



# An empirical model of amino acid transformations in an alpine soil

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## Abstract

Amino acids are potentially important nitrogen (N) sources for plants in many ecosystems. However, a quantitative understanding of organic N availability is lacking for most ecosystems. This study estimates seasonal amino acid fluxes in an alpine tundra soil using three independent data sets. In previous work in an alpine dry meadow ecosystem in the Front Range of the Colorado Rocky Mountains, we measured significant rates of amino acid production from soil peptides during the plant growing season. This suggested that proteolysis of native soil peptides could serve as a measure of amino acid availability to plants. Here we use a simple seasonal model to test the hypothesis that soil amino acid concentrations can be predicted from rates of soil proteolysis and amino acid degradation by microbes. We present seasonal data for turnover rates of the amino acid, glutamate, in soil, and experiments on the effects of temperature and moisture on amino acid fluxes in soil. We relate these studies to previously published values of proteolysis and soil amino acid concentrations in this alpine soil. Our model shows that independent measurements of proteolytic rates, microbial amino acid uptake, and soil amino acid concentrations are consistent with each other, and that most changes in the soil amino acid pool can be explained by protease and microbial uptake rates, after adjusting for environmental conditions. Immediately after snow melts in the spring and again in late summer after the onset of monsoonal rains, measured soil amino acid concentrations were slightly lower than model predictions from measured protease and microbial uptake rates. This could indicate that, at certain times, an additional sink for amino acids exists, such as rapid plant uptake or loss from the ecosystem due to leaching. We estimate amino acid production during the snow-free season at our study site to be 103 g amino acid-N m<sup>-2</sup> growing - season<sup>-1</sup>. This value is not only consistent with the three data sets used in this study, but also with previous studies of N transformations in the Colorado alpine. This flux could provide *Kobresia myosuroides*, the dominant plant in this ecosystem, with 50–100% of its annual N requirement, based on previous plant–microbe competition experiments. © 2001 Elsevier Science Ltd. All rights reserved.

*Keywords:* Alpine tundra; Amino acids; Microbial degradation; Nitrogen cycle; Organic nitrogen; Proteolysis

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## 1. Introduction

Amino acids are potentially important nitrogen (N) sources for plants in various ecosystems (Read, 1991; Jones and Darrah, 1994; Kielland, 1994; Raab et al., 1996; Schimel and Chapin, 1996; Näsholm et al., 1998; Raab et al., 1999). However, a quantitative understanding of amino acid production and turnover in natural ecosystems is generally lacking. In a modeling study of arctic plant uptake of amino acids, amino acid supply rate was the most important parameter for determining availability to plants, but was the least understood parameter and was not constrained by data (Leadley et al., 1997). The primary source of free soil amino acids in most ecosystems is probably the proteolysis of soil proteins and peptides. Proteinaceous N is the most abundant form of N identifiable in soil organic matter (Schulten and Schnitzer, 1998), and

measurements of proteolysis rates in soils are generally much higher than either net N mineralization or plant N uptake (e.g. Ladd and Paul, 1973; Chapin et al., 1988; Watanabe and Hayano, 1995). However, the published proteolysis values tend to be maximum potential values, measured at high temperature with unlimiting substrate added to the soil. These measurements cannot be used to predict quantitatively soil amino acid fluxes in natural ecosystems.

Previous studies in the Colorado Rocky Mountains have shown that amino acids probably constitute an important N source for plants in this alpine ecosystem. Measurements of net N mineralization are an order of magnitude lower than plant uptake, indicating that fluxes of inorganic N are insufficient for plant requirements (Fisk and Schmidt, 1995; Fisk et al., 1998). The alpine sedge, *Kobresia myosuroides*, efficiently takes up amino acids from hydroponic solution at concentrations found in soil pore water (Raab et al., 1996). In two studies, isotopically labeled amino acids were used to verify that this plant absorbs amino acids intact from the soil, and can compete with soil microbes for 2–4% of total

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available amino acids under natural conditions (Lipson and Monson, 1998; Lipson et al., 1999a). Amino acid-N (but very little amino acid-C) is also transferred to *K. myosuroides* by ectomycorrhizae (Lipson et al., 1999b). In protease measurements made at realistic field temperatures, and using only natural levels of endogenous soil peptides, we have observed substantial rates of amino acid production in these alpine dry meadow soils (Lipson et al., 1999c; Raab et al., 1999). Simple calculations based on these data show that yearly soil amino acid production is an order of magnitude higher than plant N uptake, and so could account for plant N needs given only a modest ability for plants to compete with microbes.

The purpose of the present study is to quantitatively describe seasonal patterns of amino acid fluxes in an alpine dry meadow soil. We combine previously published seasonal data sets of soil proteolytic rates and amino acid concentrations with new seasonal data for microbial amino acid consumption rates, using lab measurements of how these phenomena are affected by environmental factors, to produce a self-consistent description of soil amino acid flux. The three independently measured data sets (proteolysis, microbial degradation of amino acids, and soil amino acid concentration) are adjusted for physical factors and related to each other using a simple seasonal model. The purpose of the model is to show consistency between the three data sets under the assumptions of the model, and to produce an estimate of amino acid flux that is constrained by all three of the data sets. Hence it is an empirical model constructed from all available data to test the hypothesis that soil amino acid concentrations can be predicted from rates of proteolysis and microbial uptake. Each combination of two seasonal data sets is used to predict the third variable, and this prediction is compared to the actual data for that variable. Finally, a prediction that best matches all three data sets simultaneously is derived to produce a well-constrained estimate of seasonal amino acid flux. We discuss the results in terms of the environmental and microbial controls over amino acid turnover and the implications for the alpine N cycle.

