Soil Biol. Biochem. Vol. 23, No. 9, pp. 845–855, 1991
Printed in Great Britain.

0038-0717/91 $3.00 + 0.00

DENITRIFICATION: ENZYME CONTENT AND ACTIVITY IN DESERT SOILS

WILLIAM T. PETERJOHN*
Department of Botany, Duke University, Durham, NC 27706, U.S.A.

(Accepted 8 March 1991)

Summary—The content of denitrifying enzymes in upland desert soil was strongly associated with indices of N and C availability. Combinations of several predictors could explain 71% of the variance in enzyme content in Chihuahuan desert soils and 87% of the variance in soils from various deserts in the southwestern U.S.A. A significant fraction of the enzyme content in wet desert soil is derived from a persistent pool of enzymes capable of tolerating extended periods of desiccation. The synthesis of new denitrifying enzymes appears to involve a complex interaction between available C, N, and soil moisture.

The activity of denitrifying enzymes in desert soil was optimal at a pH of 7.0 and a temperature of 40°C. The Q_{10} for denitrification was 1.74, and the activation energy was about 41 kJ mol^{-1}. In addition, enzyme activity in freshly wet soil was not limited by NO_3^- availability, and only slightly limited by the availability of C. Thus, wet desert soil appears to provide optimal conditions for several variables that can influence denitrification.

INTRODUCTION

Denitrification is the microbial reduction of NO_3^- or NO_2^- to either N_2O or N_2. This reaction allows a wide variety of bacteria to use NO_3^- or NO_2^- as the terminal respiratory electron acceptor under low O_2 conditions. The bacteria capable of denitrification are generally heterotrophs with a widespread distribution (Tiedje *et al.*, 1982; Tiedje, 1988). Many factors are known to affect the rate of denitrification. These include pH, temperature, C and N availability, and the partial pressure of O_2 (Firestone, 1982; Knowles, 1982). In general, however, high rates of denitrification are associated with wet, nutrient-rich environments.

Desert ecosystems would seem unsuitable for the process of denitrification. Most desert soils are hot, dry and nutrient-poor. Surface soil temperatures can exceed 60°C in the summer and drop below freezing in the winter. Mean annual rainfall is generally <25 cm, and some regions can go for more than 12 months without precipitation. Yet, the rate of denitrification in wet desert soil is comparable to, if not greater than, that measured in more mesic ecosystems (Virginia *et al.*, 1982; W. T. Peterjohn, unpublished Ph.D. thesis, Duke University, 1990).

In ecosystems where few nutrients are lost in stream flow, the production of nitrogen gases by denitrification assumes greater importance as a vector of nutrient loss. A nitrogen budget for the Great Basin desert estimated that 19 kg N ha^{-1} yr^{-1} were lost by denitrification (West and Skujins, 1977). This amount represented 95% of annual losses and 65% of total N inputs. On a regional scale, it appears that >77% of the N inputs to the deserts of the southwestern U.S.A. are lost to the atmosphere (Peterjohn and Schlesinger, 1990). From a global perspective, emissions of N gas from arid land ecosystems may amount to 30% of the total loss from terrestrial ecosystems with most of this loss attributed to denitrification (Bowden, 1986).

Few studies have investigated denitrification in desert ecosystems despite its occurrence under apparently unsuitable conditions, and its possible significance to desert fertility. Therefore, the purpose of this study was to investigate the factors affecting denitrification in desert soils. The effect of several variables on both the production and activity of denitrifying enzymes will be considered.

MATERIALS AND METHODS

Study sites

To encompass the natural variability present in desert landscapes, I sampled 3 bajadas derived from different parent materials, in each of 3 deserts: the Chihuahuan; Mojave; and Great Basin. Soil samples were also collected from nearby playas. The location and features of each site are described in Table 1.

Field methods

I collected samples of surface soil (4–8 cm depth) along transects oriented perpendicular to the length of each bajada (Peterjohn, *loc. cit.*). Between the transects additional samples were collected for a total of 107 samples from each bajada. Soils were also collected from 8 playas. All soils were collected during the dry season, sieved (<2 mm), and stored in water-tight containers. Equal mass subsamples of the soil from each transect were mixed to form 5 composite samples for each bajada, and 1 for each playa. An additional composite sample was created for each of the bajadas in the Chihuahuan desert using equal mass from all 107 soil samples.

*Present address: The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA 02543, U.S.A.

Table 1. Location, geology, land use, and vegetation for each study site. Only the three most dominant woody perennials are listed. Important values (i.v.) are based on the relative density and dominance of each species (Mueller-Dombois and Ellenberg, 1974)

State	Site	Location	Desert	Geology		Landuse	Vegetation	
				Mountain range	Bedrock		Dominant woody perennials	i.v. (%)
N.M.	1	40 km NNE Las Cruces	Chihuahuan	Dona Ana	Rhyolite/Andesite	Mixed	*Larrea tridentata*	47
							Florensia cernua	21
							Guterizia sarothrae	10
N.M.	2	10.4 km E Anthony	Chihuahuan	Franklin	Mixed sedimentary (mostly limestone)	Grazed	*L. tridentata*	38
							Parthenium incanum	32
							G. sarothrae	16
N.M.	3	40 km NNE Las Cruces	Chihuahuan	Dona Ana Mt Summerford	Monzonite	Ungrazed	*L. tridentata*	40
							G. sarothrae	37
							Zinia acerosa	9
Calif.	1	14 km NNE Desert Center	Mojave	Coxcomb	Metasedimentary	Ungrazed	*Encelia farinosa*	38
							Ambrosia dumosa	17
							L. tridentata	12
Calif.	2	22.5 km W Desert Center	Mojave	Eagle	Gneiss	Ungrazed	*L. tridentata*	40
							A. dumosa	13
							Chrisothamnus nauseous	9
Calif.	3	9.6 km W Desert Center	Mojave	Eagle	Granite	Ungrazed	*A. dumosa*	42
							L. tridentata	27
							Hymenoclea salsola	14
Nev.	1	75.5 km N Wadsworth	Great Basin	Selenite	Quartz monzonite/ Granodiorite	Grazed	*Atriplex confertifolia*	57
							Artemisia spinescens	29
							Suaeda torreyana	6
Nev.	2	50.5 km ENE Fallon	Great Basin	Stillwater	Mixed sedimentary	Grazed	*Atriplex confertifolia*	51
							Suaeda torreyana	30
							Sarcobatus vermiculatus	16
Nev.	3	36.8 km NNW Wadsworth	Great Basin	Lake	Basalt/Andesite	Mixed	*Sarcobatus baileyi*	57
							Artemisia spinescens	25
							Sarcobatus vermiculatus	8

Laboratory methods

General procedure. To investigate factors affecting denitrifying enzyme production and denitrifying enzyme activity (DEA), a 2-step process was used. First, dry soils were incubated under controlled conditions to allow bacterial growth and the synthesis of denitrifying enzymes. Second, the enzyme content of incubated soils was fixed by adding chloramphenicol and measured by an *in vivo* DEA assay (Smith and Tiedje, 1979; Murray *et al.*, 1989). By altering the conditions of the initial incubation, differences in the amount of enzyme produced could be measured. By altering the conditions of the DEA assay itself, differences in enzyme activity could be measured.

Incubation procedure. The standard incubation procedure was to place 10 g of dry soil into a 120 ml serum bottle, with enough distilled water to achieve a matrix potential of −0.05 MPa. The bottle was capped and incubated aerobically for 3 days at 30°C. A matrix potential of −0.05 MPa was chosen because it is typical of surface soils during wet periods in the Sonoran and Chihuahuan deserts (Young and Noble, 1986; Schlesinger *et al.*, 1987). An equation generated from 53 composite soil samples was used to calculate the amount of water needed to achieve −0.05 MPa in each soil (Peterjohn, *loc. cit.*).

