

# Impacts of plant roots on soil CO cycling and soil–atmosphere CO exchange

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## Abstract

Carbon monoxide (CO) plays a major role in tropospheric chemical dynamics. Accordingly, global CO budgets have been reasonably well documented. Atmospheric CO consumption by soils contributes significantly to these budgets, with the magnitude of the sink generally considered to reflect a balance between microbial uptake and abiological production. However, assays of live fine roots showed that diverse intact plants produced carbon monoxide at net rates ranging from 2 to 3000  $\mu\text{g g dw}^{-1} \text{d}^{-1}$ . CO production was greater for legumes than nonlegumes, and primarily associated with nodules. Excised roots from woody and herbaceous plants produced CO at comparable rates. CO production rates were similar for roots of intact plants and roots excised from those plants. The magnitude of net CO fluxes from roots was determined in part by the balance between simultaneous production and consumption. Surface sterilization of roots indicated that CO consumption was due, in part, rhizoplane CO-oxidizing bacteria, but maximum CO consumption rates were typically only a small fraction of net production rates. Assays in a Brazilian agroecosystem indicated that root CO production affects soil–atmosphere CO exchange. Estimates of global CO production rates indicated that roots contribute about 170–260 Tg CO to the soil atmosphere annually, an amount comparable to current estimates of atmospheric CO uptake by soils, and much larger than estimates of net abiological soil CO production.

*Keywords:* carbon monoxide, rhizosphere, biogeochemistry

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## Introduction

Soil–atmosphere carbon monoxide (CO) exchange contributes significantly to the global CO budget, with the magnitude and direction of exchange attributed to a balance between abiological production and microbiological consumption (e.g. Conrad, 1988). Abiological production rates have been considered relatively small (10–30 Tg CO year<sup>-1</sup>; Potter *et al.*, 1996). In contrast, microbial uptake of atmospheric CO has been reported to range from 110 to 630 Tg year<sup>-1</sup>, accounting for 7–22% of estimated global sources (e.g. Sanhueza *et al.*, 1998b; King, 1999). However, results presented here indicate that soil CO dynamics are much more complex than recognized to date, and include a large and previously unknown root production term as

well as interactions between plants and microbes in the rhizoplane and rhizosphere.

Biological CO production has been well documented, and CO is now known to play an important role in cell–cell signalling analogous to that of nitric oxide (NO; Ingi *et al.*, 1996; Zakhary *et al.*, 1997; Gelperin *et al.*, 2000; Xue *et al.*, 2000). The CO sources include heme (e.g. cytochrome) oxidation (e.g. Engel *et al.*, 1972; Migita *et al.*, 1998; Kyokane *et al.*, 2001), aromatic amino acid degradation (Hino & Tauchi, 1987), and various lipid peroxidation reactions (Wolff & Bidlack, 1976). In some instances, biological production can even result in accumulation of toxic CO concentrations (Reuss & Pratt, 2000). Though ubiquitous, biological CO sources have not been considered important on a global scale. Nonetheless, because of the large standing stock of live fine roots in vegetated terrestrial ecosystems (Jackson *et al.*, 1997), even a small production per unit mass can constitute a substantial belowground CO source that

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might affect soil-atmosphere CO exchange and the microbiology of soil-CO-oxidizing microbes.

In order to address the potential significance of root CO production, we have assayed a variety of intact temperate herbaceous plants collected from the field, washed free of soil and incubated in laboratory chambers. We have compared intact plant activities with those of roots excised from select plants. We have also determined CO production rates by excised fine roots of several tropical and temperate trees. In addition, we have assessed rates of rhizoplane CO consumption, and demonstrated the potential for roots to affect soil-atmosphere CO exchange in a Brazilian agroecosystem. Collectively, the results of these studies indicate that root CO production likely exceeds  $170 \text{ Tg year}^{-1}$  on a global basis, a value comparable to the best estimates for atmospheric CO consumption by soils.

## Materials and methods

### Root assays

Intact plants were excavated from field sites at or near the Darling Marine Center, Walpole, Maine, USA during June–September 2000. With the exception of plants sampled over the growing season, samples were obtained during flowering. Within 1 h after collection, soil was gently but thoroughly washed from roots using nonchlorinated tap water. After blotting, roots were sealed into  $330 \text{ cm}^3$  aluminium-foil-covered glass chambers fitted with neoprene rubber stoppers through which the stems protruded. Modelling clay formed a seal between the stems and stoppers. Stems and leaves were misted with deionized water and incubated with ambient laboratory lighting and temperatures. Triplicate root chambers for each plant species were subsampled at 3–4 min intervals for up to 120 min. for headspace CO and  $\text{H}_2$  analysis by gas chromatography using a either a Trace Analytical RGA3 or RGD2 fitted with a reduced gas detector (Trace Analytical Inc., MD, USA). Operating conditions and standardization have been described previously (Rich & King, 1998; King, 1999a, 2000; Hardy & King, 2001). After terminating the incubations, roots were separated from stems, dried at  $110^\circ\text{C}$  and weighed. However, for select plants CO production by excised roots was assayed as described above prior to drying, thus providing a comparison between intact plant and excised root activity. Net CO production rates for intact plant and excised root incubations were calculated from temporal changes in headspace CO according to Conrad & Seiler (1980), and corrected for a small chamber blank.

