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# Plant Controls of Terrestrial Trace Gas Fluxes: Legumes, Microbes, CO and Hydrogen

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**Final Report for Period:** 04/2001 - 03/2005**Submitted on:** 09/06/2005**Principal Investigator:** King, Gary M.**Award ID:** 0089900**Organization:** University of Maine**Title:**

Plant Controls of Terrestrial Trace Gas Fluxes: Legumes, Microbes, CO and Hydrogen

**Project Participants****Senior Personnel****Name:** King, Gary**Worked for more than 160 Hours:** Yes**Contribution to Project:****Post-doc****Name:** Dunfield, Kari**Worked for more than 160 Hours:** Yes**Contribution to Project:****Name:** Tolli, John**Worked for more than 160 Hours:** Yes**Contribution to Project:****Graduate Student****Name:** Cleave, Simon**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Simon completed part of his Ph.D work collaborating on the project.

**Undergraduate Student****Technician, Programmer****Name:** Roache Johnson, Kay**Worked for more than 160 Hours:** Yes**Contribution to Project:****Name:** Yeung, William**Worked for more than 160 Hours:** Yes**Contribution to Project:****Other Participant****Name:** Murrell, Colin**Worked for more than 160 Hours:** No**Contribution to Project:**

Colin supervised a Ph.D. student at the University of Warwick who collaborated on the study.

**Research Experience for Undergraduates**

**Name:** Crosby, Heidi

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

Heidi Crosby also worked as an Honors undergraduate student.

**Years of schooling completed:** Junior

**Home Institution:** Same as Research Site

**Home Institution if Other:**

**Home Institution Highest Degree Granted(in fields supported by NSF):** Doctoral Degree

**Fiscal year(s) REU Participant supported:** 2003 2002

**REU Funding:** REU supplement

### Organizational Partners

**University of Warwick**

A Ph.D. student at Warwick completed part of his dissertation work collaborating with this project.

### Other Collaborators or Contacts

### Activities and Findings

**Research and Education Activities:**

The major research activities funded as part of this project included fieldwork at sites in Georgia and Brazil along with field and lab work in Maine to analyze interactions among plant sources of carbon monoxide, bacterial utilization of carbon monoxide, diversity of microbial CO oxidizers and exchanges of CO between soils and the atmosphere.

The research included analyses of soil-atmosphere CO exchange, assays of CO production and consumption by plant roots and the development and application of two molecular approaches for determining the distribution and diversity of CO-oxidizing microbes.

**Findings:**

Although anthropogenic landuse has major impacts on soil-atmosphere gas exchange in general, relatively little is known about impacts on carbon monoxide. We compared soil-atmosphere CO exchanges as a function of land use, crop type and tillage treatment on an experimental farm in Par na, Brazil representative of regionally important agroecosystems. Results showed that cultivated soils consumed CO at rates between 3 and 6 mg CO m<sup>-2</sup> d<sup>-1</sup>, with no statistically significant effect of tillage method or crop. However, CO exchange for a pasture soil was near zero, and an unmanaged woodlot emitted CO at a rate of 9 mg CO m<sup>-2</sup> d<sup>-1</sup>. Neither nitrite, aluminum sulfate or methyl fluoride additions affected CO consumption by tilled or untilled soils from soybean plots, indicating that CO oxidation did not depend on ammonia oxidizers, and that it differed in part from patterns reported for forest soils. The appKm for CO uptake, 5-11 ppm, was similar to values reported for temperate forest soils; Vmax values, approximately 1  g CO gdw<sup>-1</sup> h<sup>-1</sup>, were comparable for woodlot and cultivated soils, in spite of the fact that the latter consumed CO under ambient conditions. Short-term (24 h) exposure to elevated CO (10%) partially inhibited uptake at lower concentrations (i.e., 100 ppm), suggesting that the sensitivity to CO of populations active in situ differs from known carboxydotrophs. Soil-free soybean and corn roots consumed CO when incubated with 100 ppm concentrations, produced CO

when incubated with ambient concentrations. These results document for the first time a role for cultivated plant roots in the dynamics of agroecosystem CO.

