Oecologia (2002) 132:175–180
DOI 10.1007/s00442-002-0954-0

ECOPHYSIOLOGY

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Physiological response curve analysis using nonlinear mixed models

Received: 1 October 2001 / Accepted: 15 April 2002 / Published online: 1 June 2002

Abstract Nonlinear response curves are often used to model the physiological responses of plants. These models are preferable to polynomials because the coefficients fit to the curves have biological meaning. The response curves are often generated by repeated measurements on one subject, over a range of values for the environmental variable of interest. However, the typical analysis of differences in coefficients between experimental groups does not include a repeated measures approach. This may lead to inappropriate estimation of error terms. Here, we show how to combine mixed model analysis, available in SAS, that allows for repeated observations on the same experimental unit, with nonlinear response curves. We illustrate the use of this nonlinear mixed model with a study in which two plant species were grown under contrasting light environments. We recorded light levels and net photosynthetic response on anywhere from 8 to 10 points per plant and fit a Mitscherlich model in which each plant has its own coefficients. The coefficients for the photosynthetic light-response curve for each plant were assumed to follow a multivariate normal distribution in which the mean was determined by the treatment. The approach yielded biologically relevant coefficients and unbiased standard error estimates for multiple treatment comparisons.

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Keywords Nonlinear models · Response curve analysis · Mixed models · Light curves

Introduction

Many ecological and physiological studies include the analysis of response curves, commonly constructed by plotting a measured variable against a range of a factor that affects the variable. For example, soil incubation studies measure reaction rates, microbial activity, or microbial biomass in soil ecosystems over time (Coleman and Crossley 1996). Plant physiological ecologists measure photosynthetic rates in response to a variety of environmental variables, including light, temperature, humidity, or carbon dioxide concentration (Larcher 1995).

Physiological response curves that are measured in response to an experimental treatment are often nonlinear and contain repeated measurements on the same experimental unit. As a result, these data require the use of a nonlinear function with a statistical treatment that will include both fixed (treatment) and random (experimental unit) effects. Fixed effect analyses are one of the most common statistical inference techniques used in cases where the researcher designates the levels of interest. For example, temperature and species would be fixed effects in an experiment where plants from two different species are grown in different temperature regimes set by the investigator. In contrast, random effects, or experimental unit effects, are those factors whose variation is due to membership of some larger population of potential subjects. When multiple observations are recorded on the same unit, any factor or coefficient that may vary from unit to unit is regarded as random. So a mean of a population is fixed because all units from that population have that mean in common. However, individual units (e.g., plants or leaves) may exhibit random deviations from that population mean. In the previous example, random effects would stem from samples of different leaves from the same plant, or from repeated measurements on the same leaf over time. A mixed model is one that incorpo-

rates both fixed and random effects. Repeated measurements often incorporate both random effects because measurements are taken on the same experimental unit (e.g., the same plant, or the same leaf) and fixed effects since most researchers are interested in making inferences about a treatment group on the basis of the subjects sampled. Potvin et al. (1990) recognized that such response curves should utilize a repeated measures approach. They suggested several techniques, including repeated measures ANOVA, multivariate repeated measures ANOVA, and nonparametric split-plot analysis. The preferable analysis depended upon assumptions of each model. However, models suggested by Potvin et al. (1990) were linear, and were therefore only able to test treatment differences. Lost was the ability to test biologically meaningful parameters from common nonlinear functions (e.g., *k* from a Michaelis-Menten relationship).

This influential paper gave direction to ecophysiologists when analyzing response curves. To date, Potvin et al. (1990) has been cited 265 times according to the Science Citation Index. However, numerous studies since the publication of Potvin et al. (1990) still fail to treat response curves with a repeated measures approach. We conducted a survey of 16 ecological journals that published papers since 1991 that reported the results of photosynthetic responses to light and CO_2. We found 56 papers that did not cite Potvin et al. (1990), and only one of these papers used a repeated measures linear model. Thirty-five used common curve-fitting programs lacking a repeated measures approach (e.g., Sigma Plot or Systat) to fit data to a nonlinear function. Five others dissociated curves into linear portions and used least squares linear regression techniques. Twelve papers calculated means and their respected standard errors at each CO_2 or light level to examine treatment differences and three papers fitted higher order polynomials to the data.

