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Research Article

Temporal Variation of N Isotopic Composition of Decomposing Legume Roots and Its Implications to N Cycling Estimates in ^{15}N Tracer Studies in Agroforestry Systems

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Below-ground residue of agroforestry trees is an important N source for associated crops. Several studies have shown that its isotopic signature (δ^{15}N) may change after tree pruning, which makes it difficult to study below-ground N inputs from pruned trees by isotopic techniques. We studied how temporal variation of legume root residue δ^{15}N could be explained by considering differential decomposition kinetics and ^{15}N content of residue fractions. A mathematical model on the isotopic patterns of soil and a N recipient plant during root decomposition was developed and applied for testing assumptions about residue characteristics against two experimental datasets. Observed ^{15}N patterns of the recipient plants could be satisfactorily simulated only when the residue was assumed to consist of at least two fractions with distinct δ^{15}N and decomposition rates depending on their C : N ratio. Assuming δ^{15}N of residue constant over time resulted in substantial underestimates of N derived from low-quality residue (%Ndfr) by the recipient plant when compared with experimental data. Results of this study suggest that residue fractionation can help improve estimation of %Ndfr in isotopic studies, as an alternative or complementary method to assuming or aiming at homogenous isotopic composition of N sources.

1. Introduction

Pruning of trees is a common practice in agroforestry systems. In legume-based systems the main purpose of pruning is to provide nitrogen to soil and the associated crops from green manure and below-ground residue. Timing and intensity of pruning can be varied to adjust the N inputs with the requirements of the crops, and amount and optimal timing of these inputs have been the interest of numerous studies [1, 2]. Although most studies have concentrated on N release from above-ground biomass, management of N release from roots may be much more important for crop nutrition, since up to 50–60% of total plant N of frequently pruned agroforestry trees may occur in roots [3].

Isotopic techniques are commonly applied for studying the fate of N in agroforestry systems and the mechanisms involved in its cycling. They can be especially useful for studying below-ground N cycling processes that are difficult to trace otherwise. The techniques require measurement or a reasonable estimate of the N isotopic compositions of the N sources. However, recent research suggests that estimating N cycling in intercropping systems with isotopic techniques becomes difficult after management interventions which affect root turnover. After shoot harvest or pruning of the N donor plant, isotopic ratio of the N recipient plants peaks rapidly within a few days and then decreases slowly over time. Such patterns were observed in different experimental setups and N donor species: in studies applying

both ^{15}N natural abundance and ^{15}N enrichment methods for the legume tree *Gliricidia sepium* under field conditions [4, 5] and in pot culture [6], and in a study applying the ^{15}N natural abundance method for the herbaceous legume *Canavalia ensiformis* in pot culture [7]. The similarity of the results from different setups suggests that the unexpected isotopic patterns of the N recipient plants represent a general phenomenon independent of N donor species and the ^{15}N tracing technique applied.

A key reason for difficulties in analysing the fate of N from organic sources with isotopic techniques certainly is the nonuniform isotopic composition of these sources, which as such is well established. Discrimination against ^{15}N in biochemical processes results in variation of ^{15}N natural abundance between plant organs and their nitrogenous compounds, including soluble proteins, amino acids, and nitrate [8, 9]. Numerous ^{15}N enrichment studies have also demonstrated that ^{15}N labelling techniques and ^{15}N allocation within the plant may result in different plant parts becoming very differently enriched with ^{15}N [10, 11].

Isotopic variation between nitrogenous compounds of a plant could be further enhanced by their different decomposition rates, which are well known from residue decomposition research [12, 13] yet not frequently considered in ^{15}N tracer studies. Together these two factors may result in considerable temporal variation in the isotopic patterns of N released from organic inputs (e.g., roots decomposing after pruning), which is subsequently reflected in isotopic composition of the N recipient pools such as soil compartments, soil microbial biomass, or associated N recipient crops. Studies applying modelling approaches suggest that isotopic composition of N released from decomposing roots of a legume tree differs from that of living roots just before pruning, both with ^{15}N natural abundance and ^{15}N enrichment methods [6] and that N uptake from ^{15}N-enriched, surface-applied residues is best explained when decomposition rate constants are estimated separately for labile and stable residue fractions [14]. However, because isotopic heterogeneity is considered difficult to evaluate over time, N uptake or N transfer studies often assume that isotopic composition of an organic N source remains constant over the length of an experiment. Although homogeneity of ^{15}N labelling has improved through methodological development (reviewed in [15]), it remains difficult to achieve over time, especially at compound level (cf. e.g. [10, 16]). Moreover, inconsistent isotopic signatures can impair N cycling estimates also in ^{15}N natural abundance studies where isotopic compositions of N sources cannot be controlled [4, 6]. Alternative or complementary approaches to more reliably quantify N cycles in agroforestry systems are, therefore, needed.

Our objectives in this study were to (i) quantify how temporal variation in the isotopic composition of decomposing, ^{15}N-enriched legume roots affects the estimates of the fate of recycled N and (ii) study how this variation could be accounted for by considering both the isotopic heterogeneity of residue compounds and their differential decomposition kinetics. To analyse the effects of heterogeneity of decomposing roots, we measured the isotopic patterns of a N recipient plant, which served as integrator of the biological processes involved in N cycling in the soil-plant system. A dynamic model on the isotopic composition of soil pools and the N recipient plant during residue decomposition was developed and then applied for testing the study assumptions against two experimental datasets. The objective of the simulations was not to find the actual parameter values for the specific residues and soil used in the experimental studies, but instead explore which overall patterns and general assumptions about residue characteristics were necessary to explain the observed temporal changes in isotopic composition of the N recipient plants. Options for improving experimental design and interpretation of results in ^{15}N tracer studies and achieving more reliable estimates of the amount and fate of recycled N are then discussed.

2. Material and Methods

2.1. Experimental Design. Two separate datasets were used for evaluating the performance of the residue decomposition and fractionation model under different experimental designs. The first dataset was from a pot experiment where a fodder grass *Dichanthium aristatum* Poir C.E. Hubbard was grown alone in greenhouse, and ^{15}N-enriched fine root and nodule residue of the legume tree *Gliricidia sepium* (Jacq.) Kunth ex Walp. were applied in the grass pots (residue application experiment, RA in the following). Evolution of the N isotopic signature (δ^{15}N) of *D. aristatum* was then studied for 10 weeks. The experiment is described in the following. The second dataset was that described by Sierra et al. [6], where *D. aristatum* and *G. sepium* were grown together in pots in greenhouse (full interaction experiment, FI, in the following). *Gliricidia sepium* trees were labelled with ^{15}N through foliar application and pruned 12 weeks thereafter to induce root turnover. Evolution of δ^{15}N of *D. aristatum* was then studied for 48 weeks. For this study we used the data for the first 24 weeks, after which the N source from the decomposing residue appeared exhausted [6]. *Dichanthium aristatum* shoots were harvested every 4–8 weeks during the FI experiment.

The RA experiment was conducted at the greenhouse facilities of the Antillean Research Centre of INRA, Guadeloupe, Lesser Antilles (16°12′N, 61°39′, 125 m a.s.l.). Soil and plant material originated from a cut-and-carry fodder production system of *G. sepium* and *D. aristatum*. The site was established in 1989 and managed thereafter by frequent tree pruning and grass cutting. The soil on the site is Vertisol with 80% of clay, pH of 7.8, organic carbon content 33.1 g kg^{-1}, and organic N content 3.1 g kg^{-1} [6]. For detailed descriptions of the field site and the soil, see Daudin and Sierra [4] and Sierra et al. [17].

