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REGULAR ARTICLE

Root-derived respiration and nitrous oxide production as affected by crop phenology and nitrogen fertilization

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Abstract In annual crops, the partitioning of photosynthates to support root growth, respiration and rhizodeposition should be greater during early development than in later reproductive stages due to source/sink relationships in the plant. Therefore, seasonal fluctuations in carbon dioxide (CO_2) and nitrous oxide (N_2O) production from roots and root-associated soil may be related to resource partitioning by the crop. Greenhouse studies used ^{13}C and ^{15}N stable isotopes to evaluate the carbon (C) partitioning and nitrogen (N) uptake by corn and soybean. We also measured the CO_2 and N_2O production from planted pots as affected by crop phenology and N fertilization. Specific root-derived respiration was related to the ^{13}C allocated to roots and was greatest during early vegetative growth. Root-derived respiration and rhizodeposition were greater for corn than soybean. The ^{15}N uptake by corn increased between vegetative growth, tasseling and milk stages, but the ^{15}N content in soybean was not affected by phenology. A peak in N_2O production was observed with corn at the milk stage, suggesting that the corn rhizosphere supported microbial communities that produced N_2O. Most of the ^{15}N-NO_3 applied to soybean was not taken up by the plant and negative N_2O production during vegetative growth and floral initiation stages suggests that soybean roots supported the reduction of N_2O to dinitrogen (N_2). We conclude that crop phenology and soil N availability exert important controls on rhizosphere processes, leading to temporal variation in CO_2 and N_2O production.

Keywords Corn · Soybean · Carbon dioxide · Nitrous oxide · Rhizosphere processes

Abbreviations

ANOVA	analysis of variance
DAS	days after seeding
HSD	honestly significantly different

Introduction

The transport of photosynthates to the plant rhizosphere supports root growth and respiration, while

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acting as a driving force for below-ground processes such as water transport, nutrient mobilization and soil organic matter decomposition (Killham and Yeomans 2001). These rhizosphere processes support heterotrophic respiration, which represents about 50% of the total annual CO_2 released from soils in temperate ecosystems (values range from 16% to more than 90%; Subke et al. 2006). The CO_2 emissions from agroecosystems are of particular interest, especially those systems producing grain and other products for human/animal consumption and bioenergy. Managing such agroecosystems to preserve or even increase the size of the soil organic C pool, thereby mitigating CO_2 emissions from agriculture, remains a compelling challenge (Lal 2008).

Temporal variation in soil CO_2 emissions from agroecosystems is often studied in relation to environmental conditions (temperature, moisture, soils) and agricultural management (e.g., tillage, organic amendments and crop rotations; Reicosky and Lindstrom 1993; Rochette and Gregorich 1998; Rochette et al. 2000; Drury et al. 2008). It is also important to consider the seasonality in CO_2 respired from the crop, especially the roots. Source/sink relationships affect the allocation of photosynthates to roots and root exudates during crop development, suggesting that crop phenology could partially explain the seasonal fluctuation in soil CO_2 emissions. Although photosynthate allocation in relation to crop phenology is not typically monitored in the field, estimates of the annual flux of photosynthates to the root system are available. In corn agroecosystems, the quantity of C transferred to root biomass and rhizodeposits in corn represents between 60% and 117% of the C fixed annually in aboveground biomass C, including grain (Buyanovsky and Wagner 1997; Rochette and Flanagan 1997; Wilts et al. 2004). The root-derived CO_2 flux, which includes the actual root respiration plus the CO_2 produced from soil microorganisms in the immediate vicinity of the roots (Gavrichkova and Kuzyakov 2008), may represent 30–80% of soil CO_2 emissions from natural ecosystems (Hanson et al. 2000) and up to 50% in corn fields (Rochette et al. 1999). This indicates that crop roots have a considerable influence on soil respiration and consequently CO_2 emissions, but the seasonal variation in these processes is not well known.

Nutrient availability is another factor that can affect root-derived respiration (Hütsch et al. 2002). Therefore, N fertilization and the soil mineral N concentration could influence root-derived respiration, as well as N_2O production. In the presence of non-leguminous plants like corn, denitrification is often negligible due to competition between plant roots and denitrifying bacteria for NO_3-N (Guenzi et al. 1978). Leguminous crops that fix N_2 from the atmosphere also release N-rich exudates and increase the soil mineral N pool, thereby providing additional substrate for N_2O production through nitrification and denitrification processes (Rochette et al. 2004). Differences in the composition of rhizodeposits and fertilizer N use efficiencies of non-legumes and legumes may therefore affect N_2O emissions from agroecosystems (Singh 2004). Currently, not much is known about how crop phenology affects N_2O production in soil with non-legumes and legumes, or whether this N_2O is further reduced to N_2 gas before it is emitted from soils.

The objective of this study was to determine how root-derived CO_2 and N_2O produced in soil under a non-legume (corn) and a legume (soybean) were affected by crop phenology and N fertilization. Stable isotope tracers (^{13}C, ^{15}N) were used in two greenhouse studies to i) quantify C partitioning and ii) evaluate N uptake in roots and shoots, in relation to specific root-derived respiration and N_2O production.