Isolates belonging to six genera not previously known to oxidize CO were obtained from enrichments with aquatic and terrestrial plants. DNA from these and other isolates was used in polymerase chain reaction assays of the gene for the large sub-unit of carbon monoxide dehydrogenase (coxL). CoxL and putative coxL fragments were amplified from known CO oxidizers (e.g., *Oligotropha carboxidovorans* and *Bradyrhizobium japonicum*), from novel CO-oxidizing isolates (e.g., *Aminobacter* str. COX, *Burkholderia* str. LUP, *Mesorhizobium* str. NMB1, *Stappia* strains M4 and M8, *Stenotrophomonas* str. LUP, *Xanthobacter* str. COX), and from several well-known isolates for which the capacity to oxidize CO is reported here for the first time (e.g., *Burkholderia fungorum* LB400, *Mesorhizobium loti*, *Stappia stellulata* and *Stappia aggregata*). PCR products from several taxa, e.g., *O. carboxidovorans*, *B. japonicum* and *B. fungorum*, yielded sequences with a high degree of identity (>99.6%) to those in Genbank or genome databases. Aligned sequences formed two phylogenetically distinct groups. Group OMP contained sequences from previously known CO oxidizers, including *O. carboxidovorans* and *Pseudomonas thermocarboxydovorans*, plus a number of closely related sequences. Group BMS was dominated by putative coxL sequences from genera in the Rhizobiaceae and other ?-Proteobacteria. PCR analyses revealed that many CO oxidizers contained two coxL sequences, one from each group. CO oxidation by *M. loti*, for which whole genome sequencing has revealed a single BMS-group putative coxL gene, strongly supports the notion that BMS sequences represent functional CO dehydrogenase proteins that are related to but distinct from previously characterized aerobic CO dehydrogenases.

Plants contribute to atmospheric CO fluxes, although their contributions vary among species. In particular, legume nodules produce large amounts of CO, which may account for the diversity of Rhizobaeaceae that have been found to oxidize CO and that may play a role in soil-atmosphere CO fluxes. We have used a molecular approach to explore the diversity of CO oxidizers associated two legumes (peanut and soybean) and two non-legumes (cotton and corn). We sampled rhizosphere and bulk soil from an agricultural field site in Georgia with peanut, soybean, cotton and corn crops in a long-term rotation. Primers specific for two forms of CODH (coxL) were used to amplify genomic DNA extracts; PCR products were cloned and sequenced. Clone sequences were analyzed phylogenetically and statistically. The former indicated that the four libraries were comprised of closely related taxa, even though few sequences were shared among libraries. Phylogenetic analyses also revealed that most of the sequences were derived from Proteobacteria, with only a small proportion occurring in lineages with no clear affiliation. Results from LIBSHUFF and Arlequin (AMOVA) also suggested that the four libraries were similar. Collectively, these results showed that rhizosphere CO oxidizer diversity was not strongly affected by plant type. However, comparisons of cotton rhizosphere and bulk soil indicated that roots influenced local population structure. Comparisons between bulk soil from cotton and an unmanaged pine stand also revealed strong influences of land use, probably related to impacts on bulk carbon contents.

Since many CO-oxidizing bacteria are lithotrophic in nature, we have developed and pursued a second molecular approach to measure their distribution and diversity. Obligate lithotrophs (e.g., ammonia oxidizers) and facultative lithotrophs (e.g., CO and hydrogen oxidizers) collectively comprise a phylogenetically diverse functional group that contributes significantly to carbon and nitrogen cycles in soils, and plays important roles in trace gas dynamics (e.g., carbon monoxide and nitrous and nitric oxides) that affect tropospheric chemistry and radiative forcing. In spite of their diverse physiologies, facultative and obligate lithotrophs typically possess the Calvin-Benson-Bassham cycle enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisCO). In an effort designed to understand the structure of lithotrophic communities in soil, genomic DNA extracts from surface (0-2 cm) and sub-surface (5-7 cm) soils have been obtained from two sites in a Georgia agroecosystem (peanut and cotton plots) and an unmanaged pine