Significant advancements in computing power and statistical software have been made since 1990. These advances now make possible a nonlinear regression analysis of response curves that was only briefly mentioned in Potvin et al. (1990). At that time, this technique had numerous limitations, including the necessity of a fixed design matrix (i.e., fixed values for the independent variable) and the inability of the analysis to handle unbalanced or incomplete data. Here, we illustrate an analysis of response curves that overcomes the problems Potvin et al. (1990) pointed out when using nonlinear models. We combine the advantages of a mixed model analysis with the straightforward interpretability of nonlinear curves. We used procedures available in a widespread statistical package (SAS 2000), to apply nonlinear mixed models to photosynthetic light response curves that are typical of many physiological plant ecology studies where light levels are not fixed. We then compare the results of the nonlinear mixed model analysis with a fixed effects ANOVA, which was the most common analysis approach seen in our survey.

Materials and methods

Data collection and treatments

Data presented here were part of a study to examine the effects of light environment during growth on photosynthetic characteristics of two eastern North American, herbaceous legumes, *Strophostyles helvola* and *Amphicarpa bracteata* (Prichard and Forseth 1988a, b). Plants were grown in a glasshouse on the University of Maryland, College Park campus from February to June, where they were watered to saturation daily and fertilized bimonthly with Peters 20-20-20. Individuals were randomly assigned into two light treatments. Low light treatments were grown under shade cloths that reduced incident light levels over 10-fold [2.4 mol m^{-2} total daily photosynthetic photon flux (PPF, radiation between 400–700 nm) on April 30] compared to unshaded, high light treatments (34.3 mol m^{-2} total PPF on April 30).

The response of net photosynthesis (*A*) to incident PPF was measured on leaves of three randomly selected plants from each species per treatment. Two to three leaflets from the palmately compound leaves of each individual were placed within the cuvette of a Bingham Interspace (Logan, Utah) Model BI-IIdp photosynthetic system (Prichard and Forseth 1988b). Temperature, CO_2 concentration, and humidity inside the cuvette were maintained at constant levels during the measurement cycle. Steady-state values of *A* and stomatal conductance were maintained for 10–15 min at each PPF from 1,800 down to 0 μmol m^{-2} s^{-1}, at which point *A* and PPF were recorded. Light level was modified through the use of neutral density filters. Eight to 10 points per plant were measured.

Model fitting

Our approach utilizes a nonlinear mixed model in the form:

$$y = f\left(x_{ij}, \beta, u_i\right) + e_{ij} \quad (1)$$

where *f* is some nonlinear function of known vector covariates (x_{ij}) for the *j*th observation on the *i*th subject, unknown fixed effect parameters (β), an unknown vector of random effect parameters (u_i), and unknown random errors (e_{ij}) (Davidian and Giltinan 1995). For our experiment, our fixed effect (β) treatment design is a 2×2 factorial with two plant species (*S. helvola* and *A. bracteata*) and two light regimes (high and low light). Taking repeated measurements on the same leaflets at different light levels necessitates incorporation of random effects (u_i). If random effects were not in our model, the assumption of independent error terms would be violated, producing biased standard errors. The nonlinear function (*f*) we used in Eq. 1 was the Mitscherlich model equation (after Potvin et al. 1990):

$$A = A_{max}\left[1 - e^{-A_{qe}(PPF-LCP)}\right] \quad (2)$$

where A_{max} represents the asymptote of photosynthesis at high light, A_{qe} corresponds to the initial slope of the curve at low light levels, LCP denotes the *x*-intercept, when net photosynthesis is equal to zero, PPF refers to the incident photosynthetic photon flux and *A* is net photosynthesis, the response variable. Each unknown parameter has physiological meaning relating to plant performance. The three used in the model identify the light saturated rate of photosynthesis (A_{max}), apparent quantum yield (A_{qe}), and the photosynthetic light compensation point (LCP). Since the three parameters (A_{max}, A_{qe} and LCP) vary by individual plant, we assumed that the mean of these three terms varied by treatment and that these coefficients followed a multivariate normal distribution. The latter allowed A_{max}, A_{qe} and LCP to be correlated, so that a plant with an above average A_{max} might be expected to have an above average initial slope (A_{qe}).

