An empirical model of denitrification

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Bergstrom, D. W. and Beauchamp, E. G. 1993. **An empirical model of denitrification.** Can. J. Soil Sci. **73**: 421–431. We used a simple empirical model to predict denitrification rates from measurements of bulk soil properties. Boundary analysis was used to define relationships between denitrification rate and each of air-filled porosity, respiration rate and mineralizable-C content. The ratio of measured denitrifying enzyme activity to the maximum measured value was used to account for variation in amounts of enzymes and numbers of denitrifiers in soil. Nitrate content had little effect on denitrification rate and was excluded from the model. Because the model did not account for microscale variability, it did not accurately predict rates in individual soil cores. Nevertheless, population means and distributions of predicted and measured values were similar. The seasonal patterns of mean values of predicted and measured denitrification rates were also similar over the second half of the sampling period, which extended from May to November. The model did not account for appreciable denitrification on three dates in May. This discrepancy indicated that environmental regulation of denitrification may not be uniform over the season. The model was not sufficiently sensitive to factors influencing episodic events.

Key words: Denitrification rate, model, boundary line

Bergstrom, D. W. et Beauchamp, E. G. 1993. **An empirical model of denitrification.** Can. J. Soil Sci. **73**: 421–431. Nous avons utilisé un modèle empirique pour prédire le taux de dénitrification à partir des mesures des propriétés générales du sol. Une analyse de limites a servi à définir les rapports entre le taux de dénitrification, d'une part, et la porosité en air, le taux de respiration et la teneur en C minéralisable, d'autre part. Le rapport entre l'activité mesurée des enzymes dénistrifiantes et la valeur mesurée la plus élevée a permis de prendre en compte les variations observées dans les quantités d'enzymes et dans les populations d'organismes dénitrifiants présentés dans le sol. La teneur en nitrates avait peu d'influence sur le taux de dénitrification et a donc été exclue modèle. Étant donné que le modèle ne rendait pas compte de la variabilité à très petite échelle, il ne permettait pas de prédire avec précision les taux de dénitrification dans des carottes de sol. Néanmoins, les moyennes de population et la répartition des valeurs prédites et des valeurs mesurées étaient les mêmes. L'évolution saisonnière des valeurs moyennes des taux de dénitrification prédits et mesurés était également semblable tout au long de la période de prélèvement, qui s'étendait de mai à novembre. Le modèle ne permettait pas d'expliquer le taux de dénitrification appréciable observé à trois dates en mai. Cet écart laisse à penser que la régualtion de la dénitrification par les conditions du milieu ne serait pas uniforme tout au long de la saison. Le modèle n'était pas suffisamment sensible pour tenir compte des facteurs qui influent sur les mainifestations épisodiques.

Mots clés: Taux de dénitrification, modèle, ligne de séparation

Predictive models of denitrification at the field and landscape scales based upon measured soil properties are necessary for application of knowledge of denitrification to issues such as fertilizer-use efficiency, control of N_2O and NO emissions, and NO_3^- leaching from soils. Yet, spatial variability of field measurements of denitrification rate has not been adequately accounted for by measurement of bulk soil properties in previous studies (Burton and Beauchamp 1985; Myrold 1988; Grundmann et al. 1988; Parsons et al. 1991).

Grundmann et al. (1988) suggested that one cause of spatial variability was variation in microsite concentrations of substrate C and NO_3^-. Spatial variability of natural denitrification rates has also been attributed in part to the patchy dispersion of particulate organic material in soil (Parkin 1987; Parkin and Robinson 1989; Christensen et al. 1990).

Measurements of bulk soil properties have been useful in predicting relative rates of denitrification on a temporal scale. Parsons et al. (1991) used mean values of denitrification rate and determinant soil properties for each sampling date in a multiple-regression model that accounted for 74 and 91% of temporal variation in two soils. Elliott and de Jong (1992) defined regression equations that used volumetric moisture content and air temperature to predict denitrification rates for cropped and fallow fields and three distinct landscape groups. Equations were used to interpolate between monthly measurements of denitrification rate to estimate annual denitrification losses. Strong relationships have been identified between denitrification rate or activity and causal soil properties on a landscape scale (Groffman and Tiedje 1989b; Pennock et al. 1992). Nevertheless, variability of estimates of denitrification rates based on mean values is considerable.

