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The Effect of Soil Temperature on Denitrification and Ammonification

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A LABORATORY study was conducted to determine the effects of temperatures from h-1-to 30°C on denitrification and ammonificanon in anaerobic soil treated with guar or glucose. Organic C substrate as guar or glucose increased denitrification rates at all temperatures within this range and lowered the threshold temperature at which denitrification occurred. The threshold temperature for denitrification was as low as -1°C in unfrozen (supercooled) soil in contrast to most other studies where the threshold temperature was reported to be at or above 0°C. When soil was frozen at -1°C, the denitrification rate was much lower than in unforzen soil at the same temperature. A square root model was employed which showed that the square root of the denitrification rate was linearly related to temperature from -1 to 30°C. The maximum amount of NO_2^- produced during the incubation periods generally decreased from -1 to 30°C and was greatest when glucose and especially guar were added. The rate of ammonification increased with addition of guar but the quantities of NH_4^+ produced generally decreased from 30°C to -1°C.

Keywords : Denitrification, Ammonification, Threshold temperature, Frozen vs unfrozen soil, Nitrite production, Square root model.

Temperature is one of the major factors controlling denitrification in the field although relatively little attention has been given to denitrification at or close to 0°C. Denitrification has been observed at temperatures as low as 3°C (Nommik, 1956),2°C (Bremner and Shaw, 1958), 5°C (Stanford *et al.*, 1975), 4°C (Mckenney *et al.*, 1980) and 0°C (Lippold *et al.*, 1989). Smid and Beauchamps

(1976); Hassanein and El-Shebiny (2000) and El-Sayed (1995 & 1998) suggested that denitrification could occur at a temperature as low as 0°C providing a large supply of substrate C . Jacobson & Alexander (1980); Khamis & Metwally (1998) and Metwally & Kamis (1998) did not observe denitrification in a glucose - amended soil after 7 days at 1°C and suggested that denitrifiers were not capable of growth at this temperature when NO_3^- was the electron acceptor. Cho *et al.* (1979) and El-Sayed (1997 a&b) suggested that the threshold temperature for denitrification was 2.75°C. More recently, Malhi *et al.* (1990) Negm *et al.* (1998) and El-Gazzar (2000) observed relatively low rates of NO_3^- loss from soils at -4°C. It is not clear whether this loss was due entirely to denitrification or to immobilization or dissimilatorv reduction of NO_3^- to NH_4^+ occurred under anaerobic conditions (De Catanzaro *et* al., 1987 and El-Sayed & Abo El-Wafa, 2001).

A labotatory study was done to determine the threshold temperature for denitrification in soil in relation to the supply of organic carbon substrate. Thus temperature treatments from -1 to 30°C along with glucose and guar as C substrates were included in the study. In addition, the effects of freezing and supercooling on denitrification at -1°C were investigated.

Material and Methods

First experiment

This experiment was designed to determine the effect of soil temperature on denitrification with and without guar. The Ap horizon material was silt loam soil and collected on 20 July 2000, at the Farm of Faculty of Agriculture in Assiut, Al-Azhar University, after a relatively dry period. Wheat (*Tritiezirn aestivum* L.) had been grown on the site for at least five years. Roots and other oranic matter residues were discarded as the soil was passed through a 2-mm sieve. The soil was then stored in a frozen state (-15°C) until used. The soil had the following characteristic : total N, 1.25 g kg^{-1}; organic C, 11 g kg^{-1}; pH 7.8; clay, 219 g kg^{-1}; sand, 229 g kg^{-1}; NO_3^- -N, 6.15 mg kg^{-1}; NH_4^+ - N, 2.25 mg kg^{-1}; H_2O g 87 g kg^{-1}.

Samples of frozen soil (30 g oven-dry Wt basis) were weighed into 240 ml erlenmeyer flasks and sealed with serum stoppers. The bottles were aerobically incubated at 15°C for 5 days before substrate and temperature treatments were

imposed. This protocol was followed for all temperature treatments to avoid the effects of a respiratory burst following thawing (Skogland *et al.*, 1988) and to permit microbial recovery from soil freezing (Page *et al.*, 1982 and Campbell *et al.*, 1970). Cabinets with controlled temperature (± 0.5°C) were employed for all incubations (Conviron Model C 610-77, Controlled Environments, Winnipeg, MB).