We used a nonlinear mixed models procedure in SAS Version 8 (SAS 2000) to fit curves to photosynthetic data from each plant using to the nonlinear Eq. 2 with the fixed and random effects in Eq. 1. The mean of the three parameters (A_{max}, A_{qe}, and LCP) was

modeled in two different ways to incorporate our treatment design. First it was modeled analogous to a one way model in which each of the four treatments had its own mean for each of the three terms. Performing a multiple comparison procedure following such an analysis required using SAS/IML, since NLINMIXED has no contrast or multiple comparison procedures. Next it was modeled as a two-way ANOVA in which species and light regime were factors. In a 2×2 factorial, main effects and interactions for a given parameter only involve a single coefficient, so no multiple comparison procedures were required. Both methods are described below in Analysis of parameter estimates. Once our model was established by combining Eqs. 1, and 2, we needed to provide initial estimates of the parameters (A_{max}, A_{qe} and LCP) to start iterations. Seed estimates for each parameter were obtained using PROC NLIN in SAS. This procedure gives reasonable starting values for successful convergence but ignores the repeated measures aspects of the study design. It was necessary to re-scale the slope (A_{qe}) by a factor of 0.0001 due to convergence problems attributed to rounding error. Several integral approximations and optimization algorithms exist in SAS to model random effects and to minimize iteration time and memory, which are described elsewhere (Pinheiro and Bates 1995; SAS 2000).

Analysis of parameter estimates

Conventional fixed effects ANOVA models can be analyzed using a regression model based on dummy variables (Kleinbaum et al. 1988). The primary function of coding dummy variables is to examine individual levels of fixed effect treatments by assigning coefficients (e.g., 0 or 1) to different levels of treatment. In effect, a value of 0 drops that treatment level from analysis, while a value of 1 includes the level of that treatment. Normally, procedures such as PROC GLM in SAS, as well as comparable packages, do this coding automatically when the user specifies a CLASS statement. The nonlinear mixed model procedure in SAS lacks a class statement, so the user must code dummy variables for a particular treatment design. We need *k*–1 dummy variables if *k* treatment levels are to be compared (see Appendix lines 0001–0005). For each parameter in our model (i.e., A_{max}, A_{qe} and LCP), we wrote a one-way ANOVA model to analyze treatment effects:

$$A_{\max ij} = \gamma_0 + \gamma_1 Z_1 + \gamma_2 Z_2 + \ldots + \gamma_{k-1} Z_{k-1} + \text{Error} \quad (3)$$

where $A_{max\ ij}$ is the parameter estimate for the j^{th} plant in the i^{th} treatment group, γ_1 is the mean of A_{max} for the last treatment group and γ_i is the mean of A_{max} for the i^{th} treatment group minus the mean of A_{max} of the last treatment group where i=1, 2,..., k–1. Under this model, if γ_i=0 for i=1, 2,..., k–1, then all treatments have the same mean. For our example we had four treatment groups (two species at two light levels). We therefore coded 4–1, or 3 dummy variables. We arbitrarily set our treatment order to be, *S. helvola* high light as γ_0, *S. helvola* low light as γ_1, *A. bracteata* high light as γ_2 and *A. bracteata* low light as γ_3. Therefore, the mean of *S. helvola* low light is defined as $\gamma_1+\gamma_0$, the mean of *A. bracteata* high light is $\gamma_2+\gamma_0$ and so on. Thus, all other treatment groups were re-scaled from *S. helvola* high light (γ_0). For example, we obtained an A_{max} parameter estimate of 42 for *S. helvola* high light and an A_{max} parameter estimate of –27 for *S. helvola* low light from our initial model output. The actual parameter estimate of *S. helvola* low light is not –27, but must be re-scaled as a function of *S. helvola* high light. We therefore added –27 to 42, yielding a parameter estimate of 15 for *S. helvola* low light (Table 2).

