

Modeling the flow of ^{15}N after a ^{15}N pulse to study long-term N dynamics in a semiarid grassland

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Abstract. Many aspects of nitrogen (N) cycling in terrestrial ecosystems remain poorly understood. Progress in studying N cycling has been hindered by a lack of effective measurements that integrate processes such as denitrification, competition for N between plants and microbes, and soil organic matter (SOM) decomposition over large time scales (years rather than hours or days). Here I show how long-term measurements of ^{15}N in plants, microbes, and soil after a one-time addition of ^{15}N (“labeled” N) can provide powerful information about long-term N dynamics in a semiarid grassland. I develop a simple dynamic model and show that labeled-N fractions in plant and microbial-N pools (expressed as a fraction of total N in each pool) can change long after ^{15}N application (≥ 5 years). These ^{15}N dynamics are closely tied to the turnover times of the different N pools. The model accurately simulated the labeled-N fractions in aboveground biomass measured annually during five years after addition of ^{15}N to a semiarid grassland. I also tested the sensitivity of five different processes on labeled-N fractions in aboveground plant biomass. Changing plant/microbial competition for N had very little effect on the labeled-N fraction in aboveground biomass in the short and long term. Changing microbial activity (N mineralization and immobilization), N loss, or N resorption/re-translocation by plants affected the labeled-N fraction in the short term, but not in the long term. Large long-term effects on the labeled-N fraction in aboveground biomass could only be established by changing the size of the active soil-N pool. Therefore, the significantly greater long-term decline in the labeled-N fraction in aboveground biomass observed under elevated CO_2 in this grassland system could have resulted from an increased active soil-N pool under elevated CO_2 (i.e., destabilization of soil organic matter that was relatively recalcitrant under ambient CO_2 conditions). I conclude that short- and long-term labeled-N fractions in plant biomass after a ^{15}N pulse are sensitive to processes such as N mineralization and immobilization, N loss, and soil organic matter (de-)stabilization. Modeling these fractions provides a useful tool to better understand N cycling in terrestrial ecosystems.

Key words: *elevated CO_2 ; microbial N; model; N cycling; ^{15}N tracer; priming effect; pulse labeling; semiarid grassland; soil organic matter decomposition; soil organic matter destabilization; SOM.*

INTRODUCTION

Nitrogen (N) cycling plays a key role in ecosystem processes such as net primary productivity (Vitousek and Howarth 1991, Reich et al. 1997), species composition (Tilman 1982, Wedin and Tilman 1993), and carbon sequestration (Hungate et al. 2003, Pregitzer et al. 2008). Despite much effort, it still remains difficult to study many aspects of the N cycle such as N loss through denitrification, competition for N between plants and microbes, and soil organic matter decomposition and N release. A better understanding of these aspects of the N cycle is important to improve our ability to predict how terrestrial ecosystems will respond to global change.

In particular, methodological problems exist with measuring soil N mineralization under field conditions (Schimel and Bennett 2004). In many studies, soil N mineralization under field conditions has been estimated by measuring the change in inorganic N over time during in situ soil incubations, but in the absence of plants. However, plants can significantly alter N mineralization by competing for N with soil microbes (Fisher and Gosz 1986, Ehrenfeld et al. 1997, Wang and Bakken 1997) and by increasing microbial activity through providing them with labile C substrates (e.g., root exudates; De Nobili et al. 2001, Paterson 2003). Therefore, any measurement of N mineralization done in the absence of plants may not be representative of field conditions. Others have used the amount of inorganic N absorbed to resin bags or stakes buried in the soil over a period of time as a measurement of soil N availability (e.g., Binkley and Matson 1983, Johnson et al. 2005, Gill et al. 2006). Although these measurements are usually done in the presence of plants, interpretation

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can be difficult because it is not known how much of the absorbed N is affected by N supply (i.e., net N mineralization) vs. N demand rates (i.e., plant N uptake). Still others have used additions of ^{15}N to the soil and measured the ^{15}N concentration after 48 h or less in the inorganic-N pool to estimate short-term gross N mineralization and immobilization with the notion that the ^{15}N label in the soil becomes diluted with gross N mineralization (Davidson et al. 1992, Hart et al. 1994, Barrett and Burke 2000). These measurements only estimate short-term N mineralization rates (i.e., within 48 h or less) and have proven not to be very sensitive to environmental change (Zak et al. 2000, de Graaff et al. 2006, Reich et al. 2006).

A successful technique to study N cycling in terrestrial ecosystems has been the use of ^{15}N as a tracer. Adding ^{15}N to the soil in trace amounts and then measuring the ^{15}N shortly after in plants, microbes, and soil has shown to be effective for studying short-term plant–microbe–soil interactions (Kaye and Hart 1997, Hu et al. 2001, Harrison et al. 2008). In a number of studies a ^{15}N tracer has also been used to understand long-term N cycling and competition (long-term here is defined as >1 year, but note that most long-term ^{15}N tracer studies do not extend beyond 5 years; Epstein et al. 2001, Hu et al. 2005, Zak et al. 2007). In almost all ^{15}N tracer studies N cycling and competition were examined by assessing the amount of the ^{15}N recovered in plants, microbes, and soil as a percentage of ^{15}N applied. However, inferences about long-term N cycling and competition using ^{15}N recovery in plants, microbes, and soil can be confounded because of fast turnover times of some of these pools (e.g., leaves, fine roots, and microbes; Kaye and Hart 1997). For instance, significant amounts of N and ^{15}N can be lost through root death and exudation (De Graaff et al. 2007, Wichern et al. 2008) that are hard to measure and that are not accounted for when the ^{15}N in plant biomass is expressed as a percentage of ^{15}N applied.

Recently, Dijkstra et al. (2008) interpreted long-term measurements of ^{15}N in plant biomass after a one-time ^{15}N addition in a new way. Rather than calculating ^{15}N recovery (as a percentage of ^{15}N applied) in plant biomass, they expressed the ^{15}N (“labeled” N) as a fraction of total N in plant biomass (labeled-N fraction in plant biomass). They then interpreted the temporal decrease in plant biomass labeled-N fraction as a measure of how much the labeled N became diluted with N from other sources. For instance, ^{15}N becomes diluted in the plant when the fraction of labeled N in the N lost through leaf senescence, root exudation, and root death is higher than the fraction of labeled N taken up by roots. In an open-top chamber experiment, the atmospheric CO_2 concentration was manipulated (720 vs. 368 ppm) in a semiarid grassland (Morgan et al. 2004). ^{15}N was added to the soil in year 1 and measured in aboveground biomass of perennial grassland species during the next 5 years. The labeled-N fraction in aboveground biomass declined with time, but this

decline was significantly greater under elevated CO_2 (Dijkstra et al. 2008). The greater decline of the labeled-N fraction under elevated CO_2 was attributed to dilution with unlabeled soil N, most likely because of greater N mineralization (similar to short-term gross N mineralization studies already mentioned in this *Introduction*).

