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CARBON AND NITROGEN LOSSES THROUGH ROOT EXUDATION BY *AGROPYRON CRISTATUM*, *A. SMITHII* AND *BOUTELOUA GRACILIS*

MARIO BIONDINI
Department of Animal and Range Sciences, North Dakota State University, Fargo, ND 58105, U.S.A.

DONALD A. KLEIN and EDWARD F. REDENTE[1]
Department of Microbiology and Environmental Health and [1]Department of Range Science, Colorado State University, Fort Collins, CO 80523, U.S.A.

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Summary—C and N released in root exudates throughout a growing season were estimated in *Bouteloua gracilis* and *Agropyron smithii* (dominant species in the shortgrass steppe ecosystem) and *A. cristatum* (dominant species in a 40-yr-old crested wheatgrass ecosystem). The release of C and N exudate was measured with or without the presence of a rhizosphere microflora. These values were used to estimate the potential contribution of root exudates to the N cycle of both ecosystems.

Total C released through root exudates by *A. cristatum*, *A. smithii* and *B. gracilis* was estimated at 8, 17 and 15%, respectively, of C fixed. The contributions of root exudates to the N cycle were estimated to be 11 g N m^{-2} in the shortgrass steppe ecosystem and 6 g N m^{-2} in the crested wheatgrass ecosystem. The presence of rhizosphere microorganisms resulted in a significant increase in root exudates of *A. cristatum* and *A. smithii*. *A. cristatum* and *A. smithii* plants grown under axenic conditions released 60 and 17%, respectively, of the C released via root exudates by inoculated plants. No differences between inoculated and non-inoculated plants were found in *B. gracilis*, which indicated that it may be inherently insensitive to the presence of a rhizosphere microflora. These data suggested that an introduced plant species may be markedly different from native species in the shortgrass steppe in terms of exudate releases. These differences may have a significant influence upon the N cycle dynamics of both ecosystems.

INTRODUCTION

The release of organic compounds by intact roots has been studied since the time of Hiltner (1904) under a variety of conditions and methodologies (Curl and Truelove, 1986). Most of the early studies were conducted under sterile hydroponic conditions and releases of C through root exudates were reported at less than 2% of net photosynthesis (Rovira and Davey, 1974). These experiments, however, may have been misleading because of biases caused by the growth medium used. Bowen (1980) listed a series of errors that might occur when extrapolating sterile hydroponic studies to field conditions. Studies conducted under more natural conditions (use of nonsterile soils) have shown releases through exudates that range from 7 to 25% of net photosynthesis (Barber and Martin, 1976; Haller and Stolp, 1985; Milchunas *et al.*, 1985).

The release of C and nutrients through root exudates and debris constitutes the main source of substrate for the rhizosphere microflora. Haystead and Marriott (1979), Nakas and Klein (1980), Sherwood and Klein (1981), Dahm (1984a, b) and Gaskins *et al.* (1985) have shown that the rhizosphere microflora is an active component of the ecosystem that could provide nutrients for primary producers as well as act as a buffer mechanism to allow plants to cope with environment stress (Coleman *et al.*, 1983). Furthermore, rhizosphere bacteria have been shown to have as much as four to five times the ability to effect rapid biochemical changes than nonrhizosphere bacteria (Rovira and McDougall, 1967; Richards, 1972). Recent studies have also shown critical relationships between root exudates, rhizosphere microflora, and micro- and mesofauna that have the potential to accelerate nutrient cycling and enhance the ability of plants to acquire nutrients under conditions of nutrient stress (Clarholm, 1985; Ingham *et al.*, 1985).

The last decade in ecology has shown an increased emphasis on the role that nutrient cycling may play in structural and functional differences among grassland ecosystems (Klein, 1977; Woodmansee *et al.*, 1978; McGill *et al.*, 1981; Molina *et al.*, 1983). A research project was initiated in 1984 to study that N cycling characteristics of two major grassland ecosystems in the northern Great Plains: the shortgrass steppe, which is dominated by *A. smithii* and *B. gracilis*, and large tracts of croplands that were seeded in the 1940s with *A. cristatum* and have remained as virtual monocultures for more than 40 yr (Vallentine, 1971). A component of the research project was to determine the role that C and N released through root exudation may have in the overall patterns of N cycling. Our specific objectives were: (1) to determine the amount of C and N released by *A. smithii*, *A. cristatum* and *B. gracilis* through root exudates during a simulated growing season under sterile (axenic) and nonsterile (inoculation with rhizosphere microflora) conditions; and (2) to estimate these releases at the ecosystem level.