The following C substrate treatments were imposed: 1- control (no C added) and 2- dried ground guar (10 g kg^{-1} soil). The total N concentration in the guar was 30.5 g kg^{-1}. The guar contributed 0.8 µg NO_3^--N and 0.6 µg NH_4^+-N to each bottle. Distilled- deionized water containing KNO_3 was added to provide 550 g H_2O kg^{-1} and 100 mg NO_3^--Nkg^{-1}. The bottles were resealed and flushed with Ar gas for 15 min purified C_2H_2) (passed through sulfuric acid) was added to provide 10 K Pa in the headspace to block N_2O conversion to N_2.

The treated soil samples were incubated at temperature of 0, 2, 5, 20 and 30°C for periods of time which increased in length as the imposed temperature decreased. All treatments were replicated three times (Steel & Torrie, 1982 and SAS, 1985).

Nitrous oxide accumulation was determined with a Hewlett-Packard 5830 A gas chromatograph equipped with a ^{63}Ni electron capture detector. Nitrous oxide dissolved in the aquenous phase at different temperatures was taken into account (Moroahan & Buresh, 1977 & Evenhui & DeWaard, 1978). At each gas sampling time, the soil in a set of sample bottles was extracted with 2 MKCL and the extracts frozen for subsequent NO_3^-, NO_2^- and NH_4^+ (Page *et al.*, 1982).

Second experiment

A second experiment was conducted to compare guar and glucose as source of substrate C for denitrifiers at different temperatures. The substrate C treatments were: 1) 1.2mg glucose -C g^{-1} and 2) 10 mg guar tops g^{-1} soil. The availability of substrate C with the guar treatment was considered similar to that with the glucose treatment (Cottenie, 1980 and De Catanzaro & Beauchamp, 1985). The remainder of the procedures were the same as for exp. 1. The soil for this experiment was obtained on 16 May 2001 from the same site as for exp. 1 and had the following characteristics: total N, 1.25 g kg^{-1}; organic C, 8.5 g kg^{-1} pH, 7.5; NO_3^- N, 1.55mg kg^{-1}; NH_4^+-N, 1.4mg kg^{-1}; H_2O, 215 g kg^{-1}. The

temperatures studied were -1.0, 5, 15, 20 and 30°C. The soil at -1°C was unfrozen throughout this experiment.

Third experiment

The effect of freezing on denitrification at -1°C was studied in this experiment. It was observed that ice nucleation of the supercooled soil slurry at-1°C occurred after sharply hitting the side of the incubation bottle (Buoyoucos,1920 and FAO, 1980). This formed the basis for a comparison of denitrification rates of soil slurries in the frozen and unfrozen (supercooled) state at -1°C. Otherwise, the soil and substrate treatments employed were the same as for exp.2. Nitrous oxide and CO_2 in the headspace were monitored to provide an assessment of microbial activity. After 11 and 24 days of incubation, gas samples were taken. The frozen soil slurry in the incubation bottles was allowed to thaw for 30 min and then agitated to permit gas bubbles in the slurry to escape to the headspace. Separate incubation bottles were sampled at each sampling date to avoid the effects of frozen sample disturbance for the day 24 measurements.

Results

First experiment

The changes in N_2O, NO_3^-, NO_2^- and NH_4^+ concentrations over the incubation periods are presented for only the 0 and 30°C treatments because concentration patterns for the 2, 5 and 20°C treatments did not reveal any changes of particular noteworthiness (Fig. 1). Substantial denitrification occurred at 0°C especially with guar (Fig.1b) supporting the predicion by Smid and Beauehamp (1976). They suggested, however that denitrification would not occur below 5°C without added substrate C in Ap horizon material. In the current study, the denitrification rate generally decreased from 30 to 0°C in the absence of added substrate C.

Second experiment

As for exp. 1, the data for only two temperature treatments (-1and 30°C) are presented. Although the rates of N transformations were shown, there was substantial denitrification at -1°C (Fig.2 a&b). The N_2O production rates at 30°C with the guar treatment was generally more raised than with glucose during the first two days of incubation, as observed previously by Paul and Beauchamp