Thus far, we have chosen the appropriate statistical model for our data (Eq. 1), chosen an appropriate nonlinear function to fit our data (Eq. 2), and coded our treatment design (Eq. 3), thus combining Eqs. 1, 2 and 3 for the full analysis (see Appendix for syntax). To start the model iterations we used the estimates obtained in NLIN, ignoring random effects, to generate initial values of γ_1, γ_2 and γ_3 for A_{max}, and did the same for A_{qe} and LCP. NLINMIX uses an iterative approach based on these initial values to generate a solution that properly accounts for repeated measures designs. The NLINMIX procedure generated estimates of all 12 parameters (4 treatments with 3 parameters per treatment) along with an estimated covariance matrix. We fed these values into SAS/IML to test hypotheses using Wald's test (Davidian and Giltinan 1995). Wald's test can be used to test hypotheses concerning either single or multiple parameters. For example, we can ask if there are differences in the overall response curves for the four treatment groups, or if A_{max} differs significantly among treatment groups. To be consistent with NLINMIX, we modified the critical value from Wald's test to be from the appropriate *F*-distribution. Wald's statistic normally compares the square of (estimate/standard error) to a χ^2 table value for testing a single parameter. So, we chose an $F_{1,\ ddfm}$ where the denominator degrees of freedom (ddfm) are the degrees of freedom used in the *t*-tests in the NLINMIX procedure. If this modification were not made, conclusions would be sensitive to which treatment group was coded last. Users may also adjust for multiple comparisons using Bonferonni adjustments (Kleinbaum et al. 1998). Since our treatment design was also a 2×2 factorial, we substituted an alternative model for Eq. 3 to examine main effects and interactions:

$$A_{\max ij} = \gamma_0 + \gamma_1 Z_1 + \gamma_2 Z_2 + \gamma_3 Z_1 Z_2 + \text{Error} \quad (4)$$

where Z_1=1 if species is *S. helvola* and Z_1=–1 otherwise; and Z_2=1 if high light regime and Z_2=–1 otherwise; γ_1 represents the main effect of species, γ_2 is the main effect for light regime and γ_3 is the interaction.

Finally, we analyzed the parameter estimates obtained with the NLIN method (ignoring random effects) in a fixed effect ANOVA to compare the conclusions made from the fixed effect ANOVA and the nonlinear mixed model.

Results

We obtained estimates for each of the three model parameters for *S. helvola* plants grown in high light, *S. helvola* plants grown in low light, *A. bracteata* plants grown in high light, and *A. bracteata* plants grown in low light (Fig. 1). Mixed model analysis showed that *S. helvola* plants grown under high light had a significantly higher photosaturated photosynthetic rate (A_{max}) than *A. bracte-*

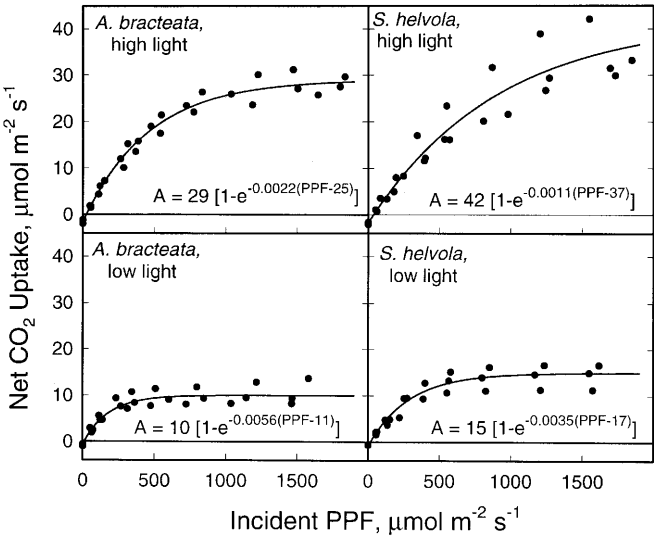

Fig. 1 Plot of net photosynthetic CO_2 assimilation against incident photosynthetic photon flux for plants of two species grown under two different light environments. Also shown are fitted curves with model equations from parameter estimates obtained from a nonlinear mixed models analysis

