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Glucose-induced nitrate assimilation in prairie and cultivated soils

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Abstract. Native prairie and grassland soils are known to accumulate little inorganic N; however, NO_3^- is constantly being formed and re-immobilized. This suggests that microorganisms in prairie soils would be highly efficient in the assimilation of NO_3^- and would regularly have the assimilatory NO_3^- reductase (ANR) enzyme in an induced and active state. Aerated slurries and static systems prepared from prairie and cultivated soils amended with glucose and NO_3^- were observed for changes in NO_3^- concentration with time. Nitrate assimilation in the presence of glucose occurred more rapidly in cultivated than in prairie soils from the same soil map unit. Nitrate assimilation rates were not affected by inoculation of prairie soil with cultivated soil. It has been reported that the addition of glucose and NO_3^- to soils results in increased peptidase activity and a release of free amino acids. Mixing, sieving, and slurrying of prairie soils followed by treatment with glucose and NO_3^- may release free amino acids and other ANR inhibitors into the prairie soil slurries. Prairie soils had higher concentrations of soluble amino-N than cultivated soils with or without glucose and NO_3^- additions. Prairie soils also had greater concentrations of total Kjeldahl N and readily hydrolyzed amino acids than corresponding cultivated soils.

Introduction

It is well established that native or established grassland soils accumulate little inorganic N, although NH_4^+ and NO_3^- are continually being formed (Woodmansee et al. 1981; Schimel et al. 1989). Nitrification has been found to account for about one third of total mineralization in a grassland soil where there is little or no NO_3^- accumulation (Schimel et al. 1989). This relatively high activity of nitrifying bacteria in grassland soils has been confirmed in other studies (Davidson et al. 1990; Both et al. 1992). The lack of NO_3 accumulation greatly reduces the potential for both NO_3^- leaching and gaseous losses of N (Woodmansee et al. 1981; Goodroad & Keeney 1984).

It has been suggested that the lack of NO_3^- accumulation in prairie or grassland soils is a function of plant uptake (Clark 1977) and microbial immobilization of NO_3^- (Schimel et al. 1989; Davidson et al. 1990). Microbes have been shown to out-compete plants for NO_3^- in aerobic short-term studies with grassland soils (Jackson et al. 1989). It is likely that microorganisms are assimilating NO_3^- because there is limited denitrification in prairie soils (Goodroad & Keeney 1984).

Studies have clearly shown that soil microorganisms preferentially assimilate NH_4^+ over NO_3^- and assimilate little NO_3^- in the presence of NH_4^+ or several amino acids (Rice & Tiedje 1989; McCarty & Bremner 1992). Rice & Tiedje (1989) present a critical level of 0.1 μg NH_4^+-N g^{-1} soil, below which NO_3^- is readily assimilated. Ammonium is generally found at greater levels than NO_3^- in grassland soils (Woodmansee et al. 1981). It was thus suggested that NO_3^- assimilation in prairie soils occurred in microsites where NH_4^+ had been exhausted by microbial activity (Davidson et al. 1990).

Laboratory studies using disturbed grassland and cultivated soils have demonstrated higher rates of gross NO_3^- immobilization in cultivated soils than in grassland soils (Schimel 1986). Greater N mineralization in disturbed prairie soils compared to cultivated soils (Keeney & Bremner 1964) may eliminate the potential for measuring gross immobilization *in-vitro* without an added energy source. To correct for this, Rice & Tiedje (1989) added glucose to soils to induce measurable NO_3^- assimilation.

Microbial assimilation of NO_3^- requires the presence of sufficient energy as C to reduce NO_3^- to NH_4^+ before incorporation into amino acids. Prairie soils have been found to have higher concentrations of labile organic C and N than their cultivated counterparts (Schimel 1986; Woods 1989; DeLuca & Keeney 1993). This pool of labile C would supply the necessary energy to account for the high rate of NO_3^- assimilation observed in prairie soils.