## 2. Methods and materials

### 2.1. Site description

The study site is located at the Niwot Ridge Long Term Ecological Research (LTER) area in the Front Range of the Colorado Rocky Mountains, US (40°03'N, 105°35'W). The site is dominated by the tussock-forming sedge, *K. myosuroides* (Vill) Paol. and Fiori. The soil is classified as a skeletal-loamy pergelic Cryumbrept. The soil in this dry meadow is particularly shallow, with a 10 cm A horizon overlaying a rocky B horizon with very few *K. myosuroides* roots. The A horizon contains 81 g C kg<sup>-1</sup> and 7.4 g N kg<sup>-1</sup> (Raab et al.,

1999) All experiments herein were performed on A horizon soil.

### 2.2. Glutamate decay rate measurements

Glutamate (glu) was chosen as the representative amino acid for this study because it is common in soil extracts (Ivarson and Sowden, 1969; Abuarghub and Read, 1988; Kielland, 1995), and because it was found that the population size of soil microbes capable of degrading glu is similar to values for many other amino acids (Lipson et al., 1999a). The decay rates of glu in soil were measured in respiration experiments. Soils (10 g) were placed in 250 ml side-arm flasks (Bellco, Vineland, NJ). Tracer quantities of <sup>14</sup>C-glu (100,000 dpm flask<sup>-1</sup>, 29 pmol glu g<sup>-1</sup> soil) were added in enough water to bring the soils to 60% gravimetric moisture content (GMC). A base trap of 1 M NaOH in the side arm of the flask was used to collect <sup>14</sup>CO<sub>2</sub>. Production of <sup>14</sup>CO<sub>2</sub> was measured by liquid scintillation. The measurements were carried out at different temperatures throughout the snow-free season so that the experimental temperatures would be close to those in the field. The <sup>14</sup>C respiration data was plotted as percent of added radioactivity respired per hour vs. time, and was fit by non-linear regression to find the first order rate constant (*R*). The rate of <sup>14</sup>CO<sub>2</sub> production (*Y*<sub>1</sub>) can be described as:

$$Y_1 = cR\beta \quad (1)$$

where *c* is the fraction of absorbed glu that is respired and *β* is the quantity of label present (Alexander and Scow, 1989). Thus,

$$d\beta/dt = -R\beta \quad (2)$$

$$\beta(t) = \beta_0 \exp(-Rt) \quad (3)$$

where *β*<sub>0</sub> is the amount of <sup>14</sup>C label initially added to the soil. Substituting this expression into Eq. (1) gives

$$Y_1 = cR\beta_0 \exp(-Rt) \quad (4)$$

when the rate of label production is expressed as a percentage of total added per hour (*Y*<sub>2</sub> = *Y*<sub>1</sub>*β*<sub>0</sub>), the expression becomes:

$$Y_2 = cR \exp(-Rt) \quad (5)$$

Thus, by fitting the data with an exponential equation, the first-order decay constant and the fraction of absorbed glu that is respired can be estimated.

### 2.3. Responses of processes to temperature and water potential

Potential protease measurements were performed as described in Lipson et al. (1999c) at temperatures ranging from 2 to 22°C. The temperature response of glu respiration was determined by measuring substrate-induced respiration (SIR) of glu over the same range of temperatures. SIR measurements were performed as described above for glu

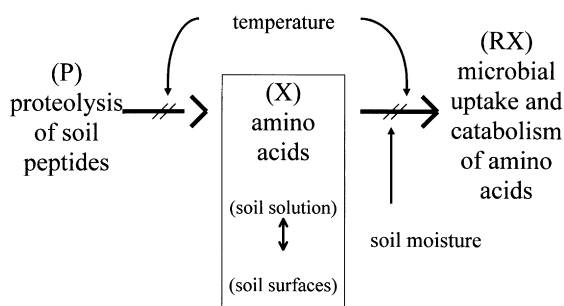


Fig. 1. Model structure.  $P$  = protease rate,  $X$  = soil amino acid concentration,  $R$  = first order decay rate of amino acids in soils.  $P$ ,  $R$ , and  $X$  are all functions of time that vary over the growing season, and are modified by the physical parameters, as indicated. Because of the method for measuring proteolysis, the effect of moisture content on proteolysis cannot be included explicitly (see discussion in text).

decay rate measurements, except sufficient non-radioactive glu was also added ( $2 \text{ mg glu-C g}^{-1}$ ) to produce maximum respiration rates. Both processes were plotted as rate vs. temperature, and were fit to an exponential curve. For reference, the  $Q_{10}$  was calculated from the exponential coefficient ( $k$ ) using the formula

$$Q_{10} = \exp(10k) \quad (6)$$

The effect of soil GMC on glu degradation in soil was determined by measuring glu SIR at a range of water contents. Soils were brought to a range of GMC from 15 to 65%. For modeling purposes, the relationship between GMC and respiration was fit with a third degree polynomial.