The experiment consisted of four potted *G. sepium* trees for providing the decomposing root and nodule residue, and eight pots of *D. aristatum* grass for studying the uptake of ^{15}N mineralised from residue. Soil for the pot experiment was collected from the topsoil layer of the field site and sieved to <1 cm aggregates while removing plant roots. Mineral N content of the soil was analysed as described by Sierra et al. [17]. *Gliricidia sepium* trees were established from cuttings in nursery bags filled with the soil. One month

after their propagation, the trees were transferred to pots of 14 L. Another two months later, *D. aristatum* swards were transplanted from the field site to similar pots as for the trees. Both series of pots were fertilised with 2 g of triple superphosphate and 2 g of K_2SO_4 at the time of planting and were irrigated daily throughout the experiment. Leaching was assumed negligible because soil inorganic N is mainly in the form of NH_4^+ [18] and fixed on soil particles in the clayey soil [19]. Daily mean of air temperature in the greenhouse varied between 25.5 and 30.5°C, with a decreasing trend towards the end of the experiment.

The trees were labelled using foliar feeding of 99% ^{15}N-enriched KNO_3 four months after their propagation. The label was applied on tree leaves with a small paintbrush at three events in equal amounts with two day intervals, allowing time for absorption of the solution [6]. The total amount of ^{15}N applied per tree was 30 mg. Foliar feeding was used because the study focused on N recycling from root and nodule turnover induced by pruning or shoot harvest of the N donor plant, common management methods in agroforestry and other intercropping systems. Labelling of above-ground plant parts with ^{15}N is the most feasible ^{15}N labelling methods to study N dynamics related to management practices in such systems. Foliar feeding also enabled comparison of the results with those of a larger study where various below-ground N transfer pathways between N donor and recipient plants were studied applying foliar feeding of ^{15}N [10].

Application of fine root residue took place when two months had passed since grass transplanting in the pots, and three weeks since tree ^{15}N-labelling. The grass shoots were first cut to approximately 2 cm height. The aim was to homogenise the initial situation and to minimise the dilution of assimilated ^{15}N within grass biomass. Cutting was also consistent with grass management at the field site, where the grass is customarily cut every 40–50 days. After grass cutting, the trees were harvested for collecting fine roots (<2 mm of diameter) and nodules which were not detached from the roots. Coarse roots were not used because they decompose slowly, and roots recycled after tree pruning are mainly fine roots [20]. Twelve holes were then carefully drilled in the soil of the grass pots, and fresh fine roots and nodules mixed with a small amount of soil were applied in the holes. The residue was not mixed homogeneously within the soil in order to avoid destructing the grass. Each pot received 3.2 ± 0.6 g of residue, which corresponded to approximately half of the fine root and nodule mass of the trees. All remaining residue material was weighed, oven-dried at 70°C for 72 h, and ground to <0.2 mm for isotopic analysis and for determining the relation of residue fresh and dry weight. Coarse roots were analysed separately.

Grass shoots were sampled for ^{15}N analysis immediately before residue application, and on days 28, 49, and 70 following it. Sampling was limited to these four events in order to avoid excessive disturbance of grass growth. At the end of the experiment on day 70, the grass was harvested and compartmented to shoot, stubble, and roots. All plant material was weighed, dried, and ground for isotopic analysis as described above. Characteristics of the labile and stable fractions were determined through model simulations as explained below. Biochemical properties of residue generally fail to predict N and C dynamics during residue decomposition [21], and the labile and stable fractions of residues are currently determined from laboratory incubations using a model to fit experimental data (e.g., [22]).

Sample N contents and isotopic ratios were determined at the Stable Isotope Facility of the University of California-Davis, US, using an element analyser interfaced to an isotope ratio mass spectrometer (Europa Integra CN; Sercon Ltd., Cheshire, UK).

The differences between the isotopic signatures (δ^{15}N) of shoot subsample and shoot total biomass at the end of the experiment were tested with Student's *t*-test, in order to estimate the sampling error for the isotopic signatures during the experiment. Correlation between grass shoot δ^{15}N and N concentration during the experiment was calculated as described by Hamlett et al. [23], taking into account that values of the variables were obtained as repeated measures of individual plants and were linked over time. Correlations were expressed as Pearson's correlation coefficients. Values of $P < 0.05$ were interpreted as indicating statistically significant differences. Results were analysed with SAS statistical analysis software, version 9.1 (SAS Institute Inc., Cary, NC, USA).

2.2. Model Structure. A dynamic model was developed for simulating N mineralisation from decomposing ^{15}N-labelled residue, its subsequent uptake by an adjacent plant, and the plant's isotopic signature (Figure 1). Residue decomposition was modelled according to the STICS residue decomposition model [24]. The STICS model considers a single fraction for the residue, which decomposes with first order kinetics at a rate determined by its C : N ratio. It has reasonably well-explained overall C and N mineralisation from plant residues in tropical environments [25], including for roots of *G. sepium* [7]. It can also be easily combined with crop or ecosystem models, as it only requires residue C : N ratio as input [26].

The model developed in this study applies a modified decomposition component with regard to STICS, in order to allow simulation of heterogeneity of residue and its isotopic composition, and the implications to soil and recipient plant in the system. Residue is divided into fractions, and δ^{15}N, N content, and C : N ratio can be specified individually for each of these. Uptake of N and its partitioning within the recipient plant was modelled according to a box model [18] which has explained well biomass accumulation and δ^{15}N in *D. aristatum* shoots [6, 18]. The final model consisted of seven types of N pools, namely, residue (N_R), microbial biomass involved in decomposition (N_B), humified organic matter (N_H), soil inorganic N originating from residue (N_{SR}), soil native inorganic N (N_{SN}), and plant roots (N_{RO}) and shoot (N_S). All pools were divided to ^{14}N and ^{15}N according to their initial δ^{15}N values. Isotopic signatures of N flows were defined by δ^{15}N of the source pool.

All residue fractions and the associated microbial biomass pools were set to either deplete or contribute to the same soil inorganic N pool, depending on the C : N ratios

of each residue fraction [24]. The model enables simulation of differential N uptake from the N_{SR} and N_{SN} pools by the recipient plant, by including a N source factor, Sf:

$$u_{SR} = \begin{cases} \min\left(u \times \dfrac{N_{SR}}{N_{SN}+N_{SR}}, N_{SR}\right), & \text{if Sf} = 0 \\ \min(u, \text{Sf} \times N_{SR}), & \text{if } 0 < \text{Sf} \le 1 \\ \max(0, \text{Sf} \times (N_{SN} - u)), & \text{if } -1 \le \text{Sf} < 0, \end{cases} \quad (1)$$

$$u_{SN} = u - u_{SR}, \quad (2)$$

where u, u_{SR}, and u_{SN} are total N uptake rate of soil N, and N uptake rates from N_{SR} and N_{SN} pools, respectively. Value of Sf in the model is fixed over time. If Sf > 0, then N is first absorbed from the N_{SR} pool and only secondarily from N_{SN}, if supply from N_{SR} is insufficient. If Sf < 0, then N is first absorbed from the N_{SN} pool.

