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Rhizosphere microorganism effects on soluble amino acids, sugars and organic acids in the root zone of *Agropyron cristatum*, *A. smithii* and *Bouteloua gracilis*

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Abstract

Three axenic and rhizosphere microorganism-inoculated shortgrass steppe plant species were evaluated for possible differences in residual organic carbon and nitrogen present as sugars, organic acids and amino acids. Introduced *Agropyron cristatum* was compared to *A. smithii* and *Bouteloua gracilis*, which are dominant species in the native shortgrass steppe. These plants, grown for 90 days in root growth chambers, showed differences in residual organic carbon and nitrogen per gram of root, and rhizosphere microbe presence resulted in additional changes in these compounds. The root biomass of *B. gracilis* was significantly increased with microbes present. The *Agropyron* species had significantly lower amino acid levels with microbes present, while under the same conditions, the *B. gracilis* showed significant decreases in residual sugars. Based on the amino acids, sugars and organic acids, the C/N ratio of the sterile *A. cristatum* was higher than for *B. gracilis*. Rhizosphere microbe presence did not result in changes in these C/N ratios. These results suggest that *A. cristatum*, with microbes present, will have lower levels of amino acids present, while *B. gracilis*, with a lower C/N ratio, will have sugars used to a greater extent by the rhizosphere microbes. This resulted in the higher levels of residual soluble organic C and N in the rhizosphere of *B. gracilis*, in comparison with the introduced *A. cristatum*. These differences may be critical in influencing the course of nutrient accumulation and plant competition in short-grass steppe communities, and in understanding basic aspects of plant-rhizosphere microorganism interactions.

Introduction

The rhizosphere is a region of intense microbial and microfaunal activity, where plant root exudates allow development of a distinct rhizosphere community (Curl and Truelove, 1986; Hiltner, 1904; Whipps and Lynch, 1986). The rhizosphere microbial community transforms and selectively utilizes exudates, which results in changes in residual carbon and nitrogen in the root environment. This, in turn, can have immediate and longer term effects on nutrient cycling and organic matter accumulation in the plant-soil system (Coleman *et al.*, 1985; Martin, 1977).

The role of the rhizosphere microorganisms in processing and retention of nutrients in plant-soil systems has been considered in general terms and in relation to broad attributes of ecosystem functioning (O'Neill and Reichle, 1980). In contrast, there has been little consideration of possible differences in residual soluble organic carbon and nitrogen of introduced and native rangeland plants, and effects of such possible differences on the long-term competitive interactions between these plant types. In this regard, large areas of the Western USA were seeded with crested wheatgrass (*Agropyron cristatum*, [L.] Gaertn.) during the 1930 period (Rogler and Lorenz, 1983). It is of interest that some of the

crested wheatgrass populations in this region have remained as virtual monocultures for more than 50 years with little or no successional trend (Vallentine, 1971).

Studies of photosynthesis and carbon allocation (Brown and Trlica, 1977; Caldwell *et al.*, 1980; Detling, 1979; Kemp and Williams, 1980; Monson *et al.*, 1983) and of plant-water relationships (Fairbourne, 1982; Sala *et al.*, 1981) have shown major differences in carbon and nutrient allocation and water utilization for crested wheatgrass and the two major components of the shortgrass steppe, blue grama (*Bouteloua gracilis*, (H.B.K.) Lag. ex Steud.) and western wheatgrass (*Agropyron smithii*, Rydb). A substantial amount of carbon (70 to 80%) is allocated to the roots by dominant native species, while crested wheatgrass allocates more carbon to aboveground photosynthetic tissue (Power, 1980).

This study was carried out to compare soluble sugars, organic acids and amino acids in the root zone of these shortgrass steppe plants grown under gnotobiotic conditions with and without an added rhizosphere microbial population. All exudate sources were measured in a previous study (Biondini *et al.*, 1988), including carbon dioxide evolved and soluble and insoluble carbon and nitrogen present in sloughed cells. In the present study, levels of residual soluble amino acids, sugars and organic acids of the various plants grown with and without rhizosphere microorganisms were evaluated.

Methods

Plant growth

The seeds of *A. cristatum*, *A. smithii*, and *B. gracilis* were germinated and grown as described by Biondini *et al.* (1988) using sterile 1000 ml pyrex growth chambers. These units had a sealed 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.

The plants were grown in inert fritted clay which had been washed four times in deionized water, with 400 ml of a 1/4 strength Hoagland's solution used in each growth chamber, when required, 10 ml of a mixture of rhizosphere microorganisms derived from each of the respective plant types was added to each unit. The inoculum was isolated and prepared according to procedures used previously (Nakas and Klein, 1980).