Materials and methods

Greenhouse experiment

Corn (*Zea mays* L. cv Cargill 2610) and soybean (*Glycine max* L. Merr. cv Cargill A0868TR) were grown in pots made from a rigid polyvinyl chloride (PVC) tube (9.75 cm internal diameter, 25 cm height), sealed at the bottom with a PVC cap to prevent nutrient loss via leaching. Pots contained field soil, a sandy loam, mixed Typic Endoaquent containing 700 g kg^{-1} of sand, 140 g kg^{-1} of silt and 160 g kg^{-1} of clay with 15.4 g organic C kg^{-1} and pH6.1. Soil was from the 0–10 cm layer of a cultivated, uncropped buffer between corn and soybean research plots, as described by Sey et al. (2008). About 300 kg of soil was collected, sieved through a 6 mm mesh to remove organic debris and rocks, homogenized and air-dried. Each pot contained 1,500 g of air-dry soil, moistened to about 25% gravimetric water content and packed in the pot at a bulk density of 1.15 g cm^{-3}, which left a headspace volume of about 562 cm^3.

Prior to seeding, we mixed 28 mg P kg^{-1} as Na_3PO_4 • 12 H_2O with the top 10 cm of soil in the pot, as a P fertilizer source. Five seeds of corn or soybean were added to each pot and thinned to a single plant per pot about one week after germination. Seed was not inoculated because the soil contained *Rhizobium* strains that induced nodulation of the soybean cultivar under field conditions. Pots were covered with lids that had an opening for the plant shoot, which was sealed during gas sampling, and a rubber septum for headspace gas sampling. Soil water content was maintained at about 60% water-filled pore space, equivalent to 32% gravimetric water content, throughout the experiment by weighing the pots every 1 to 2 days and adding distilled water when necessary. Since the mass of pots with growing plants was not constant, we estimated an increase in mass of 1% per week in corn pots and 0.5% per week in soybean pots based on the growth of these crops under optimal conditions (Fehr et al. 1971; Ritchie et al. 1986). All pots were kept in a greenhouse with natural lighting during the period November 2003 to January 2004 (plants receiving ^{15}N fertilizer) and August to October 2004 (plants with ^{13}C pulse labeling and no supplemental N fertilizer).

Experimental design

The experiments were conducted in two greenhouse trials: (1) corn and soybean received ^{13}C pulse labeling and no supplemental fertilizer (Study 1), and (2) corn and soybean received ^{15}N fertilizer (Study 2). The experimental design in Study 1 (^{13}C pulse labeling and no supplemental N fertilizer) was a completely randomized design with three plant treatments (corn, soybean and control soil without plants) and three sampling times (20 d, 60 d and 80 d after seeding (DAS)). There were five replicates for each treatment, for a total of 45 pots. Five replicates were selected randomly from each plant treatment for pulse-labelling at 19, 59 and 79 DAS. The ^{13}C pulse-labeling procedure was adapted from Bromand et al. (2001). Temperature in the Plexiglass chamber (120 cm long× 60 cm wide×104 cm height) was moderated by placing ice packs on the floor of the chamber, covered with cardboard. After sealing the chamber, ^{13}C-CO_2 was generated by reacting $NaH^{13}CO_3$ with 85% lactic acid and injected into the sealed chamber through a rubber septum. Uniform distribution of the $^{13}CO_2$ in the chamber was achieved through continuous air circulation with a 80 mm 4-pin sleeve bearing case fan (Antec Inc., Fremont, California, USA) powered by a 6 V battery. The first step in the pulse-labeling process involved priming the system with 30 mL of ^{13}C-CO_2 generated from $NaH^{13}CO_3$ (99% atom ^{13}C). Eight additional aliquots (35 mL each) of ^{13}C-CO_2 from $NaH^{13}CO_3$ (50% atom ^{13}C) were injected at 20 to 30-min intervals during a 4 h period, for a total input of 1,700 mg ^{13}C (calculated, assumes that all of the $NaH^{13}CO_3$ was converted to ^{13}C-CO_2). The CO_2 concentration in the chamber was monitored with a portable gas analyzer (LI-6400 CO_2 Gas Analyzer, Li-COR, Lincoln, Nebraska, USA). Typically, the CO_2 concentration in the chamber increased to between 380 ppm and 420 ppm immediately after injection of ^{13}C-CO_2 and quickly dropped below ambient levels (about 365 ppm CO_2) due to photosynthesis. When the CO_2 concentration declined to between 100 ppm and 200 ppm, another injection of ^{13}C-CO_2 was made. The portable gas analyzer was not able to detect the ^{13}C isotope, so we assumed that the decline in CO_2 concentration was proportion to $^{13}CO_2$ assimilation by plants. As soon as pots were removed from the labeling chamber, the opening in the pot lid was sealed with a layer of low melting point paraffin (m.p. 42°C) (Kuzyakov and Siniakina 2001). Headspace gas from the each pot was sampled immediately (t=0) and after 24 h. Then, plants were harvested and soil was removed from the pots for further analysis.