stand (> 50 yr old). The extracts have been used in polymerase chain reaction (PCR) amplifications of the *cbbL* gene for the rubisCO large sub-unit protein. *cbbL* PCR products were cloned and sequenced, and subjected to phylogenetic and statistical analyses. Numerous novel lineages affiliated with the form IC clade (one of four form I rubisCO clades), which is typified by facultative lithotrophs, comprised lithotrophic communities from all soils. One of the form IC clone sequences clustered with a form IC clade of ammonia-oxidizing Nitrosospira. Distinct assemblages were obtained from each of the sites and from surface and sub-surface soils. The results suggest that lithotrophic populations respond differentially to plant type and land use, perhaps forming characteristic associations. The paucity of clone sequences attributed to ammonia-oxidizing bacteria indicates that even though ammonia oxidation occurs in the various soils, the relevant populations are small compared to those of facultative lithotrophs.

#### **Training and Development:**

A Ph.D. student developed skills in molecular microbial ecology as part of his dissertation research. Similarly, an REU student gained molecular experience and skills and used them to complete her Honors thesis. Her experience helped her obtain an NSF graduate fellowship in her pursuit of a Ph.D.

Two postdoctoral research associates and two technicians also acquired considerable molecular and field experience as part of the project.

#### **Outreach Activities:**

Outreach consisted of training for an REU and Honors undergraduate student.

#### **Journal Publications**

King, G.M. and M. Hungria, "Soil-atmosphere CO exchanges and microbial biogeochemistry of CO transformations in a Brazilian agroecosystem", *Appl. Environ. Microbiol.*, p. 4480, vol. 68, (2002). Published

King, G.M. and H. Crosby, "Impacts of plant roots on soil CO cycling and soil-atmosphere CO exchange", *Global Change Biol.*, p. 1085, vol. 8, (2002). Published

King, G.M., "Molecular and culture-based analyses of aerobic carbon monoxide oxidizer diversity", *Appl. Environ. Microbiol.*, p. 7257, vol. 69, (2003). Published

King, G.M., "Uptake of carbon monoxide and hydrogen at environmentally relevant concentrations by mycobacteria", *Appl. Environ. Microbiol.*, p. 7266, vol. 69, (2003). Published

Tolli, J. and G.M. King, "Lithotrophic community structures in agroecosystem and unmanaged pine forest soils based on analyses of the large subunit of form I ribulose-1,5-bisphosphate carboxylase/oxygenase", *Appl. Environ. Microbiol.*, p. , vol. 71, (2005). Accepted

#### **Books or Other One-time Publications**

#### **Web/Internet Site**

#### **Other Specific Products**

#### **Contributions**

**Contributions within Discipline:**

We have developed two new molecular approaches for CO-oxidizing microbes and for bacterial lithotrophs, and have applied these for the first time in the field.

We have identified or documented a number of new CO-oxidizing bacteria.

We have shown that mycobacteria can oxidize CO at environmentally-relevant concentrations.

We have shown that plant CO production affects soil-atmosphere CO exchanges.

**Contributions to Other Disciplines:**

We suspect that our work will begin to add new insights to the dynamics of symbiont-host associations for legumes, which feed about 1/3 of the world's human population.

**Contributions to Human Resource Development:**

We have supported two postdoctoral research associates, two technicians and two students, including an REU/Honors student.

**Contributions to Resources for Research and Education:****Contributions Beyond Science and Engineering:****Categories for which nothing is reported:**

Any Book

Any Web/Internet Site

Any Product

Contributions: To Any Resources for Research and Education

Contributions: To Any Beyond Science and Engineering