It is not clear whether NO_3^- assimilation in the presence of added glucose would occur more rapidly in prairie or cultivated soils based on existing studies. It would appear that assimilatory NO_3^- reductase (ANR) activity would be high in NH_4^+ depleted microsites of prairie or grassland soils. Cultivated soils that accumulate NO_3^- and lack sufficient C to allow for microbial assimilation of NO_3^- would not normally have the NO_3^- reductase enzyme induced and should thus be less efficient at reducing NO_3^- than their prairie counterparts.

The objectives of this study were to observe the rate of glucose-induced assimilatory NO_3^- reduction in prairie and cultivated soils from the same soil map unit and to determine possible constraints on NO_3^- reduction beyond C limitation or inhibitory concentrations of NH_4^+.

Materials and methods

Soils were collected from paired prairie and cultivated sites in central Iowa previously described by DeLuca & Keeney (1993). All soils were sieved through a 2-mm mesh and stored at 5 °C until time of use (Table 1).

Table 1. General soil properties.

Soil	Cover	Sand	Silt	Clay	Total carbon	pH
		----------	g kg^{-1}	----------		
Kossuth[1]	Crop	180	300	520	32.6	6.1
	Prairie	250	330	420	39.0	5.9
Clarion[2]	Crop	320	460	220	29.5	5.9
	Prairie	370	460	170	40.5	5.9
Canisteo[3]	Crop	270	370	360	28.7	7.3
	Prairie	250	350	400	40.5	7.1

[1] Kossuth = Typic Haplaquolls
[2] Clarion = Typic Hapludolls
[3] Canisteo = Typic Haplaquolls (calcareous)

Assimilatory NO_3^- reduction studies in aerated soil slurry were performed as described by McCarty & Bremner (1992). To induce the NO_3^- reductase enzyme and assimilate pre-existing NH_4^+ preceding initiation of the study, field-moist 10 g (oven dried equivalent) soil samples were treated with 25 mg of C as glucose and 1.8 mg N as KNO_3, brought to 60% water-holding capacity, and then incubated at 30 °C for 16 hours. Soils were then treated with 5.0 mg C as glucose and 0.6 mg N as KNO_3 and shaken with 30 ml of distilled deionized water to obtain the soil slurries reported in this study.

The aerated soil slurries were continuously monitored for levels of NO_3^- by using a NO_3^--sensitive electrode. The soil slurries were treated with 14 mg of K_2SO_4 (to adjust ionic strength), placed on magnetic stir plates, maintained at 30 °C in a constant temperature chamber, and aerated by bubbling with a stream of air. A NO_3^- electrode (Orion model 93-07) coupled with a double-junction reference electrode (Orion model 90-02) containing 0.4 M K_2SO_4 outer filling solution was placed into each slurry. Electrodes were calibrated against KNO_3 standards prior to each analysis. The millivolt output was then continuously charted with a pen recorder connected to the electrode meter. To determine whether cross-

inoculation of soils would increase or decrease the rate of NO_3^- reduction, 9 g (oven dried equivalent) of prairie soil was amended with 1 g of cultivated soil.

To monitor the reduction of NO_3^- in soil over a 4-day period, 10 g (oven-dried equivalent) soil samples were placed in 250 ml French square bottles, treated with 25 mg C as glucose and 1.8 mg N as KNO_3, brought to 60% water-holding capacity, and allowed to incubate at 30 °C for 4 days. After 0, 1, or 4 days of incubation, 50 ml of 2 M KCl were added to each bottle, and the bottles were shaken for 1 hr. Suspensions were then filtered through Whatman number 5 filter papers and analyzed for NH_4^+ and NO_3^- with a flow injection auto analyzer (Lachat QuickChem Method No. 12-107-06-2-A and 12-107-0401B, Milwaukee, WI).