In addition, a survey of CO production was conducted using triplicate samples of various plant taxa collected from the Darling Marine Center and from field sites

adjacent to Hawaii Volcanoes National Park; the latter were collected during December 2001. For these assays, approximately 1 g fresh weight portions of freshly washed excised roots were incubated using procedures that were similar to those for intact plants, with the exception that the incubation chamber volume was  $110 \text{ cm}^3$ . CO production was measured similarly for nodules that were carefully excised from legume roots. Nodules were pooled from individual plants to yield fresh weights up to about 0.2 g, with incubations in triplicate. Dry weights for all samples were obtained as above.

CO consumption by washed excised roots was measured after the addition of pure CO to sealed chambers to yield final concentrations from 5 to 800 ppm. CO consumption was assayed using procedures similar to those for CO production. At low CO concentrations, uptake followed first order kinetics, from which first order rate constants were derived using a nonlinear curving-fitting algorithm (Kaleidagraph 3.5, Synergy Inc.). At high concentrations ( $> 400$  ppm), uptake was zero-order, and provided estimates of apparent maximum CO uptake rates. All consumption assays were conducted in triplicate.

The effects of root surface sterilization on CO consumption and production were assessed in triplicate using excised roots of *Trifolium arvense* that were incubated as before after immersion in a solution of 10 mM mercuric chloride for 5 min. followed by three rinses in sterile deionized water. Controls were immersed in deionized water only.

### Field assays

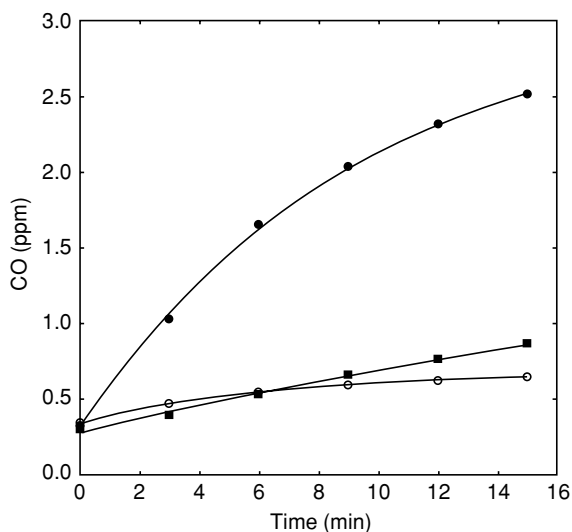
To determine the possible impact of root activity on soil-atmosphere CO fluxes, intact cores (7.5 cm inner diameter  $\times$  15 cm depth) were collected during February 2001 adjacent to stems of mature soybeans (*Glycine max*) growing in  $1 \times 2$  m plots on an experimental farm operated by EMBRAPA-Soja, Londrina, Brazil as described previously by Cattelan *et al.* (1997). The plots were fertilized with urea at levels of 0, 50, or 200 kg urea-N  $\text{ha}^{-1}$ . Triplicate cores from each treatment were returned to the laboratory within 1 h, sealed to create headspaces, and sampled for CO and  $\text{H}_2$  analysis following King, 1999a, 2000. Consumption is represented here as a positive term and emission as negative (i.e. a loss from the soil). Parallel assays of root CO production were measured as described above using triplicate plants from each treatment. Plants were carefully excavated to maximize root biomass and washed with nonchlorinated tap water to remove soil. Total root and nodule masses were estimated for individual plants after drying at  $110^\circ\text{C}$  for about 24 h. Similar assays were conducted using roots

from mature corn plants (*Zea mays* L.) and triplicate intact cores collected adjacent to corn stalks growing in plots on the EMBRAPA farm.

