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Modeling the temperature response of nitrification

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Abstract. To model nitrification rates in soils, it is necessary to have equations that accurately describe the effect of environmental variables on nitrification rates. A variety of equations have been used previously to describe the effect of temperature on rates of microbial processes. It is not clear which of these best describes the influence of temperature on nitrification rates in soil. I compared five equations for describing the effects of temperature on nitrification in two soils with very different temperature optima from a California oak woodland-annual grassland. The most appropriate equation depended on the range of temperatures being evaluated. A generalized Poisson density function best described temperature effects on nitrification rates in both soils over the range of 5 to 50 °C; however, the Arrhenius equation best described temperature effects over the narrower range of soil temperatures that normally occurs in the ecosystem (5 to 28 °C). Temperature optima for nitrification in most of the soils were greater than even the highest soil temperatures recorded at the sites. A model accounting for increased maintenance energy requirements at higher temperatures demonstrates how net energy production, rather than the gross energy production from nitrification, is maximized during adaptation by nitrifier populations to soil temperatures.

Introduction

Simulation models are necessary to predict the effects of elevated temperatures due to global warming, and elevated substrate concentrations due to atmospheric deposition, on nitrification rates. This information will help evaluate the potential for undesirable processes such as NO_3^- leaching and trace-N gas production; however, to accurately model the dynamics of nitrification in ecosystems, one must first know the nature of the relationship between environmental variables and nitrification rates.

The response of nitrification to temperature has been studied in soils from a variety of geographic regions (Russel et al. 1925; Sabey et al. 1959; Mahendrappa et al. 1966; Myers 1975; Malhi & McGill 1982), and temperature optima have been shown to vary with the environment. In all of these studies, the temperature response was described using line segments to connect points representing rates at different temperatures. The temperature optimum was defined as the incubation temperature that resulted in the highest nitrification rate. For this method, the precision of the estimate depends on the choice of temperatures for incubations. For example, a study that used incubation temperatures distributed at 5 °C intervals (e.g. 25, 30, 35, and 40 °C) would be unable to distinguish between soils with optima at 33 and 37 °C. In addition, because the estimates depend on a single point, a single spuriously high value could greatly influence the estimate of the temperature optimum. Statistical methods are rarely used to determine if a rate is significantly higher than rates at higher or lower temperatures.

A more robust method for estimating temperature optima involves using nonlinear regression to fit an appropriate equation to rate data, and then identifying the optimum based on values predicted from the equation. In this method, data points from a range of temperatures are used to estimate the temperature optimum, rather than a single data point or mean. Because software packages are now widely available that have the capability of performing nonlinear regression, it is no longer necessary to rely on linear transformations, which may produce inaccurate estimates of coefficients (Robinson 1985).

A variety of mathematical equations have been used to describe the effect of temperature on microbial processes. The effect of temperature has been described by the Arrhenius equation (Laudelout 1978), a double Arrhenius equation (Moore 1986), a generalized Poisson density function (Parton et al. 1987), an exponential function (Innis 1978), and "square root" model (McMeekin et al. 1988). It is not clear which of these models are most appropriate for describing nitrification rates in soils.

The objectives of this study were *i*) to determine the most appropriate mathematical functions for describing the effects of temperature on nitrification rates in soils from a California oak woodland-annual grassland and *ii*) to determine how the temperature optima of nitrifier communities relate to the distribution of soil temperatures at this site. Equations were fit to nitrification rate and temperature data determined in laboratory assays of soils collected from beneath oak canopies and from open grassy interspaces. Nitrifier communities in the open grassy areas at this site have temperature optima comparable to nitrifiers from tropical ecosystems, whereas the nitrifiers from beneath oak canopies have temperature optima more representative of nitrifiers from temperate ecosystems (Stark and Firestone *in press*). The nitrifier communities in the two soil types also differ more than 2-fold in absolute nitrification rates. Therefore, the fit of equations to data from these two soils should provide a good test of the ability of the equations to accurately predict the effect of temperature on nitrification rates in a variety of soils.