Table 1 Statistical comparison of photosaturated photosynthesis (A_{max}), apparent quantum yield (A_{qe}), and light compensation point (*LCP*), parameter estimates modeled as a one-way ANOVA and as a two-way factorial with main effects and interactions. Modified *F*-tests ($F_{1, 9}$) were performed for each parameter estimate using the estimated covariance matrix (see text for details)

	A_{max}	A_{qe}	LCP
Species effects within light regimes (*Strophostyles helvola* vs *Amphicarpa bracteata*)			
High light	**F*=7.9	**F*=4.3	*F*=0.2
	P<0.05	*P*<0.05	*P*>0.05
Low light	*F*=2.6	*F*=0.8	*F*=0.04
	P>0.05	*P*>0.05	*P*>0.05
Light effects within species (high light vs low light)			
S. helvola	**F*=34.6	*F*=4.2	*F*=0.4
	P<0.05	*P*>0.05	*P*>0.05
A. bracteata	**F*=42.6	*F*=2.5	*F*=0.3
	P<0.05	*P*>0.05	*P*>0.05
2×2 factorial analysis			
Species	**F*=10.6	*F*=2.4	*F*=5.1
	P<0.01	*P*>0.05	*P*>0.05
Light	**F*=70.8	**F*=6.1	*F*=0.19
	P<0.0001	*P*<0.05	*P*>0.05
Species×Light	*F*=1.9	*F*=1.2	*F*=0.78
	P>0.05	*P*>0.05	*P*>0.05

*Significant at *P*<0.05

Table 2 Summary of parameter estimates with statistical comparisons for the nonlinear mixed model and the fixed effect ANOVA model. Means are presented with standard errors for each of the three parameter estimates. Statistical comparisons for the nonlinear mixed model are based on probabilities in Table 1. The fixed effect analysis is based on LSD pair-wise comparisons. Different letters assigned to means in the same column designate statistically significant differences at the 0.05 level, the same letters indicate nonsignificance

Treatment	Photosaturated photosynthesis (A_{max})		Apparent quantum yield (A_{qe})		Light compensation point (LCP)	
	Mixed	Fixed	Mixed	Fixed	Mixed	Fixed
S. helvola, High light	42±4.3[a]	42±2.7[a]	0.0011±0.00026[a]	0.0011±0.00018[a]	40±21[a]	41±4.6[a]
S. helvola, Low light	15±4.7[c]	15±2.7[c]	0.0035±0.00096[a]	0.0036±0.00018[b]	20±30[a]	17±4.6[b]
A. bracteata, High light	29±4.8[b]	29±2.7[b]	0.0022±0.00044[a]	0.0022±0.00018[c]	30±26[a]	25±4.6[b]
A. bracteata, Low light	10±4.7[c]	10±2.7[c]	0.0056±0.00215[a]	0.0059±0.00018[d]	10±30[a]	12±4.6[b]

ata plants grown under high light. Both species grown in high light had significantly higher photosaturated photosynthetic rates than either species grown in low light treatments, yielding a main effect of light (Table 1, $F_{1, 9}$=70.8, *P*<0.0001). In addition, *S. helvola* had significantly higher A_{max} values compared to *A. bracteata* producing a main effect of species (Table 1, $F_{1, 9}$=10.6, *P*<0.01). The main effect of light was also significant for apparent quantum yield (Table 1, $F_{1, 9}$=6.1, *P*<0.05). The low light treatments, however, were not significantly different across species (Table 1). No significant differences were found for the parameter estimates for apparent quantum yield (A_{qe}) and dark respiration (LCP) within species or light regime (Table 1). There were also no interaction effects (Table 1).

