Inter-hemispheric comparison of soil C quality responses to global change manipulations in rangeland ecosystems.

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PROJECT SUMMARY

Instructions:
The summary is limited to 250 words. The names and affiliated organizations of all Project Directors/Principal Investigators (PD/PI) should be listed in addition to the title of the project. The summary should be a self-contained, specific description of the activity to be undertaken and should focus on: overall project goal(s) and supporting objectives; plans to accomplish project goal(s); and relevance of the project to the goals of the program. The importance of a concise, informative Project Summary cannot be overemphasized.

Title: Inter-Hemispheric Comparison Of Soil Carbon Quality Responses To Warming And Elevated Atmospheric [Co2] Manipulations In Temperate Grasslands

PD: Pendall, Elise	**Institution:** University Of Wyoming
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CO-PD: PD/PI 6 Name (Last, First, MI)	**Institution:**
CO-PD: PD/PI 7 Name (Last, First, MI)	**Institution:**

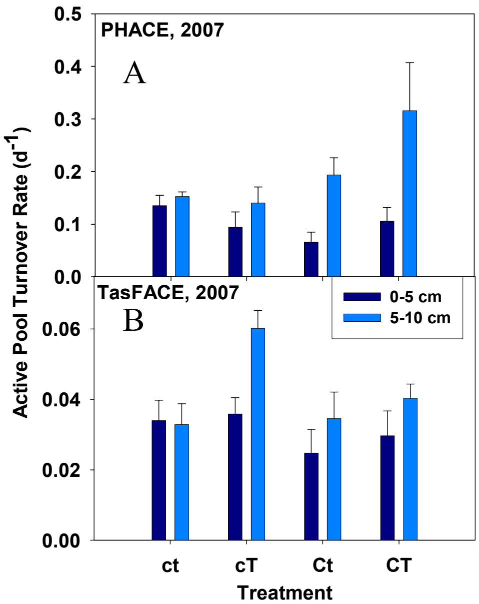
This proposal seeks funding to support a sabbatical leave with the main goal of examining organic matter chemistry across the decomposition continuum from root tissues to particulate organic matter to mineral-associated organic matter, as altered by elevated [CO2] and warming in native grasslands in southeastern Australia and Wyoming, USA. Future sustainability of agricultural productivity depends on adequate soil resources, including stocks of soil organic matter (SOM) that provide long-term C sequestration and potentially mineralizable N. Stability of SOM pools is related to their chemical composition, and may be enhanced or diminished by climate changes including elevated [CO2] and warming, depending on feedback processes between plant litter chemistry and microbial decomposition. Advances in solid state 13C nuclear magnetic resonance (NMR) spectroscopy can potentially improve predictive understanding of changes in SOM quality under future climate change. Application of quantitative NMR techniques to SOM studies was pioneered at the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Adelaide, Australia, and has elucidated mechanisms of SOM formation and stabilization. Stable forms of SOM have been identified by NMR to be related to alkyl C especially associated with suberin derived from roots. Decomposition of roots supplies the majority of organic matter to SOM pools in grasslands. By investigating chemistry and decomposition of roots and SOM pools, this research will fill a major gap in understanding feedbacks between climate change and soil C stability. Long-term transfer of NMR technology to soil studies in the US will be facilitated by additional collaborations at Rice University.