The plants were grown for 90 days in an environmental chamber, with microcosms 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 hours) at Cheyenne, Wyoming. Filtered compressed air was flushed two times a week for 15 minutes through the root growth chambers during the 90-day growth period to replenish oxygen levels and remove CO_2, using flow rates equivalent to those used previously (Barber and Martin, 1976). Sterility checks were conducted during the first three weeks of the experiment.

Soluble exudate collection

At the end of the growth period, the chambers were opened, and the roots were carefully washed free of fritted clay with 400 ml of distilled water. The same water was then used to wash the fritted clay four separate times. The wash solution was filtered using a 0.45 um membrane filter to remove particulates and the resulting volume was measured. The filtered exudates were dried by rotary evaporation at 40°C, dissolved in 30 ml of distilled water and 30 ul of chloroform was added to prevent microbial growth.

The concentrated filtrates were separated into free sugar, organic acid and amino acid fractions (Kraffczyk *et al.*, 1984). Eight ml of the concentrate were passed successively through cation and anion exchange resins. The amino acids were eluted from a Dowex 50 w × 8 cation exchange column with 2 *N* NH_4OH. The carboxylic acids were eluted from a Dowex 1 × 8 anion exchange column with 5 *N* formic acid. The neutral solution contained the sugars.

The three fractions were dried by rotary evaporation at 40°C. Sugars and organic acids were rehydrated in 7 ml of distilled water, and 6 ml were pipetted into test tubes and frozen. Amino acid fractions were dissolved in 4 ml of distilled water, and 3 ml pipetted into test tubes and frozen. The

fractions were lyophilized for 8 hrs and further dried over P_2O_7 for 24 hours.

The sugars were dissolved in pyridine and derivatized using hexamethyldisilazane and trimethylchlorosilazane (Kraffczyk *et al.*, 1984). The organic acid fraction was dissolved in pyridine and derivatized using N-methyl-N-trimethylsilyl-trifluoracetamide. The sugars and organic acids were analyzed using a 183 × 0.3 cm glass column packed with 80/100 chromosorb GAW (Supelco, Bellefonte, PA) using FID and nitrogen as a carrier gas at 20 ml per minute. Adonitol (0.4 mg) was added to sugar samples as an internal standard. As an internal standard for organic acids, 0.4 ml of heptanoic acid, diluted 1:250 in pyridine, was added to the organic acid solutions. A Varian 3700 gas chromatograph was used in all analyses. Injector and detector temperatures of 270 and 300°C, respectively, were used. For sugar analyses, the temperature was programmed from 120 to 260°C at 8 C min^{-1}, and held at the final temperature for 9 minutes. The unit was programmed to run from 80 to 250°C at 4 degrees min^{-1} for the organic acids.

The amino acids were derivatized using acetonitrile and bis(trimethylsylyl)trifluoroacetamide with decanoic acid as an internal standard (Gehrke and Leimer, 1971). A 6-m × 2-mm ID glass column of 10% OV 11 on 100–120 Supelcoport (Supelco, Bellefonte, PA) was used with an N_2 flow of 30 ml min^{-1}. The temperature was held for 5 min at 100°C after injection, then programmed to rise at 3° C min^{-1} to a final temperature of 230°C.

Five sugars (arabinose, fructose, α-glucose, β-glucose and sucrose), ten organic acids (benzoic, glycoxylic, succinic, fumaric, glutarate, malate, tartrate, oxalic, aconitic, and citric) and twenty amino acids and derivatives (alanine, glycine, valine, leucine, isoleucine, proline, serine, threonine, hydroxyproline, aspartate, methionine, cysteine, arginine, glutamate, phenylalanine, lysine, tyrosine, histidine, tryptophan and cystine) could be detected. Simulated rhizosphere soluble exudate mixtures of varying concentrations were analyzed in the same manner as the exudate samples to determine minimum detection limits and extraction recoveries for each component. Final concentrations of actual exudate components were calculated from these recovery curves, and results from each plant for a given component were used as a single datum in the statistical analyses.

Statistical analysis

The data were log transformed (ln (1 + x)), and all subsequent statistical analyses were performed on the transformed data. For statistical purposes, when a component was determined to be present in one or more replicates within a treatment group, missing values of that component within the treatment group were given 1/2 the calculated minimum detection limit. Significant effects of plant species and rhizosphere inoculum (F statistic at $P = < 0.05$) were evaluated by the least significant difference method.