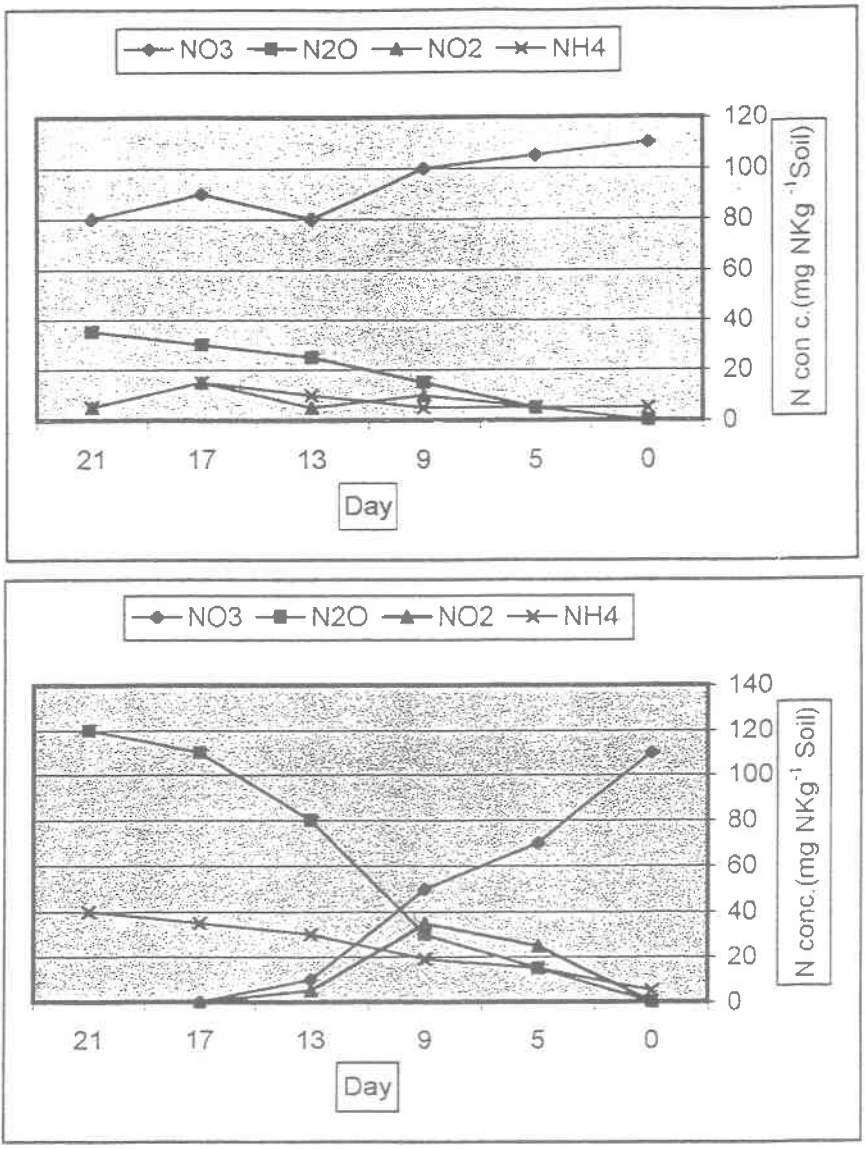
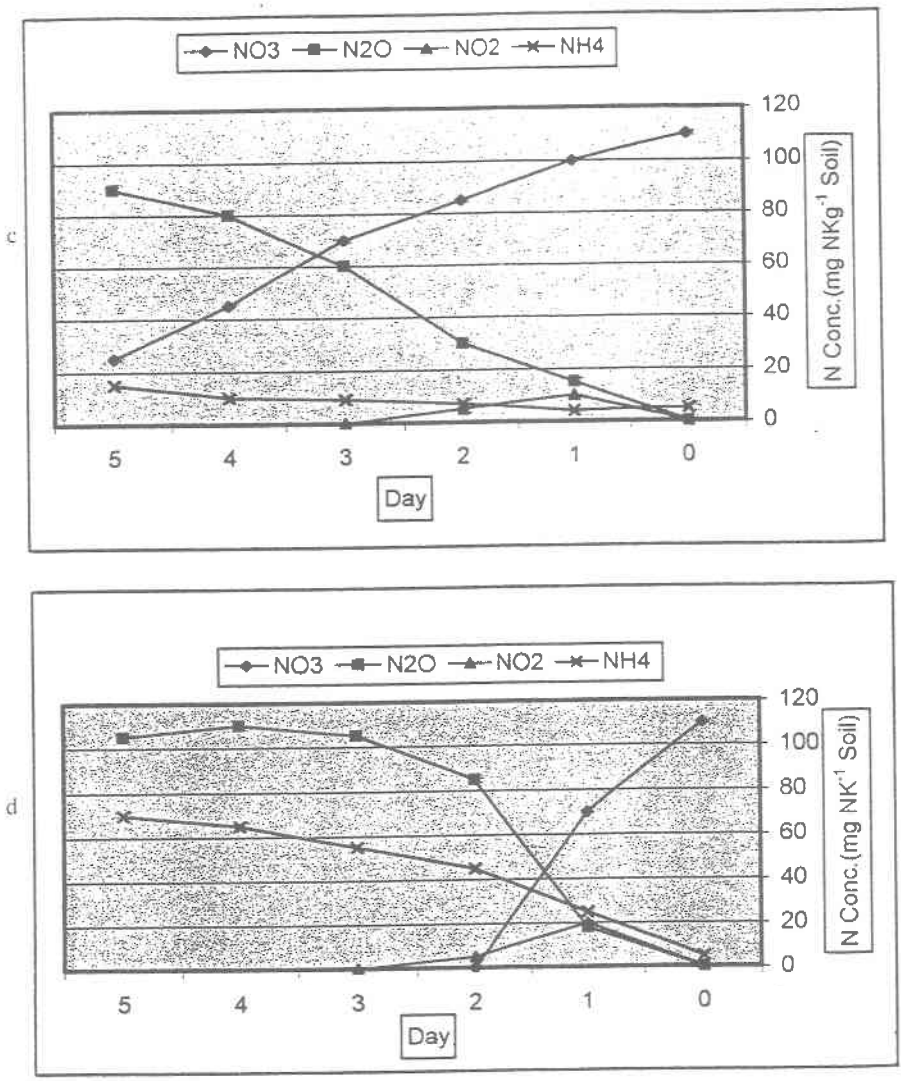