Elliott and de Jong (1993) modelled denitrification using boundary analysis (cf. Webb 1972; Livingston and Black 1987). Such an approach is applicable to denitrification-rate measurements in soil because at present inadequate knowledge of environmental regulation precludes mechanistic modelling.

Webb (1972) based the concept of a boundary line on biological rather than strictly mathematical concepts. In biological experimentation, study of the relationship between a dependent and an independent variable is often confounded by other interacting factors. In graphic presentation, data appear as an array of points, and deviation of points from a line or curve results not only from errors of measurement and variability of the biological material, but also from interactions with other factors. If the data set is large enough, the upper edge of the array can be identified as the line representing the relationship between the dependent and the independent variable when unaffected by other variables or when least affected. Webb defined this edge as the boundary line. Points lie below the line when the relationship is influenced by other variables. In practice, defining the upper edge of the array as the boundary line includes the errors of measurement and variability of the biological material with deviations attributed to interactions. Hence the boundary line is not precisely determined. Nevertheless, boundary lines may be particularly useful in disassembling denitrification-rate measurements into components dependent on particular soil properties. Such information can be used to subsequently develop mechanistic models and may identify landscape properties in geographic information systems that can be used to predict denitrification on a regional scale.

To better understand environmental regulation of denitrification, we used a simple empirical model to predict denitrification rates from measurable soil properties at a field site. We subsequently compared mean measured and predicted denitrification rates to test for uniformity of environmental regulation of denitrification over the sampling period.

MATERIALS AND METHODS

Site Description and Sampling Procedure

The sampling site was located at the Elora Research Station, 20 km north of Guelph, on Maryhill silt loam (Orthic Humic Gleysol). The site was seeded to barley (*Hordeum vulgare*) on 2 May 1990. Soil was sampled on 27 dates from May to November (1990), using metal rings (5 cm i.d., 5 cm deep). On each sampling date, 10 cores were collected along a randomly located 1-m transect at 10-cm spacings. Cores were collected from the 2.5–7.5-cm layer.

Biological and Chemical Analyses

Soil cores were immediately weighed upon arrival at the laboratory, and their denitrification and respiration rates were measured. Cores were incubated aerobically at room temperature in the presence of acetylene (approximately 6% vol vol^{-1}: Ryden et al. 1987) in 250-mL Mason jars with lids fitted with serum stoppers (Suba Seal, Barnesley, U.K.). After 7 h, headspace concentrations of N_2O and

CO_2 were determined. On the following day, each soil core was removed from the metal ring, thoroughly mixed and subsampled for analysis. Moisture content was determined gravimetrically using 15 g moist soil. Air-filled porosity was calculated from core volume, core weight, gravimetric moisture content, and an assumed particle density (2.65 Mg m^{-3}).

Moist soil (25 g) was extracted with 50 mL 0.05 mol K_2SO_4 L^{-1} and shaken for 30 min (Black and Waring 1978). Filtered extracts were refrigerated overnight, and NO_3^- concentration of extracts was determined by electrode on the following day. Mineralizable C was determined by aerobic incubation of 15 g moist soil in 250-mL amber bottles sealed with serum stoppers. Headspace concentrations of CO_2 were measured after 7 d. Because the soil was at field moisture content, the mineralizable-C determination was as much an assessment of microbial activity and numbers (as influenced by moisture content) as one of readily available C. Denitrifying enzyme activity was determined on 50 g moist soil with 40 mL buffer solution (50 mmol K_2HPO_4 L^{-1}, 10 mmol KNO_3 L^{-1}, 10 mmol glucose L^{-1}, 100 mg chloramphenicol L^{-1}, pH 7.0) by the method of Martin et al. (1988). Soil was weighed into 250-mL plastic bottles. Bottles were sealed with lids fitted with serum stoppers. Headspace atmosphere was displaced with argon, and acetylene was added (10% vol vol^{-1}). Gas samples were collected in 2-mL Vacutainers (Becton Dickinson, Rutherford, NJ) and analyzed on the following day for N_2O.