Introduction: Background and goals of applicant

This proposal seeks funding to support a sabbatical visit to Australia by Dr. Elise Pendall, a biogeochemist who has focused on understanding the influence of environmental change on soil C cycling. Her recent work using stable isotope techniques and CO_2 flux measurements demonstrated that elevated CO_2 in a Colorado grassland led to a doubling of rhizodeposition (Pendall et al. 2004b), enhanced decomposition rates (priming) of older soil organic matter (SOM) (Pendall et al. 2003) and accumulation of labile C despite increased SOM turnover rates (Pendall and King 2007). Presently she is a lead investigator on the Prairie Heating and CO_2 Enrichment (PHACE) experiment in native grassland near Cheyenne, Wyoming, which became fully operational in 2007 and will run through 2010. This experiment is unique in that it combines the latest technology in ecosystem warming (Kimball 2005), Free-Air CO_2 Enrichment (Miglietta et al. 2001) and precipitation manipulation in an intact rangeland ecosystem (www.phace.us). Pendall is applying isotope partitioning methods to elucidate biogeochemical mechanisms regulating the C cycle under the various global change manipulations at the PHACE site, with funding from NRI-Soil Processes.

About two years ago, an opportunity arose to collaborate with Dr. Mark Hovenden on soil C cycling on his global change experiment in southeastern Australia, TasFACE, which has important similarities and interesting differences in comparison with the PHACE experiment. TasFACE uses very similar technology to simulate future atmospheric [CO_2] and temperature conditions in native grassland (Hovenden et al. 2006). Species composition is similar, with co-dominant C3 and C4 grasses and smaller proportions of herbaceous and woody dicots. In both experiments, global change treatments are altering plant species composition (Williams et al. 2007; Hovenden et al. 2007; Morgan et al. 2007), with important implications for the quality of organic matter inputs (Milchunas et al. 2005a). Although both sites are semiarid, at PHACE, the climatic regime is cooler (7ºC vs. 12 ºC MAT) and drier than TasFACE (340 mm vs. 510 mm MAP). Soils at both sites have surface C contents >2%, as is typical of native grasslands, but at PHACE the soil type is fine loamy, mixed mesic Aridic Argiustoll (Stevenson et al. 1984) and at the TasFACE site the soil type is a fine textured Vertisol (Hovenden et al. 2006). Nitrogen concentration is lower in TasFACE than PHACE soils.

Preliminary results from the first comparison of TasFACE and PHACE laboratory incubation experiments showed that warming enhanced labile SOM turnover rates (**Figure 1).** At PHACE, enhanced productivity under the [CO_2] plus warming treatment (CT) offset C losses due to warming, and at TasFACE enhanced productivity of a C4 grass offset C losses due to warming (**Figure 1**; Pendall et al. 2007). These initial results demonstrate the potential for enhancing understanding of C cycling processes under global change by applying a comparative approach. Our current grants provide funding to evaluate microbial mechanisms associated with turnover rates, but not to evaluate the chemistry associated with changing plant litter quality that are also likely driving these changes. The goals for this upcoming sabbatical are to become familiar with grassland soil and ecosystem responses to global change in Australia, to establish SOM fractionation procedures at TasFACE, to learn ^{13}C NMR techniques from a pioneer in the field, and to apply these techniques in both global change experiments. *The present career enrichment proposal seeks funds to investigate soil C quality changes in the TasFACE and PHACE experiments, using ^{13}C-NMR, as an ideal complement to ongoing research at both global change experiments.*



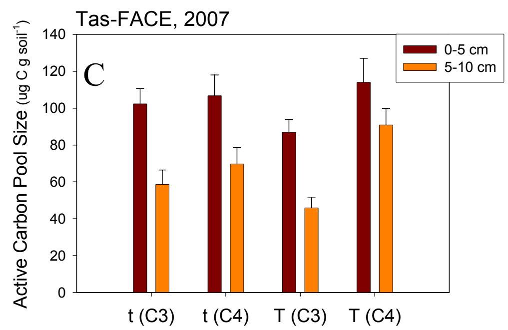


Figure 1. Active SOM turnover rates from laboratory incubations at A. PHACE and B. TasFACE in control (ct), warmed (cT), elevated CO_2 (Ct) and CO_2 plus warming (CT) treatments. C. At TasFACE, productivity by a C4 grass compensated for soil C losses due to warming. I propose to learn ^{13}C NMR techniques to investigate chemical changes in organic matter associated with these observations.