#### 2.4. Model structure

In the model (Fig. 1), soil amino acids ( $X$ ) are produced by proteolysis ( $P$ ), and are absorbed by soil microbes at a first-order rate ( $RX$ ). Amino acids interact with the soil matrix, and the rates of proteolysis and amino acid uptake are modulated by environmental factors. In the model, the adsorption of amino acids onto the soil matrix is included by adjusting the measured amino acid concentration of water extracts to reflect the actual bulk soil concentration, as

detailed below. The measured rates of proteolysis and amino acid uptake are adjusted to match field conditions using the experimentally derived relationships described above. In the simplest version of the model, the pseudo-steady state model (PSS), it is assumed that amino acid concentrations rapidly reach the steady state equilibrium concentration ( $X_{\text{eq}} = P/R$ ). This assumption is not unreasonable because, in this study, measured turnover rates were much more rapid than seasonal changes in the process rates. The steady-state assumption is also used to calculate the initial  $X$  in the more complex models, because there is no other a priori method of calculating this value from the other variables, and because the solutions quickly approached the equilibrium concentration regardless of the initial value of  $X$  used.

The ordinary differential equation (ODE) for the model is

$$dX/dt = P(t) - R(t)X \quad (7)$$

A computer program (PRXMOD) was written in C++ to solve the model using Euler's method (Blanchard et al., 1996). PRXMOD compares the predicted values with the data and calculates the sum of squared error (SSE). Because each variable has different units, the error is first normalized by dividing by the standard deviation around the seasonal mean for that variable. The null hypothesis that the variation in the variable is random is tested by comparing the SSE for the model with the SSE of the variable around its mean.

Because of the simplicity of the model, any of the three variables can be solved in terms of the other two. The 2-parameter models force the solutions through two of the data sets and calculate error between the resulting prediction and the remaining data set. In the 2-parameter models, the prediction for the third variable is generated solely from data for the first two variables, environmental conditions and the experimentally derived environmental effects on the first two data sets. Therefore, the model prediction and the remaining data set are completely independent. In order to simultaneously test the model against all three data sets, a program was written in C++. This 3-parameter model finds the seasonal trajectories of  $P$ ,  $R$  and  $X$  that satisfy the model

Table 1  
Model inputs

Input description	Units	Reference
$P$ (proteolysis rate in soil)	( $\text{nmol g}^{-1} \text{h}^{-1}$ )	Lipson et al. (1999c)
$R$ (amino acid decay rate)	( $\text{h}^{-1}$ )	This study
$X$ (soil amino acid concentration)	( $\text{nmol g}^{-1}$ )	Lipson et al. (1999c)
Average daily air temperature	( $^{\circ}\text{C}$ )	LTER database
GMC (soil gravimetric moisture content)	( $\text{g H}_2\text{O g}^{-1}$ soil)	Lipson and Monson (1998)
$K_d$ (solid-liquid partition coefficient)	( $\text{l kg}^{-1}$ )	Raab et al. (1999)
Soil amino acid respiration $Q_{10}$	–	This study
Soil proteolysis $Q_{10}$	–	This study
GMC effect on $R$	–	This study

Table 2

First order rate constant ( $R$ ) for glu turnover in soil, measured in the laboratory and adjusted for field conditions

Date	In laboratory		Field conditions		Field-adjusted <sup>a</sup>	
	$R$ ( $\text{h}^{-1}$ )	$T$ ( $^{\circ}\text{C}$ )	$T$ ( $^{\circ}\text{C}$ )	GMC	$R$ ( $\text{h}^{-1}$ )	MRT ( $\text{h}$ ) <sup>b</sup>
May 7	0.160	5	4.05	0.686	0.146	6.85
May 31	0.222	5	0.8	0.621	0.148	6.76
June 26	0.209	16	9.68	0.53	0.109	9.17
July 28	0.430	16	9.58	0.165	0.048	20.83
August 27	0.427	16	6.58	0.258	0.092	10.86
September 23 <sup>c</sup>	0.427	16	3.91	0.462	0.122	8.20

<sup>a</sup> Daily average air temperatures and soil moisture conditions for the sampling dates were used to arrive at the adjusted  $R$  and MRT shown.

<sup>b</sup> Mean residence time of glu in the soil ( $1/R$ ).

<sup>c</sup>  $R$  was not measured on September 23. For modeling purposes, the August 27 value was used.

equation while minimizing SSE across all three variables. In other words, a seasonal trajectory is found that most closely matches both the prediction for a variable based on the other two data sets and the data set for that variable. Therefore, in the 3-parameter model predictions are not independent of the data set they are predicting. The purpose in this case is to find the best fit of all the data under the model assumptions. This is done reiteratively with a bisection method (Press et al., 1986). The convergence criteria for minimization of the SSE are met when the marginal decrease in SSE drops below 0.001. Initial values for the variables were interpolated linearly between sampling dates. Because each sampling date value is adjusted for field conditions, this offers the simplest simulation of process rates, temperature and GMC. The model used in this study used a time step of 1 h.