Soluble amino-N was determined in soils following pre-treatment of soils with glucose and KNO_3 as described above or with water. Soil samples (10 g oven dried equivalent) were wetted to 60% water holding capacity with either distilled water or with water plus 25 mg of C as glucose and 1.8 mg N as KNO_3. After 16 hours the water treated soils were amended with 30 ml of distilled water and the glucose treated soils were amended with 30 ml of distilled deionized water containing 5.0 mg C as glucose and 0.6 mg N as KNO_3. Samples were then shaken for 30 minutes and then the reaction stopped by adding 30 ml of 4 M KCl to all samples (to obtain a 2 M KCl extract). The soil slurries were then shaken for 5 minutes and filtered through 1.2 μm glass fiber filter paper and analyzed for amino-N as described in DeLuca & Keeney (1993).

An assay of amino acids that are readily hydrolyzed was determined by placing 3 g of 100-mesh sieved oven-dried soil in a long Kjeldahl tube and adding 2 drops of octyl alcohol and 20 ml 1 N HCl. The tubes were then heated to 125 °C for 1 hr. The soil hydrolysate was filtered through Whatman number 42 filter paper, and the pH of the filtrate was slowly adjusted to 5.0 by using 1 N NaOH. The filtrate was then quantitatively transferred to a 100 ml volumetric flask and brought to volume. The hydrolysate was then analyzed for total amino acids (Stevenson 1982).

Total Kjeldahl N was determined by using a semimicro Kjeldahl method (Bremner & Breitenbeck 1983). Total C in soils was determined by the Walkley-Black method (Nelson & Sommers 1982). Particle size distribution was determined by hydrometer (Gee & Bauder 1986).

Results and discussion

Nitrate reduction occurred readily in the cultivated soils, but appeared greatly inhibited in the prairie soils (Fig. 1). The average rate of NO_3^-

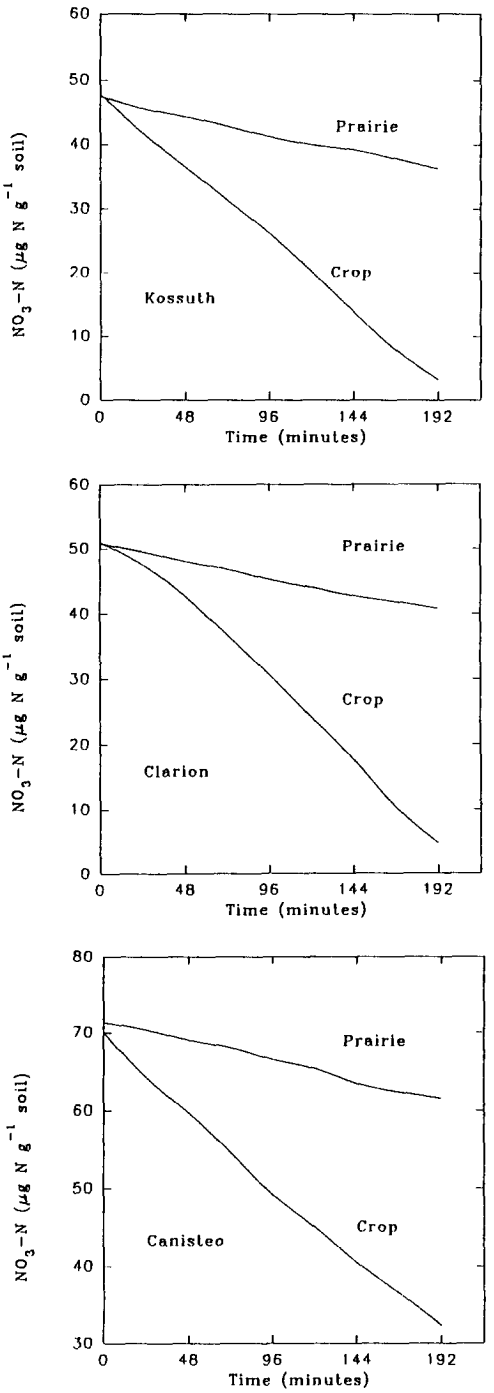

Fig. 1. Change in NO_3^- concentration with time in Kossuth, Clarion, and Canisteo prairie and cultivated soil slurries treated with glucose and KNO_3 and incubated at 30 °C.