Net mineralisation of soil native organic N (m_{SO}) was simulated as a function of soil temperature according to the equation proposed for the same soil and site as in the experimental datasets by Sierra et al. [17]:

$$m_{SO} = 0.073T - 0.28, \quad 20^{\circ}\text{C} < T < 30^{\circ}\text{C}. \quad (3)$$

Because N_{SN} represents net N mineralisation from soil organic matter, it takes into account N immobilisation during mineralisation. However, further N immobilisation from N_{SN} may occur when N demand for growth of the microbial biomass decomposing residues is greater than N supplied from N_{SR} (Figure 1) [24]. Therefore, growth of microbial biomass decomposing residues is primarily supported by organic N in residues, then by N_{SR} and then by N_{SN}.

Ambient temperature and soil humidity affect mineralisation of both residue and microbial N pools [24] and soil native organic N. Mineralisation of humified organic matter originating from the residue was considered negligible as N source for the recipient plant, in comparison to the decomposing residue and soil inorganic N (cf. [27]). Approximately 95–98% of inorganic N in the soil used for the experimental studies is in the form of NH_4^+, possibly because some tropical grasses, including *D. aristatum*, release compounds which reduce the populations or activity of nitrifying bacteria [18, 28]. Negligible volatilisation of NH_3 has been observed in a non-N-fertilised Vertisol similar to that used in the experimental studies [29], and frequent irrigation was assumed to further reduce concentration of NH_4 in the soil. Therefore, denitrification and volatilisation were excluded from the model.

Daily uptake of soil inorganic N by the recipient plant was modelled as linear over the time span of the experiments and considering that grass shoots were regularly harvested in the FI experiment. Linear N uptake was based on the results of the FI experiment and previous observations for the N recipient grass [6, 30].

2.3. Calculations of N Uptake by the Recipient Plant. The enrichment of a sample with ^{15}N in relation to the atmospheric standard is expressed as

$$\delta^{15}N_{sa}(‰) = \frac{\text{atom\%}^{15}N_{sa} - \text{atom\%}^{15}N_{atm}}{\text{atom\%}^{15}N_{atm} \times 1000}, \quad (4)$$

where the subscripts sa and atm refer to the sample and the atmospheric ^{15}N atom-% (0.3663%), respectively. Proportion of N derived from residue (%Ndfr) per total N of the recipient plant was calculated as

$$\%\text{Ndfr}_t = \frac{\delta^{15}N_{P0} - \delta^{15}N_{Pt}}{(\delta^{15}N_{SN} - \delta^{15}N_{SRt}) \times 100}. \quad (5)$$

Subscripts *P*, SN, and SR refer to the recipient plant and to N derived from soil native N and residue, respectively, 0 to the initial values before ^{15}N enrichment, and t to the point in time. Amount of N derived from residue (N_u) in the recipient plant was expressed as

$$N_u = \%\text{Ndfr} \times N_P, \quad (6)$$

where N_P denotes plant N content. Proportion of residue N uptaken by the recipient plant ($\%N_u$) was obtained from

$$\%N_u = \frac{N_u}{N_R} \times 100, \quad (7)$$

where N_R is the total N content of the residue.

2.4. Model Parameterisation and Simulations. We assumed that the fine root residue consisted of two fractions, labile and stable. The following constraints were imposed for the labile fraction: (i) the minimum C : N ratio was set to 3.0, and the maximum N content to 30% of total residue N. These are in the range of values measured for water-soluble N in plant roots [22, 31, 32]. (ii) The maximum $\delta^{15}N$ was set to 600 for the RA experiment, and to 495 for the FI experiment. With the maximum N content and respective $\delta^{15}N$ of the labile residue fraction, $\delta^{15}N$ of the stable fraction would equal to 1 in each dataset.

Input parameters for total N content, C : N ratio, and $\delta^{15}N$ of the residue in the RA experiment were the values measured for fine roots and nodules. In the FI experiment the input parameters corresponded to the amount of N recycled after pruning, and total C : N ratio and $\delta^{15}N$ of roots (including nodules; Table 1). Rate constant equations were common for the two datasets (Table 2). Temperature in the simulations was daily mean temperature in the greenhouse during the experimental studies, and soil humidity was set equal to field capacity as the grass pots were irrigated daily.

The model was run for a period of 70 days for the RA experiment and 168 days for the FI experiment. Three simulation steps were performed for each dataset to evaluate the assumptions about residue characteristics. The steps considered (i) one single residue fraction, that is homogenous residue, (ii) two residue fractions with equal isotopic composition, and (iii) two residue fractions with different isotopic composition. The aim was to test the hypotheses that

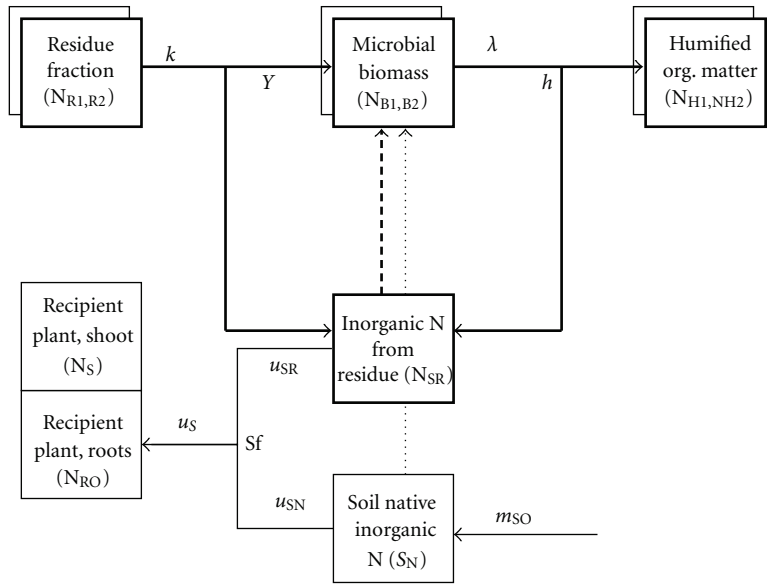

Figure 1: Model of residue decomposition and subsequent uptake of residue N by a recipient plant. Each pool is divided into ^{14}N and ^{15}N. Subscripts R1 and R2, B1 and B2, and H1 and H2 refer to the pools related to the two residue fractions in the residue, microbial biomass, and humified organic matter, respectively. Parameters k and λ are decomposition rate constants for residue and microbial biomass, respectively, Y is assimilation yield of C from residue by microbial biomass, and h is humification rate of microbial biomass. Parameter u is N uptake rate by the recipient plant, and its subscripts SR and SN refer to the uptake rates from the two soil inorganic pools (Table 2). Parameter Sf is N source factor which determines the relative N uptake rates from the two soil N pools. Parameter m_{SO} is mineralisation rate for soil organic N. Pools and processes indicated with bold lines are simulated according to Nicolardot et al. [24].

Table 1: Parameters of the residue decomposition model for the residue application (RA) and full interaction (FI) experiments. Where $\delta^{15}N$ is not given, it is defined by $\delta^{15}N$ of the source pool.