### 2.5. Model inputs

The sources for the model inputs are listed in Table 1. Seasonal measurements of protease rates were adjusted for temperature, and seasonal glu decay rate data were adjusted for temperature and soil water potential according to experimentally derived relationships. To reflect a time-integrated

measure of temperature, average daily air temperatures for each sampling date were used. These values were similar to the monthly averages. By using the average daily temperature, it is tacitly assumed that the temperature responses of the modeled processes are linear rather than exponential. However, given the small daily temperature fluctuations, this assumption causes only a 4% underestimate in process rates. Temperature data were provided by the Niwot Ridge LTER database. The protease and amino acid concentration data sets extend until September 23, while the glu decay rate measurements were carried out only until August 27 (Table 2). For modeling purposes, the rate on August 27 was used with the field conditions on September 23 to provide a value for that date. As can be seen from Table 2, most of the variation in the decay rate was due to environmental variables, so the error from this extrapolation should not be large.

Because amino acids interact strongly with the soil matrix, water extractions only liberate a fraction of free amino acids from soils. In a previous study, the solid–liquid partition coefficient ( $K_d$ ) for amino acids in alpine dry meadow soils was reported (Raab et al., 1999). The total concentration of amino acids in the soil ( $X$ ) was calculated from the concentration in water extracts ( $C$ ), using the formula

$$X = C(K_d + V + \text{GMC}) \quad (8)$$

where  $V$  is the volume of water used per soil mass ( $5 \text{ ml g}^{-1}$ ) (Sposito, 1989). This is the only aspect of the model that explicitly accounts for soil adsorption phenomena, as diffusion is not explicitly modeled. However, the effect of GMC on microbial amino acid uptake is included in the model, and probably has a strong diffusive component.

### 2.6. Sensitivity analysis

The sensitivity of model output to changes in the data set was tested by varying each parameter, individually, from 0 to 64%, and recording the change in the predicted variables. The data was varied by simultaneously altering all the data points along the seasonal time

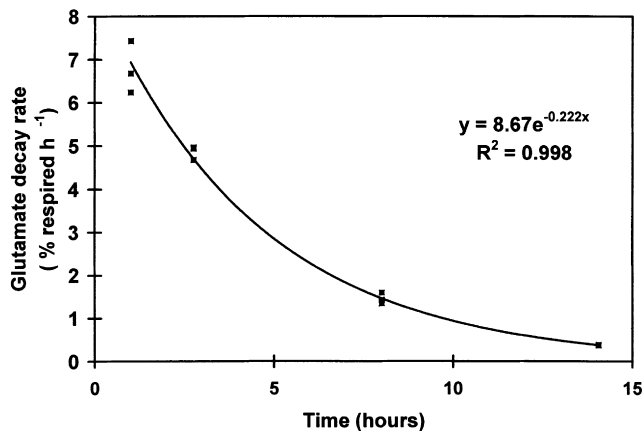


Fig. 2. Sample amino acid (glu) turnover data. The experiment shown was performed at  $5^{\circ}\text{C}$  with soil collected on May 31 1996.

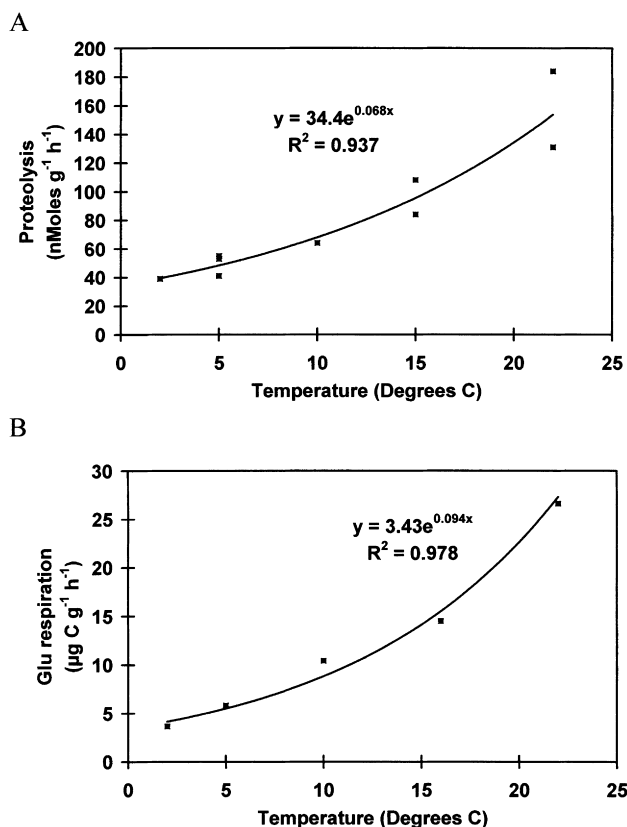


Fig. 3. The response of (A) proteolysis and (B) glu respiration in soil to changes in temperature.

course, and the corresponding change in the model output was measured as the change in the average seasonal value for that variable, relative to the output using the original data. The results are presented as slopes for the regressions between changes in the dataset and changes in the model output.

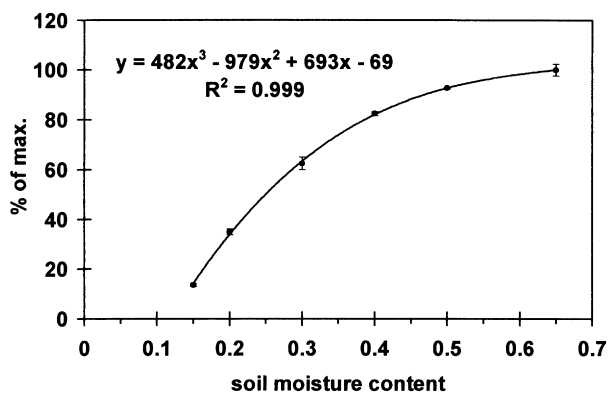


Fig. 4. Relationship between soil gravimetric water content and respiration of glutamate. Each point is the mean of three replicates. Error bars show the standard error of the mean. The cubic relationship shown above was used to adjust laboratory measurements of  $R$  for moisture contents in the field.