The fixed effect ANOVA model generated the same statistical conclusion for photosaturated photosynthetic rates (A_{max}) (Table 2). However, the standard error estimates are all equal (due to the assumption of homogeneity of variances in the ANOVA model) and smaller than the repeated measures analysis (Table 2). In contrast to mixed model results, significant treatment differences were found for quantum yield (A_{qe}) and dark respiration (LCP) (Table 2). This is due primarily to smaller standard error estimates. Therefore, the results given in the fixed effects ANOVA may be misinterpreted as being significantly different as a result of biased standard errors due to incorrectly accounting for the correlation of data points within each curve.

Discussion

Advantages of combining mixed models with nonlinear equations

Software to model linear models with repeated measures has progressed considerably since Potvin et al. (1990). With procedures available in SAS and S-plus, unbalanced and unequally replicated repeated measures designs can be accommodated. In addition, variability and correlation among observations may be modeled. Even correlation of data replicated in time and space can be accommodated to some degree in these readily accessible procedures (Littell et al. 1996). However, these procedures fail to allow nonlinear equations to enter into the model.

Nonlinear mixed models are not new and have been used in pharmacokinetic research for many years (Vonesh and Chinchilli 1997). For example, simple compartment models are used to derive nonlinear equations describing changes in blood concentration over time. However, nonlinear mixed model analysis is underused in the ecological literature. We could find only two studies that used this technique (Piepho 1999; Myers et al. 2001). Piepho (1999) fitted nonlinear models to environmental response variables for different genotypes, emphasizing the biological meaning of parameter estimates. Myers et al. (2001) determined biologically reasonable parameter estimates for carrying capacities of North Atlantic cod populations using nonlinear mixed model regression. In addition, Scheiner and Gurevitch (2001) discuss repeated measures and nonlinear responses as separate analyses, but do not address the utility of combining the two.

Nonlinear mixed models combine several useful features of previously available analyses. They can account for both repeated measures ANOVA (Potvin et al. 1990) and nonlinear responses of test subjects (Ratkowsky 1983). The nonlinear mixed models procedures in SAS and in S-plus are flexible, allowing the user to decide which parameters are fixed and which are random. An appropriate coding of dummy variables (see Kleinbaum et al. 1988) can easily accommodate many treatment designs. In addition, fitting an appropriate nonlinear model lends biological meaning to estimated parameters. Of particular interest to physiological ecologists is the use of hypothesis testing for differences in parameter estimates (i.e., slopes, intercepts, asymptotes). For example, biochemical models of photosynthesis relate curve parameters to specific limitations in the photosynthetic pathway (Farquhar and Sharkey 1982). Being able to obtain parameter estimates for those models and doing tests of significance is an extremely useful tool for treatment comparisons.

Potvin et al. (1990) pointed out that a fixed design matrix (i.e., fixed levels of the independent variable) for each experimental unit had to be used, due to limitations in computing power in nonlinear curve analysis. Any variability in the independent variable would lead to loss of precision of the parameter estimates. For example, Currie (1982) determined that variability in the design matrix influenced the precision of the parameter estimates of the Michaelis-Menten equations. Here again, advances in statistical software, particularly integration of maximum likelihood algorithms, and computing power eliminate any problems with the values assigned to the design matrix. These iterative algorithms allow for the values assigned independent variable to be continually updated for each experimental unit (Pinheiro and Bates 1995). Typically, photosynthetic response curves do not have fixed independent values due to methodology or instrument limitations. In the photosynthetic data we used here, neutral density filters were implemented to modify PPF levels, which consequently varied for each measurement cycle – producing different levels of PPF for each plant, i.e., the design matrix.