Parameter	Code	Initial value		Unit	Source	
		RA	FI		RA	FI
Residue[a]						
N content	N_R	97	1332	mg pot^{-1}	This study	[6]
C : N	R	14.5	30.2		This study	[6]
$\delta^{15}N$	$\delta^{15}N_R$	180	318	‰	This study	[6]
Microbial biomass						
N content	N_B	0	0	mg pot^{-1}	This study	[6]
C : N	R_B	$R_B = 16.1 - 123/R$, $R_B = 7.8$ when $R < 14.8$				[24]
Newly formed humified organic matter						
N content	N_H	0	0	mg pot^{-1}	This study	[6]
C : N	R_H	10.5				[33]
Inorganic N from residue						
N content	N_{SR}	0	0	mg pot^{-1}	This study	[6]
Soil native inorganic N						
N content	N_{SN}	21	17	mg kg^{-1}	This study	[6]
$\delta^{15}N$	$\delta^{15}N_{SN}$	4.3	7.2	‰	This study	[6]
Plant biomass						
N content[b]	N_P	52	68	mg pot^{-1}	This study	[6]
Shoot $\delta^{15}N$	$\delta^{15}N_S$	4.3	19.8	‰	This study	[6]
Total N uptake rate	u	3.2	3.0	mg d^{-1}	This study, est.	[6]

[a]Corresponding to fine roots and nodules for RA, and all roots and nodules for FI.
[b]Including shoot and root N.

Table 2: Common parameters in the residue decomposition model for the two experimental datasets.

Parameter	Code	Value	Unit	Source
Decomposition rate constant of residue	k	$0.07 + 1.94/R^a$	$nday^{-1}$ [b]	[24]
Decomposition rate constant of microbial biomass	λ	0.0110	$nday^{-1}$ [b]	[24]
Assimilation yield of C from residue by microbial biomass	Y	0.62		[24]
Humification rate of microbial biomass	h	$1 - 0.326R/(11.2 + R)$		[6]

[a]C : N ratio of residue.
[b]nday refers to a "normalised day" (25°C and optimum water content). In this study, we used 25°C as the reference temperature, instead of 15°C in Nicolardot et al. [24].

simulating the observed trends of δ^{15}N of the N recipient plants requires more than one residue fraction and that the fractions differ in terms of δ^{15}N. Each simulation step was run with two options for the N source factor: N uptake proportionally equal to the size of each inorganic N pool (Sf = 0, (1)), and optimising the factor in the simulation. In simulation step 3 for the RA experiment, N content of the labile fraction was fixed to 29.0, the value obtained in all previous simulations, in order to limit the number of optimised parameters.

Agreement with simulations and experimental observations was evaluated using the coefficient of variation of the root mean square error [34]:

$$\mathrm{CV(RMSE)} = \frac{1}{\overline{y}}\sqrt{\frac{\sum (y_i - \hat{y}_i)^2}{n}}, \quad (8)$$

and values <0.05 were defined to represent satisfactory model performance. Sensitivity of the model outputs was then studied by varying the values of input parameters and analysing the subsequent changes in the model outputs.

After finding the parameter values which allowed satisfactory simulation of the experimental observations, a fourth simulation step was performed to study the impacts of isotopic heterogeneity of decomposing residue on the N uptake estimates by the N recipient plant. Nitrogen uptake from the residue was estimated according to (5), using two options for δ^{15}N of N derived from the residue: (i) total δ^{15}N of the residue at the time of residue application, which corresponds to the assumption of a homogenous residue over time (uptake denoted $\%Ndfr_R$), and (ii) simulated δ^{15}N of N derived from residue ($\%Ndfr_S$), which can vary over time if residue fractions are differently enriched with ^{15}N and decompose at different rates.

The model was built using the Simile software, version 4.9 (Simulistics Ltd., Edinburgh, UK). Optimal fit was searched by using the PEST software (Model-Independent Parameter Estimation and Uncertainty Analysis, version 11.8; Watermark Numerical Computing, Australia). For detailed description of the software and the optimisation method, see the PEST manual [35].

3. Results

3.1. Experimental Data of the RA Experiment. Fine root and nodule residue of *G. sepium* applied in the grass pots had C : N ratio of 14.5 ± 0.6, N content of 97.1 ± 2.0 mg pot^{-1}, N concentration of 3.1 ± 0.1%, and δ^{15}N of 180 ± 15.

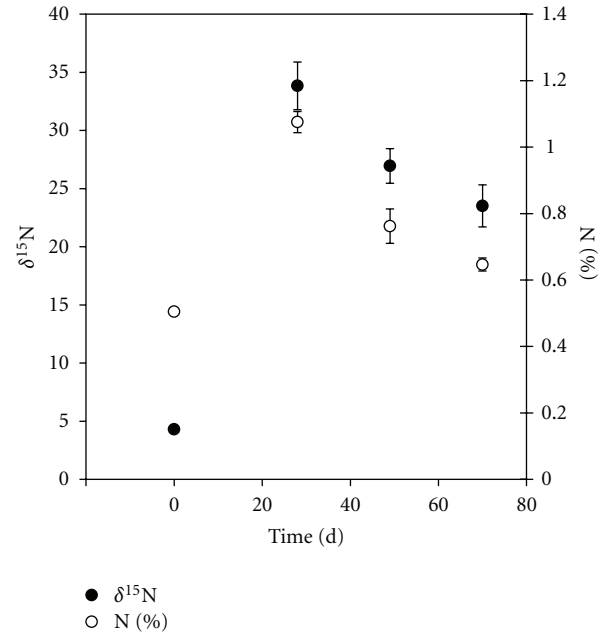

Figure 2: Observed change of δ^{15}N and N concentration in grass shoots during the RA experiment. Vertical bars indicate the standard error of mean ($n = 8$).

Shoot δ^{15}N of the N recipient grass increased rapidly at the beginning of the experiment from 4.3 to 33.8 ± 2.1 at the end of the first study period and decreased thereafter during the last two periods (Figure 2). The coefficient of variation for shoot δ^{15}N varied between 12 and 22% during the experiment. Grass shoot N concentration peaked simultaneously with shoot δ^{15}N (Figure 2) and correlated statistically significantly with it ($r = 0.38$, $P < 0.001$; $n = 32$). The values of δ^{15}N of shoot subsamples did not differ statistically significantly from δ^{15}N of total shoot biomass at the end of the experiment (23.5 ± 1.8 versus 21.7 ± 1.9, resp.). Value of δ^{15}N of coarse roots of *G. sepium* at the time of root harvest and residue application was 499 ± 38.