Nitrous oxide was measured on a Hewlett-Packard 5830A gas chromatograph equipped with a ^{63}Ni electron-capture detector. Dissolved N_2O was accounted for by the method of Moraghan and Buresh (1977). Carbon dioxide was measured using a Gow-Mac gas chromatograph equipped with a thermal-conductivity detector. Nitrate was measured with an Orion nitrate electrode (model 93-07).

Boundary Analysis and Model Definition

We used a simple empirical model that combined the approaches of Parkin and Robinson (1989) and Elliott and de Jong (1993). Boundary analysis was used to define relationships between denitrification rate and four bulk soil properties: air-filled porosity, respiration rate, mineralizable-C content and NO_3^- content. We followed a procedure similar to that of Elliott and de Jong, except that we used untransformed denitrification rates of individual soil cores. Denitrification-rate measurements were converted to fractional values (F values) of the maximum. The maximum denitrification rate of 23.2 ng N_2O-N h^{-1} g^{-1} soil was measured on November 7 and August 27 (days 239 and 311). Scattergrams of F values versus measured values of independent variables were plotted for air-filled porosity, respiration rate, mineralizable-C content and NO_3^- content. For each independent variable, a boundary line was defined based on the upper edge of the data array.

Measurements of denitrifying enzyme activity (DEA) were used to account for variation in enzyme content and numbers of denitrifiers among soil cores. Parkin and Robinson (1989) similarly used DEA measurements in a stochastic model of denitrification. Measured values of DEA were expressed as a fraction of the maximum measured DEA, and the fraction was used to reduce the model estimates in proportion to the amount of enzymes and numbers of denitrifiers present. The maximum DEA of 1.80 μg N_2O-N h^{-1} g^{-1} soil was measured on 7 November. Another high value of 1.74 μg N_2O-N h^{-1} g^{-1} soil was measured on 14 August. In the previous year, which was drier, a maximum value of 1.31 μg N_2O-N h^{-1} g^{-1} soil was measured at an adjacent site. Hence, the value of 1.80 μg N_2O-N h^{-1} g^{-1} soil was considered a plausible maximum.

The maximum denitrification rate was multiplied by F values obtained from boundary-line equations and the DEA fraction to estimate denitrification rate, as in the following:

$$D_{est} = D_{max} \times F_a \times F_r \times F_n \times d \qquad (1)$$

where D_{est} is the estimated denitrification rate; D_{max} is the maximum measured value; F_a, F_r and F_n are F values derived from boundary-line equations for air-filled porosity, respiration rate and NO_3^- content, respectively; and d is the DEA fraction. In this manner, for every measured denitrification rate, an estimated denitrification rate was calculated using measured values of the independent variables. Mineralizable C was substituted for respiration rate in a second set of calculations. The multiplicative model was used, assuming that predictive variables were independent of each other.

Model calculations were performed with a personal computer, using conventional software (CoHort).

RESULTS AND DISCUSSION

Boundary Analysis and Model Definition

The model was constructed with pooled data from 27 sampling dates (8 May to 15 November).

The two maximum denitrification rates were much greater than most of the other measured denitrification rates and may not have represented a maximum rate for the population of measured values (Fig. 1). Although we recognized this possibility, we used these points to define the maximum rate. If these two points were in fact outliers, the model would tend to overestimate denitrification rates. Boundary lines were fitted by regression to selected points along the upper edge of the data arrays for air-filled porosity, respiration rate and mineralizable C (Fig. 1; Table 1). On each scattergram three outliers were discounted in definition of the boundary lines. Boundary lines that included these outliers grossly overestimated denitrification rate. The outliers derived from four cores collected in May and will be discussed later. A boundary line was not fitted for NO_3^- content. The maximum denitrification rate was measured at NO_3^- contents of 4.6 and 2.1 μg N g^{-1} soil. Hence, denitrification was apparently not limited by NO_3^- at concentrations above approximately 2 μg N g^{-1} soil. A limiting relationship (boundary line) could not be clearly defined over the narrow range of NO_3^- concentrations below this threshold.