Assay procedure. The standard procedure was to add 10 ml of an assay solution to the incubated soil, make the headspace anaerobic by alternately evacuating and flushing it with Ar, and then overpressure the sealed bottles with 20 ml of CaC_2-generated C_2H_2 and 60 ml of Ar. The assay solution contained 100 mM NaH_2PO_4 buffer (pH = 7.00), 35.2 mM KNO_3, 6.94 mM dextrose, and 1.5 g chloramphenicol l^{-1}. Preliminary work demonstrated that these concentrations of N and C did not limit enzyme activity. Serum bottles were kept at 30°C, and 20 ml gas samples were removed at four, 1 h intervals. Gas samples along with certified standards were stored in evacuated vials for later analysis of N_2O using a gas chromatograph equipped with a ^{63}Ni electron capture detector (Peterjohn, *loc. cit.*). At the end of the assay procedure the air volume in each bottle was determined using a pressure transducer (Parkin *et al.*, 1984). All DEA values were corrected for N_2O dissolved in the assay solution (Wilhem *et al.*, 1977; Tiedje, 1982), the dilution due to the overpressure, and the amount of N_2O removed by each sampling event. Since the DEA was determined under non-limiting conditions and in the presence of chloramphenicol, differences in activities represent differences in the content of denitrifying enzymes present in the soil immediately prior to the addition of the assay solution (Smith and Tiedje, 1979; Tiedje, 1982; Tiedje *et al.*, 1989).

Enzyme content and production studies

Predictors of enzyme content. The standard soil incubation and DEA assay procedures (described above) were used to determine the denitrifying enzyme content. Soil variables potentially associated with denitrifying enzyme content were also measured in each soil (Peterjohn, *loc. cit.*). The variables included: % sand, silt, and clay; pH; % total N; % total C; % carbonate C; % organic C; nitrate-N concentration; ammonium-N concentration; 7-day nitrification potential; wet soil CO_2 production; and the C:N ratio. Bivariate plots between the denitrification enzyme content and the other soil variables were used to identify outliers, and any linear or non-linear relationships. Simple linear regression and stepwise multiple linear regression procedures were then used to establish the strength of these relationships. This analysis used all soil samples from the Chihuahuan

desert, and the composite samples for each transect in the Mojave and Great Basin deserts.

Effect of C and N on enzyme production. Composite samples from the 3 bajadas in New Mexico were used in a factorial experiment to investigate the effect of water, C, and N availability on denitrification enzyme production. The factorial experiment consisted of assigning three subsamples from each soil to 1–5 treatments: (1) 1 ml of distilled water resulting in a water potential of about −0.05 MPa; (2) 1 ml of a solution containing 134.8 mg N l^{-1} as KNO_3; (3) 1 ml of a solution containing 2940 mg C l^{-1} as dextrose; (4) 1 ml of a solution containing both C and N in the same amounts used in treatments 2 and 3 (C:N = 21.8); and (5) a control with no additions to the dry soil. After mixing in treatment solutions, the soils were incubated aerobically for 24 h at room temperature (23°C). By altering the conditions of the initial incubation, the effect of these variables on enzyme production could be studied. Following the incubation period, DEA was determined using the standard assay procedure as previously described.

A second factorial experiment was performed using the composite sample from a single bajada in New Mexico (NM 3; Table 1). The purpose of this experiment was to determine whether the manner of the treatment application could effect enzyme production. This experiment was identical to the first with 2 exceptions: soils were incubated in perforated 10 ml plastic syringes rather than in 120 ml serum bottles; and 5 ml of the treatment solutions were added to the top of each soil column rather than mixing 1 ml of the solutions into the soil. These conditions were chosen because they approximate those of a field experiment conducted on intact soil cores taken from the same site (Peterjohn, *loc. cit.*). After the incubation, soils were transferred to serum bottles and the DEA assayed in the standard manner.

Enzyme persistence in dry soil. A final experiment examined the ability of denitrifying enzymes to persist in dry desert soil. All soils in this study were collected during the dry season and stored in this condition (*ca.* 2% water) for over 2 yr. Comparison was made between the denitrifying enzyme content of these soils and the enzyme content of the same soils after 3 days of simulated wet season conditions (−0.05 MPa and 30°C). The enzyme content of the dry soil was measured by the standard DEA assay without any preliminary incubation period. The enzyme content of wet soil was measured following the standard incubation and assay procedures. All composite soil samples from each transect in the Mojave and Great Basin deserts were used. Soils from the Chihuahuan desert were not included because the factorial experiments performed on those soils already included a dry vs wet soil comparison.

Enzyme activity studies

Separate experiments examined the effects of pH, temperature, and N and C availability on denitrifying enzyme activity. The general procedure involved incubating subsamples of soil to provide replicates with equivalent enzyme contents, and then altering the conditions of the DEA assay in order to measure the effect of a variable on enzyme activity. The specific incubation conditions depended on the experiment, so each will be described separately. All experiments used composite soils (107-sample composite) from each bajada in the Chihuahuan Desert.

Effect of pH on DEA. To investigate the effect of pH on DEA, 5 g subsamples of each composite soil were incubated anaerobically at −0.05 MPa for 3 days at 30°C. Following incubation, subsamples from each composite soil were randomly assigned to 1 of 11 assay solutions, and DEA measured. Except for the pH buffer, the assay solution was identical to the standard solution described previously. Different pH levels were attained by titrating a modified universal buffer (MUB; Skujins *et al.*, 1963; Tabatabai, 1982) to one of the following pH values: 3, 4, 5, 6, 6.5, 7, 7.5, 8, 8.5, 9 and 10. Since MUB is a relatively weak buffer, 20 ml of the assay solution were added to the soil and the final pH of the slurry was measured. The final pH of the soil slurry was often higher than the pH of the assay solution due to the abundance of $CaCO_3$ in desert soil. Three replicate samples for pH levels 6, 7 and 8 were analyzed for each composite soil. One replicate was analyzed for the remaining pH levels.

Effect of temperature on DEA. To investigate the effect of temperature on DEA, 5 g subsamples of each composite soil were incubated anaerobically at −0.05 MPa for 3 days at 30°C. Following incubation, subsamples from each composite soil were randomly assigned to 1 of 9 assay temperatures, and DEA was measured. The assay solution contained MUB (pH = 6.00), but was otherwise identical to the standard assay solution. Adjusting the buffer to a pH of 6.00 ensured that the final soil slurry (20 ml solution:5 g soil) had a pH between 7.06 and 7.27. The assay temperatures were in the range 10–85°C (see Fig. 8). Three replicate samples for the 20, 40 and 60°C treatments were analyzed for each composite soil. One replicate was analyzed for the remaining temperatures.

Effect of C and N on DEA. A final factorial experiment examined the effect of C and N availability on enzyme activity in wet desert soil. Sixteen, 10 g subsamples from the composite soil of each site were incubated aerobically for 3 days at −0.05 MPa and 30°C. Following incubation, 4 subsamples from each site were randomly assigned to 1 of 4 treatments. The treatments included: (1) an assay solution with supplementary C (6.94 mM dextrose) but no supplementary N; (2) an assay solution with supplementary N (35.2 mM KNO_3) but no supplementary C; (3) an assay solution with both supplementary C and N in the same forms and amounts used in treatments 1 and 2; and (4) a control assay solution with no supplementary C or N. All assay solutions contained 100 mM NaH_2PO_4 buffer (pH = 7.00), and 1.5 g chloramphenicol l^{-1}. The amounts of C and N, when added, were sufficient to saturate denitrification enzyme activity. All subsamples were slurried with 10 ml of the appropriate assay solution before the DEA was measured.