3.2. Simulations. Simulations with a single residue fraction resulted in low δ^{15}N values for the N recipient plant in both experimental datasets (Sf > 0, Figures 3(a), and 3(b)). Neither the trends nor the ranges of plant shoot δ^{15}N corresponded to the observations. When the residues were divided into two fractions with different C : N but equal δ^{15}N, a decreasing trend of shoot δ^{15}N after an initial peak

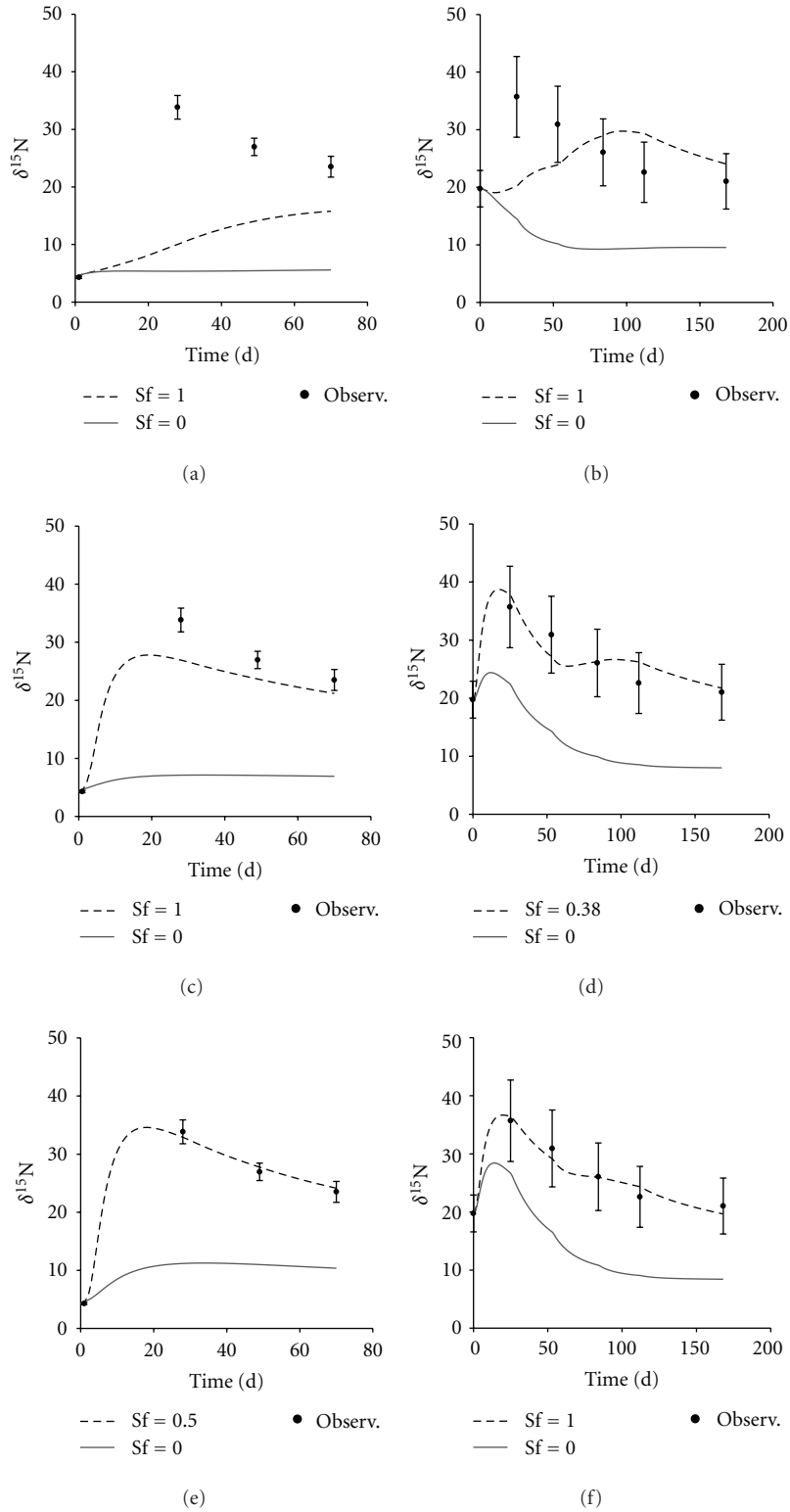

Figure 3: Simulated change of plant δ^{15}N after input of ^{15}N-labelled decomposing residue in the residue application (RA; a, c, e) and full interaction experiments (FI; b, d, f), when the residue is considered to consist of (a, b) a single fraction with uniform C : N and δ^{15}N, (c, d) two fractions with different C : N but uniform δ^{15}N, and (e, f) two fractions with different C : N and δ^{15}N. Simulations were conducted with proportionally equal N uptake from soil inorganic N sources (Sf = 0) and by optimising the N source factor in the simulations. Vertical bars indicate the standard error of mean for the observations ($n = 8$ and $n = 4$ for the RA and FI experiments, resp.). For explanations and parameter values, see text and Table 2.

Table 3: Simulation steps, optimal parameter values and performance of the residue decomposition model. Values indicated in bold were obtained through model optimisation; other values were fixed in the respective simulation steps. Residue parameter values are for labile fraction, except for the final simulation step for which values are given for both labile and stable fraction. For CV (RMSE), asterisk (∗) indicates statistically significant fit between observed and simulated δ^{15}N values of the N recipient plant (<0.05).

Step	Fraction	Characteristics				CV (RMSE)
		C : N	N (mg)	δ^{15}N (‰)	N source factor (Sf)[a]	
Residue application experiment (RA)						
(i) Single residue fraction	labile	14.5	97	180	0.0	0.820
	labile	14.5	97	180	**1.0**	0.579
(ii) Two fractions with equal δ^{15}N	labile	**3.0**	**29**	180	0.0	0.764
	labile	**3.0**	**29**	180	**1.0**	0.164
(iii) Two fractions with different δ^{15}N	labile	**3.0**	**29**	**600**	0.0	0.630
	labile	**3.3**	29	**273**	**0.50**	0.029*
	stable	**19.3**	68	**140**		
Full interaction experiment (FI)						
(i) Single residue fraction	labile	30.2	1332	318	0.0	0.553
	labile	30.2	1332	318	**1.0**	0.454
(ii) Two fractions with equal δ^{15}N	labile	**3.0**	**400**	318	0.0	0.538
	labile	**3.4**	**41**	318	**0.38**	0.093
(iii) Two fractions with different δ^{15}N	labile	**3.0**	**400**	**495**	0.0	0.482
	labile	**3.3**	**66**	**167**	**1.0**	0.047*
	stable	**31.6**	**1266**	**330**		

[a] 0 corresponds to proportionally equal uptake from the two soil inorganic N pools, and 1 to preferential uptake from N mineralised from residue over soil native N (1).

was obtained for both datasets, as also observed in the experimental studies. However, simulations deviated too much from the observations for satisfactory results (Figures 3(c), and 3(d); Table 3). Best agreement with observations for both datasets was reached when δ^{15}N of the two residue fractions were allowed to differ (Sf > 0, Figures 3(e), and 3(f); Table 3). Characteristics of the residue fractions in those simulations are given in Table 3.

When N uptake was considered proportional to the size of each soil inorganic N pool (i.e., Sf = 0), plant shoot δ^{15}N remained low in all simulations for both experimental datasets, regardless of the number of residue fractions and their δ^{15}N values. When the N source factor was optimised in the simulations, best fit for both datasets was obtained when proportionally more N was absorbed from the inorganic N pool originating from residue, in comparison to soil native inorganic N (Sf > 0; Table 3).

According to the simulations, 10.3% of N in the N recipient plant originated from the residue at the end of the RA experiment (Figure 4(a)). This corresponded to 25 mg of N and 26% of initial residue N. Nitrogen uptake estimates calculated assuming homogenous residue (%Ndfr$_R$) differed only slightly from the simulated N uptake (%Ndfr$_S$), except for the first week after residue application (Figure 4(a)). In the FI experiment N originating from decomposing residue constituted 8.1% of N of the recipient plant 25 days after tree pruning (Figure 4(b)), corresponding to 12 mg and 3% of initial residue N. Nitrogen originating from residue rapidly diluted in the plant biomass thereafter as a result of frequent shoot harvests. Nitrogen uptake estimates calculated assuming homogenous residue were 40–51% lower than simulated N uptake during the first 10 days, and 36–39% lower during the rest of the experiment, with the difference slowly decreasing with time (Figure 4(b)).