Because of the skewed frequency distribution of denitrification-rate measurements, on

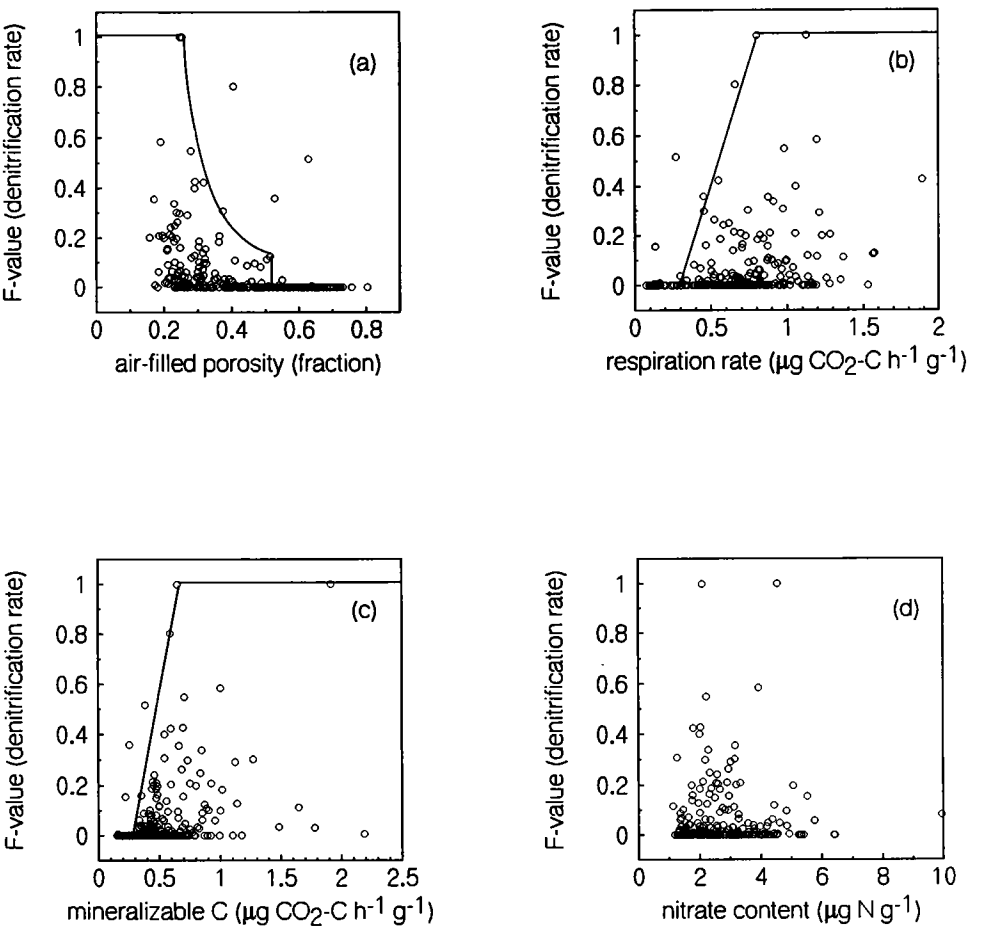

Fig. 1. Scattergrams of (a) air-filled porosity, (b) respiration rate, (c) mineralizable-C content and (d) nitrate content.