Results

Plant growth responses

After 90 days of growth, the presence of a rhizosphere inoculum only resulted in significant changes in root responses and not in shoot responses (Table 1). The *B. gracilis* had lower root dry weights than the *A. cristatum* and *A. smithii*, and did show a significant increase in dry weight with rhizosphere microorganisms present. The roots did not differ in gross morphology with and without rhizosphere microorganisms present.

Exudate analyses

The concentrations of sugars, organic acids, amino acids and the total of these compounds with-

Table 1. Shoot and root dry weight of gnotobiotic and rhizosphere-inoculated *A. cristatum*, *A. smithii* and *B. gracilis* grown for 90 days in root growth chambers.[a]

Plant type	Microbe status	Plant component	
		Shoot	Root
A. cristatum	Sterile	0.14 ax[b]	0.46 abx
	Non-sterile	0.22 ax	0.60 abx
A. smithii	Sterile	0.36 ax	0.89 ax
	Non-sterile	0.34 ax	0.66 abx
B. gracilis	Sterile	0.02 ax	0.03 bx
	Non-sterile	0.14 ax	0.16 by

[a] mg g^{-1} dry weight root material.

[b] Means in a column followed by a different letter (a, b) are significantly different at $p = < 0.05$ by LSD test. Two means for sterile and non-sterile conditions followed by different letters (x, y) are significantly different at $p = < 0.05$ by LSD test.

Table 2. Comparison of soluble amino acids, sugars and organic acids of sterile and nonsterile *Agropyron cristatum* (Agcr), *A. smithii* (Agsm) and *Bouteloua gracilis* (Bogr), after 90 days of growth[a]

Plant	Microbe status	Soluble component, mg g^{-1} dry wt roots			
		Am. acids	Sugars	Org. acids	Total
Agcr	Sterile	31.68abx	6.74ax[b]	2.75ax	43.45ax
	Non-sterile	9.86by	3.50bcx	1.04by	14.40by
Agsm	Sterile	22.72abx	4.15abcx	1.77abx	28.64abx
	Non-sterile	10.76by	4.59abx	0.86by	16.21by
Bogr	Sterile	43.42ax	3.35bcx	< 0.48	46.77ax
	Non-sterile	26.25abx	1.54cy	< 0.48	27.79abx

[a] mg g^{-1} dry weight root material.
[b] Means in a column followed by a different letter (a, b, c) are significantly different at $p = < 0.05$ by LSD test. Two means for sterile and non-sterile conditions followed by different letters (x, y) are significantly different at $P = < 0.05$ by LSD test.

in the root zones of the three species, with and without rhizosphere microorganisms present, are summarized in Table 2. Without rhizosphere microbes present, *A. cristatum* had higher levels of residual sugars in the root zone, in comparison with the sterile *B. gracilis*. There was no difference in the levels observed with the corresponding nonsterile plants. With rhizosphere microbes present, the *B. gracilis* had significantly lower levels of residual sugars, in comparison with the gnotobiotic plants. The organic acid data also indicated that there were differences in the responses of the plants. The only organic acid which was detected, and the only one in the exudate from the *Agropyron* species, was malic acid, and significant decreases occurred with rhizosphere microorganisms. No organic acids, among the ten which we were able to detect, were observed with *B. gracilis*.

The amino acid responses indicated that the highest overall levels, per gram of root material, occurred with *B. gracilis*. With rhizosphere microorganisms present, significant decreases in the residual amino acid levels occurred with both of the *Agropyron* species, but not with *B. gracilis*, similar to responses for the total exudates (Table 2).

The calculated carbon-nitrogen ratios also indicated that there were major differences in residual exudate characteristics between the plants, with and without a rhizosphere microbial community present (Table 3). In terms of the soluble amino acids, the sterile *A. smithii* had a higher C/N ratio than the sterile *B. gracilis*. The C/N ratio of the sterile and nonsterile *Agropyron* species showed interesting responses. All of the average C/N ratios for the *Agropyron* species were above 2.5, while the *B. gracilis* values were less than 2. The sterile *A. cristatum* had a significantly higher total exudate C/N ratio than observed for the *B. gracilis*. The non-sterile plants showed a similar trend.

The data for the sugars and organic acids provided additional information with which to interpret these changes in carbon and nitrogen dynamics. As noted in Table 4, the sugars detected included fructose, α- and β-glucose, and sucrose. Malic acid was only detected with the *Agropyron* species. With the *Agropyron* species, rhizosphere microbe presence did not result in lower residual sugar levels, while with *B. gracilis* a significant decrease occurred. For the malic acid, significantly lower values occurred in the two *Agropyron* species with rhizosphere microbes present.