Proposed Sabbatical Research

Overview

This proposal seeks funding to investigate the effects of experimentally imposed climate change on the molecular composition and decomposability of organic matter in two grassland ecosystems. A mechanistic understanding of soil organic matter (SOM) decomposition is required to accurately forecast soil quality and future agricultural sustainability. This study will address the chemical basis of C stabilization in soil in the context of climate change by applying solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy to organic matter fractions exposed to elevated atmospheric [CO_2] and warming.

Rationale and significance

Future sustainability of agricultural productivity depends on adequate soil resources, including stocks of SOM that can provide long-term C sequestration and a source of mineralizable N. Climate changes including elevated [CO_2] and warming may enhance or diminish SOM quality depending on feedback processes between plant litter chemistry and microbial decomposition. Advances in NMR spectroscopy have the potential to improve predictive understanding of changes in SOM quality under future climate change. SOM molecular composition is directly related to plant chemistry (Baldock et al. 1992), which has been shown to change with elevated CO_2 and warming. The proposed research builds on an investment of nearly $3M in funding from multiple agencies in the US and Australia to build and operate the TasFACE and PHACE facilities. Studies of the molecular composition of organic matter funded by this proposal will complement ongoing investigations of microbial community structure and enzyme activity at both sites. Characterization of the molecular composition of organic materials (litter and SOM) will also complement and benefit ongoing work in the Pendall laboratory on the decomposition rates and stable isotopic composition of different SOM pools from these global change experiments. *The proposed research will improve understanding of* *organic matter chemistry resulting from global changes and reduce uncertainties in predicting future sustainability of productivity and C sequestration potential of rangeland ecosystems.*

Effects of elevated CO_2 and warming on SOM quality

A meta-analysis of elevated [CO_2] experiments shows that soil C pools increased on average by 5.6% (Luo et al. 2006); likewise, our previous grassland research demonstrates greater soil C input rates (Pendall et al. 2004b). Warming, however, causes a rapid loss of labile substrates, which may make up ~10% of the total SOM pool, followed by slower mineralization of resistant SOM pools (Ineson et al. 1998a, Ineson et al. 1998b, Loiseau and Soussana 1999; Melillo et al. 2002, Kirschbaum 2004, Eliasson et al. 2005). Continued soil C storage under future climates is contingent upon adequate soil N availability (Reich et al. 2006; van Groenigen et al. 2006), which depends on SOM mineralization rates. The interacting effects of elevated [CO_2] and warming on stability of SOM pools clearly lead to major uncertainties in predicting future C sequestration rates and potential feedbacks to atmospheric [CO_2] and climate change.

Uncertainties in future soil C stability are partly due to insufficient understanding of how SOM and litter quality may respond to elevated CO_2 and warming. Elevated [CO_2] produces grass tissues with lower substrate quality (higher C:N ratios, lignin and cellulose content), leading to lower digestibility in ruminants (Milchunas et al. 2005a). Elevated [CO_2] did not affect substrate quality or decomposability of senesced tree leaf litter (King et al. 2001) but did reduce tree root litter quality (Parsons et al. 2003). Warming tended to decrease C:N ratios in tree roots (King et al. 1997), whereas it increased C:N in C3 and C4 grasses (An et al. 2005), leading to decreases in soil N mineralization rates (Wan et al. 2005). Temperature sensitivity of SOM decomposition is inversely related to SOM quality (Leifeld and Fuhrer 2005; Fierer et al. 2005, 2006), as predicted from thermodynamic theory (Davidson and Janssens 2006). A temperature gradient study suggested that SOM quantity and quality may decrease significantly with global warming (Fissore et al. 2008). However, stability of SOM appears to increase with increasing mineral N availability (Berg et al. 1996), which may be decreased under elevated [CO_2] but enhanced under experimental warming (Hovenden et al. 2008 in press; Pendall et al. unpublished data). Uncertainties in how feedbacks between litter quality, decomposability, N mineralization and plant growth may be altered by climate change limit our predictive understanding of C cycling in potentially altered future climates. *This proposal investigates the effects of elevated CO_2 and ecosystem warming on SOM quality, to complement ongoing studies evaluating N mineralization and SOM decomposition rates* (e.g., **Figure 1**).