Fig. 1. (Above and on facing page.) Concentrations of exchangeable ammonium, nitrate, nitrite and nitrous oxide over time with (a) OC, no C substrate addition, (b) OC, guar, (c) 30°C, no C substrate and (d) 30°C, guar (exp. 1).



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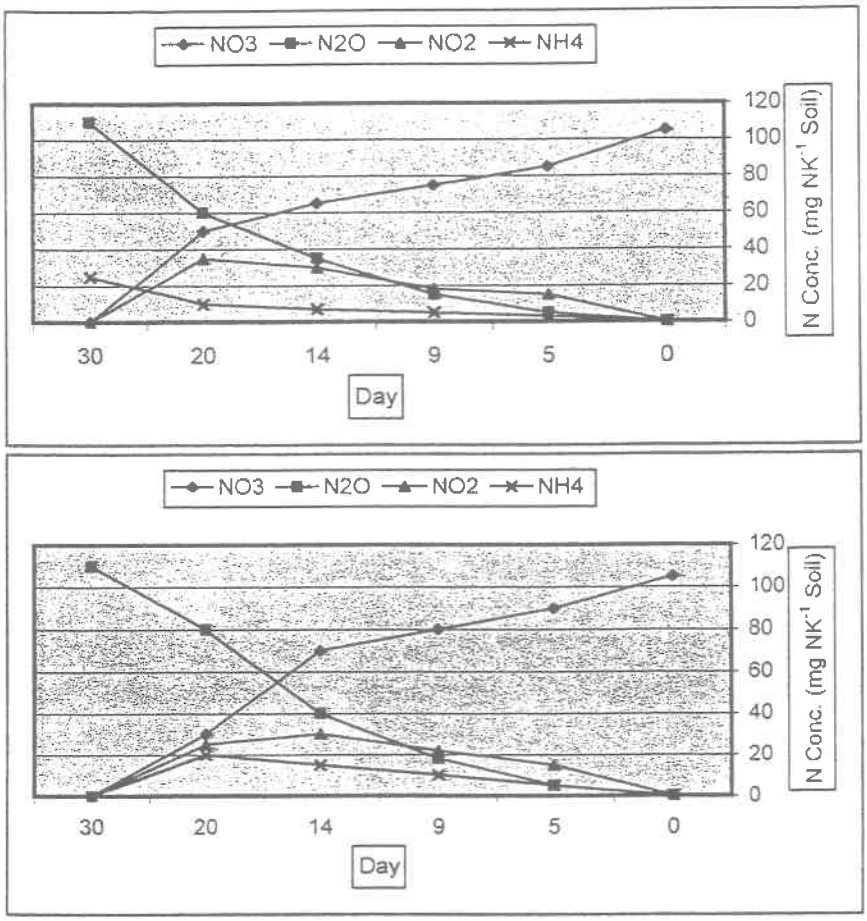
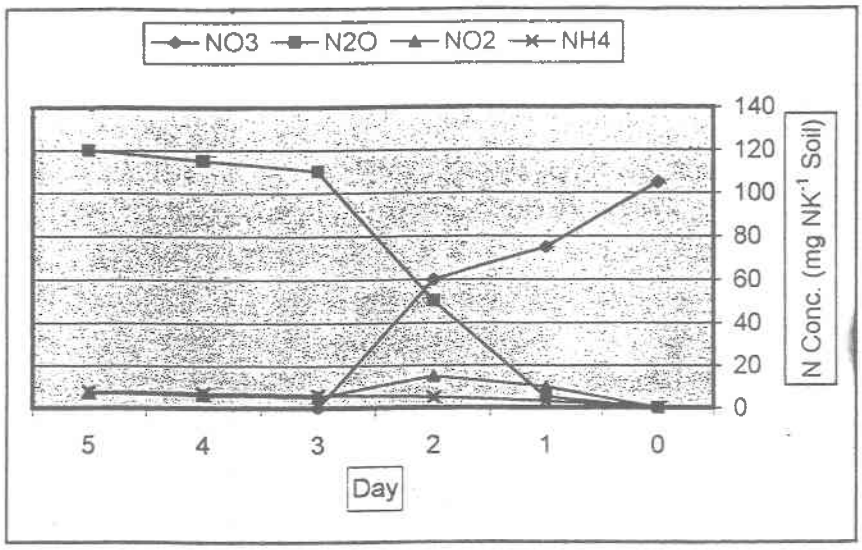