### 3. Results

In the soil glu decay rate experiments, the rate of <sup>14</sup>CO<sub>2</sub> production declined over time, following first order decay kinetics (Fig. 2). The rates of glu respiration in soils were sensitive to changes in temperature ( $Q_{10} = 2.57$ ) (Fig. 3B), and in GMC (Fig. 4). When measured values were adjusted for field temperature and soil moisture content, variation occurred over the snow-free season of 1996 (Table 2). Mean residence times of glu at field conditions ranged from 6.8 to 20.8 h. The temperature response of soil proteolytic activity had a  $Q_{10}$  of 1.98 (Fig. 3A).

The seasonal data sets of soil protease activity, amino acid turnover and amino acid concentration are consistent with each other under the assumptions of the model. All the versions of the model produced seasonal means that were surprisingly close to the measured means (Table 3). Recall that the pseudo-steady state and 2-parameter models produce estimates of each variable that are completely independent of the measurements for that variable. The models also simulated seasonal patterns well. Using the simplest version of the model (pseudo-steady state), each pair of data sets produced a reasonable prediction of the third dataset (Fig. 5). In the case of  $P$  and  $X$ , the model error was lower than the error from seasonal variation in the measured data (Table 4). The non-steady-state 2-parameter models (Fig. 6) were almost identical to the pseudo-steady state models, except for a slight reduction in error (Table 4). Because the 2-parameter models force predictions through two of the data sets, all the error ends up around the third data set. Therefore, a 3-parameter model was used to simultaneously fit all three data sets with the best fit possible while satisfying the model assumptions. As can be seen in Fig. 7, there is a trajectory for the three variables that satisfies the model and comes quite close to the measured values. The 3-parameter model is statistically reliable at very low  $P$ -values (Table 4). The 3-parameter model predictions were within the standard errors of the measurements for all but five instances: in the soil amino acid concentration time course on May 7 and August 27, and in the protease time course on May 7, July 28, and August 27 (Fig. 7).

The results of the sensitivity analysis for the 2-parameter models are predictable from first-principles.  $P$  is directly related to  $R$  and  $X$ , and  $R$  is inversely related to  $X$  (Table 5). In the 3-parameter model, the results are qualitatively

Table 3

Measured seasonal means for protease rate ( $P$ ), glu first order decay rate ( $R$ ) and amino acid concentration ( $X$ ) and those simulated by the models (PSS = pseudo-steady state, 2P = 2-parameter, 3P = 3-parameter)

	Seasonal Mean (data)	95% CI <sup>a</sup> (Low, high)	Seasonal mean (model)		
			PSS	2P	3P
$P$	32.8	(10, 55)	30.4	30.4	31.0
$R$	0.111	(0.072, 0.15)	0.127	0.127	0.111
$X$	265	(110, 420)	268	272	267

<sup>a</sup> 95% confidence interval.

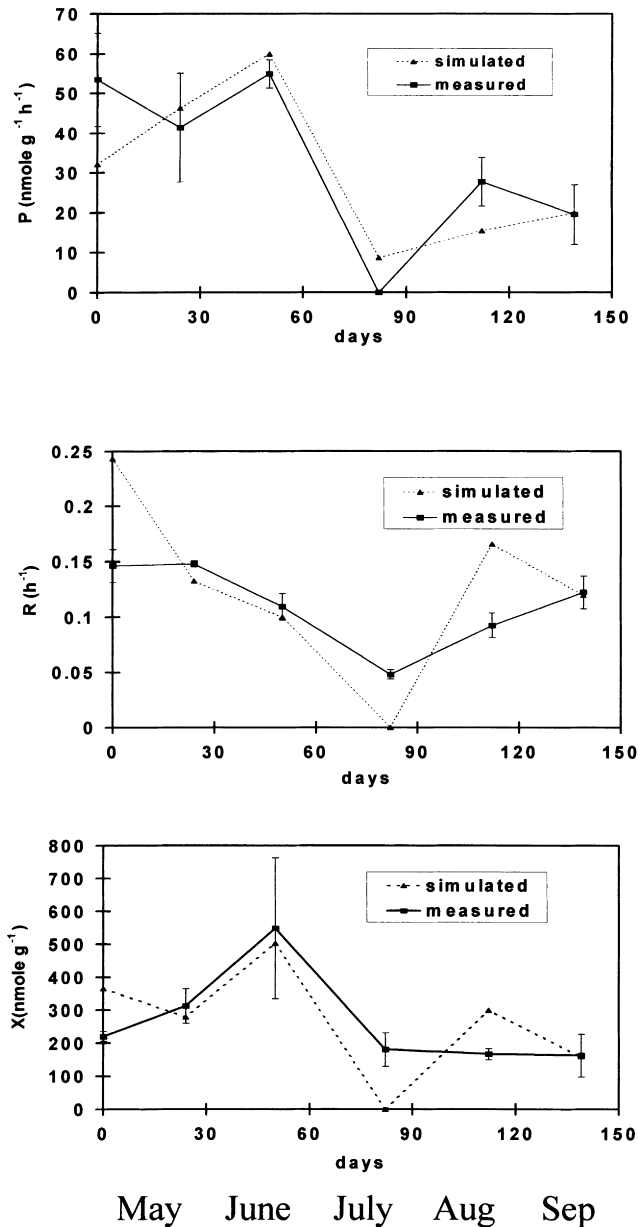


Fig. 5. Predicted and measured values of soil proteolytic rate ( $P$ ), amino acid decay rate ( $R$ ), and amino acid concentration ( $X$ ). The predictions shown were generated by the pseudo-steady state model, in which data for two variables are used to predict the third on each sampling date using the relation  $P = RX$ .