RESULTS

Enzyme content and production studies

Predictors of enzyme content. Indices of C and N availability were strong predictors of the denitrifying

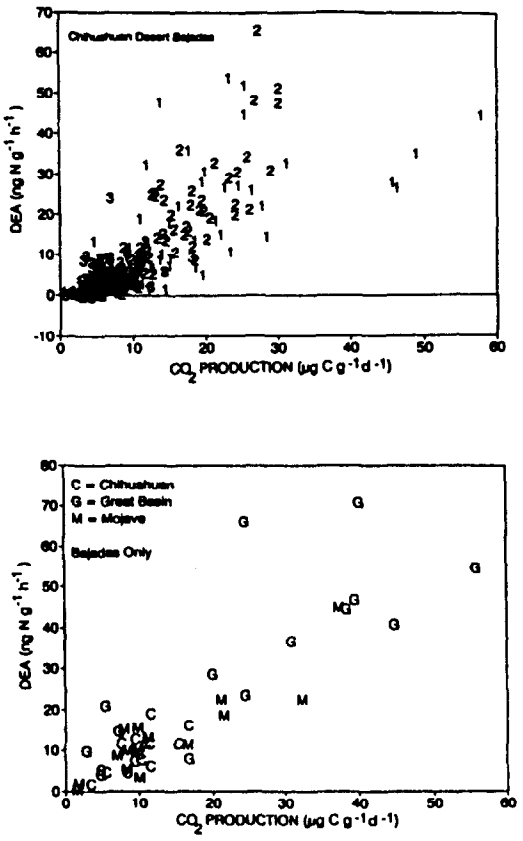


Fig. 1. Relationship between denitrifying enzyme content (measured by DEA) and C availability (measured by CO_2 production) in bajada soils. Relationship for Chihuahuan desert soils (upper graph) has 2 outliers removed. Numbers identify the site. Relationship for composite soils from all deserts (lower graph) has 1 outlier removed. Letters identify the desert.

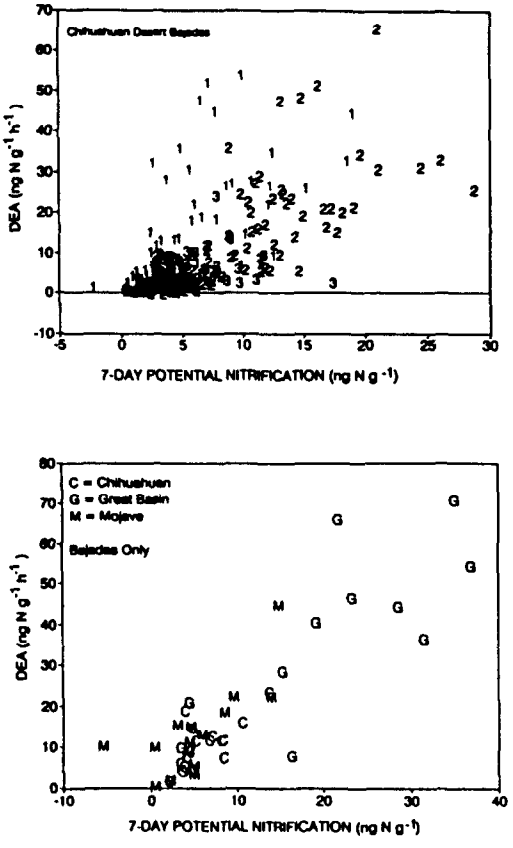


Fig. 2. Relationship between denitrifying enzyme content (measured by DEA) and NO_3^- availability (measured by potential nitrification) in bajada soils. Relationship for Chihuahuan desert soils (upper graph) has 2 outliers removed. Numbers identify the site. Relationship for composite soils from all deserts (lower graph) has 1 outlier removed. Letters identify the desert.

enzyme content in wet bajada soils, both within the Chihuahuan desert and between the major deserts of the southwestern U.S.A. (Figs 1 and 2). In the Chihuahuan desert, enzyme content correlated strongly with CO_2 production, potential nitrification, and total N (Table 2). For all deserts, enzyme content in composite samples correlated strongly with potential nitrification, CO_2 production, and silt content (Table 2). Various combinations of predictors could explain 71% of the variation in enzyme content for Chihuahuan desert soils, and 87% of the variation for composite samples from all deserts (Table 3). Although pH was not strongly associated with denitrifying enzyme content, high values (DEA > 15 ng N g^{-1} h^{-1}) were found only in soils with a pH ranging between 7.5 and 8.2. This suggests that pH limits the production of denitrifying enzymes beyond a certain range.

Playa soils frequently deviated from the patterns found when only bajada soils were considered (Fig. 3). Playas tended to have finer soil textures, higher pH values, and extreme (high or low) denitrifying enzyme content. When all playas were considered, no strong predictors of denitrifying enzyme content were identified. However, one of the playas in the Chihuahuan Desert exhibited a strong association between enzyme content and CO_2 production ($r = 0.722$; $P < 0.0120$).

Effect of C and N on enzyme production. In the first experiment, where 1 ml of the treatment solutions was mixed into the soil, the following treatment effects were found to be significantly different: water + NO_3^- = water > control = water + C + NO_3^- = water + C ($P < 0.05$; Fig. 4). This pattern was found in all 3 Chihuahuan desert bajadas. In the second experiment, where 5 ml of the same treatment

Table 2. Predictors of DEA in desert soil under simulated wet season conditions based on simple linear regression. Only samples from bajadas are included

Category variate	n	Pearson correlation coefficient	Probability level
Chihuahuan desert (2 outliers removed)			
CO_2 production	319	0.795	0.0001
Potential nitrification	319	0.670	0.0001
Total N	319	0.636	0.0001
All deserts (1 outlier removed)			
Potential nitrification	44	0.863	0.0001
CO_2 production	44	0.853	0.0001
Silt	44	0.708	0.0001

Table 3. Predictors of DEA in desert soil under simulated wet season conditions based on stepwise multiple linear regression. Only samples from bajadas are included. Within each category the model r^2 values are cumulative

Cateogry variate	Partial r^2	Model r^2	Probability level
Chihuahuan desert			
(2 outliers removed)			
CO_2 production	0.632	0.632	0.0001
Potential nitrification	0.026	0.658	0.0001
Silt	0.014	0.673	0.0002
Organic C	0.027	0.700	0.0001
Carbonate C	0.004	0.704	0.0436
pH	0.006	0.710	0.0146
Clay	0.002	0.712	0.1350
All deserts			
(1 outlier removed)			
Potential nitrification	0.745	0.745	0.0001
Silt	0.063	0.808	0.0007
Organic C	0.031	0.839	0.0079
Ammonium N	0.023	0.862	0.0155
pH	0.010	0.872	0.1004

solutions were added to the top of soil columns, the following treatment effects were significantly different: water + C + NO_3^- ⋙ water + NO_3^- > water = control = water + C ($P < 0.05$; Fig. 5). Significant differences for a given site were determined by 1-way analysis of variance (ANOVA) followed by a Tukey's multiple comparison of the means (SAS, 1982).