Table 1. Boundary-line equations for air-filled porosity, respiration rate and mineralizable-C content

Variable	Equation	Range
Air-filled porosity (a)	$F = 1$	$a < 0.247$
	$F = 0.0167a^{-2.89}$	$0.247 \leq a \leq 0.514$
	$F = 0$	$a > 0.514$
Respiration rate (r)	$F = 0$	$r < 0.319$
	$F = 1.93r - 0.615$	$0.319 \leq r \leq 0.837$
	$F = 1$	$r > 0.837$
Mineralizable-C content (c)	$F = 0$	$c < 0.294$
	$F = 2.66c - 0.78$	$0.294 \leq c \leq 0.67$
	$F = 1$	$c > 0.67$

each scattergram there were very few high values with which to define the uppermost segments of the boundary lines. Furthermore, on each scattergram, points representing nil rates were clustered along the x axis. These nil rates could not be attributed to limitation by one of the four independent variables measured.

The independent variables used were intended to account for the three proximate regulators of denitrification rate identified in a conceptual model by Tiedje (1988): O_2 concentration, substrate-C supply and NO_3^- supply. In reality, the measured soil properties integrated responses of processes occurring at the microscale (cf. Johnsson et al. 1991). Moreover, they were not completely independent of each other. Air-filled porosity was used to assess soil aeration. The boundary line for air-filled porosity indicated a substantial increase in denitrification rate below a threshold value of aeration (Fig. 1) and, in this respect, was similar to relationships described by Grundmann and Rolston (1987) and Johnsson et al. (1991). It was dissimilar to the other two relationships because it indicated a maximum denitrification rate below saturation. Respiration rates of soil cores and contents of mineralizable C were used to assess substrate-C supply. Both variables also assessed microbial activity as influenced by moisture content. The boundary lines for respiration rate and mineralizable C showed saturation-type relationships (Fig. 1). All mineralizable-C measurements were affected by variability caused by subsampling of the soil core. Nitrate content was omitted from the model in part because it appeared to influence denitrification rate over only a fraction of the range of measured values of NO_3^- content ($\sim$1–2 μg N g^{-1} soil). Furthermore, other studies have indicated that NO_3^- content has little influence on denitrification rates in fertilized soils (Murray et al. 1989; Parsons et al. 1991). A calculated diffusion rate based on soil measurements may be more informative of limitation by NO_3^- than NO_3^- content alone.

Denitrifying enzyme activity is, in principle, an indirect measure of enzyme concentration (Tiedje 1982). Parkin (1987) used DEA to account for the dispersion of potentially active denitrifying enzymes in soil. Martin et al. (1988) interpreted DEA as an estimate of the biomass of bacteria that have synthesized denitrifying enzymes. We used the ratio of measured DEA to the maximum measured value in the model to account for variation in amounts of enzymes and numbers of denitrifiers among individual soil cores. A more sophisticated approach would be to use the frequency distribution of DEA to introduce variability to estimates derived from the boundary-line relationships. Such a model would include a stochastic component.

Model Predictions

Denitrification rates were estimated using eq. 1, F values calculated for air-filled porosity and respiration rate or mineralizable-C content from equations in Table 1, and the DEA fraction. A plot of predicted versus measured denitrification rates for both calculations showed considerable scatter (Fig. 2).