Here I present a model simulating the flow of ^{15}N after a one-time ^{15}N addition. The purpose of the model is twofold: (1) to illustrate that long-term measurements of ^{15}N in plant biomass after a one-time ^{15}N addition can be useful to study long-term N cycling in a semiarid grassland, particularly when ^{15}N is expressed as a fraction of total N and (2) to examine different mechanisms that could influence ^{15}N dilution in plant biomass. For the second purpose I tested the sensitivity of five different scenarios on the labeled-N fraction in plant biomass with time. I was particularly interested in if any of the five scenarios could explain the greater decline in the labeled-N fraction in aboveground plant biomass observed under elevated CO_2 . The five scenarios simulated the effects of (1) increased plant competition for N compared to microbes, (2) increased microbial activity, (3) increased microbial activity and increased activation of the soil organic matter pool, (4) increased N loss through denitrification and leaching, and (5) changes in N resorption and re-translocation.

METHODS

Model description

I developed a simple spreadsheet model to simulate the flow of ^{14}N and ^{15}N through above- and belowground plant biomass (Above Plant N and Below Plant N), microbial biomass (Microb N), inorganic N (Inorg N), and dead organic N in the soil (Soil N; Fig. 1), with the majority of the N residing in the Soil N. The model includes the following fluxes of ^{14}N and ^{15}N affecting these pools: above- and belowground plant uptake (pu_A , uptake through roots but directly transported to shoots; and pu_B , uptake through roots without further transfer), resorption (rs), re-translocation (rt), microbial mineralization or gross N mineralization (m), microbial immobilization (i), microbial uptake from the dead soil organic-N pool (mu), microbial death (md), above- and belowground plant N loss through litter and exudation (pl_A and pl_B), input from microbial fixation and atmospheric deposition (in), and output due to leaching and denitrification (out). The model simulates the flows of ^{14}N and ^{15}N over time after a one-time addition of ^{15}N (labeled N) to the inorganic-N pool at time 0. I focus on how the fraction of labeled N (expressed as a percentage of total N in each pool) changes with time. I am particularly interested in long-term effects (i.e., up to 5 years after ^{15}N addition) on the fraction of labeled N in plant biomass. I used a set of simplifying assumptions with the purpose of illustrating how changes in plant–microbial competition for N, microbial activity, resorption/re-translocation, and N loss alter the pattern in labeled-N fraction in plant biomass over time, rather

than to explicitly predict ¹⁵N flow in soil–plant systems after ¹⁵N addition.

I assume that the plant N, inorganic N, microbial N, and soil N pools do not vary in time. This steady state situation is not unrealistic for a time period of several years (the time period for the simulations presented here is 5 years), because microbial biomass correlates well to soil C and N pools and plant biomass (Zak et al. 1990) and the majority of the N in terrestrial ecosystems resides in the soil-N pool that varies little with time (Post et al. 1985). Inorganic N and microbial N vary seasonally but should show low annual variability as long as environmental conditions are the same in each year. The plant N pools (of perennial species) can vary seasonally (e.g., large loss of N during leaf senescence), but again, much less so annually. I further assume that rates of N input (in), N loss (out), plant N uptake (pu_A and pu_B), resorption (rs), re-translocation (rt), plant N loss (pl_A and pl_B), microbial mineralization (*m*), microbial immobilization (*i*), microbial uptake from dead soil organic N (mu), and microbial death (md) are all constant with time. Again, these rates can fluctuate significantly within a year but should be fairly constant annually as long as environmental conditions remain the same. I assume that N resorption before leaf senescence (rs) equals internal N re-translocation to new leaves the next year (rt; note that in the model these processes occur simultaneously). To maintain constant plant-N pools it follows that

$$pu_A = pl_A \quad (1)$$

$$pu_B = pl_B \quad (2)$$

To maintain a constant inorganic-N pool it follows that

$$pu_A + pu_B + i - m = in - out. \quad (3)$$

To maintain a constant microbial-N pool it follows that

$$m - i = mu - md. \quad (4)$$

Because N fluxes are constant in the model, the change in ¹⁵N in each pool as a function of time after a ¹⁵N addition at time 0 is as follows:

$$\begin{aligned} \text{Above Plant } ^{15}\text{N}(t)/d(t) = & [pu_A \times A(t)_{\text{InoN}} + rt \times A(t)_{\text{BPtN}} \\ & - (pl_A + rs) \times A(t)_{\text{APtN}}]/100 \end{aligned} \quad (5)$$

$$\begin{aligned} \text{Below Plant } ^{15}\text{N}(t)/d(t) = & [pu_B \times A(t)_{\text{InoN}} + rs \times A(t)_{\text{APtN}} \\ & - (pl_B + rt) \times A(t)_{\text{BPtN}}]/100 \end{aligned} \quad (6)$$

$$\begin{aligned} \text{Inorg } ^{15}\text{N}(t)/d(t) = & [m \times A(t)_{\text{McrN}} + in \times A_{\text{in}} \\ & - (pu_A + pu_B + i + out)A(t)_{\text{InoN}}]/100 \end{aligned} \quad (7)$$

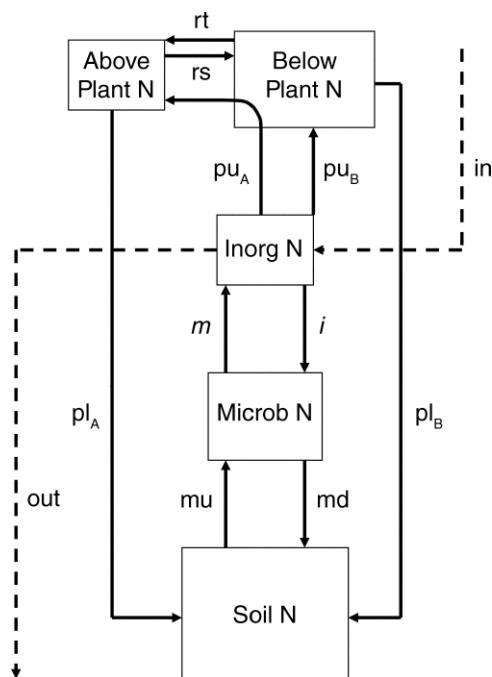


FIG. 1. Structure of the model with N pools (Above Plant N is N in aboveground plant biomass; Below Plant N, N in belowground plant biomass; Inorg N, N in inorganic soil; Microb N, N in microbial biomass; Soil N, N in dead soil organic matter) and flows (pu_A is aboveground plant uptake or uptake through roots but directly transported to shoots; pu_B, belowground plant uptake; rs, resorption; rt, re-translocation; *m*, microbial mineralization; *i*, microbial immobilization; mu, microbial uptake from Soil N; md, microbial death; pl_A, aboveground plant loss through litter; pl_B, belowground plant loss through litter; in, microbial fixation and deposition; out, denitrification and leaching).