Methods and materials

The soil samples used to evaluate the effect of temperature on nitrification rates were collected from a California oak woodland-annual grassland site at the Sierra Foothill Range Field Station, approximately 30 km northeast of Marysville, CA. The site is at an elevation of 200 m, mean annual precipitation is 644 mm, and mean annual temperature is 16.5 °C. The mean monthly minimum of 6.7 °C occurs in January during the wet season, while the mean monthly maximum of 25.6 °C occurs in July during the dry season. The vegetation consists of scattered oaks (*Quercus douglassii* H.&A. and *Q. wislizenii* A.) providing approximately 50% canopy cover and an understory of annual grasses and forbs (*Bromus* spp., *Hordeum hystrix*, *Avena barbata*, *Vulpia* spp., and *Trifolium* spp.). The soil is an Argonaut silt loam (Mollic Haploxeralf), with a pH (1:1 soil:water) of 6.4, and total C and N concentrations of 49 and 3.4 g kg^{-1}, respectively.

Six plots were established within a 0.5-ha site. Three of the plots were located beneath the canopies of oak trees, and three plots were located in open grassy areas between oak trees. Plots of the two cover types were interspersed over the entire study site. Soil temperatures beneath one oak canopy and in an adjacent interspace were monitored for 18 months with a 21X data-logger (Campbell Scientific, Logan, UT). Thermistors were placed at the litter – mineral soil interface and at 4-cm and 10-cm soil depths. Temperature readings were made at 2 min. intervals, and mean values were recorded every 2 h.

In March, samples of the 0 to 1 cm and 1 to 9 cm mineral soil layers were collected from each of the six plots and sieved (<2 mm). The effect of temperature on V_{max} of nitrifier (ammonium-oxidizer) populations was evaluated using a nitrification potential assay described by Hart et al. (1994). In this assay, soil slurries (10 g dry wt. soil + 100 mL solution) are shaken for 24 h with a buffer solution containing sufficient NH_4^+ (1 m*M*) to produce maximum nitrification rates (V_{max}) and eliminate NO_3^- assimilation. Nitrate + nitrite accumulation can then be used as the measure of nitrification rate. The weak phosphate buffer solution used in the slurries was the same as that described by Belser & Mays (1980) except that sodium chlorate was not included. This nitrification potential assay was performed at ten temperatures ranging from 5 to 50 °C. Duplicate assays were performed for each of the 12 soil samples (2 vegetation cover types × 2 soil depths × 3 plots).

Nitrifier populations from beneath the oak canopies were shown to have lower temperature optima (31.8 °C) than populations from open interspaces (35.9 °C) (Stark and Firestone *in press*). Populations from different soil depths had similar temperature optima; therefore data from the two soil depths were combined to obtain a weighted average for the 0 to 9 cm layer, where the thickness of the soil layer was used for weighting. Separate equations were fit to data for the 0 to 9 cm layer of soils from beneath the oak canopies and in open interspaces.

Five different equations were fit to the nitrification rate and temperature data. The Arrhenius equation (Tinoco et al. 1985) was fit to nitrification rates from the lowest three incubation temperatures (5 to 22 °C) to estimate activation energy (E_a) (in kJ mole^{-1}) and the pre-exponential factor (A) (in mg kg^{-1} d^{-1}):

$$V_{max} = Ae^{\frac{-E_a}{RT}}$$

where V_{max} is the rate (mg kg^{-1} d^{-1}) from the nitrification potential assay, T is the temperature (°K), and R is the universal gas constant.

A double Arrhenius model (Moore 1986) was fit to nitrification rates over the complete range of incubation temperatures:

$$V_{max} = A_1e^{\frac{-E_{a1}}{RT}} - A_2e^{\frac{-E_{a2}}{RT}}$$

This equation describes the effects of temperature on two opposing processes. The first of the two Arrhenius equations predicts the increase in rate due to a reduced energy barrier for the reaction at higher temperatures, while the second predicts the decrease in rate due to enzyme denaturation and other destructive processes that occur at higher temperatures. This equation allows simultaneous estimation of activation energies for both processes.