The model showed a short period of net N immobilisation between days 6 and 16 in the RA experiment. At the end of the simulations, all residue N would have decomposed, and 40% and 34% of it remained in soil microbial biomass and humified organic matter, respectively (Figure 5(a)). In the FI experiment the simulations indicated net N immobilisation from day 0 to day 47. Mineralisation of SOM exceeded immobilisation from day 12 onwards. At the end of the experiment all initial residue N would have decomposed, and 17% of it remained in soil microbial biomass and 76% in humified organic matter (Figure 5(b)). Values presented in Figure 5 correspond to total N content of each pool and include N immobilisation from N_{SN} in soil microbial biomass and humified organic matter.

The sensitivity analyses for the RA experiment indicated that model output (δ^{15}N of the N recipient plant) was sensitive to C : N ratio of the decomposing microbial biomass (R_B), especially during the initial phase of residue decomposition, and to N uptake rate of the recipient plant (u), especially towards the end of the experiment (Figures 6(b), and 6(d)). The model output was negligibly affected by changes in the mineralisation rate constant of soil organic matter (m_{SO}; data not shown), decomposition rate constant of residue (k), and N source factor (Sf; Figures 6(a), and 6(c))

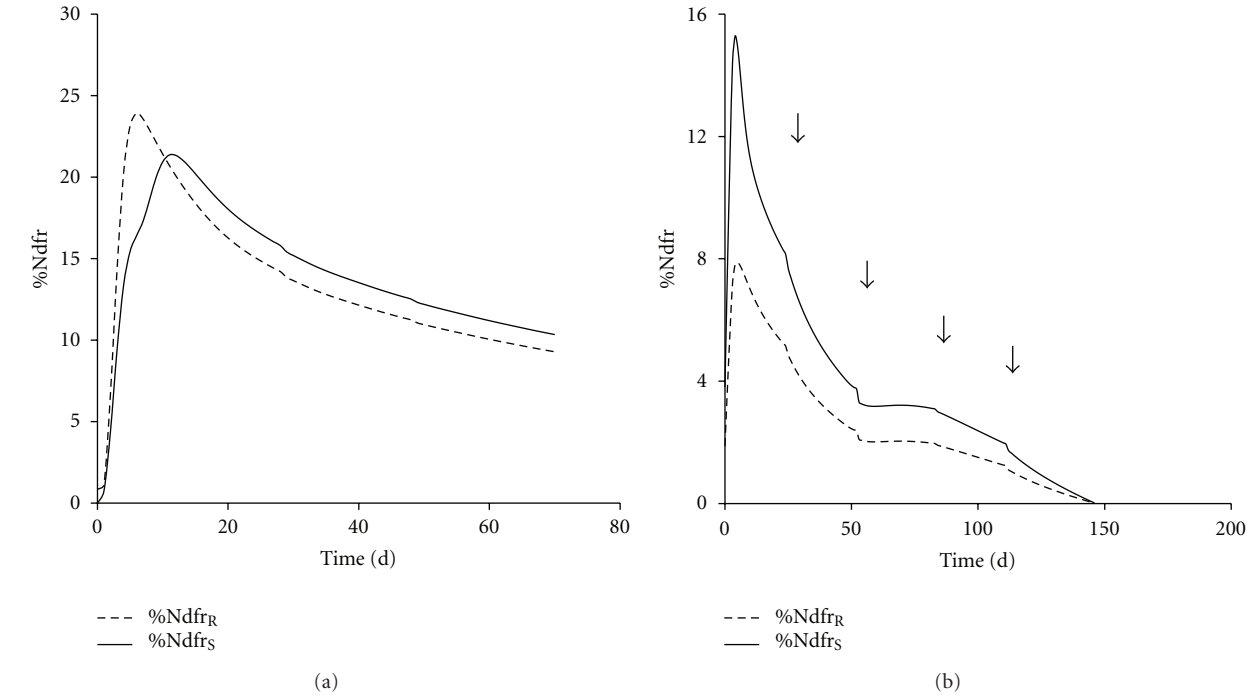

Figure 4: Effect of δ^{15}N of residue on N uptake estimates (proportion of plant N derived from residue) in (a) the residue application experiment (RA), and (b) the full interaction experiment (FI). %Ndfr$_R$: calculated with total initial δ^{15}N of residue, and %Ndfr$_S$: calculated with simulated δ^{15}N of N released from residue (%Ndfr$_S$). Arrows indicate shoot harvesting in the FI experiment.

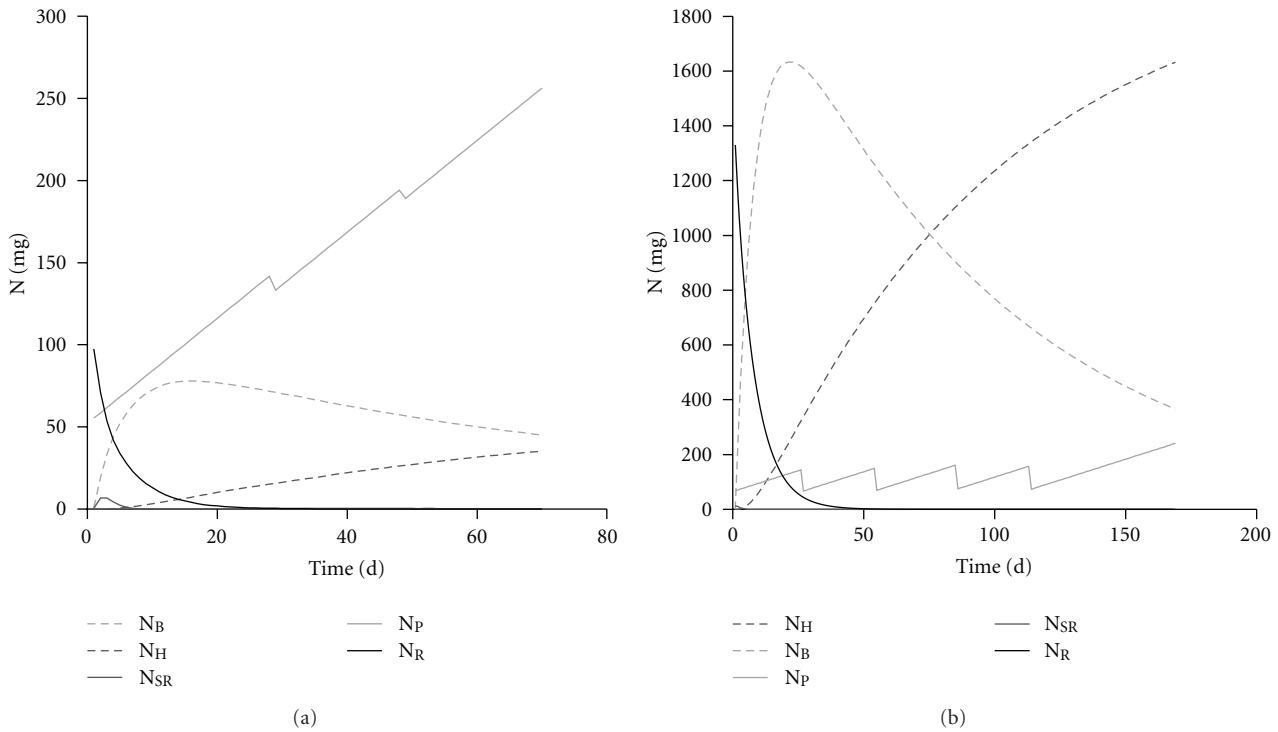

Figure 5: Simulated evolution of the N pools in (a) the residue application experiment (RA), and (b) the full interaction experiment (FI). Note that inorganic N from the residue (N_{SR}) remains close to zero for most of the time in both experiments as it is quickly absorbed by plant or microbial biomass.