$$\begin{aligned} \text{Microb } ^{15}\text{N}(t)/d(t) = & [i \times A(t)_{\text{InoN}} + mu \times A(t)_{\text{SoilN}} \\ & - (m + md)A(t)_{\text{McrN}}]/100 \end{aligned} \quad (8)$$

$$\begin{aligned} \text{Soil } ^{15}\text{N}(t)/d(t) = & [md \times A(t)_{\text{McrN}} + pl_A \times A(t)_{\text{APtN}} \\ & + pl_B \times A(t)_{\text{BPtN}} - mu \times A(t)_{\text{SoilN}}]/100 \end{aligned} \quad (9)$$

where Above Plant ¹⁵N(*t*)/*d*(*t*), Below Plant ¹⁵N(*t*)/*d*(*t*), Inorg ¹⁵N(*t*)/*d*(*t*), Microb ¹⁵N(*t*)/*d*(*t*), and Soil ¹⁵N(*t*)/*d*(*t*) are the changes in ¹⁵N in the above- and belowground plant biomass, inorganic N, microbial biomass, and dead soil organic-N pools with time, respectively, *A*(*t*)_{APtN}, *A*(*t*)_{BPtN}, *A*(*t*)_{InoN}, *A*(*t*)_{McrN}, and *A*(*t*)_{SoilN} are the ¹⁵N atom% (¹⁵N/(¹⁵N + ¹⁴N) × 100) in the above- and belowground plant biomass, inorganic N, microbial biomass, and dead soil organic N at time *t*, respectively, and *A*_{in} is the ¹⁵N atom% of N fixation and N deposition. The labeled-N fraction at time *t* (*N*_{lbi}(*t*), mg ¹⁵N/g N) in aboveground plant biomass can be

calculated with

$$N_{\text{lbl}}(t) = [A(t)_{\text{APIN}} - A(0)_{\text{APIN}}] / [A_{\text{lbl}} - A(0)_{\text{APIN}}] \times 1000 \quad (10)$$

where $A(0)_{\text{APIN}}$ is the ^{15}N atom% in the aboveground plant-N pool at time 0 and A_{lbl} is the ^{15}N atom% of the ^{15}N label. Labeled-N fractions in other N pools can be calculated similarly. Eqs. 5–10 were solved numerically using daily time steps. The amount of ^{14}N in each pool at time t was calculated as the difference between total N and ^{15}N . I assumed no ^{15}N fractionation during the transfer of ^{15}N among pools. While this assumption is not strictly valid, fractionation can be ignored when ^{15}N isotope enrichments are well above natural abundance (Stark 2000). I corroborated this assumption by simulating the effect of different fractionation processes that can occur during denitrification and plant uptake via mycorrhizae on the ^{15}N flow. When I incorporated maximum fractionation factors (β) reported in the literature ($\beta = 0.98$ for pu_A and pu_B , and 0.97 for out; Högberg 1997) in the model simulations, I observed negligible effects on ^{15}N flow (data not shown). I assumed that the ^{15}N atom% of N input would not change over time.

Case study

I used the model to simulate labeled-N fractions in above- and belowground biomass that were measured annually during five years after addition of ^{15}N to a semiarid grassland system (Dijkstra et al. 2008). In that study, atmospheric CO_2 concentration was manipulated (720 vs. 368 ppm) in open-top chambers (OTC) during a 5-year period (Morgan et al. 2004). In the first year 0.5 g/m^2 of ammonium nitrate-N, 99.9 atom% ^{15}N was added to each plot in the spring. Elevated CO_2 caused a significant increase in plant N uptake and a greater decline in the labeled-N fraction in aboveground biomass with time (Dijkstra et al. 2008). Greater plant N uptake and plant ^{15}N dilution in aboveground biomass under elevated CO_2 was attributed to increased N mineralization. Labeled-N fractions in belowground biomass were not significantly affected by elevated CO_2 . However, there was large variation among years in the belowground biomass labeled N fractions and large variation among replicates (Appendix) indicating that measurements of labeled-N fractions in belowground biomass were not as precise as the aboveground biomass measurements.

I first parameterized the model using data measured in the ambient CO_2 plots from the OTC study. For parameters not measured in the OTC study I relied on measurements from other studies of similar systems (Table 1). I used the 5-year mean amount of N in senesced aboveground plant biomass and in root biomass in the top 15 cm of the soil measured in the ambient CO_2 plots in October (Dijkstra et al. 2008) for Above Plant N and Below Plant N respectively. Plant N uptake rates (pu_A and pu_B) were calculated from plant

N biomass pools and their turnover times measured in the OTC study. Because the aboveground plant-N pool was measured in senesced plants in October (i.e., after resorption), the turnover time of the aboveground-N pool (here defined as Above Plant N/ pu_A) should be 1 year. Milchunas et al. (2005) reported a root turnover time for the ambient CO_2 plots of 7 years. Because roots can lose a significant amount of N through root exudation (De Graaff et al. 2007, Wichern et al. 2008), in reality the belowground N turnover time should be smaller. I fitted the belowground labeled-N fractions measured in the ambient CO_2 plots by adjusting the belowground N turnover time to 4.5 years. I tested the sensitivity of the size of the aboveground plant-N pool and the belowground turnover time on labeled-N fractions in above- and belowground plant biomass (*Methods: Scenarios: Scenario 1*).

I assumed that N loss through denitrification/leaching equals N input through atmospheric deposition and fixation. Since there were very few N-fixing plants in this system, atmospheric N fixation was most likely negligible. There are no accurate data available for N loss through denitrification and leaching. Mosier et al. (2002) periodically measured NO_x and N_2O fluxes and reported a mean flux of 12.6 $\mu\text{g N}\cdot\text{m}^{-2}\cdot\text{hr}^{-1}$ or 0.3 $\text{mg N}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ($\text{NO}_x + \text{N}_2\text{O}$). It is not clear how much N was lost through leaching, although N leaching in these systems is most likely small (Burke et al. 2002). Considering these uncertainties with N loss, I set N loss equal to the 1.1 $\text{mg N}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ of N deposition (wet + dry) reported by Lauenroth and Milchunas (1991) for this area. I tested the sensitivity of N loss rates on simulated labeled-N fractions in plant biomass (*Methods: Scenarios: Scenario 4*).

Much of the dead soil organic N pool is very recalcitrant and depending on the size, its contribution to the N cycle may be small. How much of the total soil-N pool is actively involved in the N cycle is unknown. In the simplified model there is only one soil-N pool that represents the active soil N only. I adjusted the size of the “active soil-N pool” to fit the labeled-N fractions in aboveground biomass. In this case, 55% of the total soil-N pool was “active.” I tested the sensitivity of the size of the active soil N pool on simulated labeled N fractions in plant biomass (*Models: Scenarios: Scenario 3*).

Net N mineralization ($m - i$) followed from Eq. 3. Microbial mineralization (m) was set three times higher than net N mineralization as reported by Barrett and Burke (2000) in a similar system. For a lack of accurate measurements of microbial biomass death rates (md) available in the literature, I used the same parameter value that was used in the model developed by Schimel and Weintraub (2003; i.e., 1.2% of the microbial-N pool is transferred to the active soil-N pool each day through microbial death). The sensitivity of different microbial parameters was tested (*Methods: Scenarios: Scenario 2*).