The decomposition continuum as shown by ^{13}C NMR

The decay continuum from plant litter to SOM has been described by Melillo et al. (1989), who demonstrated a two-stage process of mass loss by spruce needles accompanied by decreasing C:N and increasing lignin: (lignin+cellulose) ratios. Advances in solid state ^{13}C NMR spectroscopy in the last decade have allowed quantitative insight into molecular changes associated with increasing residence time (and decreasing particle size) of organic materials in soil (**Figure 2**; Baldock et al. 1992; Baldock et al. 2004; Lorenz et al. 2007). Fresh plant litter is dominated by O-alkyl C, made up primarily of cellulose and hemi-cellulose, and aromatic (lignin) C, identifiable by NMR as aryl C and O-aryl C, with lower concentrations of alkyl-C as cutins, suberins, and lipids (Baldock et al. 1997; Quideau et al. 2000, 2001; Poirer et al. 2005).

As microbes begin attacking plant biomass, their proteins and lipids are incorporated into the SOM, and plant aromatic and O-alkyl C are lost. During later stages of decomposition, alkyl C originating from plants as well as microbes accumulates in soil (**Figure 2**). Alkyl C has high mean residence time and can be considered indicative of biologically resistant SOM (Mikutta et al. 2006; Lorenz et al 2007). Quantitative NMR techniques have demonstrated that resistant SOM contains substantial alkyl C contributed from lipids such as cutin and suberin (Baldock et al. 2004; Lorenz et al. 2007), which can be selectively preserved in soils (Krull et al. 2003).

^{13}C NMR spectroscopy is replacing acid hydrolysis as the preferred technique to evaluating chemical constituents associated with stabilization of organic matter (Wang et al. 2004). Although acid hydrolysis produces a residue with relatively old mean residence times as determined by ^{14}C or ^{13}C (Paul et al. 1997; Schwendenmann and Pendall 2006), the residue also contains fresh lignin and aliphatic molecules (Preston et al. 1997; 2006). Aromatic constituents may still considered as an important component of stable SOM (Baldock et al. 2004), but lignin structures do not preferentially accumulate in soils during decomposition (Quideau et al. 2000; Grandy et al. 2007) and have not been found associated in stable mineral complexes (Mikutta et al. 2006). Increased growth of woody plants under elevated [CO_2] (Morgan et al. 2007) and warming (Harte and Shaw 1995; Hovenden et al. 2007) may be associated with increased alkyl C in comparison with graminoids they replace (Preston et al. 2000; Jalota et al. 2006).