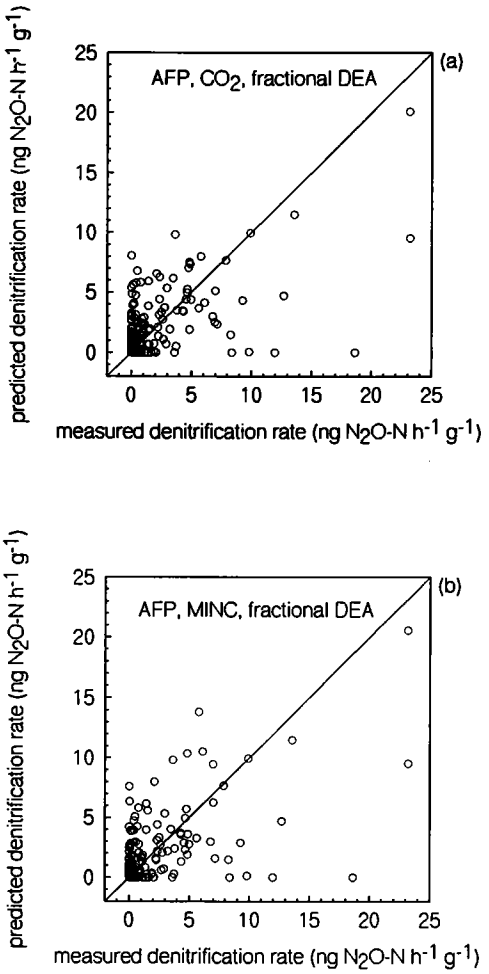

Fig. 2. Predicted versus measured denitrification rates for (a) air-filled porosity, respiration rate and DEA; and (b) air-filled porosity, mineralizable-C content and DEA.

The model made two obvious errors: it did not account for two components of the population of measured values. Points along the *y* axis represented samples in which denitrification was predicted but did not occur. Points along the *x* axis represented samples in which no denitrification was predicted but in which denitrification occurred. Points along the *y* axis indicated missing information. Denitrification did not invariably occur when bulk soil properties were favorable. Spatial arrangement of denitrifiers, substrate C and NO_3^- at anaerobic microsites may influence occurrence of denitrification. Most of the points along the *x* axis derived from the samples with no DEA but measurable denitrification rates. This discrepancy can be explained by localization of denitrifying activity in a small fraction of the soil core and exclusion of the fraction from the subsample used for analysis of DEA. Alternatively, the high NO_3^- concentration of the DEA assay solution may have inhibited activity (cf. Lalisse-Grundmann et al. 1988; Groffman

and Tiedje 1989a). Hence, the model failed to predict denitrification in some samples.

We also evaluated the model by comparing the temporal pattern of mean denitrification rates for predicted and measured values over the 27 sampling dates (Fig. 3). Mean values per sampling date were calculated arithmetically. Regression of predicted on measured mean denitrification rates gave r^2 values of 0.59 and 0.61 ($P \leq 0.001$) for calculations based on respiration rate and mineralizable C, respectively. The model more accurately predicted occurrence and magnitude of episodes of denitrification during the second half of the season, when in fact most of the denitrification occurred, than in the first half. Parsons et al. (1991) and Elliott and de Jong (1992) found bulk soil properties useful predictors of mean denitrification rates on a temporal scale.

In the first half of the season, the model failed to predict substantial denitrification on the first three sampling dates in May and overestimated denitrification on day 191.

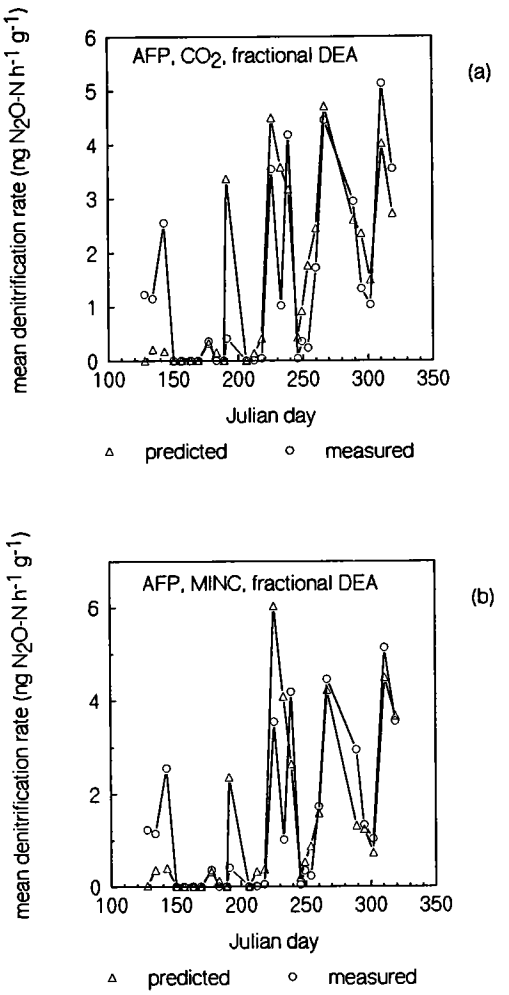

Fig. 3. Seasonal patterns of measured and predicted denitrification rates for (a) air-filled porosity, respiration rate and DEA; and (b) air-filled porosity, mineralizable-C content and DEA.