I assumed that the ^{15}N atom% of N input through fixation and deposition equals the ^{15}N atom% of

TABLE 1. Input parameters to simulate ¹⁵N flow in a mature semiarid grassland to 15 cm soil depth after a 0.4 g/m² ¹⁵N addition.

Parameter	Definition	Value	Source
Above Plant N	N in aboveground plant biomass at $t = 0$	0.8 g/m ²	OTC, Dijkstra et al. (2008)†
Below Plant N	N in belowground plant biomass at $t = 0$	7.7 g/m ²	OTC, F. A. Dijkstra et al. (unpublished data)
Inorganic N	inorganic soil N at $t = 0$	0.5 g/m ²	Schimel and Parton (1986)
Microbial N	microbial biomass N at $t = 0$	2.0 g/m ²	Kelly et al. (1996)
Active soil N	N in dead soil organic matter at $t = 0$	90 g/m ² ‡	OTC (unpublished data)
pu _A	aboveground plant uptake or uptake through roots but directly transported to shoots	2.2 mg·m ⁻² ·d ⁻¹ §	
pu _B	belowground plant uptake through roots without further transfer	4.7 mg·m ⁻² ·d ⁻¹ §	
rs	resorption	1.0 mg·m ⁻² ·d ⁻¹ ¶	
rt	re-translocation	1.0 mg·m ⁻² ·d ⁻¹ ¶	equal to rs
m	microbial mineralization or gross N mineralization	20.7 mg·m ⁻² ·d ⁻¹ #	Barrett and Burke (2000)
i	microbial immobilization	13.8 mg·m ⁻² ·d ⁻¹	follows from Eq. 3
μ	microbial uptake from Soil N	31.9 mg·m ⁻² ·d ⁻¹	follows from Eq. 4
md	microbial death	25 mg·m ⁻² ·d ⁻¹	Schimel and Weintraub (2003)
pl _A	aboveground plant loss through litter	2.2 mg·m ⁻² ·d ⁻¹	follows from Eq. 1
pl _B	belowground plant loss through litter	4.7 mg·m ⁻² ·d ⁻¹	
in	microbial N fixation and deposition	1.1 mg·m ⁻² ·d ⁻¹	Lauenroth and Milchunas (1991)
out	denitrification and N leaching	1.1 mg·m ⁻² ·d ⁻¹	equal to "in"
$A(0)_{\text{APIN}}$	¹⁵ N atom% in the aboveground plant-N pool at time 0	0.37%	OTC (unpublished data)
$A(0)_{\text{BPIN}}$	¹⁵ N atom% in the belowground plant-N pool at time 0	0.37%	OTC (unpublished data)
$A(0)_{\text{InoN}}$	¹⁵ N atom% in the inorganic N pool at time 0	28.8046%††	
$A(0)_{\text{McrN}}$	¹⁵ N atom% in the microbial biomass at time 0	0.37%	equal to $A(0)_{\text{SoilN}}$
$A(0)_{\text{SoilN}}$	¹⁵ N atom% in the dead soil organic N at time 0	0.37%	OTC (unpublished data)
A_{in}	¹⁵ N atom% of N fixation and N deposition.	0.37%	

† Open-top chamber study (OTC; Morgan et al. 2004). Pools measured in October (in senesced leaves after resorption).

‡ The total soil N pool in the top 15 cm of the OTC study was 165 g/m². Here I assumed that 55% of that pool was active.

§ Based on a turnover time of 1 year of the senesced aboveground plant N pool and 4.5 years of the belowground N pool. Plant-N turnover time is defined as plant-biomass N/plant-N uptake.

¶ Based on the difference between aboveground plant N pool measured at peak standing biomass in July and in senesced plants in October in OTC experiment (Dijkstra et al. 2008).

Barrett and Burke (2000) reported gross N mineralization rates (m) that were approximately three times higher than net N mineralization rates ($m - i = 6.9 \text{ mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$). Note that $m - i$ is defined by Eq. 3.

|| Schimel and Weintraub (2003) used 0.012 in their model as the proportion of microbial biomass that dies every day.

†† Based on 0.5 g/m² of background Inorganic N with ¹⁵N atom% of 0.3665 and 0.2 g/m² of labeled N with ¹⁵N atom% of 99.9% (assuming that 0.1 g and 0.2 g of the 0.5 g of labeled N added were immediately lost through volatilization and adsorbed to the soil N pool, respectively).

atmospheric N (0.3663) and that the ¹⁵N atom% of microbial biomass at time 0 equals the ¹⁵N atom% of the active soil N at time 0.

Five months after the 0.5 g/m² of ¹⁵N addition (as ammonium nitrate, dually labeled) to the OTC study, only 0.4 g/m² of labeled N was recovered in the plant and soil. The ¹⁵N recovery in the soil did not change after that (Dijkstra et al. 2008). I assumed that the early and rapid loss of 100 mg/m² of labeled N was due to ammonia volatilization during the addition. I did not model this volatilization but fitted the data by simulating an addition of 0.4 g/m² instead of 0.5 g/m² of labeled N at time 0. Usually between 30% and 70% of the applied ¹⁵N label ends up in the dead soil N pool within 48 h for reasons that are not well understood (Kay and Harte 1997). I fitted the soil ¹⁵N data from the OTC study by having 50% (0.2 g) of the ¹⁵N label going directly into the dead soil N pool at time 0. I assumed that the remainder of the ¹⁵N added immediately mixed with the N in the inorganic N pool. The increased availability of inorganic N with the ¹⁵N addition will cause a short-term increase in plant N uptake and microbial N immobilization. I modeled this by increasing plant N uptake (pu_A and pu_B) and microbial N

immobilization (i) during the first time step only so that after the first time step the total inorganic N pool was back to its pre-¹⁵N addition level. For the first time step pu_A, pu_B, and i were increased proportionally to their respective uptake/immobilization rates that were used for the remainder of the simulation.

I calculated goodness of fit of the model simulations with G :

$$G = \sum (O - S)^2 / S \quad (11)$$

where O is the observed labeled-N fraction and S is the simulated labeled-N fraction. A lower G indicates a better fit. When I changed the belowground N turnover time from 7 to 4.5 years while leaving all other parameter values the same as in Table 1, G decreased from 7.09 to 1.78 mg/g for the belowground labeled-N fraction (Appendix), while it only slightly affected the aboveground labeled-N fraction (G decreased from 0.10 to 0.06 mg/g).

Scenarios

After fitting the ambient CO₂ plots using parameters listed in Table 1, I tested the sensitivity of five different

TABLE 2. Parameter values to simulate ^{15}N flow in a semiarid grassland under ambient CO_2 and for five different scenarios: (1) plant/microbial competition for N, (2) microbial activity, (3) active soil N, (4) N loss, and (5) N resorption and re-translocation.