MATERIAL AND METHODS

Plant culture

Seeds from *A. smithii*, *A. cristatum* and *B. gracilis* were collected at the U.S. Department of Agriculture High Plains Grassland Research Station (HPGRS) in Cheyenne, Wyoming and surface sterilized for 1 h with a 20% clorox solution. Each individual seed was then transferred to a 60-ml bottle that contained a sterile 1/10 strength nutrient agar medium, sealed and placed in a germinator. Plants were grown inside the bottles to heights of 10 cm, and those that became contaminated with microorganisms were discarded. Noncontaminated plants were then transferred to sterile 1-l. pyrex growth containers with a sealing lid fitted with three openings; one opening was used for sterile air input, a second to allow for sterile nutrient solution input, and the third one for the plant (Fig. 1).

The plant growth medium used was fritted clay, an inert material lacking essential plant nutrients, which has excellent water-holding characteristics for plant growth and root development and closely simulates actual field conditions (van Bavel *et al.*, 1978). The fritted clay was autoclaved and leached with distilled water four times before use. The fritted clay so treated had 0.006% organic matter, 0.01 $\mu g\, g^{-1}$ of NH_4^+ plus NO_3^-, 0.02 $\mu g\, g^{-1}$ of PO_4^{-4}-P and a pH of 6.2. The chambers were filled with fritted clay and covered with a 2-cm layer of sand to help plant establishment. A total of 400 ml of a 1/4 strength Hoagland's solution, that contained a Sorensens phosphate buffer to maintain a pH of 7 was then added and the system was autoclaved three times before use. The system was buffered at a pH of 7 because soil pH in the HPGRS site ranges from 6.8 to 7.3 (top 10cm).

Each of the three plant species were grown under both sterile and nonsterile conditions. Plants for the sterile treatment were transplanted under a sterile hood from the 60-ml bottles to the sterile growth containers through an opening in the lid and sealed to the lid using a cold-curing silicon rubber (Dow Corning silastic HSRTV silicone rubber) (Fig. 1) as recommended by Barber and Martin (1976). A similar procedure was followed for the nonsterile treatments with the addition in this case of 10 ml of a mixture of rhizosphere microorganisms derived from field samples from the HPGRS. The inoculum was isolated and prepared according to the procedures described by Nakas and Klein (1980). The microbial population characteristics from the soils used to prepare the inoculum are outlined in Table 1.

Table 1. Some characteristics of the microbial populations used to prepare the rhizosphere inoculum. CW = populations derived from the crested wheatgrass ecosystem and used with *A. cristatum*. NSC = population derived from the native shortgrass ecosystem and used with *A. smithii* and *B. gracilis*

	CW	NSG
Rhizosphere fungal hyphal length (m g^{-1} soil)	28.3	22.5
Bacteria (log bact.)	9.2	9.3
Bacteria (% active)	14.4	12.7
Physiological diversity [E(s) 90 isolates $S = 0.80$]	19	14
Microbial biomass C ($\mu g\, g^{-1}$ soil)	340	510
Microbial biomass N ($\mu g\, g^{-1}$ soil)	60	80

The experiment was conducted for 90 days in a growth chamber with growth containers arranged in a completely randomized design with four replications per treatment plus four blanks (no plants). The growth chamber was programmed to simulate light and temperatures that prevail during the summer months (max 28°C, min 12°C, day length 18 h) under field conditions in Cheyenne, Wyoming. Filtered air was flushed two times a week for 15 min through the system during the growth period to remove CO_2 which in turn was captured in an alkali trap (60 ml 2N NaOH). Sterility checks (plate counts) were conducted during the first 3 wk of the experiment, and contaminated growth containers of the sterile treatments were discarded and replaced with

