

Editorials (Exemplifying Subject Area 1, Sub-Category 4)

Fingerprinting Global Climate Change and Forest Management Within Rhizosphere Carbon and Nutrient Cycling Processes

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As one of the two Principal Subject Editors for ESPR Subject Area 1 'Terrestrial Ecology and Biology / Soil and Sediments: Toxicology-related Subjects' (see pp 287–293), the senior author and his colleague, Dr Chen, present an example of sub-category 4 'Environmental studies of pesticides, air pollution, and management strategies for forestry and plant ecosystems'. Thereby, they inform the ESPR community about the new Australian research project concerning the fingerprints of global climate change (GCC) and forest management on rhizosphere carbon and nutrient cycling and, subsequently, present an overview on the GCC and forest management fingerprints.

Keywords: Australia; fingerprints; forest management; global climate change (GCC); rhizosphere carbon and nutrient cycling

The international Collaborative Research Project about the Fingerprints of Global Climate Change and Forest Management on Rhizosphere Carbon and Nutrient Cycling

A new Australian Research Council (ARC) Discovery project – 'Fingerprints of global climate change (GCC) and forest management on rhizosphere carbon and nutrient cycling' – is currently in progress, undertaken by Professor Zhihong Xu and Dr Chengrong Chen (Griffith University, Brisbane, Australia), for the period of 2006–2008, in close collaboration with

- Professor Sune Linder (Southern Swedish Forest Research Centre, Swedish University of Agricultural Sciences, Alnarp, Sweden),
- Professor Ram Oren (Nicholas School of the Environment and Earth Sciences, Duke University, Durham, USA),
- Professor John Cairney (Centre for Plant and Food Science and School of Natural Sciences, University of Western Sydney, Parramatta, Sydney, Australia), and
- Dr Sue Boyd (Centre for Forestry and Horticultural Research and School of Science, Faculty of Science, Griffith University, Nathan, Brisbane, Queensland, Australia).

The aims of the ARC Discovery project are:

- To test, develop and apply innovative rhizosphere, bio-molecular, stable isotope and nuclear magnetic resonance (NMR) techniques for quantifying impacts of global climate change and forest management on carbon (C) and nutrient cycling processes, particularly in the context of below-ground processes and their interactions with above-ground processes;

- to test and verify hypotheses that plant-soil-microbe interactions in the rhizosphere are highly sensitive to GCC and forest management, and closely linked to C and nutrient cycling;
- to examine and identify biological and chemical fingerprints of GCC and forest management on rhizosphere C and nutrient cycling for developing effective forest management and biodiversity conservation in response to GCC in Australia and elsewhere.

An Overview on Global Climate Change and Forest Management Impacts

1. Global Climate Change and Management in Forest Ecosystems

Over the last century, atmospheric CO₂ concentration [CO₂] has increased globally by nearly 30% and temperature by approximately 0.6°C, and these trends are projected to continue more rapidly (e.g. Houghton et al. 2001, IPCC 2001, Karl & Trenberth 2003, Murphy et al. 2004, Stainforth et al. 2005), particularly with more extreme climatic conditions (Cook et al. 2004, Meehl & Tebaldi 2004, Stott et al. 2004). GCC could have significant social, economic and environmental impacts on the earth (e.g. Lambeck et al. 2002, King 2004, Pierce et al. 2004, Westerling et al. 2006). Terrestrial ecosystems and the climate system are closely coupled through C cycling (Cao & Woodward 1998), and GCC can have significant potential impacts on the structure and function of terrestrial ecosystems (Root et al. 2003, Crous & Ellsworth 2004, Williams et al. 2004). In the past 20 years, impacts on different components of terrestrial ecosystems have been studied using chemical, physiological, microbiological and modelling approaches (e.g. Zak et al. 2000, Gavito et al. 2003, Feng et al. 2004). Such studies have identified globally coherent fingerprints (unique and distinct features or characteristics) of GCC impacts for 279 species across natural systems (Parmesan & Yohe 2003). It has been found that there is a consistent temperature-related shift (or fingerprint) in species ranging from molluscs to mammals and from grasses to trees (Root et al. 2003). Forests cover approximately one-third of the land surface of the Earth and account for 80–90% of plant and 30–40% of soil C (Melillo et al. 1990). The likely impacts of GCC on future structure, composition, and C and nutrient cycling in forest ecosystems deserve particular attention and