The discrepancy between predicted and measured rates on the first three dates derived from four soil cores identified as outliers in the scattergrams (Fig. 1). Appreciable denitrification occurred in these cores at relatively high air-filled porosities and relatively low respiration rates and mineralizable-C contents. The discrepancy raised the possibility of seasonal changes in environmental regulation of denitrification. Denitrifier activity can be substantial early in the season at thaw (Christensen and Tiedje 1990) and at low temperatures (Dorland and Beauchamp 1991). Higher than expected rates in May might have been a residual effect of increased denitrifier activity in late winter and spring. Furthermore, seasonal changes in the denitrifier population may occur. King and Nedwell (1984) reported seasonal selection by temperature of NO_3^--reducing bacteria in a salt-marsh sediment. A psychrotrophic component dominated by *Pseudomonas* spp. developed in winter. Alternatively, higher than expected rates in May might have resulted from tillage of the soil and fertilizer application at seeding (2 May). The overestimate on day 191 accounted for some of the points along the *y* axis in Fig. 2. Nitrate content was low on that date (1.8 μg N g^{-1}) and may have been limiting, given the very favorable conditions for denitrification indicated by the bulk soil properties.

In the second half of the season, the model overestimated the mean denitrification rate on days 226 and 233, especially when mineralizable-C content was used as a variable. The overestimate on day 233 may have derived in part from substrate depletion at microsites following a substantial denitrification event. Measurements of bulk soil properties would not account for such an effect. Groffman and Tiedje (1988) described denitrification hysteresis during wetting and drying cycles and pointed out the importance of antecedent events. Johnsson et al. (1991) concluded that their model did not adequately account for the episodic nature of denitrification. The model underestimated denitrification on the last two sampling dates, 7 and 15 November, when respiration rate was used as a variable. Because the measured soil properties used in the model were not strictly independent of each other, some underestimates may have derived from redundancy of variables in the multiplicative model.

Our model was based on macroscale measurements — that is, denitrification rates of soil cores and bulk soil properties. Consequently, it did not account for microscale variability (cf. Johnsson et al. 1991). Furthermore, subsampling of soil cores for determination of mineralizable C and DEA introduced variability into measurements. For these reasons, the model did not accurately predict rates in individual soil cores. In evaluating stochastic models of denitrification, Parkin and Robinson (1989) compared means and frequency distributions of predicted and measured values. For calculations based on respiration rate, we compared means and frequency distributions of predicted and measured values for the entire season and over the last 14 sampling dates. In both cases, means and distributions of predicted and measured values were similar (Fig. 4). Hence, the model did in part reproduce the variability of individual measured rates. Because the model overestimated denitrification rates, the frequency distribution of predicted values was less skewed than that of the measured values. Other workers have also found the simulated frequency distribution to be less skewed (Parkin 1987; Parkin and Robinson 1989; Johnsson et al. 1991).

Our model was not tested with a data set other than that used in model definition. Hence, its usefulness in estimation of denitrification rates remains to be examined for an independent data set. It is not useful in a general sense because input variables such as respiration rate, mineralizable C and DEA are not usually known. Because cores were incubated at room temperature, rates did not represent those that occurred in the field.