In Study 2 with supplemental N fertilizer, the experiment was a completely randomized design with three plant treatments (corn, soybean and control soil without plants) and three sampling times (20, 60 and 80 DAS). There were five replicates for each treatment, for a total of 45 pots. Five replicates from each plant treatment were selected randomly and received ^{15}N-labelled KNO_3 fertilizer (100 mg N pot^{-1} containing 10% atom ^{15}N) at 19, 59 and 79 DAS. Immediately after adding the fertilizer, the opening in the pot lid was sealed with a layer of low melting point paraffin (m.p. 42°C). The headspace gas from each pot was sampled immediately (t=0) and after 24 h. Then, plants were harvested and soil was removed from the pots for further analysis. In addition to pots receiving ^{15}N fertilizer described above, there were five additional pots for each plant treatment (corn, soybean and control soil without plants) that received no ^{15}N fertilizer and were destructively sampled at 80 DAS

to determine the background ^{15}N concentration in soil and plant tissues.

Gas sampling

Gas samples (20 mL) were taken with a gas-tight syringe and injected into 12-mL exetainers (Labco, Wycombe, UK) with an extra 60 mil teflon-silicone septa (National Scientific, Rockwood, TN, USA). Samples were analyzed with a gas chromatograph (Varian Model 3800, Walnut Creek, California, USA) equipped with automated valve injectors to simultaneously quantify CO_2, N_2O and O_2 concentrations in ppm v v^{-1} units (Rochette and Bertrand 2007). The CO_2 and N_2O concentrations in the headspace were calculated according to Holland et al. (1999) after converting gas concentrations from ppm (equivalent to $cm^3\ m^{-3}$) to a mass per volume concentration (d, g of C or N m^{-3}) with the ideal gas equation and the molecular mass (M, g mol^{-1}) and the C or N content (a, g mol^{-1}) of each gas (e.g., CO_2=12 g C mol^{-1} CO_2).

$$d = \frac{MaP}{RT} \quad (1)$$

where $P \approx$ atmospheric pressure (1 atm), R is the ideal gas constant (82.06 atm cm^3/mol K) and T is the average greenhouse temperature during the 24 h incubation period (303 K). Multiplying d by the headspace volume ($\approx 6.59\times10^{-4}\ m^3$) gave the mass ($C_1$, mg pot^{-1}) of CO_2-C or N_2O-N, while the gas production in the chamber headspace,f, (i.e., mg CO_2-C pot^{-1} h^{-1}) was estimated as:

$$f = {}^{(C_1 - C_0)}/_{t} \quad (2)$$

where C_0 is the gas concentration (mg pot^{-1}) when the pots were sealed and t is the incubation period (24 h).

Root-derived respiration and N_2O production

The CO_2 production in the planted pots was assumed to originate from roots (autotrophs) and soil microorganisms (heterotrophs) that mineralize plant-derived C and soil organic C to CO_2, while the CO_2 production in controls without plants was from soil microorganisms that decompose soil organic C compounds. Therefore, root-derived respiration (mg CO_2-C pot^{-1} h^{-1}) was calculated as the difference between CO_2 production in pots with plants and pots without plants (mean of 5 replicates) (Hanson et al. 2000; Gavrichkova and Kuzyakov 2008). Root-derived N_2O production (μg N_2O-N pot^{-1} h^{-1}) was the difference between N_2O emitted from pots with plants and pots without plants (mean of 5 replicates). The specific root-derived respiration (mg CO_2-C g^{-1} root h^{-1}), which is referred to as 'specific rhizosphere respiration' by Fu et al. (2002), was calculated by dividing the rhizosphere respiration by the mass of plant roots (g root pot^{-1}).

Plant and soil analyses

At harvest, the shoots (stems, leaves and other aboveground parts) were cut at the soil surface. The root-soil column was removed from the pot and most of the soil was removed by gently shaking the root mass. Fine roots were handpicked from the soil. Shoots were rinsed with distilled water and roots were washed with tapwater and distilled water to remove adhering soil particles, then dried in an oven (65°C for 48 h) and weighed. Soil was air-dried for about one week at 25°C and homogenized to obtain a representative subsample. Plant tissue and soil subsamples were then finely ground to pass through a 1 mm mesh screen and weighed into tin capsules. Total C and $\delta^{13}C$, and total N and $\delta^{15}N$ content in the samples were determined by combustion at 1,800°C with an elemental analyzer, EA 1110 (Carlo Erba Instruments, Milan, Italy) coupled with an isotope ratio mass spectrometer (DeltaPlus Advantage IRMS, Thermo Finnigan, Waltham, Massachusetts, USA) at the G.G. Hatch Isotope Laboratories (University of Ottawa, Ontario, Canada). Data was normalized using internal standards. Analytical precision was±0.02%.