assimilation across all three soil types was 13.8 (+/−1.8) μg NO_3^- hr^{-1} in cultivated soils and 3.24 (+/−0.12) μg NO_3^- hr^{-1} in prairie soils. The low level of assimilatory NO_3^- reductase (ANR) activity in the prairie soils continued throughout the experiments with no observed increase in the rate of NO_3^- reduction. Ammonium was generally at undetectable levels in all soils at the end of the slurry experiments, and addition of NH_4^+ to soil slurries rapidly inhibited NO_3^- reduction, demonstrating that the change in NO_3^- concentration with time was due to assimilation rather than denitrification of the applied NO_3^-.

The 16 hr preincubation of soils with glucose and KNO_3 generally resulted in the complete reduction of NO_3^- present in the cultivated soils, whereas much of the original NO_3^- applied to the prairie soil remained at the end of 16 hr. This suggests that the lack of NO_3^- reduction in prairie soils in the soil slurry study was not due to a missed log phase of bacterial growth on glucose and NO_3^-.

Four-day, non-slurry incubations of the prairie and cultivated soils with added glucose and NO_3^- resulted in the immobilization of most of the applied KNO_3 after 1 day of incubation in the cultivated soils and after 4 days of incubation in prairie soils (Table 2). This demonstrates that, even in the presence of limited soil microsites, ANR activity (induced by glucose) occurs more slowly in prairie soils than in cultivated counterparts.

Inoculation of prairie soil with cultivated soil did not significantly affect the rate of NO_3^- assimilation in the prairie soils (data not shown). It is likely, therefore, that the inhibition of NO_3^- assimilation in prairie soils is not due to a lack of ANR active organisms, but rather to a physical or chemical inhibition of ANR in the prairie soil.

Nitrifiers have been shown to be poor competitors for NH_4^+ in energy limited chemostats and in soil columns (Verhagen & Laanbroek 1991; Verhagen et al. 1992). It is thus unlikely that the low rate of NO_3^- assimilation in the prairie soils is a function of a high rate of nitrification in the prairie soil slurries as there would be no C-limited microsites that would give the nitrifiers a competitive advantage.

Extensive nitrification or immobilization of NH_4^+ results in the release of fixed NH_4^+ from clay lattices (Drury & Beauchamp 1991). A release of fixed NH_4^+ after glucose-induced immobilization of all exchangeable NH_4^+ might also inhibit NO_3^{-1} assimilation. However, NH_4^+ levels in prairie soils treated with glucose and KNO_3 were not significantly different from NH_4^+ levels in cultivated soils (data not shown).

Peptidase activity in soil has been found to increase immediately after treatment of soils with glucose and NO_3^-, and after 32 hr, there is an increase in nonspecific proteinase activity (Ladd & Paul 1973). Treatment

Table 2. Nitrate concentrations in three prairie and cultivated soils untreated or treated with glucose and KNO_3 and incubated at 30 °C for 0, 1, and 4 days.