Fig. 2. (Above and on facing page.) Concentrations of exchangeable ammonium, nitrate, nitrite and nitrous oxide over time with (a) -1°C, glucose, (b) -1°C, guar, (c) 30°C, glucose and (d) 30°C, guar (exp. 2).

c



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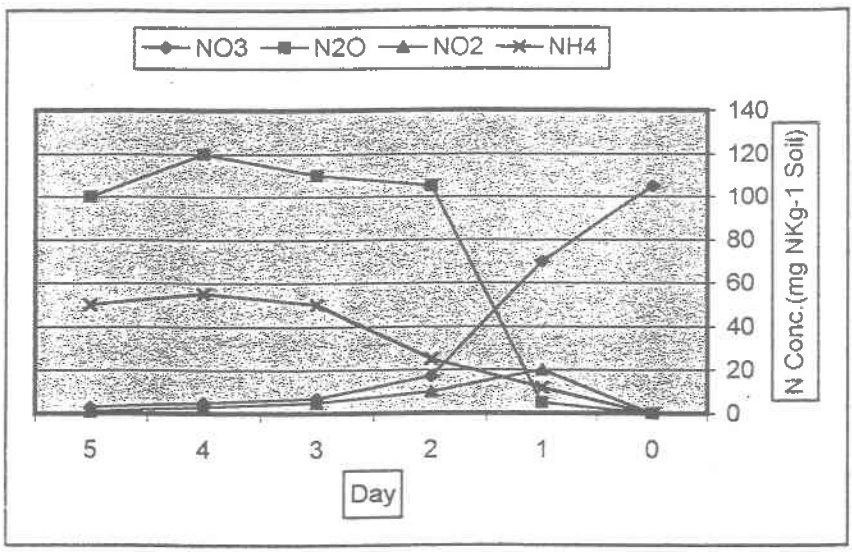

Fig. 2. Continued.

(1989). Two days were required for NO_3^- to be used up with glucose (Fig. 2c) while only one day was required with guar at 30°C (Fig. 2d).

Nitrite accumulation

Maximum NO_2^- accumulation generally decreased with increasing temperature and added substrate C as observed in exp.1 (Table 1). The accumulation with guar tended to be greater than with glucose. The presence of large quantities of NO_2^- could result in significant chemodenitrification if the soil should freeze (Christianson and Cho, 1983). On the other hand, soil pH below about 6.5 is required before freezing enhances chemodenitrification. In another study with this soil not reported here, it was observed that pH increased from 7.5 to about 7.9 over 25 days at -1°C whereas, at 30°C, the pH decreased to 6.8 -7.1 over a 5 days period of incubation.

TABLE 1. Estimated maximum concentration of nitrite and day of its occurrence during incubation with different C substrates and temperatures in exps. 1 and 2.