similar, but the predictions are more stable due to the interdependence of the three variables. In no case was a slope greater than one. This shows the model was not highly sensitive to changes in any parameter.

#### 4. Discussion

The turnover rates for the soil amino acid pool found in this study are similar to those reported in the literature (Schmidt et al., 1960; Cunningham and Wetzel, 1989;

Table 4

Summary of error statistics for all models. The SSE,  $R^2$ , degrees of freedom (d.f.) and probability ( $P$ ) values refer to the reduction in the sum of squared errors due to the model compared to the sum of squared errors of data variables around their seasonal means (SSM). All errors were normalized by dividing by the standard deviation of each variable around its seasonal mean. An asterisk indicates that the model did not reduce error relative to the SSM

Model	SSM	SSE	$R^2$	D.F.	$P$
<i>Pseudo-steady state</i>					
$P$	5.00	1.606	0.679	(1,4)	0.049
$R$	5.00	14.51	*	(1,4)	*
$X$	5.00	3.345	0.331	(1,4)	0.23
<i>2-Parameter</i>					
$P$	5.00	1.593	0.681	(1,4)	0.048
$R$	5.00	12.276	*	(1,4)	*
$X$	5.00	2.923	0.415	(1,4)	0.176
<i>3-Parameter</i>					
$P$	5.00	0.823	0.835	(1,15)	< 0.0001
$R$	5.00	0.010	0.998	(1,15)	< 0.0001
$X$	5.00	0.183	0.963	(1,15)	< 0.0001
Over all	15.00	1.016	0.932	(2,15)	< 0.0001

Hadas et al., 1992; Martens and Frankenberger, 1993; Kieland, 1995; Jones, 1999). The mean residence time of amino acids in soils in all studies is on the order of several hours to one day. Though amino acids were rapidly degraded, soil proteolytic activity was generally adequate to keep the system near steady-state. The  $Q_{10}$  for glu respiration (2.57) was somewhat higher than the  $Q_{10}$  for proteolysis (1.98). If this differential response to temperature holds true in the field, it would tend to cause greater amino acid availability when soils are colder.

Glu respiration was strongly controlled by soil water content. This effect can be attributed to diffusion rates and osmotic stress (Stark and Firestone, 1995). Water appears to be a stronger control than temperature on amino acid flux in these seasonally water-stressed dry alpine meadows; at mid-summer when soils were driest, fluxes were at a minimum, whereas fluxes were highest in the spring and autumn when soils were cold. In the present model, protease rates were not adjusted for changes in water potential. The procedure for measuring protease rates involves suspension of the soil in

Table 5

Results of sensitivity analysis of 2- and 3-parameter models. The slopes are given for the regressions of changes in model output for each variable against changes in model input (data) of a single variable. The regressions were approximately linear over the range tested (0–64% change in the data variable) (n/a = not applicable)

	2-Parameter models			3-Parameter model		
	$P$	$R$	$X$	$P$	$R$	$X$
$P$	n/a	1	1	0.54	0.02	0.5
$R$	1	n/a	-0.62	0.44	0.92	-0.31
$X$	1	-0.62	n/a	0.44	-0.05	0.51