Enzyme production (as measured by DEA) in both the first and second experiments was similar in the control (7.23 vs 8.43 ng N g^{-1} h^{-1}), water (9.59 vs 11.88 ng N g^{-1} h^{-1}), and water + C (5.69 vs 5.57 ng N g^{-1} h^{-1}) treatments. However, differences between the first and second experiments were found for the water + NO_3^- (11.91 vs 30.46 ng N g^{-1} h^{-1}), and water + C + NO_3^- (5.36 vs 89.73 ng N g^{-1} h^{-1}) treatments. In both experiments, more than 70% of the enzyme content in the water treatments was originally

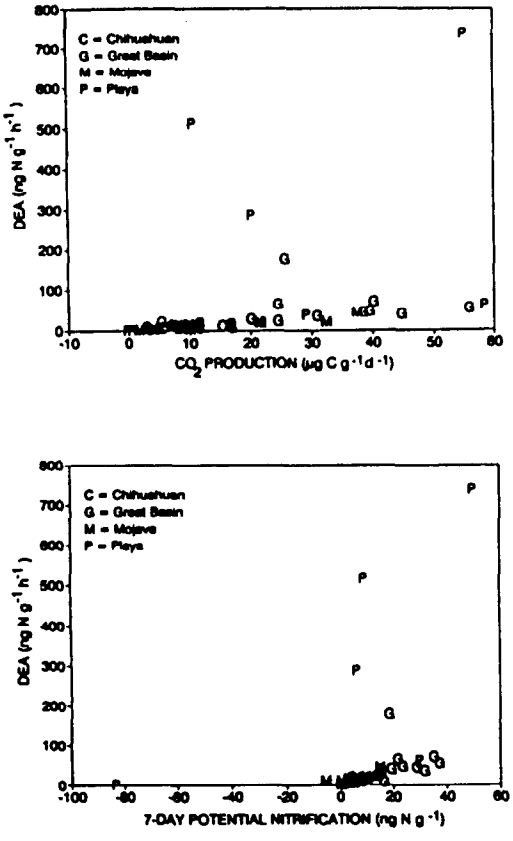


Fig. 3. Bivariate plots of CO_2 production and potential nitrification vs the denitrifying enzyme content of all soils (bajada + playa).

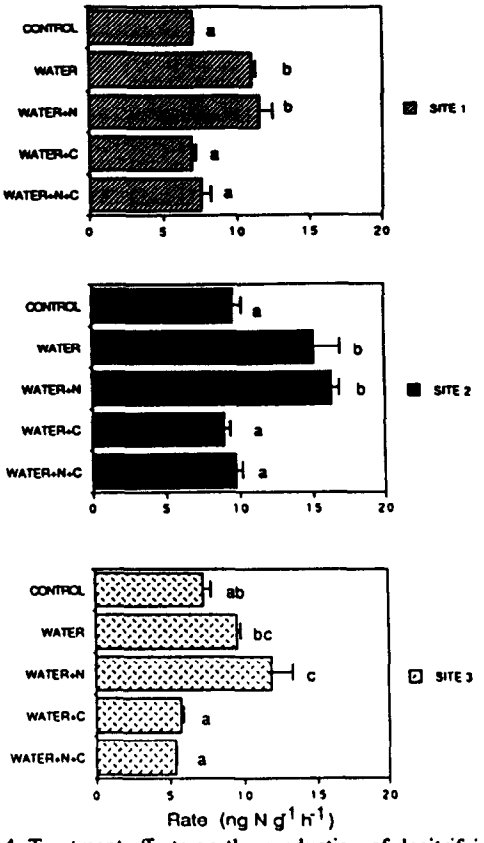


Fig. 4. Treatment effects on the production of denitrifying enzymes after 1 ml of treatment solution was mixed into soil. Soils were composite samples from 3 sites in the Chihuahuan desert. Bars with different letters indicate significant differences ($P < 0.05$). Horizontal lines represent 1 SEM.

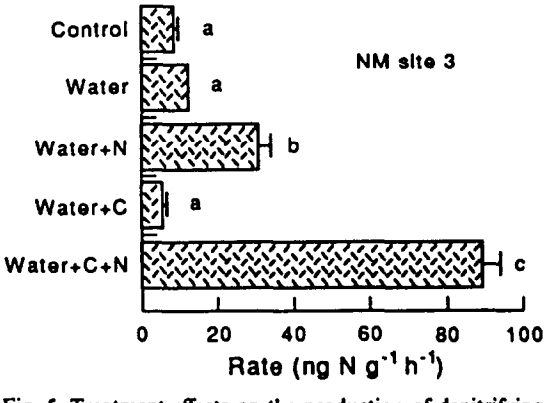


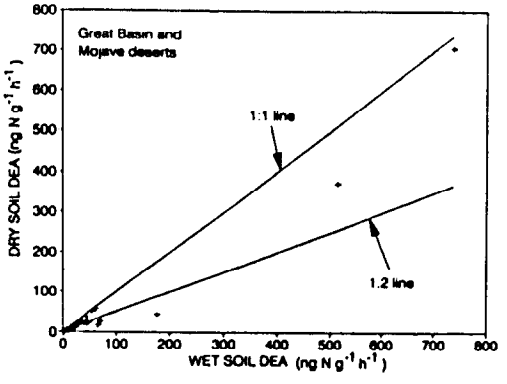
Fig. 5. Treatment effects on the production of denitrifying enzymes after 5 ml of treatment solution were poured on to soil columns. Soil was a composite sample from NM site 3. Bars with different letters indicate significant differences ($P < 0.05$). Horizontal lines represent 1 SEM.

present in the dry soil (controls) indicating that persistent denitrifying enzymes are important in the Chihuahuan desert.

Enzyme persistence in dry soil. Persistent denitrifying enzymes are also important in the Mojave and Great Basin deserts. Typically, >50% of the enzyme content in wet desert soil was present in the dry soil, even after storage for >2 yr (Fig. 6). Wet and dry soil enzyme contents were strongly correlated ($r = 0.939$; $P < 0.0001$). However, the ratio of dry to wet soil enzyme content was not strongly associated with any of the measured soil parameters. CO_2 production was the best single predictor of the ratio of dry to wet soil enzyme content ($r = 0.428$; $P < 0.0103$), and was the only variable entered in stepwise multiple linear regression.

Enzyme activity studies

Effect of pH on DEA. The dependence of DEA on the pH of the incubation solution was bell-shaped with a narrow optimum range centered around a pH of 7.0 (Fig. 7). Enzyme activity was reduced by at least 50% if the pH was <6 or >8, and very low activity was found when the pH was <5 or >9. Good agreement was found between the observed data and the equation for a diprotic system where successive pK_a values are closer than 3.5 pH units (Segel, 1975; Alberty, 1983). This equation is:



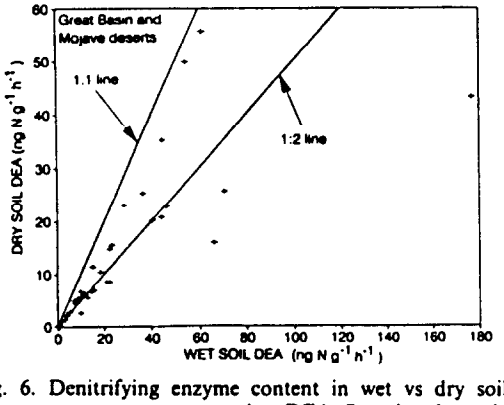


Fig. 6. Denitrifying enzyme content in wet vs dry soil. Enzyme content was measured as DEA. Samples above the 1:2 line had >50% of their wet soil enzyme content originally present in the dry soil. Only samples with a DEA of <200 ng N g^{-1} h^{-1} are plotted in the lower graph.