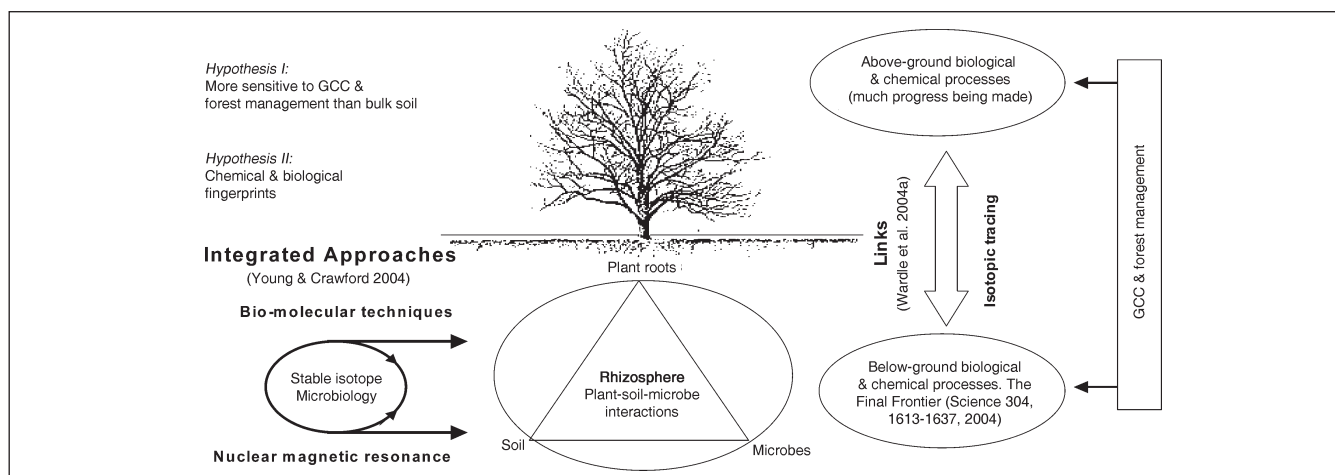


Fig. 1: Impacts of GCC and forest management on plant-soil-microbe interactions, two hypotheses

further research (e.g. Jarvis & Linder 2000, Oren et al. 2001, Melillo et al. 2002, Hungate et al. 2003, Blum 2005, Reich et al. 2006). Elevated $[\text{CO}_2]$ could increase forest growth and litterfall and fine root growth (e.g. Norby et al. 1999), and affect the stability of forest ecosystems in the long term. However, impacts of GCC on and responses in the rhizosphere are not well understood (Hungate et al. 1997, Carnol et al. 2002). Recent evidence has also shown the acclimation of soil CO_2 efflux to warming (e.g. Oechel et al. 2000, Luo et al. 2001, Strömberg 2001, Melillo et al. 2002). It is interesting to note that labile and non-labile soil organic C (SOC) pools respond similarly to global warming (Fang et al. 2005) or non-labile SOC is more sensitive to temperature than labile SOC (Knorr et al. 2005) implying that the long-term positive feedback of soil decomposition in a warming world may be even stronger than predicted by current climate models. Elevated $[\text{CO}_2]$ may also have potential impacts on soil nitrogen (N) dynamics and litter decomposition (Hungate et al. 1999, Norby et al. 2001, Williams et al. 2004, Reich et al. 2006).

Little is known about the impacts of GCC and forest management on plant-soil-microbe interactions (Sadowsky & Schortemeyer 1997). Plant-soil-microbe interactions mainly occur in the rhizosphere, which is defined as the zone of soil that is affected by the root activity of any plant species (Lynch 1982). The rhizosphere is suggested here as the 'hotspot' for plant-soil-microbe interactions – the most chemically and biologically active microsite in soil (e.g. Klironomos 2002, Seguin et al. 2004) – and represents a complex integrated ecosystem (Toal et al. 2000). The ecology in the underworld, particularly below-ground processes and their interactions with above-ground processes, has been highlighted in a recent issue of *Science*: "Soils – The Final Frontier" (*Science* 304, 11 June 2004). There is growing need for improving the understanding and management of important below-ground processes such as soil C dynamics (e.g. Lal 2004), nutrient cycling (McNeill & Winiwarter 2004, Vitousek et al. 2004, Wardle et al. 2004b) and soil-microbe interactions (Young & Crawford 2004), particularly in the context of GCC impacts on and linkages between the below-ground and above-ground processes (Wardle et al. 2004a, Yang 2004). For forest ecosystems, information about the responses of rhizosphere microbial populations and processes