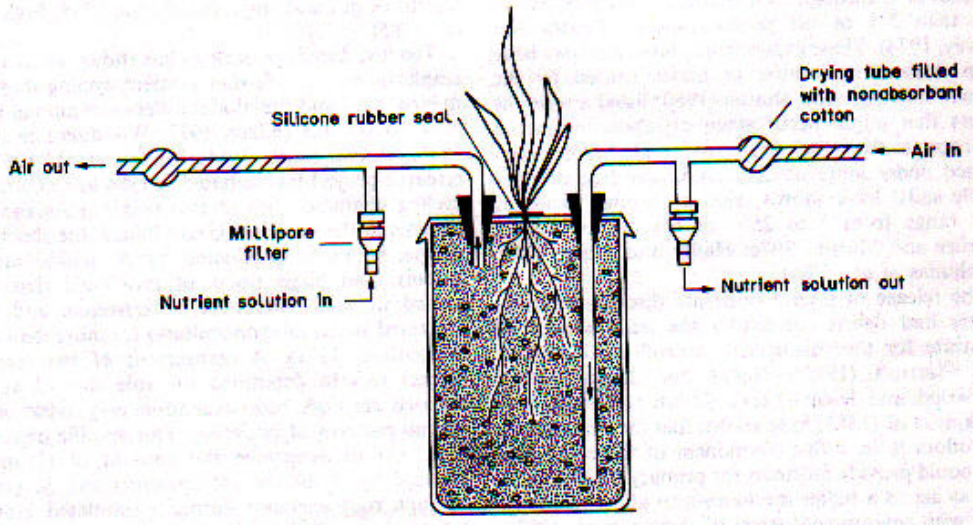

Fig. 1. Apparatus for the growth of plants in sterile and nonsterile fritted clay. Flow directions of air and nutrient solutions are shown.

new ones. Replacement plants were also grown for 90 days.

Analytical procedures

The CO_2 that accumulated in the alkali traps was determined by titration with the $BaCl_2$ method (Pramer and Schmidt, 1964). The aboveground parts of the plants were clipped at the lid level at the end of the 90-days and weighed. The growth containers were then opened and the roots were washed free of fritted clay with 400 ml distilled water, dried and weighed. The same water was then used to wash the fritted clay four separate times to remove the soluble fraction of root exudates. The soluble samples were filtered to remove particulates and the resulting volume was measured. The filtered exudates were dried by rotary evaporation at 40°C, redissolved in 30 ml deionized water with 30 μl chloroform added to prevent microbial growth. The fritted clay was then treated with 400 ml 6N HCl for 24 h to extract the insoluble root exudate fraction (Bokhari *et al.*, 1979).

Following fritted clay removal, and before drying, subsamples of roots were treated with fluorescein diacetate (FDA) to determine if there had been significant accumulation of dead root material (Heslop-Harrison and Heslop-Harrison, 1970). FDA is accumulated in living cells where it is hydrolyzed by esterases to yield fluorescein. Fluorescein is retained by living cells which fluoresce under blue–violet illumination.

Total nonvolatile organic C in the water-extracted (soluble) root exudates was determined by a modified dichromate–sulfuric acid reduction (Johnson, 1949) using titration with 0.05 N thiosulfate. Total organic C in the acid extracts (nonsoluble) were determined by colorimetry using anthrone (Morris, 1948). In both cases, glucose was used as the standard and as a consequence analytical results were obtained as glucose equivalent. The data was then transformed to mg C using the amount of C in a glucose molecule. Mycorrhizal infection in the roots of the nonsterile plants was measured in subsamples of roots stained to enhance mycorrhizal structures (Phillips and Hayman, 1970) and the extent of the infection determined by the grid-intersect method (Giovannetti and Mosse, 1980). These data were used to compare the amounts of mycorrhizal infection in the growth chamber experiment with those found under field conditions by Klein *et al.* (1987). Total N in the soluble and nonsoluble fractions was estimated using a micro-Kjeldahl method (AOAC, 1984).