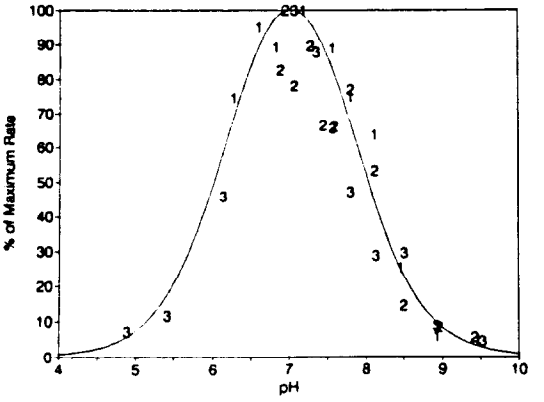


Fig. 7. The effect of pH on denitrifying enzyme activity. Soils were composite samples from the Chihuahuan Desert. Numbers identify the study site. The curve was generated from the theoretical equation for a diprotic system.

$$V_{obs} = V_{max}/(1 + ([H^+]/K_{a1}) + (K_{a2}/[H^+])), \quad (1)$$

where V_{obs} = observed rate, V_{max} = rate when all the enzyme is in the active form, $[H^+]$ = hydronium ion concentration, and K_{a1} and K_{a2} = first and second acid dissociation constants (Price and Stevens, 1982). The equation was fit using a multivariate nonlinear procedure (SAS, 1982) which estimated V_{max} to be 21.32 ng N g^{-1} h^{-1}, and K_{a1} and K_{a2} to be 5.89×10^{-7} and 1.47×10^{-8}, respectively.

Effect of temperature on DEA. Increasing the temperature of the assay solution caused an increase in DEA, up to a temperature of about 40°C. At temperatures higher than 40°C the enzyme activity rapidly decreased, and ceased altogether when temperatures exceeded 60°C (Fig. 8). In the range from 10 to 40°C, the observed activity is in good agreement with that predicted by the Arrhenius equation. This equation is:

$$\ln(V_{obs}) = -(E_a/R)(1/T) + C, \quad (2)$$

where V_{obs} = observed rate, E_a = activation energy, R = gas constant, T = temperature in °K, and C = a constant (Alberty, 1983). A linear regression procedure was used to estimate the slope (4935.7) and intercept (19.452) for Eq. 2. Since the slope estimates

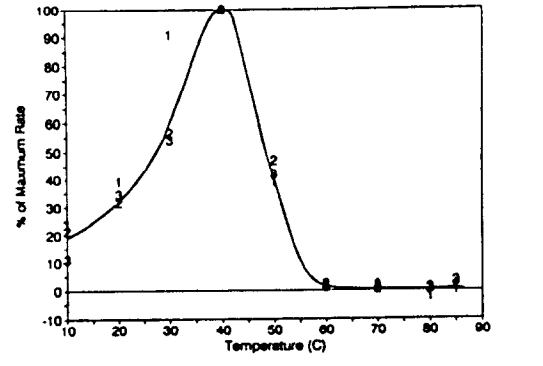


Fig. 8. The effect of temperature on denitrifying enzyme activity. Soils were composite samples from the Chihuahuan desert. Numbers identify the study site. The curve was fit by eye.

E_a/R, multiplying by the gas constant provides an estimate for the activation energy. For this study, the activation energy was about 41 kJ mol^{-1}. This value compares well with the range typical of enzyme catalysis (20.9–62.8 kJ mol^{-1}; Segel, 1975), and is very close to the value calculated by Parker *et al.* (1983) for soil respiration in a wet Chihuahuan desert soil (39.5 kJ mol^{-1}). The Q_{10} for DEA in Chihuahuan desert soil was 1.74.

Effect of C and N on DEA. Enzyme activity showed a slight increase when supplementary C was added to the assay medium, either by itself or in combination with supplementary N (Fig. 9). No increase in activity was detected when supplementary N was added to the assay medium. These results were consistent for all 3 sites in the Chihuahuan desert. Since the controls in this experiment contained only the C and N made available during the 3-day incubation period, the results indicate that under wet season conditions the availability of N does not limit the rate of enzyme activity, and the availability of C only slightly limits enzyme activity.

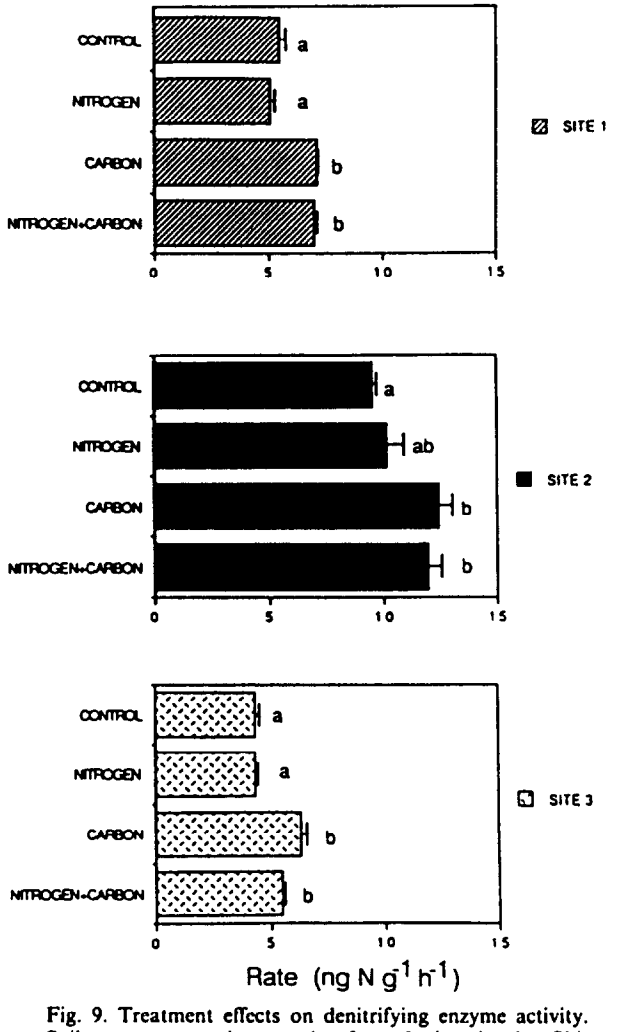


Fig. 9. Treatment effects on denitrifying enzyme activity. Soils were composite samples from 3 sites in the Chihuahuan desert. Bars with different letters indicate significant differences ($P < 0.05$). Horizontal lines represent 1 SEM.

Table 4. Comparison of the denitrifying enzyme content in soils of different habitats

Habitat	Enzyme content as measured by DEA (ng N g^{-1} h^{-1})
Forest soils*	
NC hardwood	84
MI hardwood	84
MI hardwood†	190
NM aspen	364
Venezuelan rainforest	392
Nigerian rainforest	224
Agricultural soils	
GA corn*	28
MI corn*	168
IA corn*	280
MD corn‡	126
MD corn, no-till‡	285
MD corn, no-till§	151
New Zealand pasture*	280
Freshwater sediments*	
White cedar swamp	1820
Farm stream	2100
Eutrophic, pelagic lake	6440
Desert soils¶	
NM bajadas	9
CA bajadas	13
NV bajadas	43
NM playas	192
CA playas	237
NV playas	163

*Tiedje *et al.* (1982).
†Groffman and Tiedje (1989).
‡Parkin *et al.* (1987).
§Parkin and Robinson (1989).
¶Peterjohn, *loc. cit.*

DISCUSSION

Enzyme content and production

The denitrifying enzyme content of a soil is thought to be a long-term integrative index, reflecting the history of environmental variables that change rapidly at a given site (Groffman and Tiedje, 1989; Tiedje *et al.*, 1989). The content of denitrifying enzymes in soil strongly correlates with the annual denitrification rate in forest ecosystems (Groffman and Tiedje, 1989), and has been used to predict the mean denitrification rate in an agricultural soil (Parkin and Robinson, 1989).

The content of denitrifying enzymes in upland desert soils is low (Table 4), and is strongly associated with indices of both C and N availability. This agrees with general ecosystem theories suggesting that nutrient loss is greater in systems with higher nutrient availability (Vitousek *et al.*, 1979, 1982; Matson and Vitousek, 1987). In addition, other studies demonstrate higher rates of denitrification in sites with high nutrient availability (Melillo *et al.*, 1983; Robertson and Tiedje, 1984, 1988), and that denitrifying enzyme content is strongly associated with soil carbon and microbial biomass (Myrold and Tiedje, 1985b; Tiedje *et al.*, 1982).