Experiments	Temperature (°C)	Max. conc. (mg N kg^{-1})	Day	Max. conc. (mg N kg^{-1})	Day
		Control		Guar	
Exp. 1	0	9	9	39	9
	2	7	7	37	9
	5	5	5	46	7
	20	9	2	31	2
	30	11	1	19	1
		Glucose		Guar	
Exp.2	-1	31	17	37	14
	0	26	13	31	9
	5	21	8	24	7
	15	17	3	30	3
	20	17	3	21	3
	30	11	2	19	1

Ammonium accumulation

The NH_4^+ concentration at the time in each incubation period when N_2O production reached a maximum with the guar treatment, was very low at all temperatures except -1°C with the glucose treatment in exp.2 (Table 2). On the other hand, the NH_4^+ concentration was higher and generally increased as temperature increased with the guar treatment, indicating a release (or ammonification) of the organic N in guar. Taken together with similar data from

exp. 1. it is evident that denitrification was less affected than ammonification at low temperatures.

Under the conditions of these experiments it is quite likely that NH_4^+ was immobilized by microorganisms to produce organic N. especially with glucose (De Catanzaro *et al.*, 1987). That some of the NH_4^+ could have been derived from dissimilatory nitrate reduction (De Catanzao *et al.*, 1987) does not seem to be significant in as much as the quantity of N_2O produced was stoichemetrically similar to the quantity of NO_3^- present at the beginning of each experiment. Although apparent complete recovery of NO_3^- as N_2O occurred, it was observed by Flather (1989) that acetylene partially suppresses NH_4^+ production from NO_3^- by fermenter organisms when glucose was present. The extent of acetylene interference with this process at various temperatures is not clear in this study.

TABLE 2. Ammonium concentration in soil with different C substrates at different temperatures in exps. 1 and 2.

Experiment 1				Experiment 2			
Temp. (°C)	Day[z]	Control (mg N kg^{-1})	Guar (mg N kg^{-1})	Temp. (°C)	Day[z]	Glucose (mg N kg^{-1})	Guar (mg N kg^{-1})
0	26	6	31	-1	27	21	0
2	18	7	31	0	18	2	13
5	12	7	31	5	11	2	17
20	5	7	36	15	5	3	21
30	3	7	56	20	5	2	31
				30	3	2	28

[z]The day recorded was that when N_2O production reached a maximum with the guar treatment in each experiment.

N_2O production rates vs temperature

The Arrhenius equation has been used to describe the effect of temperature on denitrification rate (Mckenney *et al.*, 1980) although Focht and Verstraete (1977) suggested its use was not appropriate for temperatures below about 20°C. McMeekin *et al.* (1988) indicated that the activation energy term (slope) of the Arrhenius equation appears to decrease as temperature increases in terms of microbial growth. They suggested that a square root model was an acceptable alternative and applied it to many data sets to demonstrate its suitability for a wide range of temperatures and growth of different microorganisms. The square root model proposed by McMeekin *et al.* (1988) is given as follows:

$$\sqrt{K} = b\,(T - T_{min})$$

Where: K = specific growth rate, b = constant (slope of linear regression line), T=temperature (K), and T_{min} = temperature (K) where the regression line intercepts the temperature axis.

Although K usually represents the growth rate of a microbial population where nutrients are not limiting, it was taken to be the rate of N_2O production where NO_3^- was not limiting in a saturated soil in the current study. Thus K values were determined for each temperature from the linear increase in N_2O concentration following the initiation of anaerobic incubation. The occasional slight lag effect was ignored. Regression coefficients for the initial linear phase of N_2O production over time were 0.90 or greater and highly significant. It is noted here parenthetically that a plot of Ln K vs. $1/T$ according to the Arrhenius equation did not result in a significant departure from expected linearity, as also observed by McKenney *et al.* (1980). It was decided to use the square root model because it provides a minimum temperature. It simply provides an empirical relationship, like the Airhenius equation, but is easier to read.

The data for exps. 1 and 2 in Fig. 3 show that the relationship between $\sqrt{K}$ temperature was linear down to - 1°C (soil unfrozen). The data for exp. 1 show that the rate of N_2O production was enhanced with the addition of guar over the whole temperature range studied.

Denitrifiers were active at 0 or 1°C and, according to Fig. 3, would be active down to a T_{min} of - 13°C. This would suggest that they were psychrotrophs according to McNleekin *et al.* (1988). These authors indicated, however, that T_{min} values may be "notional" temperatures and that microbial growth or activity may actually cease at a higher temperature. They also suggested that "water activity" could be altered (soil freezing) which would alter microbial activity or cause it to cease. In addition, it is apparent from Fig. 3 that T_{min} values are very dependent on the slope of the $\sqrt{K}$ vs. temperature relationship.

The addition of guar in exp.2, while showing a higher rate of N20 production than with glucose, produced a different slope of the v-K vs. temperature relationship compared with other data in exps. I and 2. The K values for guar from exp.2 appeared to correspond well with those obtained for experiment 1 except at 15, 20 and 30°C.