Soil/ Time (day)	Cultivated		Prairie	
	Control	Treated	Control	Treated
	μ g g^{-1}			
Kossuth				
0	17.0	69.0	3.8	45.0
1	18.0	1.9	4.6	12.8
4	18.6	1.4	5.8	1.2
LSD ($P < 0.05$) within columns = 2.9				
Clarion				
0	21.3	56.0	12.3	52.5
1	20.1	0.8	14.5	17.5
4	18.3	1.2	18.5	1.5
LSD ($P < 0.05$) within columns = 1.4				
Canisteo				
0	17.7	69.0	25.0	82.5
1	19.3	0.7	27.6	29.2
4	20.4	1.1	31.2	15.7
LSD ($P < 0.05$) within columns = 1.8				

of soils with glucose and NO_3^- results in a release of certain free amino acids (Putnam & Schmidt 1958; Paul & Schmidt 1961). If the addition of glucose and KNO_3 to soils induced peptidase activity that, in turn, caused a release of amino acids, then the increase in free amino acids or their deamination production (NH_4^+) would inhibit ANR (Rice & Tiedje 1989; McCarty & Bremner 1992).

The pool of soluble amino-N was found to be greater in the prairie soils than in the cultivated soils when incubated either with water or with glucose and NO_3^- (Table 3). Glucose and NO_3^- treatment appeared to enhance the presence of free amino-N in the cultivated soils, but prairie soils treated with just water had higher levels of soluble amino-N, than the samples treated with glucose and NO_3^-. The lack of a glucose and NO_3^- induced increase in free amino-N in the prairie soils may be a function of the high ambient levels of labile C in the prairie soils (Woods 1989) which would normally stimulate peptidase activity. Addition of glucose and NO_3^- to the prairie soils would increase microbial activity and possibly reduce the level of measurable soluble amino-N due to rapid immobilization of free amino acids in the presence of glucose. The release of free amino-N from cultivated soils following amendment with glucose and NO_3^-, may

Table 3. Soluble amino-N following treatment of soils with water or glucose and KNO_3, readily hydrolyzed amino acids, and total N in Kossuth, Clarion, and Canisteo prairie and cultivated soils.

Soil	Land use	Glucose & KNO_3 treated soluble amino-N[1]	Water treated soluble amino-N[1]	Readily hydrolyzed amino acids[2]	Total Kjeldahl N
		$\mu g\ g^{-1}$			
Kossuth	C[3]	0.56	0.16	162.0	3,033
	P	1.27	6.67	208.3	3,667
T-test		**[4]	**	**	**
Clarion	C	1.21	0.59	169.7	2,900
	P	2.42	9.92	217.7	3,733
T-test		**	**	**	**
Canisteo	C	1.14	0.48	137.0	2,933
	P	1.60	3.93	207.0	4,367
T-test		**	**	**	**

[1] Soil amino-N soluble in 2 M KCl extracts following 16 hr pre-treatment with glucose and KNO_3 or water.

[2] Soils hydrolyzed by heating soils to 125 °C in 1 N HCl for 1 hour.

[3] C = cultivated and P = prairie.

[4] ** demonstrates significant mean separation at $P < 0.01$.

have been due to a lack of available C, thus glucose addition would stimulate peptidase activity above ambient levels.

The prairie soils used in this study had significantly greater concentrations of readily hydrolyzed amino acids and total Kjeldahl N than did cultivated soils (Table 3). Prairie soils clearly have a larger pool of labile N than do corresponding cultivated soils (Keeney & Bremner 1964; Campbell & Souster 1982; Schimel 1985; Schimel 1986; DeLuca & Keeney 1993).

Both cultivated and prairie soils demonstrated ANR activity, but the prairie soils had lower ANR activity than the cultivated soils. These results are similar to laboratory investigations by Schimel (1986) and were possibly a result of a release of free amino acids or other inhibitors when the soils were mixed, sieved, and slurried with glucose and NO_3^-. Under natural conditions, NO_3^- assimilation in grassland ecosystems occurs in soil microsites void of NH_4^+ (Davidson et al. 1990). In our experiments, soil microsites were eliminated or greatly reduced (non-slurry experiment), potentially allowing free amino acids or their deamination product

(NH_4^+) to interfere with ANR. The prairie soils have the potential to release greater quantities of free amino acids than cultivated soils, but the role of these free amino acids in undisturbed soils needs further investigation.

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