In desert ecosystems, a significant fraction of the denitrifying enzyme content is derived from a persistent pool of enzymes capable of tolerating extended periods of desiccation. Such desiccation tolerance has been demonstrated in other soils and allows denitrifiers to respond rapidly to favorable conditions (Smith and Parsons, 1985; Groffman and Tiedje, 1988; Martin *et al.*, 1988). Rapid response by denitrifiers may be particularly important in desert

ecosystems where moisture availability is highly episodic. The mechanism for desiccation tolerance of denitrifying enzymes is currently not known.

The production of denitrifying enzymes in desert soils responded to experimental changes in water, C and N availability. In all cases, the addition of water stimulated enzyme production, but the addition of C along with water prevented enzyme synthesis. The effect of added C was probably due to immobilization of available N and indicates that N availability is necessary to initiate enzyme synthesis in desert soils. Laboratory studies have also demonstrated that NO_3^- is either required for or greatly enhances the production of denitrifying enzymes (Firestone, 1982; Korner and Zumft, 1989). Enzyme production in response to N and C + N additions, however, was sensitive to the exact nature of the treatment conditions. When 5 ml of treatment solution was poured onto soil columns, both significant N and C + N response occurred. However, when only 1 ml of the same solutions was mixed into the soil, enzyme production showed no significant N limitation, and the addition of C + N suppressed enzyme formation. The reason for the differential response to N and C + N additions is not known, but the large differences indicate complex interactive effects between C, N and soil moisture.

Enzyme activity

Recent studies of *in situ* denitrification have shown that the temporal variability of enzyme content in a given soil is much lower than the temporal variability of the actual denitrification rate (Smith and Parsons, 1985; Groffman and Tiedje, 1989; Parkin and Robinson, 1989). This suggests that most of the variability in the actual denitrification rate at a given site is due to changes in the activity of existing enzymes rather than changes in the enzyme content of the soil. This is likely to also be the case in desert ecosystems, since desert soils contain a significant pool of persistent denitrifying enzymes, and only small changes in enzyme content result from the addition of water.

Factors known to affect the activity of denitrifying enzymes include: pH; temperature; C and N concentration; and the partial pressure of O_2. The optimal pH for DEA in Chihuahuan desert soil is about 7.0, which agrees with other studies of denitrification (Wijler and Delwiche, 1954; Nommik, 1956; Bremner and Shaw, 1958b; Klemedtsson *et al.*, 1977; Firestone, 1982; Knowles, 1982). The pH of desert soils in the southwestern U.S.A. ranges from 5.47 to 10.91, with a median value of 7.85 for upland soils (Schlesinger and Peterjohn, 1988). Most desert soils are highly buffered by $CaCO_3$, so at any given location there is little temporal change in pH. Thus, pH may have an effect on the spatial variability of DEA in deserts, but little effect on the temporal variability.

Playas should be conducive to denitrification because they often have fine textured soils, a high moisture holding capacity, and relatively high nutrient availability. Indeed, playas in this study had a higher average denitrifying enzyme content than upland desert soils (Table 4). The enzyme content of individual playas, however, represented both extremes and was low in soils with a pH > 8.2 (Peterjohn, *loc. cit.*). In a broader survey of playas in the southwestern U.S.A., Schlesinger and Peterjohn (1988) measured pH values ranging from 8.22 to 10.91. Thus, pH may prevent significant rates of denitrification from occurring in most playa soils. Denitrifiers may adapt to the environmental pH (Parkin *et al.*, 1985), but this is unlikely to have occurred in desert soils because respiration rates in samples with a pH > 8.20 were very low (Peterjohn, *loc. cit.*).

The optimum temperature for denitrifying enzyme activity in desert soils is close to 40°C. This is substantially lower than an optimum of 65°C reported in earlier studies (Nommik, 1956; Bremner and Shaw, 1958b), but agrees with the optimum for soil respiration measured in a Chihuahuan desert soil (Parker *et al.*, 1983). Keeney *et al.* (1979) consider 65°C too high for the true optimum of biological denitrification due to chemical decomposition of NO_2^- at temperatures greater than 50°C.

During the summer, surface soils in deserts can be >10°C hotter than the air temperature, and soils experience diurnal fluctuations of >30°C (Billings, 1978; Nobel and Geller, 1987). Mean minimum and maximum temperatures during July were 22.8 and 46.2°C at a depth of 5 cm in Chihuahuan desert soils (Wierenga, 1988), and about 22.2 and 60°C at a depth of 2.54 cm in Great Basin desert soils (Billings *et al.*, 1954). For the same sites the mean minimum and maximum soil temperatures in January were 1.3 and 13.2°C in the Chihuahuan desert, and about −3.9 and 15.6°C in the Great Basin desert. Temperature differences of 10°C between exposed and shaded soils have also been reported in the Chihuahuan and Sonoran deserts (Parker *et al.*, 1983; Nobel and Geller, 1987). Thus, temperature should have a strong temporal and spatial effect on the rate of denitrification enzyme activity in desert soils. Few thermophiles are found in desert soils, and it is important to realize that most microbial activity occurs at the lower temperatures associated with greater soil moisture (Skujins, 1984). For example, a single rain storm in the Great Basin desert decreased the maximum soil temperature at a depth of 2.54 cm from 49 to 26°C in 5 days (Billings *et al.*, 1954).

In wet desert soils the availability of N was sufficient to saturate denitrification enzyme activity, and the availability of C only slightly limited enzyme activity. Michaelis–Menton constants (K_m) for NO_3^- reduction by denitrification vary by >3 orders of magnitude (Yoshinari *et al.*, 1977; Myrold and Tiedje, 1985a; Murray *et al.*, 1989). The higher K_m values reported for soils, however, may have resulted from denitrification being limited by C or the rate of NO_3^- diffusion (Firestone, 1982; Myrold and Tiedje, 1985a; Murray *et al.*, 1989). Recent work is in closer agreement with the lower values reported for isolated cultures, which indicates that low values are probably more appropriate. Low K_m values ($<140\ \mu M\ NO_3^-$) also indicate that enzyme activity is probably not limited by NO_3^- in desert soils. For example, in saturated extracts of desert soils, a conservative estimate of the NO_3^- concentration is 300 μM, which is close to saturation for the lower values of K_m that have been reported (Klemmedtson *et al.*, 1977;

Christensen and Tiedje, 1988; Murray *et al.*, 1989; Nielsen *et al.*, 1990).

Several studies demonstrate that denitrification depends strongly on the availability of C (Bremner and Shaw, 1958a, b; Bowman and Focht, 1974; Burford and Bremner, 1975; Stanford *et al.*, 1975; Westerman and Tucker, 1978; Reddy *et al.*, 1982). These studies, however, do not separate enzyme production (bacterial growth + enzyme synthesis) from enzyme activity. I found little response of enzyme activity to supplementary C and N in wet desert soils. Therefore, the large increase in total denitrification (enzyme production + activity) due to C + N additions in wet desert soils (Westerman and Tucker, 1978; Peterjohn, *loc. cit.*) is probably the result of enhanced enzyme production rather than enhanced enzyme activity.

High availability of C and N for denitrification in freshly wet desert soil is consistent with many studies that report a burst of nutrient availability and microbial activity after wetting dry soils (Myers and McGarity, 1971; McKenzie and Kurtz, 1976; Patten *et al.*, 1980; Kroeckel and Stolp, 1986; Kieft *et al.*, 1987; Groffman and Tiedje, 1988). Drying and wetting cycles are common in deserts, and they may maintain nearly optimal substrate concentrations for denitrification enzyme activity in wet desert soil.