## Results

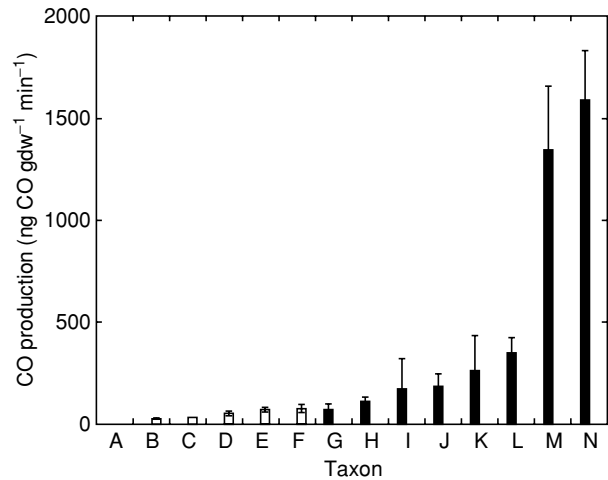
Time course assays revealed that CO accumulated in root chambers of all intact plants surveyed. Rates varied considerably among taxa, with low, often apparently linear production by nonlegumes, and rapid and nonlinear production by legumes (Fig. 1). In the latter case, CO concentrations approached a plateau of 1–5 ppm within 2 h. Net CO production rates determined from time course data ranged from about 2 to 110  $\mu\text{g gdw}^{-1} \text{d}^{-1}$ , and about 100 to 2300  $\mu\text{g gdw}^{-1} \text{d}^{-1}$  for nonlegume and legume species, respectively (Fig. 2). In spite of the variability among taxa, net CO production by legumes was significantly greater than for nonlegumes (ANOVA, log-transformed rates,  $P < 0.01$ ).

A similar trend was also observed for excised fine roots: (ranges were about 1–4  $\mu\text{g gdw}^{-1} \text{d}^{-1}$  and 10–740  $\mu\text{g gdw}^{-1} \text{d}^{-1}$  for nonlegumes and legumes, respectively). A comparison of CO production by roots of intact *Ranunculus acridis* plants and roots excised from the same plants indicated that root excision had little, if any, immediate effect. Activity was similar before and after excision (Fig. 3; mean production  $\pm 1$  standard error,  $39 \pm 1$  and  $33 \pm 4$   $\mu\text{g CO gdw}^{-1} \text{d}^{-1}$  for intact plant and excised roots, respectively;  $P = 0.55$ , two-tailed *t*-test). CO production was also comparable for excised roots of the legume, *Lotus corniculatus*, which were incubated

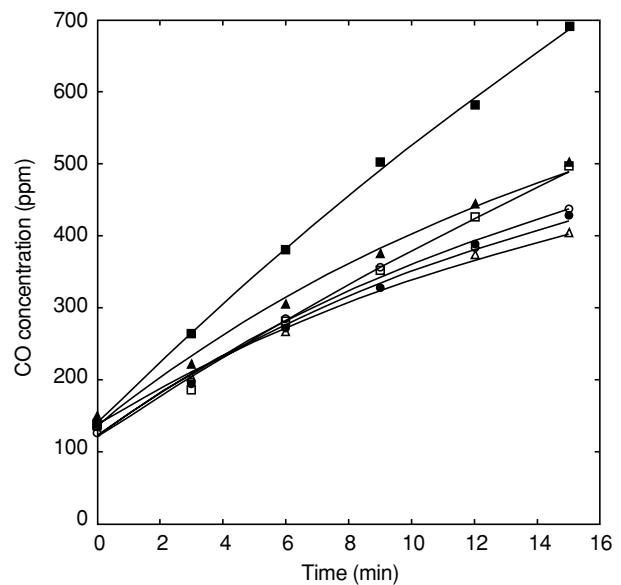


**Fig. 1** Representative time course of CO accumulation in root chambers with intact plants. Symbols represent individual vetch, *Vicia cracca*, and illustrate typical within taxa variability and nonlinear changes for legumes.

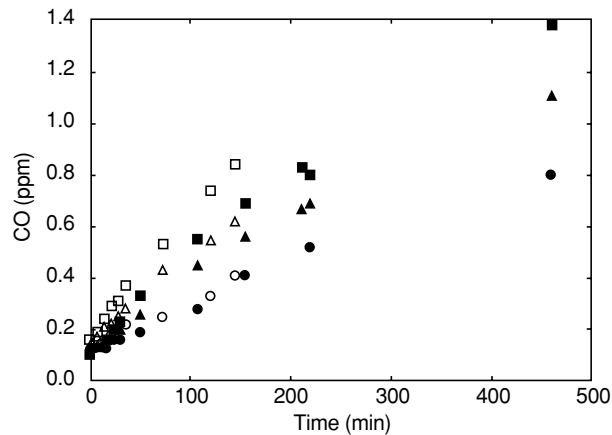
in sealed chambers for an initial period of 2 h, exposed to room air for 1 h and then re-incubated for an additional 2 h (Fig. 4). Net CO production by excised roots of *T. repens* and the nonlegume, *Solidago canadensis*, varied