The atom percent enrichment (APE) of ^{13}C or ^{15}N in plant and soil samples was the difference between the atom percent of stable isotopes measured in experimental materials and the atom percent of stable isotopes in unlabeled materials (background levels). The background levels were 1.098% ^{13}C for corn and 1.082% ^{13}C for soybean, based on whole-plant averages reported by Smith and Epstein (1971). Soil from the unplanted control of Study 1 had a background level of 1.089% ^{13}C (measured). We acknowledge that it would have been more accurate to measure ^{13}C in unlabeled plants from this study, but did not include the necessary controls in the experimental design. Pots that did not receive ^{15}N fertilizer

in Study 2 showed atom percent enrichment of 0.3686% ^{15}N in corn, 0.3666% ^{15}N in soybean and 0.3673% ^{15}N in soil. The mass enrichment m of ^{13}C and ^{15}N in plants and soil was calculated from the following equations:

$$m = M * \frac{\%C}{100} * \frac{APE^{13}C}{100} \quad (3)$$

$$m = M * \frac{\%N}{100} * \frac{APE^{15}N}{100} \quad (4)$$

where is the dry mass of the shoots, roots or soil from each pot, *%C* is the percent of total C and *%N* is the percent of total N in the plant tissue or soil sample.

Statistical analyses

Data were tested for normality (Shapiro Wilk test) and, if normal, tested for homogeneous variance (Levene test). Data were transformed or analyzed with an unequal variance ANOVA model, as appropriate (Littell et al. 2006). The effect of sampling time on the atom percent enrichment and masses of ^{13}C and ^{15}N in soil, shoots and roots, as well as CO_2 and N_2O production from pots and specific root-derived respiration, were evaluated separately for each crop using one-way ANOVA. The effect of sampling time, N fertilization and time x N fertilization interaction on rhizosphere respiration and rhizosphere N_2O production was evaluated for each crop by two-way ANOVA. Degrees of freedom for all ANOVA models were adjusted using the Kenward-Rogers methodology due to uneven replication, and analyzed with SAS statistical software (Version 9.1, SAS Institute Inc., Cary, NC) at α=0.05. Multiple mean comparisons were made with Tukey's Honestly Significantly Different (HSD) test. Spearman correlation coefficients describing the relationship between specific root-derived respiration and ^{13}C in plant roots were calculated using the PROC CORR function of SAS. Data were back-transformed to the original scale for presentation in tables and figures.

Results

Plants developed at a similar rate with and without supplemental N fertilizer in the greenhouse, so the sampling times corresponded to the same growth stage in both studies. Corn was at the early vegetative growth stage (V2) at 20 DAS, the tasseling stage (VT) on 60 DAS and the milk stage (R3) on 80 DAS, based on the growth classes described by Ritchie et al. (1986). Soybean growth stages for the same sampling times were the second node stage (V3), the flowering stage (R2) and the pod-filling (R6) stage (Fehr et al. 1971).

The ^{13}C stable isotope was used as a tracer to monitor the partitioning of recently photosynthesized ^{13}C-CO_2 in the soil-plant system. The ^{13}C content in soil, shoots and roots of corn and soybean was determined at the growth stages described above. In soil, the ^{13}C signal was above background levels in corn pots (APE ^{13}C values were positive), but not in soybean pots (Table 1). At 20 DAS, roots contained 46% of the total ^{13}C mass in corn and 44% of the total ^{13}C mass in soybeans (Table 1). This suggests that nearly half of the ^{13}C fixed through photosynthesis was allocated to roots during early vegetative growth in these plants. The mass of ^{13}C in corn roots declined after 20 DAS, while the mass of ^{13}C in corn shoots had increased significantly ($P<0.05$) by 80 DAS (Table 1). The mass of ^{13}C in soybean shoots and roots was greater at 20 DAS than 60 or 80 DAS (Table 1), which may indicate that the efficiency of ^{13}C fixation declined between vegetative and reproductive growth stages. Another possibility is that the high temperatures (up to 40°C) measured in the Plexiglass chamber during these labeling periods may have induced stomatal closure and thus reduced photosynthesis by soybean. In Table 1, we reported the CO_2 and N_2O production from the pot (roots, root-associated microorganisms and soil microorganisms) during a 24-h incubation period. There was no difference in the CO_2 production between sampling times, for either crop (Table 1). Both crops had greater N_2O production on 20 DAS than at later sampling times (Table 1).

Specific root-derived respiration was calculated with data from Study 1, which involved ^{13}C-labeling of plants. The specific root-derived respiration was greatest at 20 DAS and declined during the late vegetative and reproductive growth stages in corn and soybean (Fig. 1). Pooling the data from Study 1 for corn and soybean revealed that specific root-derived respiration was positively correlated (r=0.778, P<0.001, n=25) with the ^{13}C allocation to roots (^{13}C mass in roots/total

Table 1 Results from Study 1, an experiment with ^{13}C-CO_2 pulse-labeling of corn and soybean at vegetative and reproductive growth stages. Values are the atom percent enrichment (APE) and mass of ^{13}C in the soil, plant shoots and plant roots, sampled 24 h after pulse-labeling. Gas production from the planted pots and unplanted pots (in brackets) was evaluated during the same 24 h period. Mean values (n=5) without letters or with the same letter are not significantly different (P<0.05, Tukey's HSD test)