The third model fit to the temperature and rate data was a generalized Poisson density function of the form:

$$\text{Relative rate} = \left(\frac{b-T}{b-a}\right)^{c} \exp\left\{\left(\frac{c}{d}\right)\left[1 - \left(\frac{b-T}{b-a}\right)^{d}\right]\right\}$$

where T is the temperature (°C), a is the temperature optimum (rel. rate = 1), b is the temperature maximum (rel. rate = 0), and c and d are shape parameters for portions of the curve to the right and left of the temperature optimum. The relative rates from this equation were multiplied by a scalar (m) to convert relative rates into absolute rates of V_{max} (in mg kg^{-1} d^{-1}). While this equation has no theoretical relationship to temperature responses, it has a flexible form and allows estimation of the temperature optimum using data from the full range of temperatures. The equation has also been used previously to model temperature response (e.g. Parton et al. 1987).

The fourth equation was an exponential function that has also been used to model temperature response (Innis 1978; Wight & Skiles 1987):

$$V_{max} = e^{(a+bT+cT^2)}$$

where T is the temperature (°C) and a, b, and c are empirically determined coefficients. This exponential function has an advantage in that initial estimates of the coefficients are easily obtained by taking the natural logarithm of both sides of the equation and using linear regression to solve the resulting polynomial equation.

The fifth equation is an empirical function referred to as a "square root" model (McMeekin et al. 1988) because the square root of the rate is proportional to temperature up to the optimum. Above the optimum, the rate decreases exponentially with temperature:

$$V_{max} = a(T - T_{min})(1 - e^{b(T - T_{max})})$$

where T is the temperature (°C), T_{min} is the minimum temperature (V_{max} = 0), T_{max} is the maximum temperature ($V_{max} = 0$), and a and b are empirically determined coefficients. McMeekin et al. (1988) used this equation to model the effect of temperature on growth rates of heterotrophic microorganisms, and Prosser (1990) recommended that it be investigated for use in modeling the temperature response of nitrification.

Rate and temperature data were fit to the five models by nonlinear least squares analysis. Sum of squares errors were minimized in successive iterations using a Marquardt algorithm in a routine provided by the KaleidaGraph software package (Synergy Software, Reading, PA). Different initial values were used to verify end-point stability. The fit of the models were compared using an F-test described by Beck & Arnold (1977) and recommended by Robinson (1985).

Results and discussion

All five of the equations had significant fits to nitrification and temperature data from soils of both vegetative cover types ($p < 0.05$) (Table 1) (Figures 1 and 2). An F-test showed that the Poisson function had a significantly better fit to data than the exponential, double Arrhenius, or square root functions ($p < 0.05$). The exponential equation had a reasonable fit to rates at moderate temperatures, but tended to underestimate rates at the lowest temperature and overestimate rates at the highest temperature.

Nonlinear regression using the double Arrhenius equation sometimes produced unstable estimates of model coefficients, primarily when the equation was fit to data from soils beneath oak canopies. This equation produces a curve that is strongly skewed to the right (Figures 1 and 2). The portion of the curve to the right of the temperature optimum is so steep that, as the curve shifts to the left to fit the low temperature data, the residual sum of squares