**Fig. 2** Net root CO production rates for roots of intact plants; open bars for nonlegume and closed bars for legume species, respectively. All results are means of triplicate determinations  $\pm 1$  standard error. Taxon key: A, *Daucus carota*; B, *Ranunculus acridis*; C, *Hieracium pratense*; D, *Solidago canadensis*; E, unidentified Poaceae; F, *Chrysanthemum leucanthemum*; G, *Lotus corniculatus*; H, *Trifolium pratense*; I, *Coronilla varia*; J, *Lupinus perennis*; K, *Trifolium arvense*; L, *Trifolium agrarium*; M, *Vicia cracca*; N, *Trifolium repens*.



**Fig. 3** Time course of CO production by roots of intact *Ranunculus acridis* plants (open symbols) and excised roots from the same plants (closed symbols). Different symbols represent individual replicates.



**Fig. 4** Time course of CO production by excised roots of *Lotus corniculatus*. Open symbols represent results of incubation immediately after excision. Closed symbols represent results after a second incubation that was preceded by exposure to ambient CO levels for a period of 1 h. Different symbols represent individual replicates.

through the growing season with highest rates in mid- to late-June, and lowest rates as plants senesced in late September (Table 1). The change over time was most dramatic for *T. repens* (about 100-fold).

Net CO production was compared for sequential assays of excised roots with intact nodules from a variety of legumes and the nodules themselves after excision. Nodule activity (range 45–4700  $\mu\text{g gdw}^{-1} \text{d}^{-1}$ ) typically exceeded production for nodulated roots by a factor  $> 5$ , indicating that nodules were a major source of legume CO. CO production rates were also determined for excised fine roots of five woody species including deciduous, coniferous, temperate and tropical taxa (*Acacia koa*, *Metrosideros polymorpha*, *Myrica faya*, *Pinus strobus* and *Abies balsamea*). Activities varied considerably among taxa, but were comparable to rates for herbaceous species (Table 2).

CO consumption by excised plant roots was documented by two approaches. In one instance, roots of *T. arvense* were incubated until chamber CO concentrations approached a plateau; subsequently, CO was added to the chambers to elevate concentrations to 3–5 ppm. Time courses revealed that concentrations decreased over time and approached levels prior to CO addition (Fig. 5). Similar patterns were observed for *T. repens* (not shown).

In a second approach, roots of a variety of legumes and nonlegumes were incubated with elevated CO; time course assays revealed that CO was consumed in many cases, but not all, with considerable variation within and among taxa (Fig. 6; Table 3). No statistically significant trends were apparent for legumes and nonlegumes using

**Table 1** CO production by excised roots of a legume and non-legume assayed periodically through the growing seasons. Rates are means of triplicates  $\pm 1$  standard error (in parenthesis)

Plant	Sample date	Rate ( $\mu\text{g gdw}^{-1} \text{d}^{-1}$ )
<i>Trifolium repens</i>	26 June	57.1 (8.4)
	20 July	2.7 (0.3)
	27 September	0.5 (0.1)
<i>Solidago canadensis</i>	19 June	1.9 (0.4)
	27 July	1.1 (0.2)
	27 September	0.5 (0.2)

**Table 2** CO production rates for excised roots of tropical and temperate woody species. Rates are means of triplicates  $\pm 1$  standard error (in parenthesis)