Parameter	Ambient CO_2	Scenario									
		1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Above Plant N (g/m^2)	0.8	1.1	1.3	1.1	1.3	1.1	1.1	0.8	0.8	0.8	0.8
Below Plant N (g/m^2)	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7
Active soil N (g/m^2)	90	90	90	90	90	120	150	90	90	90	90
pu_A ($\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	2.2	3	3.6	3	3.6	3	3	2.2	2.2	2.2	2.2
pu_B ($\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	4.7	6.2	7.8	6.2	7.8	6.2	6.2	4.7	4.7	4.7	4.7
rs ($\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	1	1	1	1	1	1	1	1	1	0	2.2
rt ($\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	1	1	1	1	1	1	1	1	1	0	2.2
m ($\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	20.7	20.7	20.7	27.6	34	20.7	20.7	20.7	20.7	20.7	20.7
i ($\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	13.8	11.5	9.3	18.4	22.6	11.5	11.5	9.4	3.9	13.8	13.8
μ ($\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	31.9	31.9	31.9	42.5	52.4	42.5	53.2	31.9	31.9	31.9	31.9
md ($\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	25	22.7	20.5	33.3	41	33.3	44	20.6	15.1	25	25
pl_A ($\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	2.2	3	3.6	3	3.6	3	3	2.2	2.2	2.2	2.2
pl_B ($\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	4.7	6.2	7.8	6.2	7.8	6.2	6.2	4.7	4.7	4.7	4.7
$(\text{pu}_A + \text{pu}_B)/i$	0.5	0.8	1.2	0.5	0.5	0.8	0.8	0.7	1.8	0.5	0.5
out ($\text{mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	1.1	1.1	1.1	1.1	1.1	1.1	1.1	5.5	11	1.1	1.1
Turnover times											
Above Plant N (yr)	1	1	1	1	1	1	1	1	1	1	1
Below Plant N (yr)	4.5	3.4	2.7	3.4	2.7	3.4	3.4	4.5	4.5	4.5	4.5
Microbial N (yr)	0.12	0.13	0.13	0.09	0.07	0.1	0.09	0.13	0.15	0.12	0.12
Active soil N (yr)	7.7	7.7	7.7	5.8	4.7	7.7	7.7	7.7	7.7	7.7	7.7

Notes: Each scenario was run twice (A and B). In the B run, values of parameters changed in the A run were further increased/changed to indicate more clearly how sensitive each scenario was. Details of each scenario are discussed in *Methods: Scenarios*. Parameter values not listed are the same as in Table 1. Changes in parameter values from the ambient CO_2 simulation are in bold. Parameters were changed in such a way that the constraints shown in Eqs. 1–4 were met.

processes (hereafter called scenarios) on the labeled-N fraction in plant biomass by changing the values of specific parameters. I ran each scenario twice (A and B run) and calculated their goodness of fit with G . In the B run, values of parameters changed in the A run were further increased/changed to indicate more clearly how sensitive each scenario was. I also tested if the scenarios could explain the increased plant ^{15}N dilution observed in aboveground biomass under elevated CO_2 . Particularly for the first three scenarios, parameter values were modified to simulate specific responses to elevated CO_2 . All parameter value changes involved in the five scenarios are listed in Table 2.

Scenario 1. Plant/microbial competition for N.—I tested the effect of increased plant N uptake ($\text{pu}_A + \text{pu}_B$) relative to microbial N immobilization (i). It has been suggested that under elevated CO_2 , plants can become more competitive for N compared to microbes because of increased soil exploitation by roots (Finzi et al. 2007, Zak et al. 2007). In the A run I increased the aboveground plant N pool from 0.8 to 1.1 g/m^2 (as observed in the OTC study under elevated CO_2 ; Dijkstra et al. 2008) and reduced the belowground N turnover time by 24% (similar to the decrease in root turnover time under elevated CO_2 observed in the OTC study; Milchunas et al. 2005). As a result both pu_A and pu_B increased. I assumed that plant/microbial competition for N does not affect mineralization (m) and microbial uptake from active soil N (μ). It then follows from Eqs. 3 and 4 that immobilization (i) and microbial death (md) decreased with the same amount as the increase in $\text{pu}_A + \text{pu}_B$ (note that a decrease in i results in increased

net N mineralization). In the A run the $(\text{pu}_A + \text{pu}_B)/i$ ratio thus increased from 0.5 to 0.8. In the B run I increased the $(\text{pu}_A + \text{pu}_B)/i$ ratio to 1.2.

Scenario 2. Microbial activity.—I tested the effect of increased microbial activity on the labeled-N fraction in plant biomass. It has been suggested that microbial activity increases under elevated CO_2 (Hungate et al. 1997, Cheng 1999). In the A run I simulated increased microbial activity by increasing N mineralization (m), microbial N immobilization (i), microbial uptake from active soil N (μ), and microbial death (md) simultaneously by 33% and in the B run by 65%. Because in absolute terms m increased more than i , net-N mineralization increased in both runs. I did not want to affect plant–microbial competition for N in this scenario as I did in scenario 1. Therefore, $(\text{pu}_A + \text{pu}_B)$ and i were increased at the same rate so that the $(\text{pu}_A + \text{pu}_B)/i$ ratio did not change. This simulated increase in microbial activity also decreased microbial turnover time (here defined as Microbial N/($i + \mu$)) and active soil-N turnover time (here defined as Active soil N/ μ). This decrease in active soil N turnover time could be considered as a priming effect where increased stimulation of microbial activity results in increased organic N decomposition (Cheng 1999).

Scenario 3. Active soil N.—It has also been suggested that a priming effect can increase the active soil organic matter pool (i.e., soil organic matter that was once considered inactive or very recalcitrant becomes active or more labile with greater microbial activity; Hu et al. 2005, Dijkstra and Cheng 2007, Fontaine et al. 2007). In this scenario I tested the effect of an increase in the

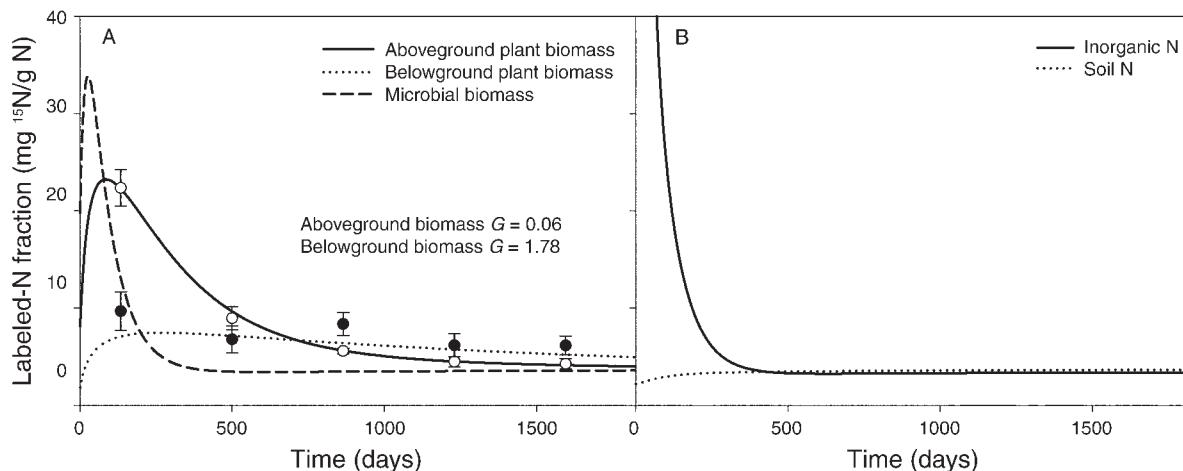


FIG. 2. Simulation (lines) and actual data (symbols) of (A) the labeled-N fraction in above- and belowground plant biomass and microbial biomass and of (B) the simulation of the labeled N fraction in the inorganic and active soil-N pools during 5 years after a 0.4 g/m^2 ^{15}N addition using parameters listed in Table 1. In panel (A), open circles show observed labeled-N fraction in aboveground plant biomass; solid circles show observed labeled-N fraction in belowground plant biomass. Error bars indicate \pm SE. Included are the goodness-of-fit G values calculated for the simulations of the labeled-N fraction in above- and belowground plant biomass.

