutrients were kept the same were possible, the only difference between the solutions being the form of nitrogen species present. The six solutions contained either double ^{15}N enriched urea, ammonium nitrate, ammonium sulfate, calcium nitrate, or ammonium nitrate All enrichments were 98% or more. Solutions were monitored for pH, electrical conductivity and temperature, and samples were taken for analysis with Ion Chromatography (IC) Once the experiment was complete, plants were first sectioned, and then oven dried at 50 degrees Celsius until a constant mass was reached Each section of the plant (leaves, branch/stems, roots), was weighed, and then ground with a mortar and pestle or grinder to obtain a powder

The decomposition experiment was undertaken using the highly ^{15}N enriched *Eucalyptus Pilularis* plant material from the hydroponics experiment Samples of finely ground leaves were mixed with 60% field capacity soil from a plantation in Imbil, southeast Queensland, and incubated for up to 8 weeks. The same experiment was repeated with mixed samples of leaves, stems and roots mixed with soil. Samples taken were dried in an oven at 70 degrees Celsius, and kept in a freezer, until needed for analysis with NMR. Hot water and KCl extractions were also taken every two weeks and frozen until analysed using IC.

Results and Discussion

The hydroponics experiment allowed us to examine the uptake of nitrogen in an ideal environment. Due to the very high enrichment of the plants grown, a qualitative ^{15}N NMR CPMAS and Bloch Decay spectrum with more peaks was observed quickly, compared to a sample with low enrichment, providing information on different nitrogen containing functional groups. The peaks observed show that a sample of *Eucalyptus Pilularis* typically contains heterocyclic N (-210 ppm), amide/peptide groups (-264 ppm), amines (-290 to -320 ppm) and amino groups (-330 to -360 ppm) It was found that the nitrogen species a plant was exposed to, had little or no difference on the composition of nitrogen (% a peak took up in ^{15}N spectrum) found in both *Pinus Elliotti* and *Eucalyptus Pilularis*, however, the amount of enrichment did vary between hydroponic solutions. These results show the plants grown find it easier to take up certain forms of nitrogen.

Preliminary ^{15}N and ^{13}C NMR results from the decomposition experiment over the 8-week period show that the ^{15}N species (Figure 1) have remained basically unchanged while ^{13}C species (Figure 2) gave a trend similar to those found by Blumfield *et al* (2004) This presentation will highlight both qualitative and quantitative information on nitrogen and carbon species during the 8-week decomposition experiments.

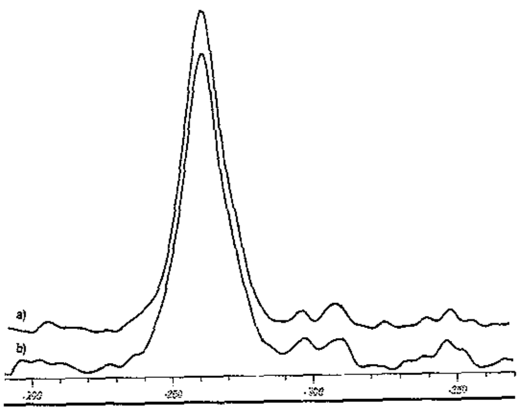

Figure 1 ^{15}N NMR spectra of decomposed *Eucalyptus Pilularis* leaves mixed with soil after a) 0 weeks, b) 8 weeks

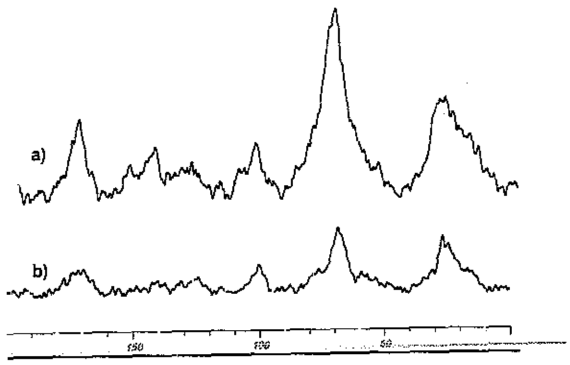

Figure 2 ^{13}C NMR spectra of decomposed *Eucalyptus Pilularis* leaves mixed with soil after a) 0 weeks, b) 8 weeks.

References

Blumfield T J, Xu Z H, Mathers N J and Saffigna P G, 2004, Decomposition of Nitrogen-15 Labeled Hoop Pine Harvest Residues in Subtropical Australia, Soil Sci. Soc. Amer. J. 68, 1751

Knicker H, 2000, Biogenic Nitrogen in Soils as Revealed by Solid-state Carbon-13 and Nitrogen-15N Nuclear Magnetic Resonance Spectroscopy, J. Eviron Qual. 29, 715

Knicker H, Schmidt M W I and Kogel-Knabner I, 2000, Immobilization of Peptides in Fine Particle Size Separates of Soil as Revealed by NMR Spectroscopy, Soil Bio.Biochem 32, 241

Knicker H and Hatcher H G, 2001 Sequestration of Organic Nitrogen in the Sapropel from Mangrove Lake, Bermuda, Org. Geochemistry, 32, 733

Kogel-Knabner I, 2000, Analytical Approaches for Characterizing Soil Organic Matter, Org Geochem 31, 609

Sjoberg G, Knicker H, Nilsson S I and Berggren D, 2004, Impact of long-term N fertilization on the structural composition of spruce litter and mor humus, Soil Bio.Biochem. 36, 609

Smernik R J and Baldock R J, 2005, Solid-state 15N NMR Analysis of highly 15N-enriched Plant Materials, Plant and Soil, 275, 271

Wilson M A, 1987, N.M.R Techniques and Applications in Geochemistry and Soil Chemistry, Pergamon Press, Sydney