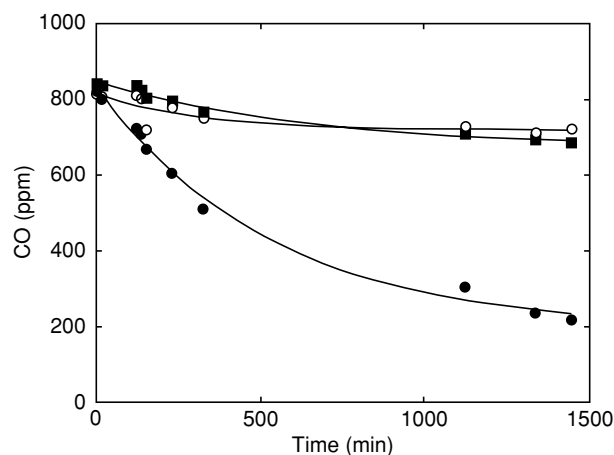
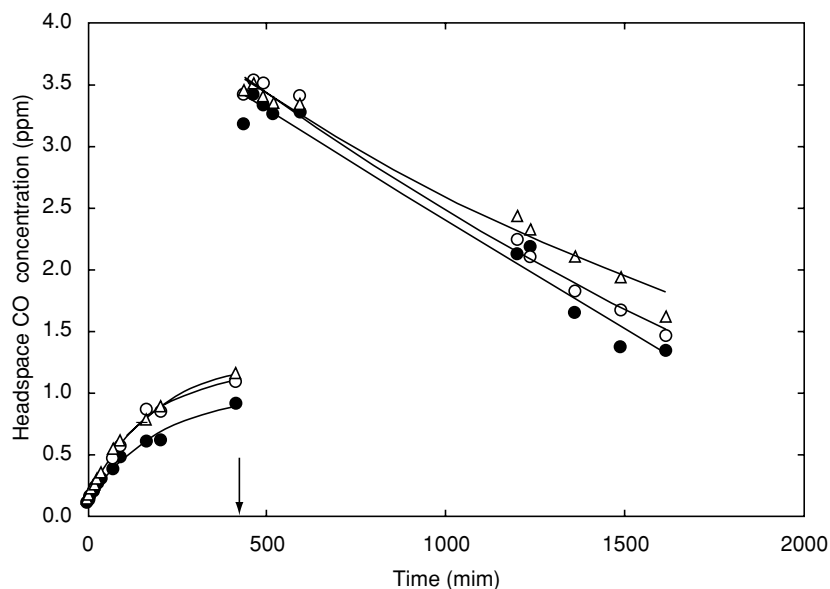
Taxon	Common name	Rate ( $\mu\text{g gdw}^{-1} \text{d}^{-1}$ )
<i>Myrica faya</i>	Fire tree	9.0 (1.2)
<i>Metrosideros polymorpha</i>	Ohia	3.5 (0.3)
<i>Acacia koa</i>	Koa	3.0 (1.2)
<i>Abies balsamea</i>	Balsam fir	58.7 (23.5)
<i>Pinus strobus</i>	White pine	9.8 (1.4)

a *t*-test ( $P = 0.27$ ), although values for legumes tended to be higher than those for nonlegumes.

Surface sterilization of *T. arvense* roots with mercuric chloride significantly increased rates of net CO production relative to nonsterilized roots (Fig. 7a; mean  $\pm 1$  standard error,  $29 \pm 8$  and  $3 \pm 1 \mu\text{g CO d}^{-1}$  for sterile and nonsterile roots, respectively;  $P = 0.02$ , one-tailed *t*-test). In addition, consumption of CO added at concentrations of about 200 ppm was strongly inhibited by mercuric chloride, while untreated roots rapidly consumed CO at these levels (Fig. 7b).

Analyses of net CO production by soybean roots indicated that rates varied as a function of nodule/root ratios and nitrogen fertilization treatment – rates tended to increase with increasing root nodulation and decrease with increasing nitrogen fertilization (Fig. 8a). In spite of the variability within treatments, the slope relating net root production and nodule/root ratios was statistically significant as determined using regression analysis ( $r^2 = 0.71$ ;  $P = 0.002$ ). In contrast, soil–atmosphere CO exchange for soybean plots was negatively correlated with root CO production (Fig. 8b;  $r = -0.97$ ,  $P < 0.001$ ); further, the highest root CO production was associated with CO emission to the atmosphere. Data from corn soils and roots were consistent with the trends from soybeans (Fig. 8b).

**Fig. 5** Time course of CO production and consumption by roots of intact *Trifolium arvense*. CO was added at the point indicated by an arrow. Different symbols represent individual replicates.



**Fig. 6** Representative time course of CO uptake by excised roots; data shown are for *Chrysanthemum leucanthemum* incubated with exogenous CO at initial concentrations of approximately 800 ppm. Different symbols represent individual replicates.

**Table 3** CO consumption rates for excised roots of various legumes and nonlegumes incubated with initial headspace concentrations of approximately 800 ppm. Data are means of triplicates  $\pm 1$  standard error (in parenthesis)