Crop	Days after seeding	Soil		Shoot		Root		CO_2 production (mg CO_2-C pot^{-1} h^{-1})	N_2O production (μg N_2O-N pot^{-1} h^{-1})
		APE ^{13}C (%)	Mass of ^{13}C (mg pot^{-1})	APE ^{13}C (%)	Mass of ^{13}C (mg pot^{-1})	APE ^{13}C (%)	Mass of ^{13}C		
Corn	20[a]	0.001	0.34	0.297	2.39^{B}	0.405^{A}	2.02^{A}	0.932 (0.278)[d]	0.574^{A} (0.239)
	60[b]	0.002	0.42	0.152	1.61^{B}	0.085^{B}	0.11^{B}	0.820 (0.025)	0.004^{B} (0.024)
	80[c]	0.002	0.47	0.143	9.93^{A}	0.090^{B}	0.32^{B}	0.923 (0.165)	0.005^{B} (0.088)
Soy	20[a]	-0.001	-0.17	0.325^{a}	2.17^{a}	0.270^{a}	1.69^{a}	0.581 (0.278)	0.642^{a} (0.239)
	60[b]	-0.001	-0.31	0.047^{b}	0.22^{b}	0.012^{b}	0.03^{b}	0.481 (0.025)	0.173^{b} (0.024)
	80[c]	-0.002	-0.39	0.008^{b}	0.03^{b}	0.003^{b}	0.01^{b}	0.274 (0165)	0.008^{b} (0.088)

[a] Early vegetative growth (V2 stage) in corn and second node (V3 stage) in soybean

[b] Tasseling (VT stage) in corn and flowering (R2 stage) in soybean

[c] Milk (R3 stage) in corn and pod-filling (R6 stage) in soybean

[d] Values in brackets are the CO_2 or N_2O production from unplanted pots, subject to the same experimental conditions

^{13}C mass in plants) as well as the ^{13}C mass in roots (r=0.685, P<0.001, n=25) (data not shown).

Study 2 involved monitoring CO_2 and N_2O production from corn and soybean plants that received ^{15}N fertilizer. The recovery of ^{15}N fertilizer was 53 to 96% in corn pots and 71 to 78% in soybean pots. The mass of ^{15}N increased significantly (P< 0.05) in corn shoots and roots, but not in soybean during the study (Table 2). Corn was efficient at assimilating nitrate, especially at 80 DAS when more than half of the ^{15}N-NO_3 was transferred into corn roots and shoots within 24 h of adding the fertilizer. Table 2 reports CO_2 and N_2O production from the roots, root-associated microorganisms and soil microorganisms after 24 h of ^{15}N fertilizer addition. The CO_2 production from corn and soybean pots was not affected by sampling times (Table 2). The N_2O production from corn pots was greater at 80 DAS than earlier sampling times, while the N_2O production from soybean pots declined between 20 DAS and later sampling times (Table 2).

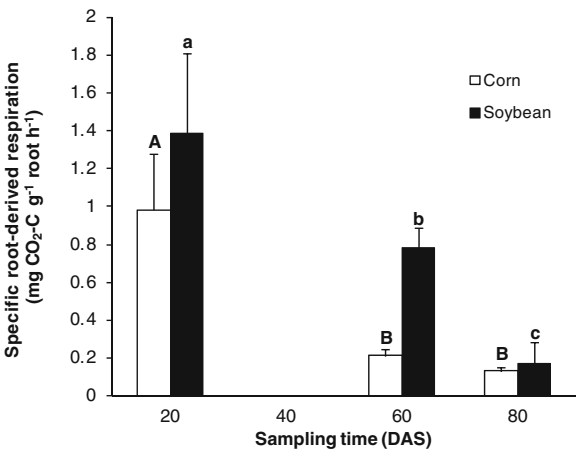

Fig. 1 Specific root-derived respiration of corn and soybean grown in pots in the greenhouse. Plant growth stages at 20 days, 60 days and 80 days after seeding (DAS) are described in the footnote to Table 1. Bars show the mean value (n=5) and standard error, while different letters on bars indicate statistical differences (P<0.05, Tukey's HSD test) between sampling times for corn (capital letters) and soybean (lowercase letters)

Although the greenhouse trials were not run concurrently, the crops exhibited similar phenological development, as evidenced by the development stage reached by each sampling date. The magnitude of CO_2 production was similar at most dates in Study 1 and Study 2, with a notable exception being CO_2 production from corn at 20 DAS (Tables 1 and 2). Based on the general similarities in crop phenology and CO_2 production, we assumed that rhizosphere processes were comparable in the two studies. Therefore, we pooled the data from these studies to evaluate the effect of sampling time and N fertilization on root-derived respiration and N_2O production, the difference between CO_2 and N_2O production in

Table 2 Results from Study 2, applying ^{15}N-fertilizer to corn and soybean at vegetative and reproductive growth stages. Values are the atom percent enrichment (APE) and mass of ^{15}N in the soil, plant shoots and plant roots, sampled 24 h after adding 100 mg $^{15}NO_3$-N (10% atom ^{15}N). Gas production from the planted pots and unplanted pots (in brackets) was evaluated during the same 24 h period. Mean values (n=5) without letters or with the same letter are not significantly different (P<0.05, Tukey's HSD test)