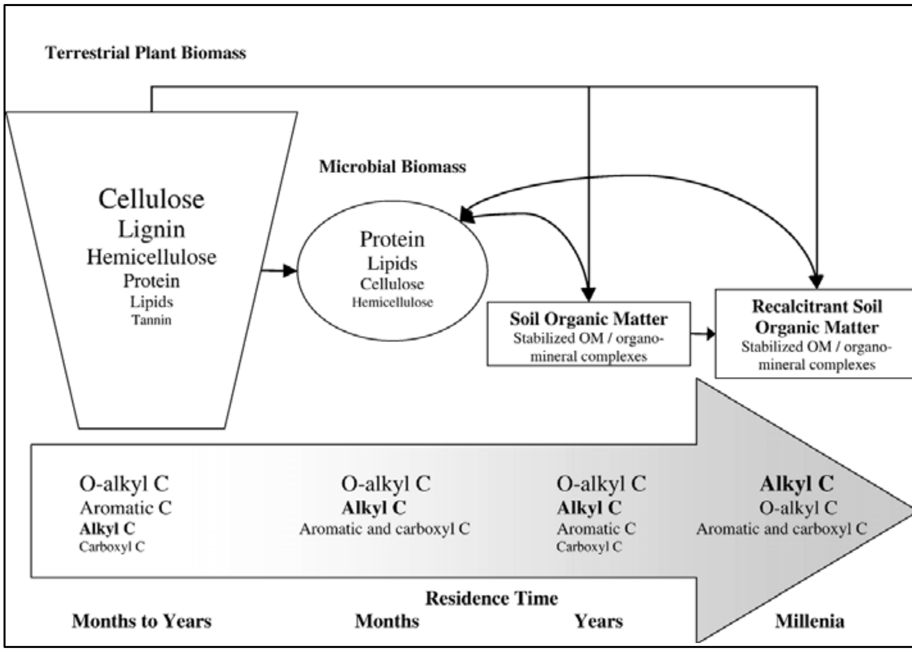


Figure 2. Molecular composition of organic materials in the decay continuum (Lorenz et al. 2007), demonstrating the accumulation of alkyl-C and loss of O-alkyl C (dominated by celluloses) and aromatic C (dominated by lignin), as demonstrated by quantitative ^{13}C NMR. SOM with short MRT is found in low-density particulate fractions, and that with long MRT is found in dense, fine fractions (Baldock et al. 1992).

As plant organic materials are incorporated into soils, the continuum of decay can be altered by interactions with mineral surfaces and protection within aggregates, such that biochemically labile compounds can be preserved from microbial attack (Baldock and Skjemstad 2000). Resistant organic biomolecules compounds comprise up to 50% of SOM and have residence times of up to thousands of years (Mikutta et al. 2006), but resistant SOM may be vulnerable to degradation in the presence of labile substrates, via priming effects (Fontaine et al. 2007). *The proposed research will use quantitative ^{13}C NMR in association with experiments designed to evaluate the role of mineral surfaces and priming effects on the chemical composition of decomposing SOM as altered by climate change.*

Importance of roots

Root biomass comprises about 75% of NPP in semiarid grasslands (Milchunas and Lauenroth 2001), thus supplying the majority of C for SOM production. Alkyl C has been identified as the most stable form of soil C, and is dominated by suberin and cutin (Lorenz and Lal 2005; Winkler et al. 2005). Suberin is a good tracer of root biomass in grasslands (Rasse et al. 2005). Roots tend to have higher lignin concentrations than aboveground plant parts (Fernandez et al. 2003), although the contribution of this lignin to stable SOM may be less important than the alkyl components. Root production is stimulated by elevated [CO_2] (Milchunas et al. 2005b) and warming (King et al. 1997), and roots generally decompose more slowly than aboveground biomass (Jalota et al. 2006). However, roots produced under elevated [CO_2] had lower lignin and higher soluble contents than under ambient [CO_2] (Milchunas et al. 2005b), suggesting higher decomposability and potential to stimulate priming (e.g., Pendall et al. 2003). Root decomposition and the contribution of root derived C to SOM have received much less attention than aboveground residues (Jalota et al. 2006; Lorenz et al. 2007). Zak et al. (2000) identified changes in biochemistry of root tissues as a major gap in understanding of changes in soil C and N cycling under global change. Root turnover rates are directly related to their biochemical composition (Silver and Miya 2001) and are considered one of the least understood aspects of the global C cycle (Strand et al. 2008). *The proposed research will examine organic matter chemistry across the decomposition continuum from root tissues to particulate organic matter to mineral-associated organic matter, as altered by elevated CO_2 and warming in two temperate grasslands.*

Experimental Objectives:

1. Establish the molecular composition of organic matter in the decay continuum as affected by elevated [CO_2] and warming using physical soil fractionation methods;
2. Determine changes in molecular composition and temperature sensitivity of root tissue during decomposition, as altered by elevated [CO_2] and warming;
3. Evaluate interactions among soil N availability, priming of SOM decomposition, and litter quality under elevated [CO_2] and warming.