Symbol	Exp	Substrate	b	r
C4	1	None	0.154	0.97**
C3	1	Guar	0.200	0.99**
C2	2	glucose	0.210	0.99**
C1	2	Guar	0.298	0.99**

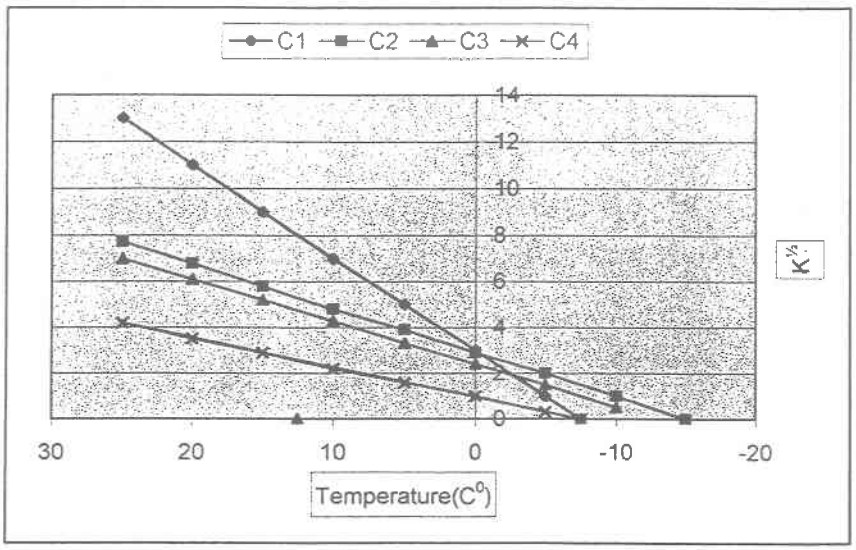

Fig. 3. Relationship between square root of rate of N_2O production, K, and temperature with data from exp.

There was no apparent change in slope of the $\sqrt{K}$ vs. temperature relationship at approximately 11°C as observed by Stanford *et al.* (1975) with a log K vs. T relationship or at 12°C as suggested by Focht and Verstraete (1977) with the Ln K vs. $1/T$ relationship.

Third experiment

Freezing of the soil at-1°C resulted in significant decreases in N_2O and CO_2 production as compared to unfrozen soil at the same temperature (Table 3). Production of N_2O increased more with the guar treatment between 11 and 24 days in the unfrozen soil than with the glucose treatment. These data showed that soil freezing at 1°C, while not preventing denitrification, certainly reduced denitrifier activity. The decrease with freezing was likely due to reduced NO_3^- availability. Reports of large N_2O emissions following soil thawing may be

relevant although anaerobic conditions normally were not considered to be involved (Goodroad & Keeney, 1984 and Christensen & Tiedje, 1990).

It is noteworthy that CO_2 production virtully ceased after day 11 whereas the rate of N_2O production continued to increase in the frozen soil. The increase in N_2O production from day 11 to 24 may be part of a lengthy lag phase or temperature adaptability of psychrotrophic denitrifiers (Zachariah and Liston, 1973).

TABLE 3. Production of N_2O and CO_2 during incubation of saturated soil with glucose or guar while frozen or unfrozen (supercooled) at -1°C.

Substrate added	Day	N_2O		CO_2	
		Forzen (mg N kg^{-1})	Unfrozen (mg N kg^{-1})	Forzen (mg C kg^{-1})	Unforzen (mg C kg^{-1})
Glucose	11	1.9 (0.5)[z]	16.3 (1.3)	3.7 (0.4)	7.1 (0.9)
	24	8.5 (2.5)	48.6 (2.1)	4.2 (1.2)	11.0 (0.5)
Guar	11	1.2 (0.5)	17.5 (0.3)	4.4 (0.2)	7.2 (0.7)
	24	15.8 (2.0)	89.0 (2.0)	5.3 (0.5)	14.2 (0.8)

[z]Standard error in parentheses.