to GCC is scant (Gough & Seiler 2004), possibly due to the high set-up cost of experiments and technical problems with rhizosphere sampling (Seguin et al. 2004). Understanding rhizosphere C and nutrient cycling processes in relation to rising $[\text{CO}_2]$ and temperature is crucial for predicting the response of forest ecosystems to GCC (e.g. Zak et al. 1996, Boone et al. 1998, Matamala et al. 2003, Reich et al. 2006). Plant-soil-microbe interactions in response to GCC are very likely to be complicated by land-use changes and forest management practices (e.g. Jarvis & Linder 2000, Luo et al. 2001, Hungate et al. 2003, Dijkstra et al. 2006, Reich et al. 2006). Since forests could act as both C sink and source of atmospheric C, it is necessary to develop sound strategies for sustainable forest management and for the possible mitigation of the onset of projected GCC. The following hypotheses are advanced here to be tested with a series of integrated approaches (Fig. 1):

- *Rhizosphere – 'hotspot' for plant-soil-microbe interactions:* Plant-soil-microbe interactions in the rhizosphere are more sensitive to GCC and forest management than in the non-rhizosphere zones, and closely linked to rhizosphere C and nutrient cycling;
- *Biological and chemical fingerprints in the rhizosphere:* There are biological and chemical fingerprints of GCC and forest management on rhizosphere C and nutrient cycling processes, which are closely linked to the above-ground processes.

2. Rhizosphere Study Techniques

The quantitative understanding of rhizosphere processes is poor, since the rhizosphere is a difficult system to physically sample and manipulate (e.g. Toal et al. 2000). Currently there are two commonly-used methodologies to physically separate rhizosphere soil from bulk soil. One is the hand-shaking method (e.g. Hendriks & Jungk 1981, Seguin et al. 2004), whereby the rhizosphere soil is separated from the bulk soil by gentle shaking. The soil adhering to the roots after shaking is taken as the rhizosphere soil. This method is simple and convenient, and has been used in both laboratory and field experiments (e.g. Seguin et al. 2004). It is interesting to note that the hand-shaking method has been recently used to separate effectively rhizosphere soils from non-rhizosphere

soils (bulk soils) and then inner rhizosphere soils from outer rhizosphere soils in forest ecosystems (Seguin et al. 2004) in the context of field spatial variability. The second approach involves direct (*in situ*) sampling of soil adjacent to roots by thin sectioning and/or placement of different sized mesh materials around roots. This method worked well only for plant roots with root hairs (e.g. Teng & Timmer 1995). Kuchenbuch & Jungk (1982) developed an improved method to sample rhizosphere soil at a known distance from the root surface. Recently, the method of Kuchenbuch & Jungk (1982) has been modified to study various aspects of rhizosphere nutrient dynamics (e.g. Chen et al. 2002). However, these methods have rarely been used to sample rhizosphere soil in forest ecosystems (e.g. Seguin et al. 2004). It is challenging, but necessary to develop sampling techniques and protocols building on the promising hand-shaking method (Seguin et al. 2004), which takes into account the spatial and temporal variability in the rhizosphere of forest ecosystems in response to GCC and management regimes.

3. Microbiological Methods

Soil microbial properties, such as microbial biomass C, N and phosphorus (P), respiration, metabolic quotient and enzyme activity, can be very sensitive to GCC and forest management (e.g. Högberg et al. 2001, Chen et al. 2002), however information about the impacts of GCC and forest management on soil microbial properties is rather limited (e.g. Sadowsky & Schortemeyer 1997, Chen et al. 2004, Bastias et al. 2006a, Burton et al. 2006). Conventional culture-dependent methods have been used for the measurement of soil microbial composition for more than a hundred years. Nevertheless, only 0.1–1% of soil microorganisms are accessible by these approaches (Hill et al. 2000). Recently, some culture-independent methods have been developed and evaluated to measure soil microbial biomass and community structure. Signature molecules, including phospholipid fatty acid (PLFA) and ergosterol, have been used to estimate soil microbial (bacterial and fungal) biomass and composition (e.g. Frostegård & Bååth 1996, Murata et al. 2002, Simpson et al. 2004, Six et al. 2006). The PLFAs are present exclusively in cell membranes, some of which are indicative of specific groups of microorganisms (e.g. Boschker et al. 1998, Boschker & Middelburg 2002). BIOLOG® microplate assay, based on assessment of utilization patterns of sole C sources and multivariate statistical analysis, has been extensively used to evaluate microbial functional diversity of soil as affected by heavy metal contamination, different plant species and growth stages, fertilization and GCC (e.g. Priha et al. 1999, Preston-Mafham et al. 2002).