Nonlinear mixed procedure can fit nonlinear models to situations where both fixed and random effects are nonlinear. PROC NLMIXED fits the model by maximum likelihood techniques. Several other algorithms and iterative approaches are available (SAS 2000), which allow different distribution assumptions for the response variable (e.g., normal, binomial or Poisson). In our model, we assumed independence across subjects, but correlation within subjects due to repeated observations on the same experimental unit. Random effects were also assumed to follow a bivariate normal distribution. These assumptions may be modified on a case-by-case basis. A more extensive discussion of nonlinear mixed models methods can be found in Davidian and Giltinan (1995), Pinheiro and Bates (1995), Vonesh and Chinchilli (1997) and McCulloch and Searle (2000).

Applications of nonlinear mixed models

Nonlinear response data are prevalent in environmental and ecological studies. Some of the most common are logistic growth curves, photosynthetic response curves (Larcher 1995), pressure volume curves (Urban et al. 1993), hydraulic conductivity analysis (Sperry et al. 1988) and numerous uses of the Michaelis-Menten kinetic equations. These models all have the advantage of having biologically meaningful parameters. They also require a rigorous statistical handling of correlated data because measurements are often taken on the same subject (Werker and Jaggard 1997), or on the same sample (Lindstrom et al. 1998). The benefit of this method is that it is not limited to a defined set of nonlinear equations or treatment design. The user defines the nonlinear model that applies to a particular data set or response curve then codes the appropriate treatment schedule with dummy variables. This technique could therefore apply to numerous areas of study, such as growth analysis (Zeger and Harlow 1987), dose-response curves (Calabrese and Baldwin 1999 and references within), survivorship curves (Karban 1997), and developmental patterns (Knight et al. 1991).

Conclusions

The advancement of statistical methodology and computing power has significantly expanded the techniques available to ecologists for data analysis. We advocate using nonlinear mixed models for a more rigorous statistical analysis of correlated data, particularly in the case of data with repeated measures, where many of the prior limitations have been alleviated (Potvin et al. 1990). Mixed model approaches are also appropriate for unbalanced or incomplete data sets, the norm in ecological studies. They allow better analysis of data collected outside of a fixed design matrix, another common problem in ecology due to instrumentation limitations, time constraints, or methodological differences. Nonlinear mixed

models have the further advantage of being able to attach biological meaning to parameters of nonlinear curves, a decided advantage when interpreting higher order functions. A final benefit of nonlinear mixed models, is that they do not require large numbers of observations on each experimental unit.

Acknowledgements We thank A.B. Griffith and two anonymous reviewers for comments on earlier versions of this manuscript.

Appendix

Nonlinear mixed model SAS syntax with a four-treatment design (SAS 2000).

- 0001 ****Coding dummy variables for *k*–1 treatment groups;
- 0002 if cat='SH' then do; *z*1=0; *z*2=0; *z*3=0; end;
- 0003 if cat='SL' then do; *z*1=1; *z*2=0; *z*3=0; end;
- 0004 if cat='AH' then do; *z*1=0; *z*2=1; *z*3=0; end;
- 0005 if cat='AL' then do; *z*1=0; *z*2=0; *z*3=1; end;
- 0006 *****Initiating nonlinear mixed model procedure;
- 0007 Proc nlmixed data=Light;
- 0008 ****Initial seed estimates from Proc NLIN procedure to start iterations;
- 0009 parms *b*01=35 *b*11=–20 *b*21=–5 *b*31=–25
- 0010 *b*02=65 *b*12=–10 *b*22=5 *b*32=–3
- 0011 *b*03=34 *b*13=–10 *b*23=–10 *b*33=–10;
- 0012 ****Modeling parameter estimates for 4 treatments using dummy variable coding;
- 0013 *b*1=*b*01+*b*11*z*1+*b*21*z*2+*b*31*z*3+*u*1;
- 0014 *b*2=*b*02+*b*12*z*1+*b*22*z*2+*b*32*z*3+*u*2;
- 0015 *b*3=*b*03+*b*13*z*1+*b*23*z*2+*b*33*z*3+*u*3;
- 0016 ****Nonlinear Mitscherlich model;
- 0017 e=exp[(–0.0001**b*2)*(*P*–*b*3)];
- 0018 pred=*b*1*(1–e);
- 0019 ****Gaussian quadrature algorithm; model A ~normal(pred, 8);
- 0020 ****Specifying random effects with mean=0 and variances=8;
- 0021 random *u*1 *u*2 *u*3 ~ normal ([0, 0, 0],[8, 8, 8, 8, 8, 8]) subject=plant; Quit;