Discussion

Kaplan *et al.* (1977) and El-Sayed (1995) studied denitrifiers response to temperature in relation to seasonal temperature at the time of sampling of salt marsh sediments. Response to temperature imposed in the laboratory increased as the temperature at sampling increased. Similar observations on seasonal temperature effects have not been made with agricultural soils. Powlson *et al.* (1988), Negm *et al.* (1998) and El-Gazzar (2000) found that soils from different climatic zones responded differently to laboratory temperatures. For example, they found that an English soil (stored at 5°C for 4 weeks) denitrified much more rapidly at low temperatures (5, 10 and 15°C) than an Australian soil (stored at 25°C for 4 weeks). They concluded that denitrifiers were adapted to their environment so that those in temperate zone soils are able to denitrify more rapidly at low temperatures than those in tropical soils. This conclusion agrees with that of Gamble *et al.* (1977) and Metwally & Khamis (1998). Kaplan *et al.* (1977) and El-Sayed (1998) suggested that physiological adaptation on a seasonal basis does not occur, contrary to Zachariah and Liston (1973). Rather, Kaplan *et al.* (1977) and Hassanein & El-Shebiny (2000) suggested that at least

two distinct populations of denitrifiers become active depending on temperature conditions. In a study involving salt marsh sediments, King & Nedwell (1984) and Khamis & Metwally (1998) observed a significant seasonal variation in the rate of NO_3^- reduction. They suggested that a psychotropic denitrifier population was mainly active during the cooler parts of the year whereas a mesotrophic denitrifier population was mainly active in the warmer seasons.

The findings of Kaplan *et al.* (1977), King & Nedwell (1984), Powlson *et al.* (1988) and El-Sayed & Abo El-Wafa (2001) suggest that the results of the current study should be interpreted with consideration of the date of soil sampling (20 July 2000 for exp. 1 and 16 May 2001 for exp.2). It may be speculated that the difference in the $\sqrt{K}$ vs. temperature relationship for the guar treatment between exps. 1 and 2 was due to sampling date. The effect of sampling date, however, could also be affected by the sample storage conditions. In this study, the samples were stored in the frozen state (-15°C) for varying lengths of time followed by an aerobic adaptation period of 6 days at 20°C before denitrification measurements commenced. Breitenbeck and Bremner (1987) and El-Sayed (1997 a&b) found that storing soil in a frozen state (-4°C) increased the denitrification rate and that the effect was not solely due to increased soil organic C availability. Thus, greater attention probably needs to be given to environmental temperatures prior to sampling and during soil storage and pretreatment temperatures in laboratory studies.

In spite of possible effects of sampling data and storage conditions, it is evident that denitrification can occur at soil temperatures at or lower than 0°C. Also, it is apparent that the rate of denitrification at any temperature was dependent on organic C substrate supply.

Conclusions

As the organic substrate supply decreased, the threshold temperature for denitrification may increase and could be higher than 0°C. This provides an explanation for some of the findings of others cited earlier who observed that the threshold temperature for the denitrification process was considerably higher than 0°C. Alternatively, the adaptability of denitrifiers from different climatic regions to low soil temperature conditions may explain differences in threshold temperature. In this study, it was evident that denitrifiers could function at -1°C providing the soil was not frozen.

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تأثير حرارة التربة على إنطلاق الآزوت والنشدرة

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صممت تجربة معملية لدراسة تأثير درجة الحرارة من -١ إلى ٣٠°م على إنطلاق الآزوت والنشدرة تحت ظروف لا هوائية فى تربة معاملة بالجوار أو الجلوكوز.

أوضحت النتائج مايلى:

* مادة التفاعل العضوية مثل الجوار أو الجلوكوز تزيد من معدل إنطلاق الآزوت فى جميع درجات الحرارة في هذا المدى المذكور، ثم تنخفض درجة الحرارة فى بداية حدوث إنطلاق الآزوت.

* بداية درجة الحرارة لإنطلاق الآزوت كانت منخفضة عند -١°م تربة غير مجمدة، وعلى العكس من ذلك فى معظم الدراسات الأخرى حيث بداية درجة الحرارة كانت مساوية أو أعلى من الصفر. عندما كانت التربة مجمدة عند -١°م،فإن معدل إنطلاق الآزوت كان منخفضا بكثرة عن التربة الغير مجمدة عند نفس درجة الحرارة. صورة الجذور المستعملة في المتر المربع لمعدل إنطلاق الآزوت كان خطا مستقيما فى درجات الحرارة مابين -١ ،٣٠°م. الكمية العظمى من NO_2^- (النتريت) المنتجة خلال فترة التحضين تتناقص علي وجه العموم من -١، ٣٠م وتكون أعلى ما يمكن فى وجود الجلوكوز وخصوصا عند إضافة الجوار.

* يزداد معدل النشدرة عند إضافة الجوار ولكن كمية NH_4^+ (الامونيوم) المنتجة تتناقص على وجه العموم من ٣٠م إلى -١م.