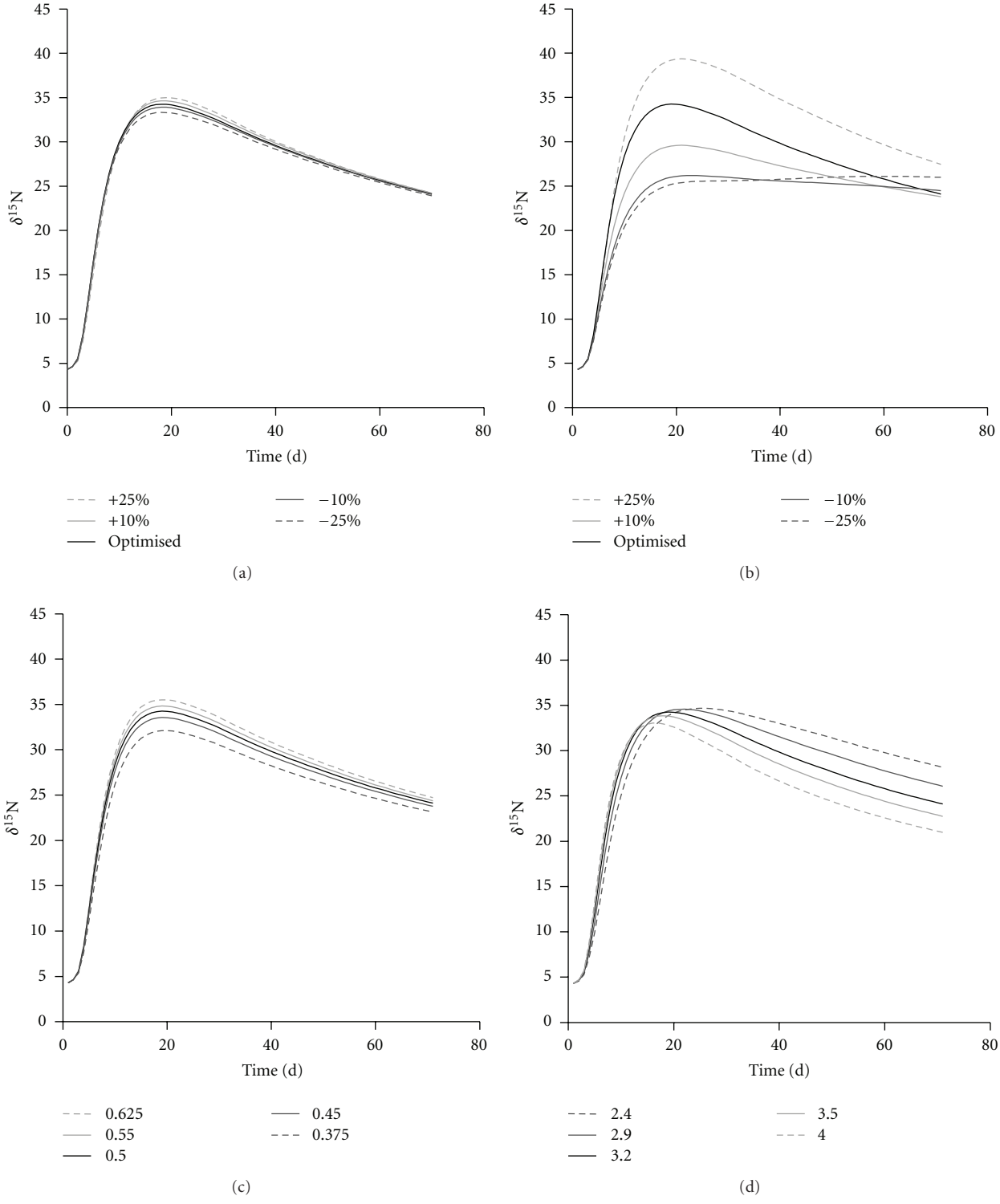

Figure 6: Sensitivity analysis for the residue application experiment (RA). Change in δ^{15}N of the N recipient plant with the change of (a) residue decomposition rate constant, k, (b) C : N of microbial biomass involved in residue decomposition, R_B, (c) N source factor for the N recipient plant, Sf, and (d) N uptake rate by the N recipient plant. The actual values in time were varied between −25% and +25% of the values with which the model was optimised. Exact values are given for those parameters which have a fixed value in the model (c, d).

within the tested range of values. Relative values of k for the two residue fractions were retained the same in the simulations, that is, δ^{15}N of N released from the residue was not altered. The model was very sensitive to changes in Sf values between 0.0 and approximately 0.3 (data not shown) but was little affected by changes at higher Sf values because of the rapid decomposition and exhaustion of N from the labile residue fraction.

4. Discussion

4.1. Residue Characteristics. We observed a rapid initial peak and a slow subsequent decrease in the isotopic composition of the N recipient plant after incorporation of organic residues in the RA experiment, similar to that obtained in other studies applying both ^{15}N natural abundance and ^{15}N enrichment methods [4–7, 14]. The simulation results show that the isotopic patterns of the N recipient plants may be explained by the combined effects of a rapid initial release of N from a labile residue fraction, and its differential isotopic composition with regard to total residue. It is noteworthy that the temporal pattern of the ^{15}N content of the recipient plant was not affected by the fact that in the RA experiment the tree roots were harvested for residue application 3 weeks after ^{15}N labelling, whereas in the FI experiment tree pruning which induced root decomposition occurred 12 weeks after labelling. Moreover, the fact that similar trends in the isotopic composition of the N recipient plant were observed in studies applying ^{15}N natural abundance [6, 7] indicates that the observed trends cannot be explained by the method of ^{15}N labelling alone.

The results are in line with the observations of Sierra et al. [6] who showed that δ^{15}N of N released from decomposing roots may not correspond to that measured from the living roots just before pruning. By fractioning ^{15}N-enriched residue into labile and stable components, Hadas et al. [14] managed to simulate fairly well the isotopic composition of the N recipient plant, except for its simulated initial peak soon after residue application. They measured isotopic composition of the recipient plant first time 47 days after residue application. This may have concealed the role of isotopic heterogeneity of the residue fractions, which became evident in the RA experiment involving earlier measurements. Recently, it was shown that nitrate content and isotopic fractionation during metabolic processes explain variation in δ^{15}N of nitrogenous compounds in rapeseed leaves (*Brassica napus* L.), where nitrate and amino acids were considerably more enriched than soluble proteins [9]. While fractionation is not of relevance in ^{15}N labelling studies, the results demonstrate that N allocation and plant metabolic processes are capable of considerably altering δ^{15}N of nitrogenous compounds and that of residue fractions as a consequence.