Distinct shifts in patterns of individual amino acids occurred when plants were grown with and without microorganisms (Table 5). For both of the *Agropyron* species, more amino acids originally detected in the root zones of the non-sterile plants could not be detected with rhizosphere microbes present. In contrast, not only was the average concentration of residual amino acids higher with the *B. gracilis*, but more amino acids (seven) could be detected with microorganisms present than in the gnotobiotic plants, opposite from results for the *Agropyron* species.

Table 3. Carbon-nitrogen ratios of soluble amino acids versus amino acids plus sugars and organic acids of *Agropyron cristatum* (Agcr), *A. smithii* (Agsm) and *Bouteloua gracilis* (Bogr) with and without rhizosphere microbes present, after 90 days of growth.

Plant	Microbe status	Carbon-nitrogen ratio	
		Amino acids	Total of amino acids, sugars, organic acids
Agcr	Sterile	1.88 abx[a]	3.28 ax
	Non-sterile	1.77 b x	2.62 abx
Agsm	Sterile	2.03 a x	2.59 abx
	Non-sterile	1.77 b y	2.71 abx
Bogr	Sterile	1.73 b x	1.92 b x
	Non-sterile	1.86 abx	1.98 b x

[a] Means in a column followed by a different letter (a, b) are significantly different at $p = < 0.05$ by LSD test. Two means for sterile and non-sterile conditions followed by different letters (x, y) are significantly different at $p = < 0.05$ by LSD test.

Table 4. Soluble sugars and organic acids of *Agropyron cristatum* (Agcr), *A. smithii* (Agsm) and *Bouteloua gracilis* (Bogr) grown with and without rhizosphere microorganisms, after 90 days of growth[a]

Compound	Plant					
	Agcr		*Agsm*		*Bogr*	
	S[b]	NS[c]	S	NS	S	NS
Sugars						
Fructose	3.14ax[d]	12.6by	1.38bx	1.64bx	3.35ax	1.44by
α-Glucose	1.65ax	1.05ax	1.05ax	1.45ax	< 0.62	< 0.62
β-Glucose	1.19ax	0.19cx	0.35bcx	0.50bx	< 0.04	0.10c
Sucrose	0.76ax	1.00ax	1.37ax	1.00ax	< 0.34	< 0.34
Total	6.74ax	3.50bcx	4.15abcx	4.59abx	3.35bcx	1.54cy
Organic acids						
Malic acid	2.75ax	1.04by	1.77abx	0.86by	< 0.48	< 0.48

[a] mg g^{-1} dry weight root material.
[b] S = sterile.
[c] NS = non-sterile, with rhizosphere inoculum.
[d] Means across a row followed by a different letter (a, b, c) are significantly different at $p = < 0.05$ by LSD test. Two means for sterile and non-sterile conditions followed by different letters (x, y) are significantly different at $p = < 0.05$ by LSD test.

Table 5. Soluble amino acids of *Agropyron cristatum* (Agcr), *A. smithii* (Agsm), and *Bouteloua gracilis* (Bogr), with and without rhizosphere microorganisms, after 90 days of growth[a]

Amino acid	Plant					
	Agcr		*Agsm*		*Bogr*	
	S[b]	NS[c]	S	NS	S	NS
Alanine	< 0.15	< 0.15	< 0.15	0.21b	< 0.15	2.19a
Glycine	< 0.13	< 0.13	< 0.13	< 0.13	< 0.13	0.36
Valine	< 0.07	< 0.07	< 0.07	< 0.07	0.62	< 0.07
Leucine	< 0.09	< 0.09	0.18	< 0.09	< 0.09	< 0.09
Isoleucine	< 0.23	< 0.23	0.35	< 0.23	< 0.23	< 0.23
Proline	< 0.20	< 0.20	0.44ax[d]	0.28ax	< 0.20	1.51a
Serine	6.99ax	1.03by	2.50abx	1.24by	3.00abx	3.07abx
Hydroxyproline	< 0.26	< 0.26	< 0.26	< 0.26	< 0.26	0.43
Aspartate	2.27a	< 1.19	4.49a	< 1.19	12.34a	< 1.19
Methionine	< 0.18	< 0.18	0.38abx	0.27bx	< 0.18	0.63a
Arginine	17.04abx	4.95bx	8.48bx	5.34by	21.71ax	12.23aby
Glutamate	< 0.20	< 0.20	0.28	< 0.20	< 0.20	< 0.20
Phenylalanine	0.72abx	0.37bx	0.37abx	0.18bx	0.89ax	0.53abx
Lysine	< 0.52	< 0.52	1.14a	< 0.52	< 0.52	0.86a
Tyrosine	< 0.22	0.39a	0.30ax	0.33ax	0.53ax	0.37ax
Histidine	0.36a	< 0.25	0.42a	< 0.25	< 0.25	0.35a
Tryptophan	0.80a	< 0.51	0.68a	< 0.51	< 0.51	< 0.51
Cystine	5.77ax	3.12ax	2.71ax	2.91ax	4.33ax	3.72ax
Totals	33.95abx	9.86by	22.72abx	10.76by	43.42ax	26.25abx