4. Bio-molecular Techniques

Recent advances in bio-molecular techniques make it possible to apply culture-independent and DNA/RNA nuclear acid-based techniques to analyze the targeted sequences of bacterial or fungal DNA directly extracted from soil (e.g. Moon-van der Staay et al. 2001, Braid et al. 2003, Anderson & Cairney 2004, He et al. 2005b, Bastias et al. 2006a, b, He et al. 2006, Zhang et al. 2006). The determination of 16S ribosomal RNA (rRNA) genes and 18S rRNA genes has proved most useful for investigating the diversity and composition of bacteria and fungi respectively since these molecules are

composed of highly conserved regions and also of regions with considerable sequence variation (e.g. Anderson & Cairney 2004, Bastias et al. 2006b, He et al. 2006, Zhang et al. 2006). A series of such techniques has been developed or adopted to analyse extracted and purified soil DNA/RNA (e.g. He et al. 2005a, Leininger et al. 2006), including polymerase chain reaction (PCR), denaturing/thermal gradient gel electrophoresis (DGGE/TGGE) (e.g. Kozdroj & van Elsland 2001, He et al. 2005b, Bastias et al. 2006b, He et al. 2006, Zhang et al. 2006), single strand conformational polymorphisms (SSCP) (e.g. Mora et al. 2000), terminal-restriction fragment length polymorphism (T-RFLP) (Horz et al. 2000, Bastias et al. 2006b), amplified rDNA restriction analysis (ARDRA) and amplified ribosomal intergenic spacer analysis (ARISA) (e.g. Anderson & Cairney 2004), length heterogeneity-polymerase chain reaction (LH-PCR) (e.g. Ritchie et al. 2000), and cloning with DNA sequencing (e.g. Anderson & Cairney 2004, He et al. 2005b, Bastias et al. 2006b, He et al. 2006, Zhang et al. 2006). Once the DNA fragments are sequenced, the sequences can be compared with known sequences in the public databases, such as GenBank. This may allow the sequences to be placed into phylogenetic groups (e.g. Lopez-Garcia et al. 2001, Acinas et al. 2004, He et al. 2005b, Bastias et al. 2006b, He et al. 2006), even if the exact species/form has not been identified previously. While some broad-scale DNA techniques have been used for examining the shift in soil microbial community as affected by GCC (e.g. Bruce et al. 2000), there have been few such studies for forest ecosystems (Klamer et al. 2002). Reporter gene technology has provided a new exciting molecular approach for investigating dynamics of C and nutrients in the rhizosphere that bypasses the inherent technical problems associated with direct extraction and analysis of DNA/RNA from soil (e.g. Killham & Yeomans 2001). A reporter gene encodes proteins that produce detectable signals and are environmentally and metabolically responsive. Currently available reporter genes, which have been introduced to rhizosphere microorganisms to monitor C flux and nutrient availability, include *lux*, *luc*, *gfp*, *lac*, *xyl*, and *ice* (e.g. Yeomans et al. 1999, Hansen & Sorensen 2000).

5. Stable Isotope and NMR Techniques

Stable isotope techniques are considered as a critical component in the studies of GCC (e.g. elevated [CO₂]) and forest management on soil C and N dynamics (e.g. Xu et al. 1993a, b, Cheng 1999, Radajewski et al. 2003, Blumfield et al. 2004, 2006, Burton et al. 2006, Chen and Xu 2006). Stable isotope techniques have been found to be a very powerful tool for advancing the understanding of important C and N cycling processes in terrestrial ecosystems (e.g. Näsholm et al. 1998, Xu et al. 2000, 2003, Binkely et al. 2004, Wilcke & Liliensein 2004, Burton et al. 2006, Deluca et al. 2006, Luxhoi et al. 2006). Recent applications of stable isotope techniques to soil biological studies have resulted in significant advances in the understanding of soil microbial processes regulating the C and N cycling in terrestrial ecosystems (e.g. Stark & Hart 1997, Hu et al. 2001). Natural ¹³C abundance technique has been successfully used by Högberg et al. (1999) and Ortega et al. (2004) to study plant-soil-microbe interactions, particularly on trophic status of fungi and host-origin of C in mycorrhizal fungi as well as photosynthesis-driven soil respiration in forest ecosystems. In one of the few studies using stable isotope ¹³C for directly linking microbial populations to spe-