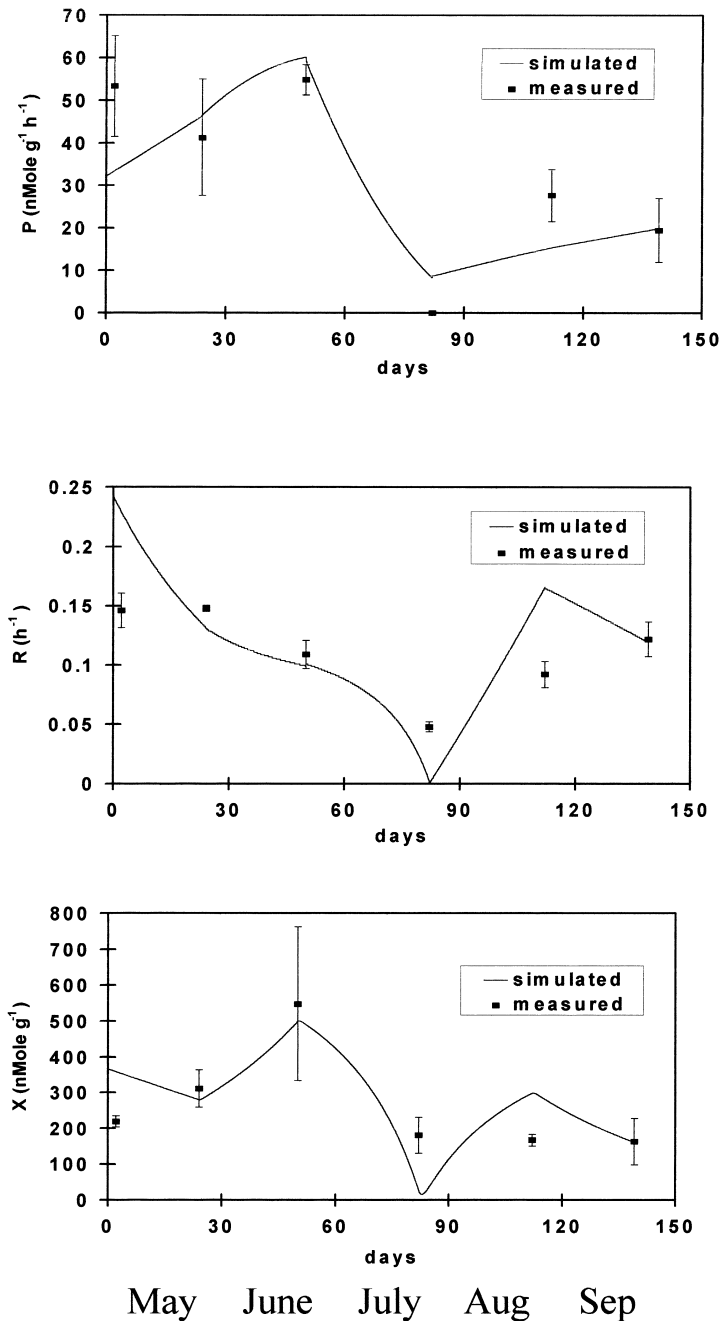


Fig. 6. Predicted and measured values of soil proteolytic rate ( $P$ ), amino acid decay rate ( $R$ ), and amino acid concentration ( $X$ ). The predictions shown were generated by the 2-parameter models, in which data for two variables are used to predict the third using Euler's method.

solution. For this reason, water content cannot be experimentally manipulated. However, there are two reasons that this may not be a large concern. The majority of measured protease activity is extracellular. Toluene is added as a microbial inhibitor, so little internal metabolism occurs during the measurements. Therefore, osmotic stress on microorganisms would not directly affect this process. Also, because proteolysis was undetectable during the driest time of the season, there is little sensitivity to moisture in the data set. This may indicate that proteolytic rates in the field

are sensitive to low water potentials, and thus, the time-integrated effects of moisture on proteolysis were included intrinsically in the seasonal dataset.

The agreement among the three independently measured data sets (protease rates ( $P$ ), glu decay rates ( $R$ ) and soil amino acid concentrations ( $X$ )) is remarkable. Even the simplest model, using a steady state assumption for each sampling date, produced seasonal averages that were very close to the measured values, and seasonal predictions that were within the standard errors of the measurements in

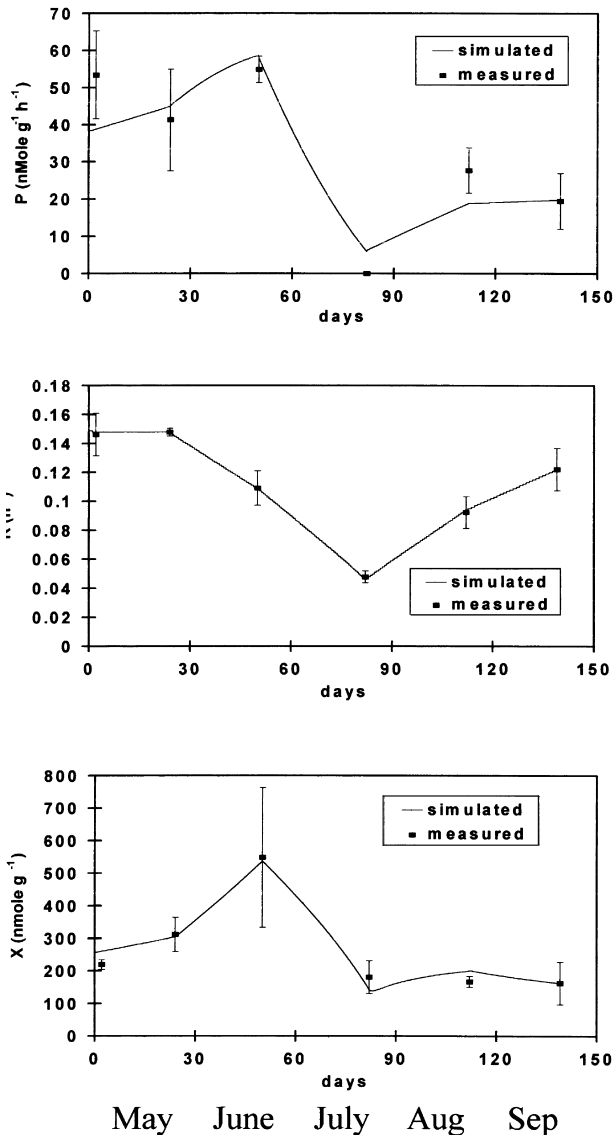


Fig. 7. Predicted and measured values of soil proteolytic rate ( $P$ ), amino acid decay rate ( $R$ ), and amino acid concentration ( $X$ ). The predictions shown were generated by the 3-parameter model, in which trajectories for all three variables are found that satisfy the model, while minimizing SSE between measured and predicted values.