The model structure provided general insights into regulation of denitrification in this soil. Aeration was combined with biological activity (assessed by respiration rate or

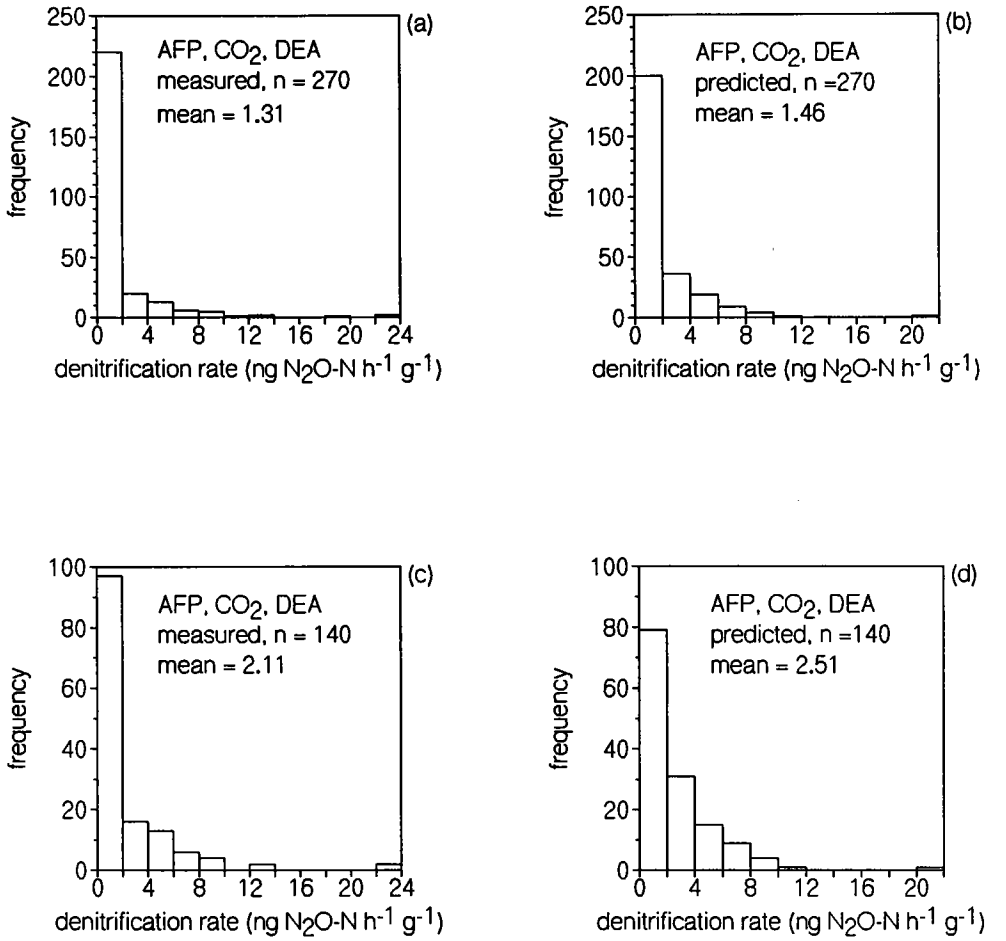

Fig. 4. Frequency distributions of denitrification rates for (a) measured values for the entire season, (b) predicted values for the entire season, (c) measured values for the second half of the season and (d) predicted values for the second half of the season, using a model combining air-filled porosity, respiration rate and DEA.

mineralizable-C content) to determine the magnitude of denitrification rates. Variation in enzyme content and numbers of denitrifiers (assessed by DEA) accounted for at least part of the small-scale variability. Nitrate content had very little effect on denitrification over most of the range of measured values. Both respiration rate and mineralizable-C content resulted in overestimates of denitrification on some dates; hence, they had similar predictive value.

CONCLUSIONS

An empirical model based on bulk soil measurements did not accurately predict denitrification rates in individual cores. It was more useful in simulating the mean and distribution of the population of measured rates and in predicting the seasonal pattern and magnitude of denitrification events in the second half of the sampling period. Discrepancies between predicted and measured denitrification rates pointed to the importance of microscale

variability in determining occurrence and magnitude of denitrification, the possibility of changes in environmental regulation over the season, and the importance of factors that influence episodic events. Annual estimates of denitrification must account for any seasonal changes in environmental regulation of denitrification and be sensitive to factors influencing episodic events.

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