Crop	Days after seeding	Soil		Shoot		Root		CO_2 production (mg CO_2-C pot^{-1} h^{-1})	N_2O production (μg N_2O-N pot^{-1} h^{-1})
		APE ^{15}N (%)	Mass of ^{15}N (mg pot^{-1})	APE ^{15}N (%)	Mass of ^{15}N (mg pot^{-1})	APE ^{15}N (%)	Mass of ^{15}N (mg pot^{-1})		
Corn	20[a]	0.357	8.79^{A}	0.634^{B}	0.32^{B}	1.303	0.48^{B}	0.315 (0.084)[e]	0.968^{B} (0.332)
	60[b]	0.135	3.98^{B}	0.845^{B}	0.57^{B}	1.730	0.70^{B}	1.086 (0.091)	1.547^{B} (0.366)
	80[c]	0.147	3.29^{B}	1.782^{A}	3.70^{A}	2.410	1.60^{A}	0.839 (0.086)	6.479^{A} (0.015)
Soy	20[a]	NA[d]	NA[d]	0.330	0.20	0.749	0.09	0.319 (0.084)	0.607^{a} (0.332)
	60[b]	0.238	6.33	0.506	0.61	0.941	0.13	0.224 (0.091)	0.019^{b} (0.366)
	80[c]	0.262	6.87	0.384	0.80	0.888	0.10	0.246 (0.086)	0.032^{b} (0.015)

[a] Early vegetative growth (V2 stage) in corn and second node (V3 stage) in soybean

[b] Tasseling (VT stage) in corn and flowering (R2 stage) in soybean

[c] Milk (R3 stage) in corn and pod-filling (R6 stage) in soybean

[d] NA = not available

[e] Values in brackets are the CO_2 or N_2O production from unplanted pots, subject to the same experimental conditions

planted and unplanted soils. For corn, root-derived respiration ranged from 0.22 to 0.87 mg CO_2-C pot^{-1} h^{-1}, with the greatest values at 60 DAS (Fig. 2a). Root-derived respiration represented 70 to 96% of the total CO_2 production from corn pots (Tables 1 and 2). With soybean, root-derived respiration was greater in pots without supplemental N fertilizer (Fig. 2b). Root-derived respiration was 40 to 95% of the total CO_2 production from soybean pots without N fertilizer and between 62 and 76% of the total CO_2 production from soybean pots receiving ^{15}N fertilizer (Tables 1 and 2). Corn that received N fertilizer exhibited a peak of 5.8 μg N_2O-N pot^{-1} h^{-1} in root-derived N_2O production at 80 DAS, with lower values at other sampling times (Fig. 3a). The root-derived N_2O production from soybean was affected by the N fertilization x time interaction, with values ranging from -0.35 to 1.24 μg N_2O-N pot^{-1} h^{-1} during the study (Fig. 3b).

Discussion

It was not possible to measure the ^{13}C-CO_2 uptake by plants due to limitations of the Li-6400 monitoring equipment. Less than 10% of the calculated ^{13}C input during pulse labeling was retained in the plant-soil system, but the signal was sufficient to be detected above background levels and provided insight into the C allocation among shoots and roots at various growth stages. During vegetative growth, nearly half of the recently photosynthesized $^{13}CO_2$-C was translocated to corn and soybean roots. Corn assimilated considerable quantities of ^{13}C during tasselling and milk stages, but more than 90% of the $^{13}CO_2$-C photosynthesized by corn at these growth stages was retained in leaves and stems, not transported to the roots. Between 75 and 88% of the $^{13}CO_2$-C fixed by soybean during the flowering and pod-filling stages was retained in leaves and stems. These results are consistent with other annual legumes (*G. max, Pisum sativum* L.) and the annual grass *Bromus madritensis* (Pausch et al. 1996; Warembourg and Estelrich 2001; Voisin et al. 2003). During early vegetative growth, photosynthates are allocated preferentially to roots, presumably to increase anchorage and nutrient and water uptake. The retention of C in the above-ground biomass to support floral development and seed (grain) production during late vegetative and reproductive stages is typical in annual plants (Warembourg and Estelrich 2001).

We detected ^{13}C in soil of pots planted with corn, which suggests that some of the recently photosynthesized $^{13}CO_2$-C was lost through corn roots through root exudation within 24 h of the pulse-

labeling event. Our inability to detect ^{13}C in pots with soybean may be due to their less extensive rooting and smaller root biomass, compared to corn. If soybean roots were in contact with a smaller fraction of the soil mass than corn roots, the ^{13}C signal from soybean root exudates could have been masked by the ^{12}C signal of the large soil organic C pool. Another possibility is that soybean roots do not secrete as much ^{13}C into the soil as corn roots. An estimated 1,330 kg C ha^{-1} comes from the root biomass and rhizodeposits of soybean, less than half of the 3,800 kg C ha^{-1} input from corn roots and rhizodeposits (Johnson et al. 2006). The C-rich rhizodeposits from these crops can stimulate the growth and respiration of root-associated microorganisms and