many instances. The pseudo-steady state and 2-parameter models inspire confidence in the data, because they compare predictions from two data sets with a third independent data set. The 3-parameter model is more useful for simultaneously testing the model assumptions against all three data sets. The model confirms the hypothesis that amino acid concentrations in alpine dry meadow soil can generally be predicted from field-adjusted proteolytic rates and the first order decay constant. However, there were five points at which the 3-parameter model predictions were outside the standard errors of the measurements (Fig. 7). One of these occurred in the protease time course on July 28, when measurements were below detection limits, and therefore no standard error bars are available. However, it may be

significant that on May 7 and August 27, measured soil amino acid concentrations were lower than model predictions from measured proteolysis and microbial uptake rates. This can be seen most dramatically as a departure from the pseudo-steady state model (Fig. 5). On these two dates, measured amino acid uptake rates would have to be increased by about 66% to be consistent with measured protease rates and soil amino acid concentrations. This could indicate a sink for amino acids that was not included in the model, such as uptake by plant roots and mycorrhizae or leaching from the ecosystem due to melting snow in May and monsoonal rains in late August. The seasonal measurements of microbial amino acid uptake were done on soils from which plant roots were excluded, and from which leaching was prevented. The measurements of amino acid concentration, on the other hand, would have been affected by these factors in the field prior to sampling. Both leaching and rapid plant uptake are plausible sinks; export of N from Colorado alpine watersheds during spring runoff has been reported (Campbell et al., 1995; Williams et al., 1996), and *K. myosuroides* is capable of much higher maximal amino acid uptake rates than its average seasonal rate of N uptake (Raab et al., 1996).

By integrating the predicted proteolysis rates over the entire season, a yearly N budget can be estimated. Assuming the average amino acid contains  $20.3 \text{ g N mol}^{-1}$ ,  $103 \text{ g N m}^{-2}$  was produced between May 7 and September 23 1996. If plant roots were active for this entire time, they could receive 50–100% of their annual N budget from amino acids, assuming a 2–4% competitive rate based on past studies (Lipson and Monson, 1998; Lipson et al., 1999a), and a  $4.1 \text{ g N m}^{-2}$  plant requirement (Fisk et al., 1998). Our estimate of annual amino acid flux is larger than the figure of  $42 \text{ g N m}^{-2}$  arrived at by Raab et al. (1999) using a simple calculation. This is partly because Raab et al. (1999) used a shorter growing season and a lower basal proteolytic activity. The amino acid budget produced in this study is consistent with reported values of N transformations in the Colorado alpine. Based on the values of microbial biomass N measured at the site (Lipson et al., 1999c), the mean residence time of amino acid-N in microbial biomass is 0.035 y, similar to the residence time of 0.05 y for microbial biomass N reported by Fisk et al. (1998). Using previously reported rates of gross mineralization (Fisk et al., 1998), we estimate that about 33% of amino acid-N taken up by microbes is secreted as ammonium.

A question raised by the large calculated annual flux of amino acids in this ecosystem is the source of substrate for soil proteases. In earlier work we observed that microbial biomass declined immediately after snowmelt, releasing protein into the soil (Lipson et al., 1999c). Soil proteases were saturated by this pulse of protein just after snowmelt, but later became substrate-limited. The present study supports our previous conclusion that there is a large pulse of amino acids available early in the growing season, and this is probably linked to the turnover of microbial



biomass after snowmelt. However, based on the calculations above, a single catastrophic event could not liberate enough protein from the microbial biomass to supply the entire season's proteolytic activity. As the season progresses, there must be continual recycling of protein from microbes and fine roots. This view is supported by the observation that microbial biomass is quite dynamic during the growing season (Fisk and Schmidt, 1995; Lipson et al., 1999c).

A limitation of the present model is that amino acids were treated homogeneously. Previous work showed that most amino acids support similar soil microbial populations, but that glycine (gly) is used by a smaller microbial population, and is degraded 61% as fast as glu (Lipson et al., 1999a). The current model would therefore overestimate the rate of gly uptake by microorganisms, although it should work well for most other amino acids. If gly represents a large portion of the free amino acid pool, then the measured *R* would be over-estimated, and the soil amino acid concentration would be under-estimated. The amino acid composition of soil peptides is generally consistent across soil types (Schulten and Schnitzer 1998). In one study, glycine made up 8–9% of total amino acids in soil peptides (Senwo and Tabatabai, 1998). Using decay rate values for gly of 0.61 times that of glu, and assuming that liberation of gly from soil peptides is 0.08 times the total protease rate, the model produces only 5% higher total amino acid concentrations. However, the gly concentrations simulated using these inputs are 65% higher than those assuming the same *R* for gly. This could be significant for plant uptake, as plants generally take up gly more rapidly than other amino acids (Kielland 1994; Schmidt and Stewart, 1997; Lipson et al., 1999a).

The current model was based on a limited spatial scale, however, the results of the present study can be extended to larger spatial scales within the alpine ecosystem using the established relationships among protease rates, extractable protein and soil organic matter (Raab et al., 1999). Alpine tundra may be unique in having high levels of substrate available for proteolysis in the soil and plants with low N requirements, allowing organic N to play an important role in this ecosystem. The approach used in this study could be useful for quantifying organic N availability in other systems.

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