Calculations

Total C exudate for the sterile plants was calculated as the sum of the soluble and nonsoluble components and expressed as mg C g^{-1} root dried wt for the 90-day growth period. The difference in the C that was released as respiration (expressed in mg C g^{-1} root dry wt) between sterile and nonsterile plants was assumed to be of microbial origin. This assumption was used by Haller and Stolp (1985) to partition aerobic respiration between roots and rhizosphere bacteria in maize plants. The C source for microorganisms in the growth containers was almost entirely from root exudates because the growth medium (fritted clay) has very little background C (C values from blanks were subtracted in our calculations) and only small amounts of dead roots had acccumulated during the short growth period. Results from the FDA method indicated an average accumulation of dead root material of 2.6% (nonsterile *A. cristatum* had the highest with 3.1% and *A. smithii* the lowest at 1.5%). The sum of microbial respiration and extracted soluble and nonsoluble exudates was used as the total root exudates produced by nonsterile plants.

Estimation of C and N releases through root exudates at the ecosystem level

Net primary productivity (NPP) was estimated in 1985 for both the native shortgrass steppe and the introduced crested wheatgrass ecosystem at the HPGRS. Live and dead above- and belowground biomass in both ecosystems was estimated on four sampling occasions between May and October. Within each ecosystem 20 randomly located rectangular (0.5 × 1 m) quadrats were used to estimate aboveground biomass. The major components of the aboveground compartment sampled were live material, standing dead and litter. Root biomass was estimated using methods outlined by Bohm (1979). Twenty soil cores (5.2 cm dia, 1 m depth) were used for each ecosystem in each sampling occasion. The cores were taken within each of the quadrats used for aboveground biomass and subdivided upon root concentration. Hand sorting and the FDA method (Heslop-Harrison and Heslop-Harrison, 1970) were used to estimate live and dead root material. Above- and belowground NPP were estimated (Sala *et al.*, 1981).

Total root exudate C and N from the nonsterile plants in the growth chamber experiment were expressed as a percentage of NPP. These values were then multiplied by the field estimated belowground NPP of each species to calculate total release of C and N at the ecosystem level.

RESULTS

Total C and N released through root exudates by sterile (axenic) plants throughout a simulated growing season were significantly higher ($P < 0.05$) for *B. gracilis* compared with *A. smithii* and *A. cristatum* (Table 2). No significant differences in total exuded C and N were found between the two *Agropyron* species. A similar pattern was observed in root respiration (Table 2).

Total C and N released through root exudates by nonsterile *B. gracilis* and *A. smithii* plants during the simulated growing season were significantly higher ($P < 0.05$) than for the nonsterile plants of *A. cristatum* (Table 2). A similar pattern was also observed for microbial respiration.

The presence of rhizosphere microbial populations significantly increased ($P < 0.05$) releases of C through root exudation from both *A. smithii* and *A. cristatum*. The amount of C released as root exudates under sterile conditions was 17% of that under nonsterile conditions for *A. smithii* and 60% for *A. cristatum* (Table 2). No significant differences were found, however, in *B. gracilis*, where the amount of C g^{-1} root dry wt exuded for the 90-day simulated

Table 2. Summary of C and N released through root exudates, plant respiration, microbial respiration and mycorrhizal infection for the sterile and nonsterile (inoculated with rhizosphere microorganisms) treatments. The data represent the total for a 90-day simulated growing season

Species	Total root exudates (90 days) C (mg C g^{-1})[1] Sterile	Nonsterile	Organic N (mg N g^{-1}) Sterile	Nonsterile	Respiration (mg C g^{-1} 90 days) Plant	Microbial	Mycorrhiza infection at 90 days (%)
A. cristatum	39a[2]x	65ay	3ax	5ay	137a	58a	9.5a
A. smithii	21ax	120by	2ax	8by	82a	108b	9.8a
B. gracilis	120bx	123bx	9bx	10bx	209b	105b	11.0a

[1] g^{-1} dry root wt.

[2] Means in a column followed by a different letter at the beginning of the alphabet (a,b) are significantly different at $P < 0.05$. Two means for sterile and nonsterile conditions followed by different letters at the end of the alphabet (x, y) are significantly different at $P < 0.05$.