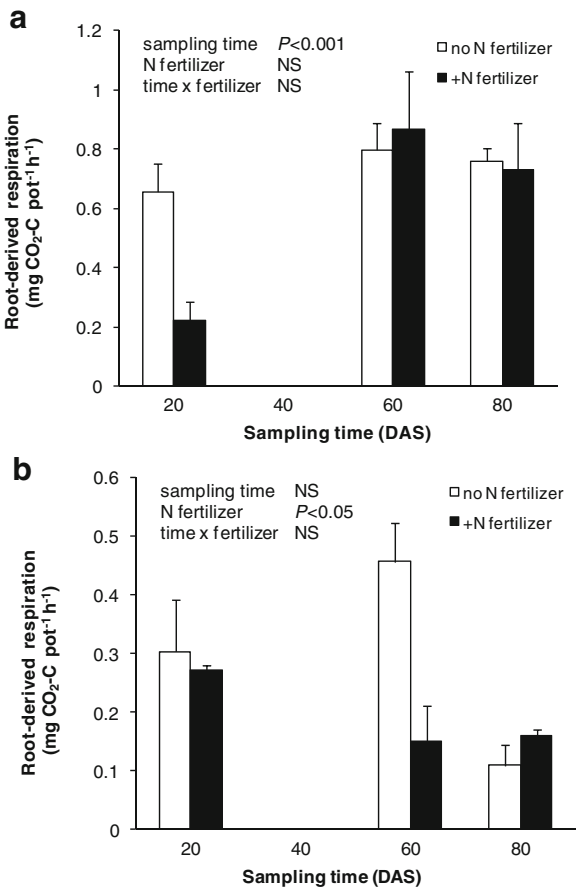

Fig. 2 Root-derived respiration of **a** corn and **b** soybean grown in pots in the greenhouse that received N fertilizer or no supplemental N fertilizer. Plant growth stages at 20, 60 and 80 days after seeding (DAS) are described in the footnote to Table 1. Bars are the mean value (n=5) and standard error. Significant results from the analysis of variance are shown

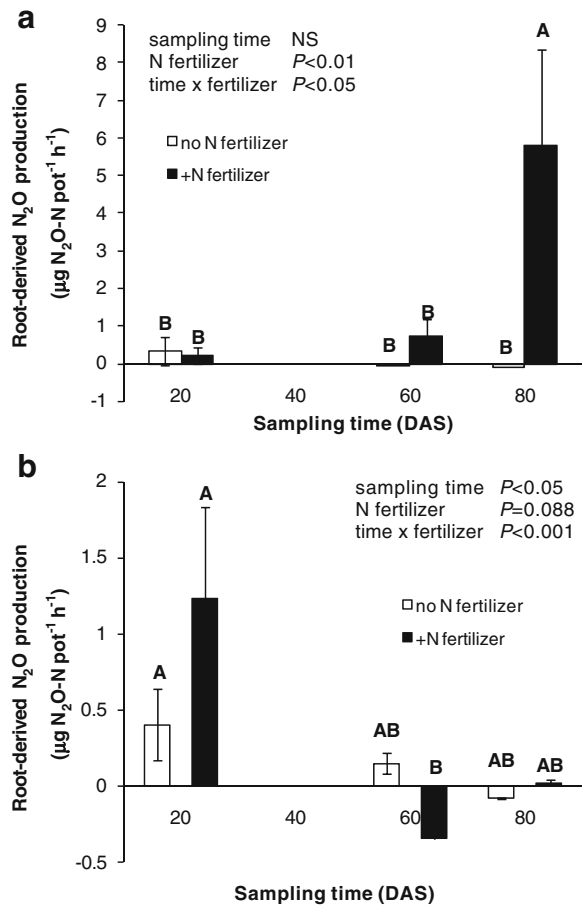

Fig. 3 Root-derived N_2O production from **a** corn and **b** soybean grown in pots in the greenhouse that received N fertilizer or no supplemental N fertilizer. Plant growth stages at 20 days, 60 days and 80 days after seeding (DAS) are described in the footnote to Table 1. Bars show the mean value (n=5) and standard error, while different letters on bars indicate statistical differences (P<0.05, Tukey's HSD test) due to the sampling time x N fertilizer interaction

their predators (protozoa, bacterial-feeding and fungal-feeding nematodes), thus contributing to CO_2 production. We did not attempt to quantify the ^{13}C in root exudates or follow their transformations by soil biota into stable soil organic C. Since annual crops transfer 20 to 50% of their photosynthesized C to roots and root exudates (Kuzyakov and Domanski 2000; Johnson et al. 2006), such research would be a logical next step.

The term "rhizosphere respiration" is often used to describe root respiration plus CO_2 produced from the rhizosphere priming effect that stimulates microbially-mediated soil organic C decomposition in the vicinity

of roots (Cheng 2008). Here, we have used the term "root-derived" respiration and N_2O production to account for the contribution of roots and soil microorganisms to gas production (Gavrichkova and Kuzyakov 2008). Root-derived respiration accounted for 70 to 96% of the CO_2 production from corn pots and between 40 and 95% of CO_2 production from soybean pots, but no clear pattern emerged in relation to crop phenology. Root-derived respiration from corn was estimated to be 30 to 80% of total soil respiration in greenhouse studies (Martens 1990; Kuzyakov and Cheng 2004; Werth and Kuzyakov 2005; Ding et al. 2007), although field-based studies with ^{13}C natural abundance show that root-derived respiration from corn increases during the growing season but does not exceed 40-50% of the total soil respiration (Rochette et al. 1999). Root-derived respiration of soybean grown in the greenhouse ranged from 53% to more than 90% of total soil respiration (Fu et al. 2002; Yang and Cai 2006). Variability in root-derived respiration reflects differences in growing conditions (nutrient cycling, water regimes, temperature) and possibly constrained root growth in greenhouse pot studies. Comparing the root-derived CO_2 efflux from planted soil with bare soil is also problematic because of the interaction between growing roots and soil microbial communities that stimulates soil organic C decomposition (Cheng 2008), however this method gives results that are similar to the ^{14}C pulse labeling method (Gavrichkova and Kuzyakov 2008).