References

Calabrese EJ, Baldwin LA (1999) Reevaluation of the fundamental dose-response relationship. BioScience 49:725–732

Coleman DC, Crossley DA (1996) Fundamentals of soil ecology. Academic Press, New York

Currie DJ (1982) Estimating Michaelis-Menten parameters: bias, variance and experimental design. Biometrics 38:907–919

Davidian M, Giltinan DM (1995) Nonlinear models for repeated measurement data. Chapman and Hall, New York

Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. Annu Rev Plant Phys 33:317–345

Karban R (1997) Evolution of prolonged development: a life table analysis for periodical cicadas. Am Nat 150:446–461

Kleinbaum DG, Kupper LL, Muller KE, Zam AN (1988) Applied regression analysis and multivariable methods. Duxbury Press, Boston

Knight AL, Beers EH, Elsner EA (1991) Modeling postdiapause egg and nymphal development of the white apple leafhopper (Homoptera: Cicadellidae). Environ Entomol 20:1659–1664

Larcher W (1995) Physiological plant ecology, 3rd edn. Springer, Berlin Heidelberg New York

Lindstrom JE, Barry RP, Braddock JF (1998) Microbial community analysis: a kinetic approach to constructing potential C source utilization patterns. Soil Biol Biochem 30:231–239

Littell R, Milliken F, Stroup W, Wolfinger R (1996) SAS system for mixed models SAS Institute, Cary, N.C.

McColloch CE and Searle SR. 2000. Generalized, linear and mixed models. Wiley, New York

Myers RA, MacKenzie BR, Bowen KG, Barrowman NJ (2001) What is the carrying capacity for fish in the ocean? A meta-analysis of population dynamics of North Atlantic cod. Can J Fish Aquat Sci 58:1464–1476

Piepho HP (1999) Fitting a regression model for genotype-by-environment data on heading dates in grasses by methods for nonlinear mixed models. Biometrics 55:1120–1128

Pinheiro JC, Bates DM (1995) Approximations to the Log-likelihood Function in the Nonlinear Mixed-effects Model. J Comp Graph Stat 4:12–35

Potvin C, Lechowicz MJ, Tardif S (1990) The statistical analysis of ecophysiological response curves obtained from experiments involving repeated measures. Ecology 71:1389–1400

Prichard JM, Forseth IN (1988a) Rapid leaf movement, microclimate and water relations of two temperate legumes in three contrasting habitats. Am J Bot 75:1201–1211

Prichard JM, Forseth IN (1988b) Photosynthetic responses of two heliotropic legumes from contrasting habitats. Plant Cell Environ 11:591–601

Ratkowsky D (1983) Nonlinear regression modeling. Dekker, New York

SAS (2000) Version 8. Cary, N. C.

Scheiner SM, Gurevitch J (2001) Design and analysis of ecological experiments. Oxford University Press, New York

Sperry JS, Donnelly JR, Tyree MT (1988) A method for measuring hydraulic conductivity and embolism in xylem. Plant Cell Environ 11:35–40

Urban L, Jaffrin A, Chraibi A (1993) Analysis of pressure-volume curves of leaves of *Rosa hybrida cv. Sonia*. J Exp Bot 44:605–613

Vonesh E, Chinchilli V (1997) Linear and nonlinear models for the analysis of repeated measurements. Dekker, New York

Werker AR, Jaggard KW (1997) Modelling asymmetrical growth curves that rise then fall: applications to foliage dynamics of sugar beet (*Beta vulgaris* L.). Ann Bot 79:657–665

Zeger SL, Harlow SD (1987) Mathematical models from laws of growth to tools for biological analysis: fifty years of growth. Growth 51:1–21