Hypotheses

1.a. Roots produced under elevated [CO_2] and warming will have higher O-alkyl C and C:N ratios and higher decomposition rates than ambient roots.

1.b. Particulate and mineral associated SOM exposed to 6 years of elevated [CO_2] and warming will have higher concentrations of alkyl C than ambient soils due to faster decomposition of carbohydrate-rich roots.

1.c. The effects of elevated [CO_2] and warming will be additive in terms of SOM quality and decomposability.

2.a. Roots produced under elevated [CO_2] and warming will have lower temperature sensitivity than ambient roots

2.b. Lignin and cellulose in roots will decompose faster under heated treatments, preferentially concentrating more alkyl C.

2.c. Priming by root exudation is more important than elevated [CO_2] or warming in determining root decomposition rates

3.a. Differences in soil N availability between the two grassland global change experiments will lead to differences in SOM quality, decomposition rates and priming effects

Experiment 1: Establish the chemical composition of organic matter in the decay continuum in TasFACE soils using physical soil fractionation methods

Since 2001, the TasFACE facility has been exposing native grassland to conditions expected to occur later this century (Hovenden et al. 2006). Six 1.5-m diameter plots are exposed to a [CO_2] of 550 μmol mol^{-1} and six more are controls. Half of the elevated [CO_2] plots and half of the control plots are exposed to an additional 140 W m^{-2} of infrared radiation, providing a warming of 2.0°C at the canopy and 0.8°C at the soil. The experimental site is a species-rich native grassland dominated by perennial grasses *Themeda triandra* (C4) and *Austrodanthonia caespitosa* (C3) and is thus typical of native grassland for much of southeastern Australia.

Soils will be sampled from all 12 plots in Sept.-Oct. 2008 by sampling triplicate cores over depth increments of 0-5, 5-10 and 10-20 cm. Roots will be removed by wet sieving at 250-μm. Fresh root pieces >1-cm in length will be picked by hand and washed thoroughly. Subsamples of remaining soils will be subjected to fractionation by wet sieving at 20-μm and 2-μm, after ultrasonic dispersion. The light particulate (>20 μm) and fine silt (2-20μm) fractions will be separated by floating in NaI at 1.8 g cm^3 (Baldock et al. 1992). The whole clay (<2-μm) fraction will be used without density separation, will be treated with 6% NaOCl and 10% HF (Mikutta et al. 2006). Leaf litter, root and light SOM fractions, and a subset of clay fractions, will be analyzed for NMR analysis in Adelaide, as well as C, N, ^{13}C and ^{15}N analysis at UW.

Experiment 2: Determine changes in molecular composition and temperature sensitivity of root tissue during decomposition

Exp. 2a. Under controlled laboratory conditions, evaluate differences in decomposition rate, temperature sensitivity, and changes in molecular composition of root tissues grown in TasFACE

Using roots collected from all 12 treatments in 2008, laboratory incubation experiments will be established by adding 150-mg tissue clipped to 1-cm length to low-C, root-free soil (sieved to 2-mm and picked). Soil with low C (<0.2%) content is used to reduce potential priming effects. Two temperature treatments will be used, 10° and 30°C, and four subsamples will be used for each of the 12 field treatments. Temperature sensitivity will be determined at four times (2, 4, 8 and 16 weeks), following Fierer et al. (2005), after which destructive harvest of one subset will take place. The light particulate fraction (>20μm, <1.8 g cm^3) will be isolated for NMR analysis at CSIRO, and for C, N, ^{13}C and ^{15}N analysis at UW. Curve fitting for decomposition rates will follow Pendall and King (2007).