The fact that satisfactory simulation of the observations for both experimental datasets in this study was reached with low C : N values of the labile fraction of 3.3 suggests that the fraction consisted of water-soluble components, for example amino acids and possibly inorganic N [31, 32]. Simulated proportion of labile N was much smaller in the FI experiment than in the RA experiment because residue characteristics in the FI experiment corresponded to those of total roots instead of fine roots only. Total N concentration of the root residue in the FI experiment also was approximately a third lower than in the RA experiment (data not shown).

Labile residue fraction appeared more enriched than the stable fraction in the RA experiment, but the opposite was true for the FI experiment in which ^{15}N labelling of the trees took place much earlier. The ^{15}N label in plants may initially occur as metabolically active N or be stored predominantly in coarse roots. In contrast, it can be assumed that N bound in structural components during their formation is generally not replaced over their life time. Therefore, labelling would not affect the isotopic composition of these components, if they were formed before ^{15}N labelling, as presumably was the case for majority of the fine roots of *G. sepium* in the RA experiment. Relative ^{15}N enrichment of root fractions in the RA experiment (coarse roots > labile fraction of fine roots > stable fraction of fine roots) is in line with these assumptions about the fate of applied ^{15}N within the plant. Relative ^{15}N enrichment of the fractions can be assumed to change over time, as metabolically active and stored ^{15}N are converted to structural components during biomass growth. Differential ^{15}N enrichment in the FI experiment may also partly be explained by the fact that stable fraction corresponded to both the structural components of the fine roots, and coarse roots as a whole, including stored ^{15}N. Method of ^{15}N labelling may importantly affect the relative ^{15}N enrichment of residue fractions [15]. An early start of ^{15}N labelling would help to enrich structural components of the plant more evenly, but may in turn disturb the system more through repeated interventions and need of higher ^{15}N inputs.

4.2. Nitrogen Availability from the Residue to the Associated Plant. The observed correlation between shoot N concentration and δ^{15}N of the N recipient grass after the incorporation of ^{15}N-labelled residue in the RA experiment indicates that grass obtained proportionally more N from decomposing residue than from soil native N pools. This was supported by the results of the modelling study, where proportionally higher N uptake of N mineralised from residue compared to soil native N was necessary for obtaining a satisfactory agreement with the experimental observations (Figure 3). Residue application appeared, therefore, to enhance grass N nutrition instead of merely substituting soil native N as N source (cf. [36]).

Plant N uptake is affected by the availability of N in the proximity of roots [37]. The physicochemical properties of the very clayey soil in the experimental studies restrict solute flow [38, 39]. Therefore, it can be assumed that spots of high N concentration emerged in soil as a result of root decay, and that the N recipient plant absorbed proportionally more N from these spots. Such spots are also more porous as the surrounding soil, which may have facilitated colonisation by grass roots and subsequent uptake of N originating from residue [40, 41]. Soil in the holes where the residue in the RA experiment was applied was more loosely packed than the elsewhere in the pots, which may have simulated

the effect of increased soil porosity. Small diameter of the holes, and the fact that grass roots had already effectively colonised the pots, likely contributed to the rapid capture of N from the residue. In the FI experiment roots of *G. sepium* and *D. aristatum* were mixed together in the pots, and N release from decomposing root residue could be assumed to have been spatially more homogenous than in the RA experiment. Intraspecific competition for soil N could explain why according to the simulations grass would have absorbed proportionally more N originating from residue than in the RA experiment. Grasses are generally more effective competitors for soil N than legumes [42]. Although heterogenous distribution of residue influences N uptake from decomposing roots also in natural systems [41], it was probably more pronounced in the experimental datasets because of experimental factors. The differences in N uptake between the two sources do not, however, affect the results about residue fractionation and distinctive ^{15}N enrichment of labile and stable residue fractions discussed above (cf. Figures 3(e), and 3(f)).

According to the simulations, the N recipient plant had obtained 26% of initial residue N at the end of the RA experiment, and 3% of initial residue N during the first 25 days of the FI experiment. These were on the lower side of the values measured in previous studies, which range from 10 to 100% of total residue N taken up by the associated plants [43–46]. Variation in the observations may result for example from residue type and method of application, biological or environmental conditions facilitating or restricting decomposition, capacity of the soil to sequester organic inputs, synchrony with the nutrient needs of the recipient plant, methods for estimating N uptake, and the length of the observation period. Nitrogen from below-ground residues appears to decompose initially faster than that of above-ground residues, and more of it is also taken up by subsequent crops [12, 43, 47]. Very little research, however, has focused on recycling of plant below-ground N, especially in tropical agroecosystems.

4.3. Implications of the Findings to N Cycling Estimates. Quantification of N cycling in agroforestry systems using isotope techniques crucially depends on the determination of the isotopic signature of the N sources. We used simulation methods to evaluate how the common assumption of homogenous isotopic composition and decomposition rates of organic N sources affects estimates of N recycling from decomposing residue, when the residue fractions actually differ in both parameters. Accuracy of the estimates depends on the quality of the residue, relative sizes, and ^{15}N enrichment of its fractions. Nitrogen recycling estimates may not be largely affected by isotopic heterogeneity when only relatively high-quality residues (e.g. fine roots of legume trees) are concerned, which decompose entirely over a short time and N originating from the residue fractions become effectively mixed in the system. In contrast, substantial errors in N recycling estimates can be obtained for lower-quality residues with large, differentially ^{15}N-enriched stable fractions, if isotopic composition of released N is assumed constant. Role of the residues in crop N nutrition in such cases may be underestimated as was the case for the FI experiment where the labile residue fraction was less enriched with ^{15}N than the stable fraction. Depending on the timing or method of ^{15}N labelling, N recycling might also be overestimated if labile residue fraction is more enriched with ^{15}N than the stable fraction (cf. the RA experiment).

While homogenous ^{15}N labelling of the N source is commonly assumed in ^{15}N enrichment studies, it is generally difficult to achieve over time. Assimilation of N from soil and its allocation within the plant during growth, and in legumes also N_2-fixation, result in dilution of the label at different rates for different organs [16, 48]. Moreover, in ^{15}N natural abundance studies the isotopic composition of the N sources cannot be controlled [9]. Analysis of temporal isotopic variation of the N sources and reasons affecting it could provide an alternative or complementary method aiming at, or simply assuming, homogenous isotopic composition in ^{15}N tracer studies. Measuring δ^{15}N and N content of labile and stable residue fractions separately can be a useful first step in evaluating whether isotopic heterogeneity is likely to result in too small or large estimates for N recycling from organic sources in the concerned system. Water-soluble N may be a good approximation for the labile fraction for this purpose (cf. [2, 31]).

If no information of δ^{15}N of residue fractions in ^{15}N enrichment studies is available, caution should be exerted in estimating N recycling and uptake from the δ^{15}N values of the recipient pools in the short term. This is especially the case after discrete events in time such as pruning or green manure application, or under pronounced seasonality, if there are large inputs of residue from senescing biomass over a short-time period. Previous studies indicate that these considerations are important also for studies using the ^{15}N natural abundance method [4, 6, 7, 9], and the results explained here reveal that the role of residue fractionation in explaining isotopic patterns in ^{15}N natural abundance studies merits research.

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