Oxygen inhibits both the synthesis of denitrifying enzymes and their subsequent activity (Firestone, 1982; Knowles, 1982; Parkin and Tiedje, 1984; Bonin *et al.*, 1989; Korner and Zumft, 1989). Desert soils are often dry, poorly aggregated, and aerobic. Thus, deserts would seem unsuitable for denitrification. However, the fact that denitrification occurs shortly after wetting indicates that anaerobic sites must become established quickly in desert soils. Although the effect of O_2 was not studied directly, it seems likely that the formation of anaerobic sites in desert soils is closely coupled to soil moisture, temperature, and available C. Soil moisture decreases the diffusion rate of O_2 by 4 orders of magnitude, and increased temperatures can significantly decrease the solubility of O_2 in water (Smith, 1980). Soil moisture, increased temperature, and available C all enhance respiration rates and thus accelerate O_2 depletion. In addition, organic matter is important in the formation of soil aggregates where anaerobic microsites can occur (Sexstone *et al.*, 1985; Tiessen and Stewart, 1988). Further research is needed before the role of O_2 in controlling denitrification in desert ecosystems is fully understood.

From my investigations of denitrification in desert ecosystems, deserts seem more suitable for this microbial process than it would appear at first. Although the enzyme content of upland desert soils is low when compared to that in other habitats, the denitrification rates in wet desert soils are $\geqslant$ than those measured in more mesic ecosystems (Peterjohn, *loc. cit.*). Thus, the nearly optimal conditions that exist for several factors that influence enzyme activity in wet soil apparently compensate for the low enzyme content. From this study it is also evident that denitrification in desert ecosystems is triggered by rainfall and is tightly coupled to the complex interplay between soil moisture, carbon and nitrogen availability, pH, temperature, and O_2.

Acknowledgements—I thank Tony Rathburn, Scott Bridgham, Larry Yungk, Jay O'Neill and Wes Willis for field and laboratory assistance. Valuable advice was provided by William Schlesinger, Tim Parkin, Jim Siedow, Phil Robertson and Pam Matson. Generous logistical support was provided by Carol Wells, Curt Richardson, Bruce Corliss, Walt Whitford and Paul Fonteyn. William Schlesinger and Lisa D. Schlesinger provided valuable editorial comments. Financial support came from a National Science Foundation Dissertation Improvement Award, and a NSF grant awarded to the Jornada Long Term Ecological Research program.

REFERENCES

Alberty R. A. (1983) *Physical Chemistry*. Wiley, New York.

Billings W. D. (1978) *Plants and the Ecosystem*. Wadsworth, Belmont.

Billings W. D., Humphreys M. K. H. and Darling J. B. (1954) Environmental studies in the cold deserts and semideserts of the western Great Basin of North America. Report of Contract DA 44-109-qm-1261, Quartermaster Research and Development Command, U.S. Army, Natick.

Bonin P., Gilewicz M. and Bertrand J. C. (1989) Effects of oxygen on each step of denitrification in *Pseudomonas nautica*. *Canadian Journal of Microbiology* **35**, 1061–1064.

Bowden W. D. (1986) Gaseous nitrogen emissions from undisturbed terrestrial ecosystems: an assessment of their impacts on local and global nitrogen budgets. *Biogeochemistry* **2**, 249–279.

Bowman R. A. and Focht D. D. (1974) The influence of glucose and nitrate concentrations upon denitrification rates in sandy soils. *Soil Biology & Biochemistry* **6**, 297–301.

Bremner J. M. and Shaw K. (1958a) Denitrification in soil I. Methods of investigation. *Journal of Agricultural Science* **51**, 22–39.

Bremner J. M. and Shaw K. (1985b) Denitrification in soil II. Factors affecting denitrification. *Journal of Agricultural Science* **51**, 40–52.

Burford J. R. and Bremner J. M. (1975) Relationships between the denitrification capacities of soils and total, water soluble and readily decomposable soil organic matter. *Soil Biology & Biochemistry* **7**, 389–394.

Christensen S. and Tiedje J. M. (1988) Sub-parts-per-billion nitrate method: use of an N_2O-producing denitrifier to convert NO_3^- or $^{15}NO_3^-$ to N_2O. *Applied and Environmental Microbiology* **54**, 1409–1413.

Firestone M. K. (1982) Biological denitrification. In *Nitrogen in Agricultural Soils* (F. J. Stevenson, Ed.), pp. 289–326. Soil Science Society of America, Madison.

Groffman P. M. and Tiedje J. M. (1988) Denitrification hysteresis during wetting and drying cycles in soil. *Soil Science Society of American Journal* **52**, 1626–1629.

Groffman P. M. and Tiedje J. M. (1989) Denitrification in the north temperate forest soils: relationship between denitrification and environmental factors at the landscape scale. *Soil Biology & Biochemistry* **21**, 621–626.

Keeney D. R., Fillery I. R. and Marx G. P. (1979) Effect of temperature on the gaseous nitrogen products of denitrification in a silt loam soil. *Soil Science Society of America Journal* **43**, 1124–1128.

Kieft T. L., Soroker E. and Firestone M. K. (1987) Microbial biomass response to a rapid increase in water potential when dry soil is wetted. *Soil Biology & Biochemistry* **19**, 119–126.

Klemedtsson L., Svensson B. H., Lindberg T. and Rosswall T. (1977) The use of acetylene inhibition of nitrous oxide reductase in quantifying denitrification in soils. *Swedish Journal of Agricultural Research* **7**, 179–185.

Knowles R. (1982) Denitrification. *Microbiological Reviews* **46**, 43–70.

Korner H. and Zumft W. G. (1989) Expression of denitrification enzymes in response to the dissolved oxygen level and respiratory substrate in continuous culture of *Pseudomonas stutzeri*. *Applied and Environmental Microbiology* **55**, 1670–1676.

Kroeckel L. and Stolp H. (1986) Influence of the water regime on denitrification and aerobic respiration in soils. *Biology and Fertility of Soils* **2**, 15–21.

McKenzie E. and Kurtz L. T. (1976) Effect of pretreatment on loss of nitrogen-15-labelled fertilizer nitrogen from waterlogged soil during incubation. *Soil Science Society of American Journal* **40**, 534–537.

Martin K., Parsons L. L., Murray R. E. and Smith M. S. (1988) Dynamics of soil denitrifier populations: relationships between enzyme activity, most-probable-number counts, and actual N gas loss. *Applied and Environmental Microbiology* **54**, 2711–2716.

Matson P. A. and Vitousek P. M. (1987) Cross-system comparisons of soil nitrogen transformations and nitrous oxide flux in tropical forest ecosystems. *Global Biogeochemical Cycles* **1**, 163–170.

Melillo J. M., Aber J. D., Steudler P. A. and Schimel J. P. (1983) Denitrification potentials in a successional sequence of northern hardwood forest stands. *Ecological Bulletins* **35**, 217–228.

Mueller-Dombois D. and Ellenberg H. (1974) *Aims and Methods of Vegetation Ecology*. Wiley, New York.

Murray R. E., Parsons L. A. and Smith M. S. (1989) Kinetics of nitrate utilization by mixed populations of denitrifying bacteria. *Applied and Environmental Microbiology* **55**, 717–721.

Myers R. J. K. and McGarity J. W. (1971) Factors influencing high denitrifying activity in the subsoil of solodized solonetz. *Plant and Soil* **35**, 145–160.

Myrold D. D. and Tiedje J. M. (1985a) Diffusional constraints on denitrification in soil. *Soil Science Society of American Journal* **49**, 651–657.

Myrold D. D. and Tiedje J. M. (1985b) Establishment of denitrification capacity in soil: effects of carbon, nitrate and moisture. *Soil Biology & Biochemistry* **17**, 819–822.