active soil N pool that could have occurred under elevated CO_2 . In the A run I increased the active soil N pool from 90 to 120 g/m^2 , at the same rate (33%) as the increase in μ simulated in the A run of scenario 2, so that the active soil N turnover time did not change. Because activation of previously inactive soil N could be due to increased soil exploitation by roots, I also increased the $(\text{pu}_A + \text{pu}_B)/i$ ratio as in scenario 1A to simulate increased plant competitiveness for N. As a result, m remained the same as simulated for the ambient CO_2 plots. In the B run I increased the active soil N pool to 150 g/m^2 .

Scenarios 4 and 5. N loss and resorption/re-translocation.—I tested the sensitivity of the model to changes in some of the parameters that were not affected by the three scenarios above. In particular, I was interested in how sensitive the model output was to changes in N loss (out) and resorption and re-translocation (r_s and r_t), because these processes were not accurately measured in the OTC study. To increase loss of N through denitrification/leaching while maintaining constant inorganic N and microbial N pools in the model, I decreased microbial immobilization (i) and microbial death (md) with the same amount as the increase in “out” (see Eqs. 3 and 4). Note that a decrease in i resulted in an increase in the $(\text{pu}_A + \text{pu}_B)/i$ ratio, while a decrease in md resulted in a loss in the active soil N over time. I used the same parameters as fitted for the ambient CO_2 plots (Table 2), but simulating a five times (A run) and 10 times (B run) higher rate of N loss. I further simulated the effect of the two most extreme cases possible for resorption and re-translocation: zero resorption and re-translocation (A run) and maximum resorption and re-translocation equal to total aboveground plant N uptake (B run).

RESULTS

I first ran the model with the parameters listed in Table 1. After the ^{15}N addition at time 0, the labeled-N fractions in microbial and plant biomass initially increased and then declined with time (Fig. 2A). Nitrogen pools with shorter turnover times (e.g., 0.12, 1, and 4.5 years for microbial N, aboveground plant N, and belowground plant N, respectively) exhibit greater spikes and more rapid subsequent declines in labeled-N fractions. The labeled-N fractions in the plant N pools with relatively high turnover times (i.e., root N) remained dynamic after five years of ^{15}N addition. The initial increases in the labeled-N fractions in microbial and plant N were caused by uptake of N from the inorganic N pool that began with a high percentage of ^{15}N but that declined rapidly with time (Fig. 2B). The labeled N added to the inorganic soil N pool at time 0 dissipated with time to the other N pools and was partially lost through denitrification and leaching. The labeled-N fraction in the active soil-N pool slowly increased because of microbial immobilization of ^{15}N and eventually the labeled-N fraction equilibrated with the labeled-N fraction in the inorganic soil N pool (Fig. 2B). The model simulated the labeled-N fractions in aboveground biomass from the OTC study quite well (Fig. 2A). Particularly for aboveground biomass (where ^{15}N measurements were more precise than for belowground biomass) the model fitted the data well ($G = 0.06$ compared to $G = 1.78$ for belowground biomass).

I observed the following by testing the five different scenarios described in the *Methods* section. Because the labeled-N fraction measured in belowground plant biomass was highly variable with time and was difficult to model, and because these measurements were not very precise, I focused on relating modeled results to