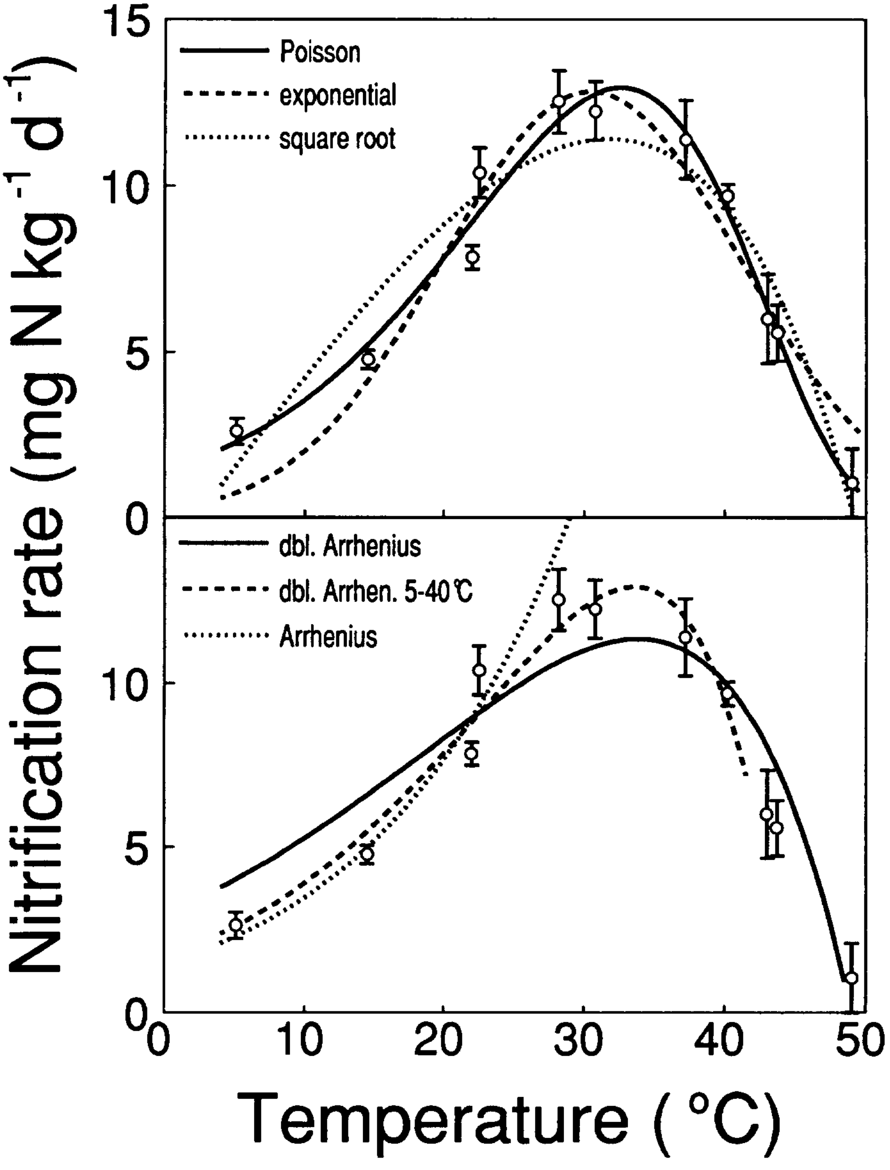

Figure 1. Fit of models to temperature response data for nitrification rates in soils from beneath oak canopies. Data points and error bars represent means and standard errors ($n = 3$) for samples collected from the 0 to 9 cm soil layer of three plots in March.

rapidly approaches infinity. Therefore, the rates at high temperatures tend to have a strong influence on the model coefficients. When the high temperature data (>41 °C) were excluded, the double Arrhenius equation had a much