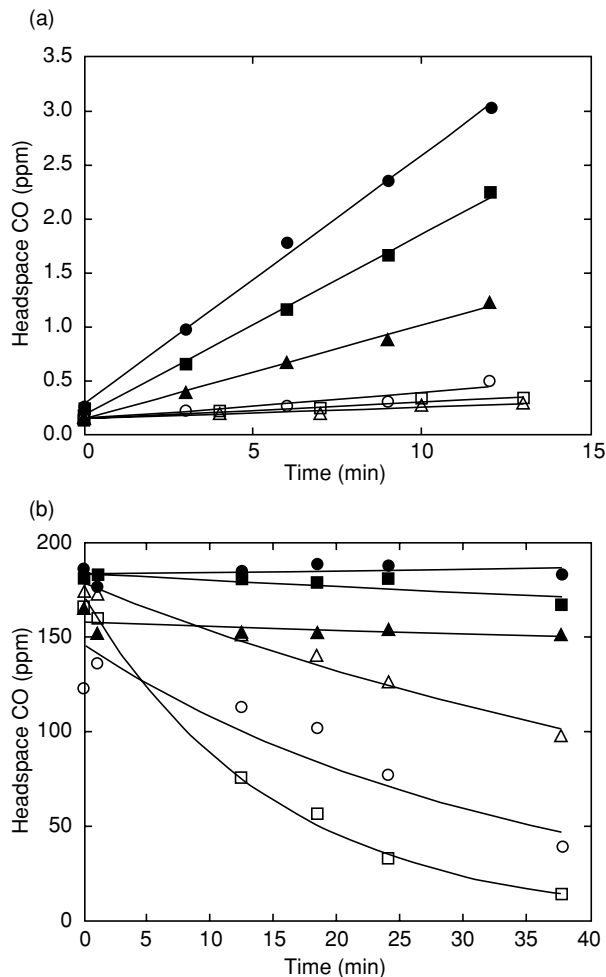
Taxon	Common name	Rate ( $\mu\text{g gdw}^{-1} \text{d}^{-1}$ )
<i>Trifolium repens</i>	White clover	3.3 (0.5)
<i>Trifolium pratense</i>	Red clover	1.4 (1.3)
<i>Trifolium agrarium</i>	Hop clover	0.4 (0.02)
<i>Coronilla varia</i>	Crown vetch	0.2 (0.1)
<i>Lotus corniculatus</i>	Birds-foot trefoil	0.1 (0.04)
<i>Lupinus perinus</i>	Common lupine	0.1 (0.01)
<i>Vicia cracca</i>	Common vetch	0.0 (0.0)
Unidentified Poaceae	Grass	0.4 (0.3)
<i>Hieracium pratense</i>	Hawkweed	0.4 (0.1)
<i>Daucus carota</i>	Wild carrot	0.2 (0.1)
<i>Chrysanthemum leucanthemum</i>	Daisy	0.0 (0.0)
<i>Solidago canadensis</i>	Goldenrod	0.0 (0.0)

## Discussion

CO production by the plant canopy has been recognized for some time, with global estimates of atmospheric flux on the order of 100–200 Tg year<sup>-1</sup> (Khalil, 1999). Canopy CO production primarily involves photochemical reactions that depend on light with wavelengths < 400 nm (e.g. Schade, 1997). CO is also derived from other plant sources. For example, certain macroalgae accumulate concentrations up to 10% in their pneumatocysts (float bladders); macroalgal CO production appears to occur by both enzymatic and photochemical reactions (see King, 2001 and references therein). In addition, dried plant

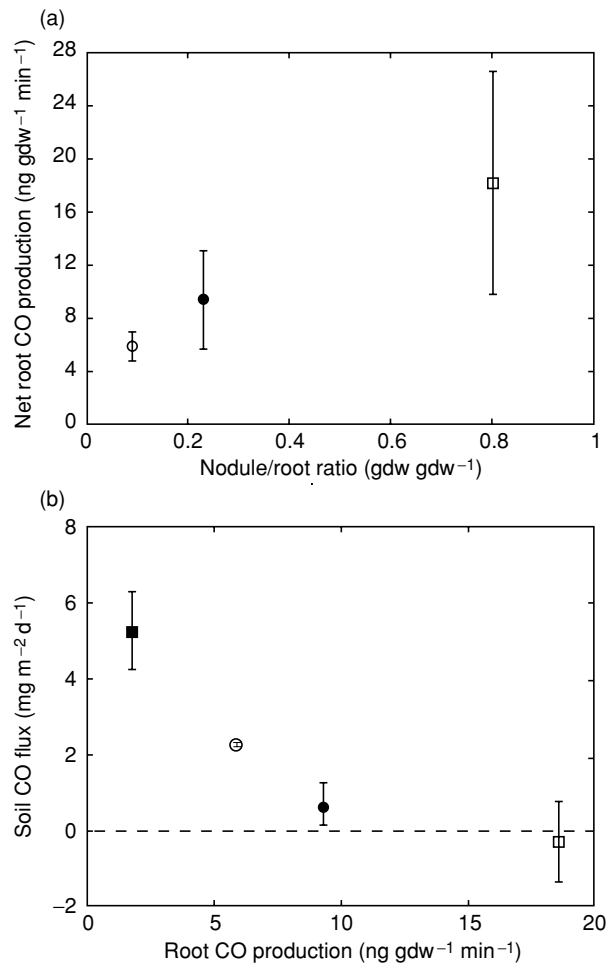
seeds have been reported to produce CO, with rates by canola that are comparable to those reported here for nonlegume roots (Reuss & Pratt, 2000). The mechanism for seed CO production is not clear, but presumably involves various enzymatic processes. However, neither seed nor macroalgal CO sources appear globally significant largely owing to their relatively small biomass.