Specific rhizosphere respiration is considered an unbiased indicator of root-derived respiration because it is normalized with root biomass (Fu et al. 2002). Our results demonstrated that specific root-derived respiration is greatest during early vegetative growth, corresponding to the period when C allocation to roots is high in both corn and soybean. A decrease in the root activity with plant maturity has been reported for many annual crops including wheat, sorghum, soybean, amaranthus and sunflower (Keith et al. 1986; Swinnen et al. 1994; Fu et al. 2002). Since plant root biomass is smaller during early vegetative growth, we might expect total CO_2 production (soil plus roots) to be low at this stage and increase as plants reach reproductive growth stages. Corn receiving N fertilizer followed this trend (Table 2), but we observed no difference in total CO_2 production during the study from pots with corn and no supplemental N fertilizer (Table 1), soybean plus N fertilizer (Table 2) and noted a decline in total CO_2 production between vegetative and reproductive growth stages in soybean without supplemental N fertilizer (Table 1). In these cases, there may be (1) more root-derived respiration or (2) greater stimulation of soil microbial respiration from rhizodeposition during vegetative than reproductive growth stages. Root-derived respiration tended to be higher at the tasselling or floral initiation stages in this study and in other reports (Yang and Cai 2006; Fu et al. 2002), so it seems likely that rhizodeposition may stimulate CO_2 production from root-associated soil during early vegetative growth, as indicated by Qian et al. (1997). However, Cheng et al. (2003) reported a strong rhizosphere priming effect on soil microbial communities growing in pots with wheat and soybeans during late vegetative growth and floral initiation stages. Further investigation is needed to understand how rhizodeposition by corn and soybean at different growth stages, with and without supplemental N fertilizer, may affect the CO_2 emissions from agricultural soils.

The recovery of ^{15}N fertilizer was 53 to 96% in pots with corn and soybean, and about 81% in the control soil (pots without plants). While the recovery of ^{15}N in corn pots at 60 DAS seemed particularly low, gaseous losses of 2 to 19% of ^{15}N-NO_3 from soil collected from corn agroecosystems occurred during a short incubation study without plants (Liu et al. 2007). The N uptake by corn was constant between the early vegetative growth stage and the tasseling stage but increased significantly between the tasseling and milk stages. This is similar to findings from Sadler and Karlen (1995), who reported sharp increases in the N uptake of corn during the onset of tasseling and milk stages (VT and R3 stages). Soybeans assimilated less than 10% of the ^{15}N fertilizer added, probably because they were well nodulated and did not require supplemental N. The negative feedback between N fertilization and N_2 fixation in nodules is well known (Lyons and Earley 1952), but a 24-h period may be insufficient to inhibit N_2 fixation and permit much uptake of ^{15}N-NO_3. While not significant, there was a slight increase in ^{15}N in soybean shoots during the study period, consistent with the expected increase in shoot N during soybean growth (Sadler and Karlen 1995).

The effect of N fertilization on N_2O production should be interpreted with caution, as the data came from a separate greenhouse study. The results suggest that nitrogen fertilization affected N_2O production by

the soil and root-associated microorganisms in corn and soybean pots. When no supplemental N fertilizer was applied, root-derived N_2O production was not affected by plant phenology in corn or soybean. When N fertilizer was added, there was an increase in root-derived N_2O production as corn plants reached the milk stage, consistent with the pattern of N_2O production from corn plants reported by Qian et al. (1997). Lower N_2O concentrations in the soybean rhizosphere suggest denitrification, in other words the conversion of N_2O to N_2 gas. Biological nitrogen fixation is thought to stimulate N_2O production (O'Hara and Daniel 1985), however some strains of *Rhizobium* are capable of reducing N_2O to N_2 (Hoch et al. 1960, Coyne and Focht 1987; Freney 1997). We also note that the apparent transformation of NO_3 from fertilizer to N_2 occurred within 24 h of N fertilizer addition. Under field conditions, Rochette et al. (2004) also found that negligible N_2O production occurred in alfalfa and soybean fields, despite the accumulation of mineral N (NH_4 plus NO_3) and high rainfall. This is also agrees with Rochette and Janzen (2005), who concluded that biological N fixation per se is not necessarily a significant source of soil N_2O from legume crops.

We conclude that specific root-derived respiration from corn and soybean was related to the greater C allocation to roots during vegetative rather than during reproductive growth stages. Corn had greater root-derived respiration and root exudation than soybean in this controlled study. The rhizosphere N_2O production was negligible in pots with soybean, but much greater with corn. The processes controlling short-term N fertilizer transformations were not examined in this study and deserve further attention. A better understanding of the seasonal fluctuations in CO_2 and N_2O emissions in agroecosystems can be achieved by considering the interactive effects of crop phenology and soil N availability on rhizosphere processes.

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