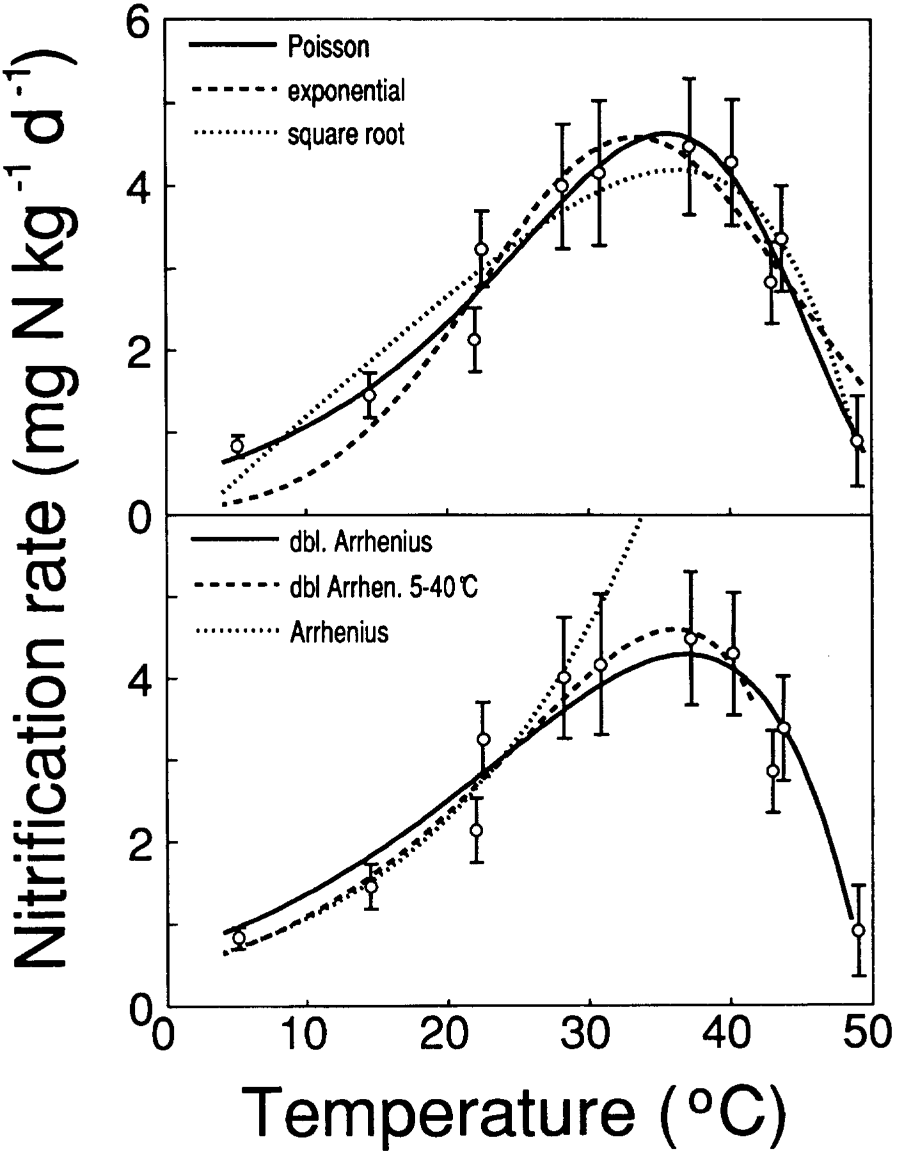

Figure 2. Fit of models to temperature response data for nitrification rates in soils from open grassy interspaces. Data points and error bars represent means and standard errors (n = 3) for samples collected from the 0 to 9 cm soil layer of three plots in March.

better fit to the remaining data. The R^2 values for this limited data set were 0.949 and 0.953 for fits to rates from canopy and open sites, respectively, compared to R^2 values of 0.856 and 0.927 for the fit of the original equation to data for temperatures <41 °C.

Table 1. Coefficients for various temperature response functions fit to nitrification rates in soils collected from beneath oak canopies and open grassy interspaces in a California oak woodland-annual grassland.