Table 3. Estimates of net primary production (NPP) and C and N released as root exudates at the ecosystem level

Plant community	Aboveground NPP (g m^{-2})	Belowground NPP (g m^{-2})	Total NPP (g m^{-2})	C released through root exudates (g C m^{-2})	N released through root exudates (g N m^{-2})
Introduced crested wheatgrass	164a[1]	1115	1279	72a	6a
Native shortgrass	114b	1209	1323	147b	11b

[1] Means with different letters in the same column are significantly different at $P < 0.05$.

growing season amounted to 120 mg C g^{-1} root dry wt under sterile conditions and 123 mg C g^{-1} root dry wt under nonsterile conditions (Table 2). A similar pattern was observed for the three species in relation to total N losses through exudation under sterile and nonsterile conditions (Table 2). The level of mycorrhizal infection was similar for the three species grown under nonsterile conditions averaging 10.1% (Table 2). This value is slightly lower than the 11.8% for a similar crested wheatgrass site and 12.7% for a similar native shortgrass site reported by Klein *et al.* (1987).

Aboveground NPP was significantly higher ($P < 0.05$) in the introduced crested wheatgrass ecosystem than in the native shortgrass ecosystem (Table 3). No significant differences were found in below ground NPP between both ecosystems (Table 3). The calculated potential release of C and N through the root exudate pathway was significantly higher ($P < 0.05$) in the native shortgrass ecosystem than in the introduced crested wheatgrass ecosystem (Table 3).

DISCUSSION

The total C released through root exudates by nonsterile *A. cristatum*, *A. smithii* and *B. gracilis* was estimated as 8, 17 and 15% of net C fixation, respectively. These results are consistent with values of 12% reported by Haller and Stolp (1985) in maize (*Zea maize*) and 22% for wheat (*Triticum aestivum*) reported by Milchunas *et al.* (1985). For *B. gracilis*, Milchunas *et al.* (1985) reported C exudation to be 6% of net C fixation. Our results suggested that larger amounts of C and N are released by intact roots into the surrounding medium than was previously reported. When our results were extrapolated to the ecosystem level, 6 g N m^{-2} in the crested wheatgrass ecosystem and 11 g N m^{-2} in the native shortgrass ecosystem were contributed by root exudates to the decomposable substrate.

Coleman *et al.* (1983) have postulated two major pathways for substrate decomposition: the "fast" and the "slow" pathways. The "fast" pathway is concerned with the utilization by rhizosphere microorganisms and fauna of low mol weight C and low C-to-N ratio (an average of 13 in our study) sources released primarily by root exudates (including sloughing of cells and decay of root hairs) such as amino acids and various mono- and oligosaccharides. These exudates are important for the maintenance of the rhizosphere microflora and allow subsequent grazing of the microbes by protozoa and nematodes (Anderson *et al.*, 1981). Ingham *et al.* (1985) and Clarholm (1985) have clearly shown that the grazing activity of nematodes and amoebae increase bacteria turnover in the rhizosphere making more N available for plant growth. Data from Ingham *et al.* (1985) also indicate that more than 70% of the total microbial and grazing fauna biomass is concentrated in the rhizosphere zone. As a result, the amount and rates of root exudates, which directly affect the "fast" decomposition pathway, could be an important factor in the overall N cycle processes of grassland ecosystems (Coleman *et al.*, 1983).

The "slow" pathway for decomposition is concerned with the utilization of less labile organic matter, its major source being dead plant material. The mineralization rate will be heavily dependent on the C-to-N and the lignin-to-N ratios (Hunt, 1977; Melillo *et al.*, 1982). High C-to-N and lignin-to-N ratios would cause lower N mineralization and greater N immobilization rates (by microflora) with less mineral N available for plant growth.

Our data indicate that the introduced *A. cristatum* releases lower amounts of root exudates than the dominant grass species in the shortgrass steppe ecosystem. Klein *et al.* (1987) have shown that aboveground substrate quality in the crested wheatgrass ecosystem is significantly lower than in the native shortgrass ecosystem (lignin-to-N ratio of 10.6 vs 7). Similarly Klein *et al.* (1987) have presented some data that show lower N and glucose mineralization rates in the crested wheatgrass ecosystem when compared

to the native shortgrass ecosystem. We believe that these overall patterns of differences may significantly affect the size of the "fast" vs "slow" decomposition pathway and thus the N cycle dynamics of both ecosystems.

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