Root CO production likely involves a suite of processes similar to those previously described for animals, fungi and bacteria, e.g. heme oxidation, lipid peroxidation, aromatic amino acid degradation (e.g. Simpson *et al.*, 1959; Engel *et al.*, 1972; Wolff & Bidlack, 1976; Hino & Tauchi,



**Fig. 7** (a) Time course of CO production by excised *Trifolium arvense* roots before (open symbols) and after mercuric-chloride-surface-sterilization (closed symbols). Different symbols represent individual replicates; (b) CO uptake by nonsterile (open symbols) and mercuric-chloride-surface-sterilized (closed symbols) excised *T. arvense* roots after CO addition to 150–200 ppm. Different symbols represent individual replicates.

1987; Migita *et al.*, 1998; Kyokane *et al.*, 2001). Variability in rates among plant taxa (e.g. Fig. 2, Table 2) presumably reflects species-specific variations in the relative significance of these processes as well as their absolute magnitude. For example, comparatively high CO production by legume nodules likely reflects high concentrations and turnover of heme as leghaemoglobin, and peroxidative turnover of the extensive peribacteroid membrane (Puppo *et al.*, 1991; Herrada *et al.*, 1993; Moreau *et al.*, 1996). In addition, oxygen radicals derived from ferri-leghaemoglobin may result in CO production from a variety of cellular organics (Moreau *et al.*, 1996; Mathieu *et al.*, 1998). At present, it is not clear which of these processes predominates CO production by nodules or



**Fig. 8** (a) Net CO production for soybean roots vs. nodule/root ratios (gdw gdw<sup>-1</sup>); □ = no fertilization, ● = +50 kg/ha urea-N, ○ = +200 kg/ha urea-N. All data are means of triplicates ± 1 standard error; (b) soil-atmosphere CO exchange vs. net root CO production, symbols as before, ■ = *Zea mays* L. plots. Data are means of triplicates ± 1 standard error.

nonlegume roots, and how these mechanisms might vary among taxa or over time.

Nonetheless, nodule CO production is noteworthy because leghaemoglobin plays a critical role in oxygen transport and the metabolism of nitrogen-fixing bacteroids (Appleby, 1984). Accordingly, leghaemoglobin contributes to the overall efficiency of nitrogen fixation. However, leghaemoglobin binds CO with a high affinity (Martin *et al.*, 1990), which implies that the dynamics of CO production and loss constrain in part leghaemoglobin availability for binding oxygen and supporting bacteroid metabolism. In addition, CO may affect nitrogen fixation by inhibiting bacteroid respiratory proteins (e.g. cytochrome oxidases). These previously unrecognized constraints could exacerbate known inefficiencies arising from hydrogen evolution by nitrogenase (Schubert &

Evans, 1976; Rasche & Arp, 1989) and direct inhibition of nitrogenase by CO (George *et al.*, 1997).

Irrespective of the mechanisms responsible for CO production and their cellular impacts, transfer of root-derived CO to the soil atmosphere is limited in part by rhizoplane or endophytic microbes, possibly including nitrogen-fixing symbionts (Lorite *et al.*, 2000), which consume CO. During incubation of either excised roots or intact plants in root chambers, CO accumulated nonlinearly with an initially rapid rate of increase followed by a slower change as headspace concentrations approached a plateau (e.g. Figs 1–5). A plateau could indicate either a change in root physiological status leading to decreased CO production, an exponential decrease in availability of a CO precursor, or a steady state with equal production and consumption rates.

However, CO was produced at similar rates by *R. acridis* roots before and after excision (Fig. 3), and by *L. corniculatus* roots during sequential extended incubations with an intervening period of exposure to ambient CO levels (Fig. 4). These results are inconsistent with a significant change in root physiological status or an exponential decrease in CO precursor availability. In addition, CO was consumed rapidly when added to root chambers in which headspace CO concentrations had stabilized, and headspace concentrations returned to levels observed prior to CO addition (Fig. 5). Moreover, when added at high concentrations (i.e. 800+ ppm) CO is readily consumed by many, though not by all roots (Fig. 6, Table 3). Collectively, these observations indicate that root CO production and consumption occur simultaneously as has been documented repeatedly for bulk soil (Conrad, 1996), and that the plateaus attained during root incubations reflect CO concentrations at which production and consumption rates are equivalent.