Poisson function		Exponential function		Arrhenius equation		Double Arrhenius equation		Square root function	
Canopy	Open	Canopy	Open	Canopy	Open	Canopy	Open	Canopy	Open
a = 32.6	35.6	a = −1.55	−3.09	A = 4.69 × 10^{10}	5.12 × 10^{9}	A_1 = 2.34 × 10^{11}	8.41 × 10^{11}	a = 0.622	0.154
b = 59.8	61.4	b = 0.269	0.277	E_a = 54.9	52.5	E_{a1} = 52.9	60.9	b = 0.0562	0.118
c = 20.1	97.7	c = −0.00442	−0.00415			A_2 = 6.49 × 10^{11}	8.94 × 10^{12}	T_{min} = 2.32	2.21
d = 0.326	0.0625					E_{a2} = 55.6	67.2	T_{max} = 49.1	50.0
m = 12.9	4.64								
R^2 = 0.976	0.949	0.918	0.875	0.927	0.835	0.861	0.912	0.905	0.902
predicted relative rate[1]:									
1.00	1.00	0.78	0.87	0.97	1.05	1.26	1.08	1.19	1.09

[1] Rates predicted by the equations for temperatures that normally occur in the 0 to 1 cm soil layers beneath oak canopies and in open grassy interspaces (shown in Figure 3). All values are expressed relative to the rates predicted by the Poisson function.

The square root function had a reasonable fit at moderate to high temperatures, but a poor fit at low temperatures (Figures 1 and 2). This was because the curve to the left of the temperature optimum tended to be concave downward, whereas the rate data showed a concave upward relationship with temperature.

When only the three lowest temperatures ($\leq$22 °C) were considered, the simple Arrhenius equation had a significantly better fit than any other equation ($p < 0.05$). This equation had a reasonable fit to data from temperatures as high as 28 °C; however, at temperatures near the optimum or higher the equation had a poor fit.

For many modeling applications, it may not be necessary to use an equation that predicts the response at temperatures higher than the optimum. This is because temperature optima may be higher than the temperatures that normally occur in the environment. In almost all cases, nitrifier populations in the soils of the oak woodland-annual grassland had temperature optima that were higher than even the most extreme soil temperatures (Figure 3).

While the Poisson equation had the best fit over the entire range of temperatures assayed, the Arrhenius equation had an excellent fit over much of the temperature range that normally occurs in this soil, and temperatures where the Arrhenius equation had a poor fit (>28 °C) occurred at a relatively low frequency (Figure 3). The five equations were used to predict nitrification rates, given the frequency distribution of temperatures in the 0 to 1 cm soil layers. Rates predicted by the Arrhenius equation were within 5% of the rates predicted by the Poisson equation (Table 1), while rates predicted using the other equations were 8 to 26% higher or lower than the Poisson equation.

The observation that the temperature optima for nitrification are higher than the maximum soil temperatures is somewhat surprising, considering that nitrification rates would be maximized if the temperature optima corresponded to the mean soil temperature during the active period. A lack of overlap between temperature optima and soil temperatures may occur because the energetic cost of operating near the temperature optimum is greater than the benefit produced from higher rates. Temperatures above the temperature optima depress rates by enzyme denaturation and other destructive processes (VanDemark & Batzing 1987). Replacement of these denatured enzyme systems increases maintenance energy requirements. At some point the increased energy production from higher nitrification rates will be offset by increased maintenance energy requirements. In fact, it is not high rates of gross energy production that should be selected for during adaptation to temperature, but high rates of net energy production (gross energy production minus maintenance energy requirement), since net energy production represents the amount of energy that is available for growth and reproduction. Figure 4 shows how