Nielsen L. P., Christensen P. B., Revsbech N. P. and Sorensen J. (1990) Denitrification and oxygen respiration in biofilms studied with a microsensor for nitrous oxide and oxygen. *Microbial Ecology* **19**, 63–72.

Nobel P. S. and Geller G. N. (1987) Temperature modelling of wet and dry desert soils. *The Journal of Ecology* **75**, 247–258.

Nommik H. (1956) Investigations on denitrification in soil. *Acta Agriculturae Scandinavica* **6**, 195–228.

Parker L. W., Miller J., Steinberger Y. and Whitford W. G. (1983) Soil respiration in a Chihuahuan desert rangeland. *Soil Biology & Biochemistry* **15**, 303–309.

Parkin T. B. and Robinson J. A. (1989) Stochastic models of soil denitrification. *Applied and Environmental Microbiology* **55**, 72–77.

Parkin T. B. and Tiedje J. M. (1984) Application of a soil core method to investigate the effect of oxygen concentration on denitrification. *Soil Biology & Biochemistry* **16**, 331–334.

Parkin T. B., Starr J. L. and Meisinger J. J. (1987) Influence of sample size on measurement of soil denitrification. *Soil Science Society of America Journal* **51**, 1492–1502.

Parkin T. B., Sexstone A. J. and Tiedje J. M. (1985) Adaptation of denitrifying populations to low soil pH. *Applied and Environmental Microbiology* **49**, 1053–1056.

Parkin T. B., Kaspar H. F., Sexstone A. J. and Tiedje J. M. (1984) A gas-flow soil core method to measure field denitrification rates. *Soil Biology & Biochemistry* **16**, 323–330.

Patten D. K., Bremner J. M. and Blackmer A. M. (1980) Effects of drying and air-storage of soils on their capacity for denitrification of nitrate. *Soil Science Society of America Journal* **44**, 67–70.

Peterjohn W. T. and Schlesinger W. H. (1990) Nitrogen loss from deserts in the southwestern United States. *Biogeochemistry* **10**, 67–79.

Price N. C. and Stevens L. (1982) *Fundamentals of Enzymology*. Oxford University Press, New York.

Reddy K. R., Rao P. S. C. and Jessup R. E. (1982) The effect of carbon mineralization on denitrification kinetics in mineral and organic soils. *Soil Science Society of American Journal* **46**, 62–68.

Robertson G. P. and Tiedje T. M. (1984) Denitrification and nitrous oxide production in successional and old-growth Michigan forests. *Soil Science Society of America Journal* **48**, 383–389.

Robertson G. P. and Tiedje J. M. (1988) Deforestation alters denitrification in a lowland tropical rain forest. *Nature* **336**, 756–759.

SAS (1982) *User's Guide: Statistics*. SAS Institute, Cary, N.C.

Schlesinger W. H. and Peterjohn W. T. (1988) Ion and sulfate ratios in arid soils subject to wind erosion in the southwestern U.S.A. *Soil Science Society of America Journal* **52**, 54–58.

Schlesinger W. H., Fonteyn P. H. and Marion G. M. (1987) Soil moisture content and plant transpiration in the Chihuahuan Desert of New Mexico. *Journal of Arid Environments* **12**, 119–126.

Segel I. H. (1975) *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Sytems*. Wiley, New York.

Sexstone A. J., Revsbech N. P., Parkin T. B. and Tiedje J. M. (1985) Direct measurement of oxygen profiles and denitrification rates in soil aggregates. *Soil Science Society of America Journal* **49**, 645–651.

Skujins J. (1984) Microbial ecology of desert soils. *Advances in Microbial Ecology* **7**, 49–91.

Skujins J. J., Braal L. and McLaren A. D. (1963) Characterization of phosphatase in a terrestrial soil sterilized with an electron beam. *Enzymologia* **25**, 125–133.

Smith K. A. (1980) A model of the extent of anaerobic zones in aggregated soils and its potential application to estimates of denitrification. *Journal of Soil Science* **31**, 263–277.

Smith M. S. and Parsons L. L. (1985) Persistence of denitrifying enzyme activity in dried soils. *Applied and Environmental Microbiology* **49**, 316–320.

Smith M. S. and Tiedje J. M. (1979) Phases of denitrification following oxygen depletion in soil. *Soil Biology & Biochemistry* **11**, 261–267.

Stanford G., Vander Pol R. A. and Dzienia S. (1975) Denitrification rates in relation to total and extractable soil carbon. *Soil Science Society of America Proceedings* **39**, 284–289.

Tabatabai M. A. (1982) Soil enzymes. In *Methods of Soil Analysis, Part II* (A. L. Page, R. H. Miller and D. R. Keeney, Eds), pp. 903–947. Soil Science Society of America, Madison.

Tiedje J. M. (1982) Denitrification. In *Methods of Soil Analysis, Part II* (A. L. Page, R. H. Miller and D. R. Kenney, Eds), pp. 1011–1026. Soil Science Society of America, Madison.

Tiedje J. M. (1988) Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In *Biology of Anaerobic Microorganisms* (A. J. B. Zehnder, Ed.), pp. 179–244. Wiley, New York.

Tiedje J. M., Simkins S. and Groffman P. M. (1989) Perspectives on measurement of denitrification in the field including recommended protocols for acetylene based methods. *Plant and Soil* **115**, 261–284.

Tiedje J. M., Sexstone A. J., Myrold D. D. and Robinson J. A. (1982) Denitrification: ecological niches, competition and survival. *Antonie van Leeuwenhoek Journal of Microbiology* **48**, 569–583.

Tiessen H. and Stewart J. W. B. (1988) Light and electron microscopy of stained microaggregates: the role of organic matter and microbes in soil aggregation. *Biogeochemistry* **5**, 312–322.

Virginia R. A., Jarrell W. M. and Franco-Vizcaino E. (1982) Direct measurement of denitrification in a *Prosopis* (mesquite) dominated Sonoran Desert ecosystem. *Oecologia* **53**, 120–122.

Vitousek P. M., Gosz J. R., Grier C. C., Melillo J. M. and Reiners W. A. (1982) A comparative analysis of potential nitrification and nitrate mobility in forest ecosystems. *Ecological Monographs* **52**, 155–177.

Vitousek P. M., Gosz J. R., Grier C. C., Melillo J. M., Reiners W. A. and Todd R. L. (1979) Nitrate losses from disturbed ecosystems. *Science* **204**, 469–474.

West N. E. and Skujins J. (1977) The nitrogen cycle in North American cold-winter semi-desert ecosystems. *Oecologia Plantarum* **12**, 45–53.

Westerman R. L. and Tucker T. C. (1978) Factors affecting denitrification in a Sonoran desert soil. *Soil Science Society of America Journal* **42**, 596–599.

Wierenga P. J. (1988) Weather data: daily and monthly means, March 1983–October 1987, Jornada LTER site. Research Report 88-ss-02, NSF. Project No. BSR-8612106, Department of Agronomy and Horticulture, New Mexico State University, Las Cruces.

Wijler J. and Delwiche C. C. (1954) Investigations on the denitrifying process in soil. *Plant and Soil* **5**, 155–169.

Wilhem E., Battino R. and Wilcock R. J. (1977) Low-pressure solubility of gases in liquid water. *Chemical Reviews* **77**, 219–262.

Yoshinari T., Hynes R. and Knowles R. (1977) Acetylene inhibition of nitrous oxide reduction and measurement of denitrification and nitrogen fixation in soil. *Soil Biology & Biochemistry* **9**, 177–183.

Young D. R. and Nobel P. S. (1986) Predictions of soil–water potentials in the north-western Sonoran Desert. *Journal of Ecology* **74**, 143–154.