Exp. 2b. Under field conditions in treatment plots, determine decomposition rates, temperature sensitivity, and changes in molecular composition of standard root tissues in PHACE.

At the PHACE experiment, atmospheric [CO_2] is elevated to 600 ppm during daylight hours of the growing season (starting April 2006), and temperature is elevated by 1.5°C during daytime and 3°C at night, year round (starting March 2007), to simulate conditions expected during the coming century (IPCC 2001). Two irrigation treatments examine soil-plant water relations likely to be altered by elevated [CO_2] and warming, and provide opportunities to investigate potential effects of precipitation change on these water-limited ecosystems. Each of the six treatments is replicated in five rings, for a total of 30 rings.

Root tissues from two dominant species (*Bouteloua gracilis*, C4, and *Stipa comata*, C3) grown at ambient conditions were placed in Ankom ® pouches at 5-cm depth in each of the 30 treatment plots in Nov. 2007. Litter pouches will be collected from the field after 4, 8, 16, 24 and

36 months of exposure to field treatments. A split-plot treatment was imposed to remove growing plants and root exudation where half the litter pouches are buried. Subsamples of decomposing root litter will be analyzed for molecular composition at Rice University after Pendall's return to the US. Analysis for C, N, ^{13}C and ^{15}N will be done at UW. Decomposition rates will be reported on an ash-free basis. Temperature sensitivity will be estimated based on curve fitting of mass loss of each chemical constituent against accumulated degree days of exposure in the experimental plots.

Time Line:

Sept. 1, 2008 to Jan. 31, 2009: Collect soil, root and leaf samples from TasFACE experiment in Hobart, Australia; conduct Experiment 1 (SOM physical fractionations) and Experiment 2a (laboratory incubation with added tissue substrates) in collaboration with Dr. Hovenden.

Feb. 1, 2009 to Mar. 15, 2009: Travel to Adelaide to analyze organic matter quality in soil and tissue samples from Experiments 1 and 2a using NMR techniques in collaboration with Dr. Baldock.

Mar. 15 to May 31, 2009: Model carbon compound proportions generated by NMR using Molecular Mixing Model (Baldock et al. 2004); prepare manuscripts.

June 1 to July 31, 2009: Return to Laramie, prepare Experiment 2b samples from PHACE field decomposition experiment samples for analysis at Rice University in collaboration with Dr. Masiello.

Aug. 1 to Aug. 15, 2009: Analyze organic matter quality in PHACE samples at Rice University.

Future Research Goals

Future research will begin with Objective 3, examine the relationships among N availability, priming and SOM quality, by integrating results generated during the sabbatical with ongoing experiments in a multi-author manuscript. Additional soil and plant analysis will continue in subsequent years. Pendall will bring ^{13}C-NMR expertise into the soils and ecological research programs at University of Wyoming, in collaboration with Dr. Carrie Masiello at Rice University. Pendall and Masiello have also discussed potential application of NMR to Pendall's ongoing research in sagebrush rangelands subjected to prescribed burning, where char is likely responsible for substantial C sequestration.

Enhancement of Research Capabilities

Pendall's biogeochemistry and stable isotope expertise and understanding of global change in grasslands will be an asset to the TasFACE experiment. Pendall will be learning cutting-edge ^{13}C nuclear magnetic resonance (NMR) spectroscopy techniques from Dr. Baldock for quantifying the chemical composition of plant tissues and soil organic matter (Baldock et al., 2004). The ^{13}C-NMR methods pioneered by Dr. Baldock are rarely used in the US due to a lack of infrastructure and training among ecologists here. An active soil science research group is expanding at University of Wyoming, and awareness of the potential applications of NMR techniques to rangeland sustainability issues will be promoted through seminars and discussions upon Pendall's return to UW. This inter-hemispheric comparison will improve understanding of rangeland responses to global change in Australia and in the US, and will provide insights into ecological processes that regulate feedbacks between climate and CO_2 that are applicable globally.

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