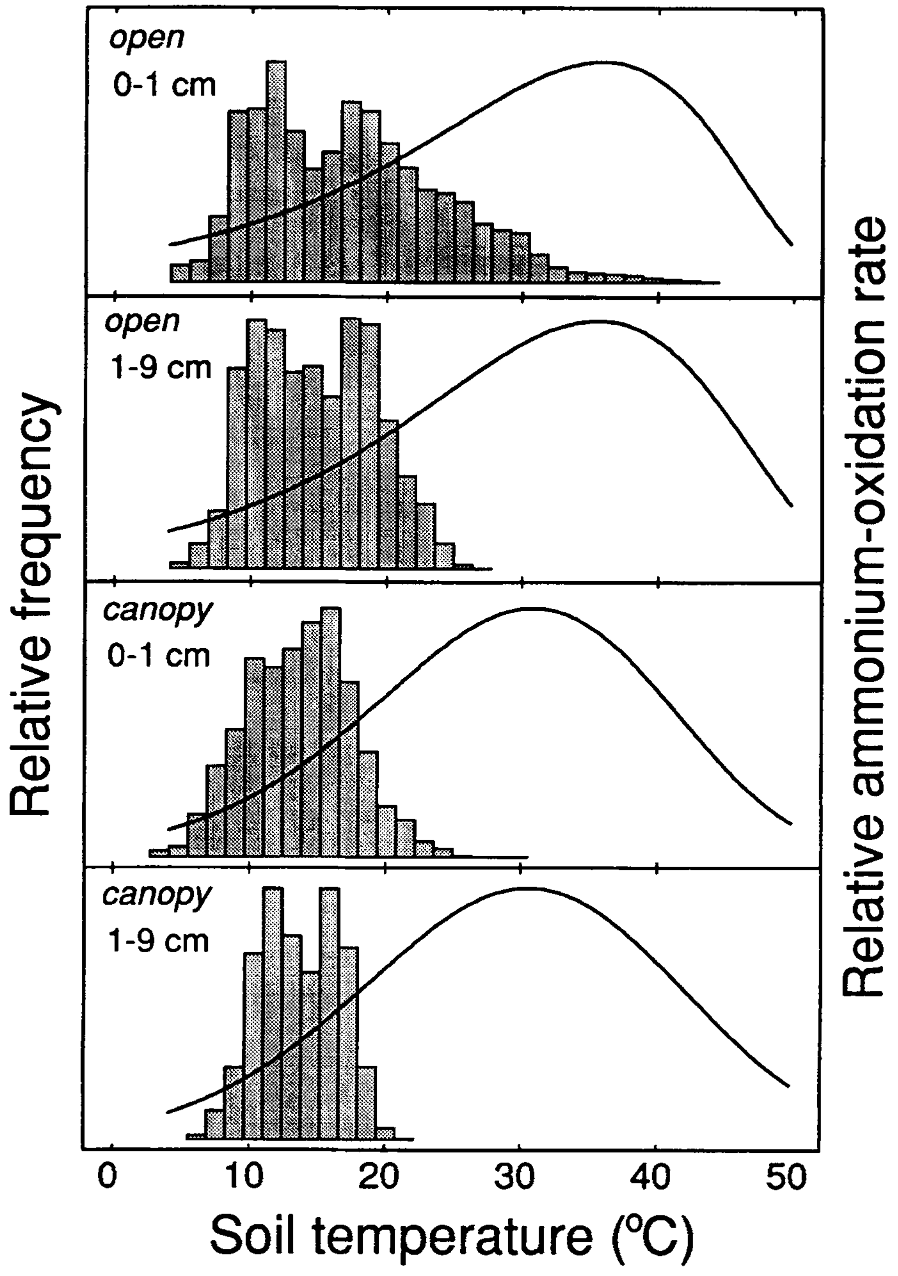

Figure 3. Relative frequency histograms of soil temperatures (2-h means) occurring between January and May, and temperature response curves for nitrifier populations in the 0 to 1 and 1 to 9 cm soil layers beneath oak canopies and in open grassy interspaces.