[a] mg g^{-1} dry weight root material.
[b] S = sterile.
[c] NS = non-sterile, with rhizosphere inoculum.
[d] Means across a row followed by a different letter (a, b) are significantly different at $p = < 0.05$ by LSD test. Two means for sterile and non-sterile conditions followed by different letters (x, y) are significantly different at $p = < 0.05$ by LSD test.

Discussion

Differences in residual soluble exudate sugars, organic acids, and amino acids, and in carbon and nitrogen retention in these compounds by three grasses considered to be introduced (*A. cristatum*) and native (*A. smithii*, *B. gracilis*) to the shortgrass steppe were observed in this study. These differences were not only related to plant type, especially between *A. cristatum* and *B. gracilis*, but also whether rhizosphere microorganisms were present. The increased retention of nitrogen and carbon in these soluble compounds by *B. gracilis*, and especially in comparison with the introduced *A. cristatum*, suggests that this climax species would have a greater potential for soluble organic matter accumulation, and especially of amino acids, to occur in the root environment. There is an extensive body of literature available concerning exudate carbon effects on microbial community responses in the rhizosphere (Curl and Truelove, 1986; Newman and Watson, 1977; Smucker and Safir, 1986) and concerning succession, litter quality and belowground processes (Heal and Dighton, 1986). However, it does not appear that there has been a documentation of comparative residual soluble organic carbon and nitrogen present as amino acids, sugars and organic acids in the root environment of plants which may compete in a particular environment.

A. cristatum, particularly, had a higher C/N ratio based on these analyses of sugars, organic acids, and amino acids (*A. smithii* showed a similar trend) in comparison with the *B. gracilis*. The microbes in the rhizosphere of the two *Agropyron* species thus may have been more nitrogen-limited, resulting in greater relative amino acid use and a tendency to use carbohydrates to a lesser extent. The *B. gracilis* microbes, in comparison, with a lower C/N ratio residual exudate, tended to use more carbohydrates (significant decreases occurred) while placing a lesser demand on the amino acids. A trend towards accumulation of amino acids has been observed in artificial root exudate systems (Odham *et al.*, 1986). Such accumulation of nitrogen-rich exudates, and lesser degradation of these exudates by *B. gracilis*, represents a major energetic cost which did not appear to occur to the same extent with the *A. cristatum*. This lesser allocation to accumulation of belowground resources by *A. gristatum* in comparison with *B. gracilis* may contribute, together with differences in temperature relationships and water use, to the continued maintenance of *A. cristatum* as distinct introduced plant communities which have not undergone major successional changes.

Newman and Watson (1977) in a computer model of microbial growth in the rhizosphere, provided a summary of prior literature on soluble organic materials exuded from roots under sterile conditions. A range of 6 to 250 mg of total materials released per gram of root for various lengths of plant growth were reported, including values of 1–100 mg for soluble exudates per gram of roots (Newman, 1985). The values for soluble exudates found in our study, approximately 28–46 mg g^{-1} roots under sterile conditions, and 14–28 mg g^{-1} roots under non-sterile conditions, thus are in ranges comparable with prior literature values. Unfortunately, nitrogen was not considered in these earlier exudate studies. This nutrient is very important in grassland plant interactions, both in terms of better understanding competition between introduced and native plants, and in the dynamics of longer-term successional processes.

As discussed in recent reviews (Heal and Dighton, 1986; Smucker and Safir, 1986), soil microbial populations may play distinct roles in influencing the rate and possibly the course of plant community development (Heal and Dighton, 1986). Information on comparative nutrient retention in the root environment, as carried out in this study, should assist in better understanding interactions of introduced and native plants in shortgrass steppe communities and in providing concepts which will be useful in analyses of other plant-soil systems.

Although the microbial populations which developed in the rhizosphere were not characterized in this preliminary study, these results suggest that investigations where rhizosphere inocula are exchanged between introduced and native plants, and where defined inocula would be used, could provide additional insights into mechanisms of carbon and nitrogen retention in the rhizosphere and ultimately in soil organic matter.

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