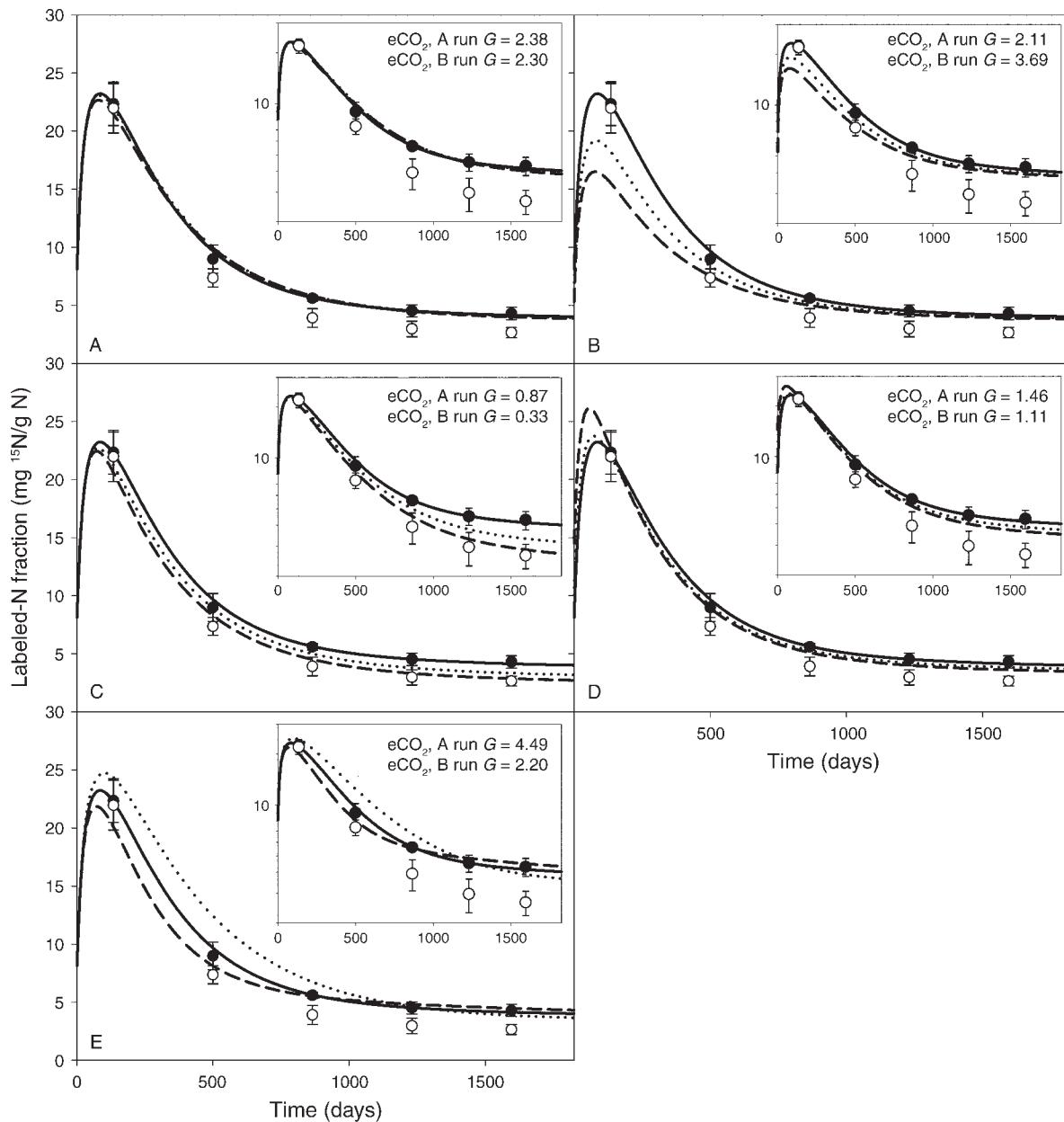


FIG. 3. Simulation (lines) and actual data (symbols) of the labeled-N fraction in aboveground plant biomass during 5 years after a $0.4 \text{ g/m}^2 \text{ }^{15}\text{N}$ addition using parameters listed in Table 2. Simulation of the labeled-N fraction in aboveground biomass for (A) scenario 1, plant/microbial competition for N; (B) scenario 2, microbial activity; (C) scenario 3, active soil N; (D) scenario 4, N loss; and (E) N resorption and re-translocation. Each panel shows the actual data of the ambient CO₂ plots (solid circles) and elevated CO₂ plots (eCO₂, open circles), and the model simulation of the ambient CO₂ plots (solid lines), A runs (dotted lines), and B runs (dashed lines). Insets show the same data, but now with the y-scale log-transformed to amplify labeled-N fractions during the later years. Error bars show standard error. Included are the goodness-of-fit *G* values calculated for the simulations of the labeled-N fraction in aboveground plant biomass in the elevated-CO₂ plots.

observed results for aboveground plant biomass. Modeled and observed results of labeled-N fractions for belowground plant biomass are shown in the Appendix.

Scenario 1. Plant/microbial competition for N.—Increasing the $((p_{uA} + p_{uB})/i)$ ratio to 0.8 (A run) or 1.2 (B run) hardly affected the labeled-N fraction in aboveground biomass (and thus poorly fitted observed

labeled-N fractions under elevated CO₂; Fig. 3A). The labeled-N fraction did not change much because the turnover time of the aboveground plant N pool was not affected in this scenario. On the other hand, the reduced turnover time of the belowground N pool resulted in faster movement of the label through this pool compared to the ambient CO₂ simulation, as was

expressed in a higher spike and faster subsequent decline (Appendix).

Scenario 2. Microbial activity.—By increasing overall microbial activity by 33% (A run), but not plant/microbial competition for N to simulate the effect of elevated CO_2 , I observed a greater initial dilution of the labeled N in the aboveground plant N pool. This dilution effect decreased with time and disappeared completely after five years of simulation compared to the ambient CO_2 simulation (Fig. 3B). Consequently, observed labeled-N fractions in aboveground biomass during the latter three years under elevated CO_2 were poorly simulated. Increasing microbial activity by more than 33% only resulted in greater ^{15}N dilution early on in the aboveground biomass, but again converged to the ambient CO_2 simulation after five years (B run in Fig. 3B).

Scenario 3. Active soil N.—By increasing the active soil-N pool by 33% in combination with a 33% increase in microbial uptake of dead soil N (thus no change in the turnover time of the active soil N pool), and increased plant competitiveness for N as in scenario 1 (A run), this resulted in increased labeled N dilution in aboveground plant biomass that persisted in the long term (Fig. 3C). In contrast to scenarios 1 and 2, the labeled-N fraction in aboveground biomass did not converge by year 5, but remained lower. Increasing the active soil N pool by 67% (B run) simulated long-term labeled-N fractions in aboveground plant biomass measured under elevated CO_2 relatively well ($G = 0.33$; Fig. 3C).

Scenarios 4 and 5. N loss and resorption/re-translocation.—The effect of N loss on labeled-N fractions in above- and belowground plant N was small. A fivefold increase in “out” (A run) caused a slight short-term increase and a slight long-term decrease of the labeled-N fraction in aboveground plant N (Fig. 3D). These effects were slightly higher with a 10-fold increase in “out” (B run). Because i needed to decrease with an increase in “out” to maintain a constant inorganic N pool, the short-term effect of the increase in “out” on the labeled-N fraction in plant N was due to increased plant competition for N (i.e., $(pu_A + pu_B)/i$ ratio increased). When changing resorption and re-translocation to their minimum (0, A run) and maximum (pu_A , B run) values, the effects on long-term labeled-N fractions in plant-N pools were small, but they were substantial in the short-term (Fig. 3D).

DISCUSSION

The model presented here provides new insights into the use of a ^{15}N tracer to study long-term N cycling in a semiarid grassland. I have shown that temporal dynamics of ^{15}N fractions (labeled-N fractions) in plant and microbial biomass are closely tied to the turnover time of these N pools. Once added, the labeled N in the inorganic N pool dissipates quickly to the microbial N pool and more slowly to the plant N pools. The labeled-N fraction peaks earlier in the microbial biomass than in

the plant biomass due to a much shorter turnover time of the microbial N. Thus, short-term measurements of labeled N in plant and microbial biomass, and long-term measurements of labeled N in plant biomass after ^{15}N labeling can provide useful information about N cycling in the short- and long-term. It has been suggested that long-term measurements of ^{15}N after labeling are confounded by short turnover times of microbes, leaves, and fine roots (Kaye and Hart 1997). For instance, one may come to the wrong conclusion to suggest that microbes compete less for N than plants when one observes in the long-term a lower ^{15}N recovery (amount of ^{15}N expressed as a percentage of ^{15}N applied) in microbial biomass compared to plants. Here I argue that long-term measurements of ^{15}N after labeling should be interpreted as a measure of ^{15}N dilution with non-labeled sources. The labeled-N fraction in microbes with a faster N turnover time than plants is expected to decrease faster after the initial spike because microbes lose ^{15}N and take up unlabeled N much faster. Similarly, the labeled-N fraction in plant biomass is expected to decrease with time as well, but at a much smaller rate than for microbes, because the N turnover time in plants is longer.

I used the model to simulate labeled-N fractions that were measured each year for five years in aboveground biomass under ambient and elevated CO_2 after a one-time ^{15}N addition in a semiarid grassland (Dijkstra et al. 2008). Despite its simplicity (e.g., only one active soil-N pool with one turnover time and constant N pools and fluxes), the model provided a good fit of the ambient CO_2 data, suggesting that the model incorporated the important mechanisms affecting the long-term N cycle in this grassland system.