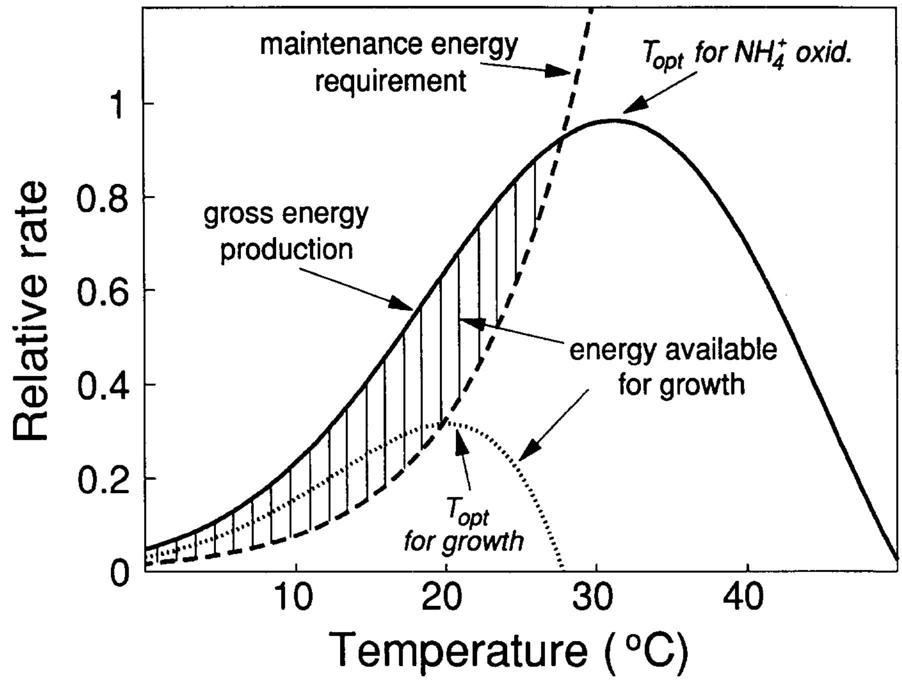

Figure 4. Theoretical effect of temperature on maintenance energy requirements and gross energy produced from nitrification. The difference between these two curves (indicated by the vertical lines and plotted as the dotted curve) represents the amount of energy available for growth. The effect of temperature on nitrification rates was modeled by the Poisson equation using the coefficients determined for populations from the 0 to 1 cm soil layer beneath oak canopies. The effect of temperature on the maintenance energy requirement was modeled by the Arrhenius equation. Coefficients for the Arrhenius equation ($A = 1.3 \times 10^{17}$ mg kg^{-1} d^{-1}, $E_a = 98.7$ kJ mole^{-1}) were obtained using an optimization procedure to maximize the energy available for growth at the temperatures occurring in this soil (see Figure 3). In this scenario, the temperature optimum for nitrification is 30 °C, but the temperature optimum for growth is 20 °C.

differences in the temperature responses of nitrification rates and maintenance energy requirements could result in maximal growth rates at the soil temperatures that commonly occur.

The nitrifier population from the surface of open interspaces was the only population that had temperature optima below the maximum soil temperatures encountered (Figure 3). This may be because the evolutionary limit of temperature tolerance has already been reached by the enzymes involved. The temperature optimum of this population (35.9 °C) is near the maximum values reported in the literature even for tropical soils (35 to 37 °C) (Myers 1975), while the temperature optimum of populations beneath oak canopies (31.8 °C) is in the middle of the range of reported values (20 to 37 °C) (Malhi

& McGill 1982; Haynes 1986). If the maximum temperature optimum for nitrification is 35 to 37 °C, then soil temperatures in warmer climates may frequently exceed the temperature optimum. In these cases, the Poisson function would provide more accurate estimates of nitrification rates than the simple Arrhenius equation.

Of the models describing temperature response, the generalized Poisson function fit nitrification rates best over the full range of temperatures (5 to 50 °C); however, it may not be necessary to model temperature effects over a range this wide. At this site, soil temperatures fall within a range that can be adequately modeled using the simple Arrhenius function (5 to 28 °C). In fact, for the range of soil temperatures encountered at the study site, the Arrhenius equation more closely predicted nitrification rates in soil slurries than did the Poisson function. However, if one wishes to model growth rates, nitrification rates in warmer climates, or the effects of more drastic changes in environmental conditions such as those that might result from climate change or habitat destruction (e.g. elimination of the oak canopy) where soil temperatures reach or exceed temperature optima, the Poisson function may provide more reliable predictions of temperature responses.

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