cific biogeochemical processes, Boschker et al. (1998) have identified specific functional groups of bacteria involved in two important biogeochemical processes: sulphate reduction coupled to acetate oxidation in estuarine and brackish sediments, and methane oxidation in a freshwater sediment. It is very exciting to see the combined use of stable isotope and bio-molecular techniques in recent studies (e.g. Radajewski et al. 2000, Shen et al. 2001, Manefield et al. 2002, Lueders et al. 2004), which have identified specific microorganisms that are actively involved in particular metabolic processes. Therefore, the usefulness of stable isotope techniques can be significantly enhanced when combined with innovative bio-molecular and NMR techniques.

NMR techniques have been increasingly used in soil science, geochemistry and environmental science (e.g. Wilson 1987, Mathers et al. 2000, Hedges et al. 2001, Turner et al. 2003, Chen et al. 2004, Huang et al. 2005, Johnson et al. 2005, Blumfield et al. 2006). In particular, ^{13}C NMR has been widely used to improve the understanding of soil organic matter (SOM) quality and composition in relation to terrestrial C and N cycling processes (e.g. Guinto et al. 1999, Chen et al. 2004, Johnson et al. 2005, Blumfield et al. 2006). Recent developments in ^{13}C cross polarisation / magic angle spinning (CP/MAS) spectral editing techniques have enabled the NMR spectrum to be edited into C-, CH-, CH_2 - and CH_3 - subspectra according to their attached proton multiplicity (Mao et al. 1998). Natural abundance ^{15}N CP/MAS NMR spectra of SOM have been obtained by Knicker et al. (1993), indicating that almost all signal intensity is in the chemical shift region assigned to peptide/amide N. In the first application of ^{14}N -NMR to soil humic acid (HA) studies, we have discovered the existence of nitrate-N in soil HA, with the HA nitrate-N closely related to soil N availability and rather responsive to ecosystem management (Mao et al. 2002). There are numerous other NMR techniques that may be applied to study soil chemical and biological processes. These include solution ^1H , ^{13}C and ^{31}P NMR and diffusion measurements using pulse field gradient techniques. These advanced NMR techniques need to be assessed for their potential in improving the understanding of rhizosphere C and nutrient cycling, particularly when combined with stable isotope and bio-molecular techniques (e.g. Knicker 2002, 2003).

6. Concluding Remarks

There is increasing evidence of GCC linking to human influences, but GCC impacts on critical C and nutrient cycling processes in forests are not well understood, particularly in the context of the below-ground processes and their interactions with the above-ground processes as highlighted in a special issue of *Science* (11 June 2004): 'Soils – The Final Frontier'. An improved understanding of GCC and management impacts on important C and nutrient cycling in forest ecosystems is therefore urgently required for developing effective forest management and biodiversity conservation, which meet economic, environmental, and social expectations of our and future generations.

There are two interesting and exciting hypotheses to be tested in this ARC Discovery project:

(a) **rhizosphere – 'hotspot'**: plant-soil-microbe interactions in the rhizosphere are more sensitive to GCC and forest

management than in the non-rhizosphere zone, and closely linked to rhizosphere C and nutrient cycling; and

(b) **chemical and biological fingerprints of GCC and forest management**: There are biological and chemical fingerprints of GCC and forest management on rhizosphere C and nutrient cycling processes, which are closely linked to the above-ground processes. This represents an exciting attempt to integrate the use of innovative rhizosphere, bio-molecular, stable isotope and NMR techniques for studying impacts of GCC and management on rhizosphere C and nutrient cycling in long-term forest manipulation experiments and short-term laboratory experiments.

The project will lead to: (a) improved rhizosphere, bio-molecular, stable isotope and NMR techniques for studying rhizosphere C and nutrient cycling in forest ecosystems in response to GCC; and (b) identified biological and chemical fingerprints of GCC and management on rhizosphere C and nutrient cycling for developing effective forest management and biodiversity conservation in Australia and elsewhere.

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