Several lines of evidence indicate that root-associated microbes, rather than plant cells *per se*, consume CO. First, surface sterilization inhibits CO consumption (Fig. 7b), which is accompanied by a stimulation of CO production (Fig. 7a). Second, bacterial enrichment cultures derived from legume and nonlegume roots readily consume CO (G.M. King, unpublished results), an observation that establishes CO-utilizing bacteria on (or in) roots. Third, *Bradyrhizobium japonicum*, an important soybean symbiont, and a *Bradyrhizobium* strain isolated from peanut nodules consume CO (Lorite *et al.*, 2000; G.M. King, unpublished results). The latter observations suggest that root CO production plays a role in the dynamics of root-microbe interactions, potentially affecting the abundance and diversity of nitrogen-fixing symbionts.

Although root-derived CO appears to be largely consumed within the soil matrix, a comparison of urea-fertilized and unfertilized soybean (*G. max*) treatments in a Brazilian field study indicates that roots can affect

the magnitude and direction of soil-atmosphere CO exchanges. Relative to unfertilized treatments, urea addition substantially reduces the mass of nodules present on roots and rates of CO production (Fig. 8a). Though rates within treatments vary, atmospheric CO uptake increased with increasing urea addition and varied inversely with root CO production rates (Fig. 8b). Relatively high soil-atmosphere CO fluxes for corn (*Zea mays* L.) and relatively low rates of corn root CO production further support the trend observed with soybeans (Fig. 8b). Since, previous studies have shown that nitrogen fertilization either has no effect (King, 1999a) or results in only a small stimulation of CO consumption (Bender & Conrad, 1994), changes in root CO production and not responses to nitrogen best account for the patterns observed among treatments.

The magnitude and significance of belowground root CO production can be assessed using rate data from intact herbaceous plants (Fig. 2) and estimates of global live fine root biomass ( $40.8 \times 10^9$  Mg), the most abundant and important live root fraction (Jackson *et al.*, 1997). Assuming that average net CO production rates observed for nonlegumes in this study ( $60 \pm 17 \mu\text{g gdw d}^{-1}$ ) are broadly representative, that nonlegumes account for 99% of all roots, and that observed activities extend for a 100 day growing period, global belowground CO production by nonlegumes amounts to about  $240 \pm 69$  Tg year<sup>-1</sup>. Using a median value for herbaceous nonlegumes ( $40 \mu\text{g gdw d}^{-1}$ ) reduces this estimate by one-third. However, data from excised roots of several woody species (Table 2) indicate that extrapolations based only on nonwoody plants may be conservative, and underestimate production in forested systems, which account for about 40% of the global live fine root mass.

The fraction of total live fine root biomass attributable to legumes is unknown, but legumes are widely distributed and are the most speciose plant family (Moulin *et al.*, 2001). Assuming that legumes account for 1% of total live fine root biomass, and that average net CO production rates observed here ( $510 \pm 210 \mu\text{g gdw d}^{-1}$ ) are broadly representative, global belowground legume CO production for a 100 day growing period amounts to about  $20 \pm 9$  Tg year<sup>-1</sup>. Using a median value from the legume data set ( $270 \mu\text{g gdw d}^{-1}$ ) reduces the estimate by about one-half. Collectively, these estimates suggest that total global belowground CO production likely ranges between about 170 and 260 Tg year<sup>-1</sup>.

Data obtained at intervals during a growing season (70–100 day) from two noncultivated plants in a Maine meadow (*T. repens* and *S. canadensis*; Table 1) and from four cultivated species in a Georgia agroecosystem (*Zea mays* L. and *Gossypium hirsutum*, nonlegumes; *G. max* and *Arachis hypogaea*, legumes; data not shown) indicate that

CO production decreases as plants mature and senesce. Since most of the CO production rates used in this study were obtained from flowering plants, the global estimates calculated above may need to be reduced to better reflect seasonal averages. On the other hand, use of a 100 day growing season for global extrapolations significantly underestimates the period of plant activity in tropical and subtropical systems (about 38% of global fine root mass), and likely underestimates the active period for many temperate systems as well.

Accordingly, plant roots represent a substantial and important source of belowground CO with a magnitude of about 170–260 Tg year<sup>-1</sup> that augments inputs from atmospheric deposition (best estimates are 200–300 Tg year<sup>-1</sup> globally; Khalil, 1999) and abiological production, which is relatively small. Because they are similar in magnitude, atmospheric deposition and root CO production may play similar roles in supporting the high-affinity CO oxidizers that have been proposed to account for atmospheric CO uptake (Hardy & King, 2001). However, roots also introduce CO at locally high concentrations to depths well below the soil surface where atmospheric CO is primarily consumed. Locally high CO concentrations may select for low-affinity CO oxidizers, and help to explain the routine isolation of organisms for which ecological and biogeochemical roles have thus far been enigmatic (Conrad *et al.*, 1981).

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