The above- and belowground-N pools did fluctuate among years due to relatively dry (fourth year) and wet years (third year) during the experiment. Total N in senesced aboveground biomass ranged between 0.68 and 1.16 g/m^2 during the 5-year period of the experiment. Nevertheless, the labeled-N fraction in aboveground plant biomass showed a clear declining pattern with time, suggesting that annual fluctuations in total aboveground N had a relatively small effect on its ^{15}N dilution with time. The simulated labeled-N fractions in aboveground plant biomass were also insensitive to an increase in the aboveground-N pool from 0.8 to 1.3 g/m^2 (Scenario 1; Fig. 2A). In order to fit the data, I had to simulate a smaller belowground plant N turnover time than has been reported for root biomass for this system (Milchunas et al. 2005) and an active soil-N pool that was smaller than the total soil N pool. Recently it has been suggested that N loss through root exudation can be substantial (De Graaff et al. 2007, Wichern et al. 2008), indicating that root N turnover time should be smaller than root biomass turnover time. Because I did not know the fraction of soil N that was actively involved in the N cycle, I fitted the size of this pool. In reality, fractions of soil N have different degrees of

activity and recalcitrance. In other models this issue has been addressed by separating soil organic matter in multiple pools with multiple turnover times (Parton et al. 1988) or by defining organic matter quality as a measure of substrate availability (Ågren and Bosatta 1998). The model here could be improved by a more detailed description of active soil N to better understand the role of soil-N pools and their turnover times on the N cycle.

In Dijkstra et al. (2008) we suggested that the greater decline of the labeled-N fraction in aboveground plant biomass under elevated CO₂ was caused by greater net N mineralization, most likely due to improved soil water conditions under elevated CO₂. Here I explored in more detail potential mechanisms that caused greater ¹⁵N dilution in aboveground plant biomass under elevated CO₂.

Elevated CO₂ can result in increased soil exploitation by growing more roots (Finzi et al. 2007, Zak et al. 2007), which could increase plant competitiveness for N relative to microbes. Increased plant competition for N compared to microbes without affecting N mineralization (but reducing microbial N immobilization and thus increasing net N mineralization, scenario 1) could potentially have caused the observed increase in aboveground plant-N pool and increased belowground N turnover under elevated CO₂ (Milchunas et al. 2005, Dijkstra et al. 2008). However, increased plant competition for N had no effect on the labeled-N fraction in aboveground plant biomass in the model, suggesting elevated CO₂ changed more of the N cycle than just by increasing plant competitiveness or soil exploitation for N.

It has been suggested that microbial activity increases with elevated CO₂ because of greater supply of labile substrates to microbes (Hungate et al. 1997, Cheng 1999; scenario 2). This could then also lead to greater soil organic matter decomposition and net N mineralization (Cheng 1999). But as with scenario 1, the model simulations of increased microbial activity (i.e., increased *m*, *i*, *mu*, and *md*) poorly fitted the long-term effect of elevated CO₂ on the labeled-N fraction in plant biomass. No matter how much the microbial activity was increased, the simulated long-term labeled-N fraction in aboveground plant biomass never diverged from the ambient CO₂ simulation, but only resulted in a short-term decrease. This suggests that other mechanisms were involved in the increased long-term decline in labeled-N fraction in aboveground plant biomass under elevated CO₂.

Recently it has been suggested that an increase in the supply of labile substrates to microbes can result in increased destabilization of soil organic matter (Fontaine et al. 2007). Thus, an increase in microbial activity may not only increase decomposition (or *mu* in the model), but could also convert recalcitrant soil organic matter into more active soil organic matter. Similarly, soil N should then become more active with an increased

supply of labile substrates and increased microbial activity (Hu et al. 2005; scenario 3). This increase in active soil N could occur when elevated CO₂ increases soil exploitation by roots and thereby affects more soil organic matter through root-induced processes. Indeed, Hagedorn et al. (2008) observed increased leaching of old dissolved organic matter under elevated CO₂ in an alpine system, suggesting that elevated CO₂ increased destabilization of soil organic matter. When the active soil N pool in the model was increased at the same rate as the increase in *mu* (i.e., the turnover time of the active soil-N pool remains unchanged), the long-term simulated labeled-N fraction in plant biomass dropped. With a greater active soil-N pool, more unlabeled N ended up in the N cycle, diluting the plant-biomass-labeled N in the long-term. Note that microbial mineralization (*m*, or gross N mineralization) did not change in this scenario, despite an increase in *mu*. This is in agreement with the notion that gross N-mineralization rates are often non-responsive to elevated CO₂ (e.g., see review by Zak et al. 2003). When I increased the active soil N pool, the model still did not accurately simulate short-term labeled-N fractions in aboveground biomass measured under elevated CO₂. However, the increase in the active soil-N pool showed a significant improvement of simulating long-term labeled-N fractions compared to scenarios 1 and 2, where long-term labeled-N fractions in plant biomass could not be lowered.

Dijkstra et al. (2008) suggested that the increased ¹⁵N dilution of aboveground plant biomass under elevated CO₂ may have been a result of improved soil moisture conditions that increased net N mineralization under elevated CO₂. The scenario 3 simulations imply that an increase in active soil N (or greater destabilization of soil organic matter) due to increased soil exploitation by roots may have been an important factor in causing lower labeled-N fractions in aboveground biomass under elevated CO₂ of this semiarid grassland. Nitrogen fixed in long-lived plant biomass and soil organic matter under elevated CO₂ could reduce soil N availability in the long-term thereby constraining plant productivity, also referred to as the concept of progressive N limitation (PNL; Luo et al. 2004). However, by increasing destabilization of soil organic matter relatively rich in N, plants grown under elevated CO₂ may avoid PNL in this semiarid grassland.

Long-term labeled-N fractions could not be lowered substantially by increasing N loss (scenario 4) or by changing resorption/re-translocation rates of N between above- and belowground plant biomass (scenario 5). There is no evidence that elevated CO₂ increased N loss through denitrification in this system (Mosier et al. 2002), and in other grassland and forest systems no or reduced N loss through leaching has been observed (Niklaus et al. 2001, Hagedorn et al. 2005, Dijkstra et al. 2007). There is also no evidence that elevated CO₂ affects N resorption (Norby et al. 2001, Billings et al. 2003). Although I did not have accurate measurements

of N loss (i.e., N leaching) and resorption/re-translocation, the model simulations indicate that changes in N loss and resorption/re-translocation could not have caused the long-term effect of elevated CO₂ on the labeled-N fraction in aboveground plant biomass.

The simulations of each of the five scenarios gave distinct temporal patterns. Increased plant competition for N had no effect on the labeled-N fraction in aboveground plant biomass. Increased microbial activity decreased the labeled-N fraction in aboveground biomass in the short-term, but not in the long-term. Only by increasing the active soil N pool did the model simulate a lower fraction of labeled N in the long-term as was observed under elevated CO₂ in a semiarid grassland. The model presented here provides a useful tool to evaluate the mechanisms by which experimental perturbations affect N cycling in semiarid grasslands. It remains to be seen how well the model performs in other ecosystem types.

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APPENDIX

Effects of the five scenarios on labeled-N fractions in belowground plant biomass (*Ecological Archives* E090-152-A1).