Dear Dr. DeAngelis,

We appreciate this opportunity to revise our paper and we are glad that you appreciate its value to the Ecosystem audience. We believe that the paper has been much improved in response to your suggestions and those of the reviewers. You will find our responses below, in blue, and our changed text is reported in green. Three sections of the paper that were substantially revised are included here in full, namely the first paragraph of the introduction and the sections on selecting and applying error terms.

SUBJECT-MATTER EDITOR'S COMMENTS
Subject-Matter Editor: DeAngelis, Donald
Comments to the Author:
This paper presents a simple approach to estimating the error in calculating the N content of vegetation. The goal of the authors is to make the calculation of error “less daunting for ecosystem ecologists”. The authors use a Monte Carlo approach in this relatively brief paper.

The reviewers differed somewhat in their assessments of the quality of this paper, one giving it 3 excellents and 4 goods, while the other gave it 3 goods, 3 fairs, and 1 poor. The second reviewer notes that the methodology described here is not new, but rather well known. That reviewer also criticizes the paper for being parochial in the work it cites; especially not citing work outside of the US. The parochial nature of the paper is what Reviewer 1 focuses on, as well. In that reviewer’s opinion, the paper is oriented too much around Hubbard Brook, not just in the example calculations, but throughout the paper.

I agree with both reviewers. The topic of the paper is estimating uncertainty in ecosystem budget calculations, and should be of general use for all ecosystems, at least all forest ecosystems. While it is perfectly appropriate to use one specific ecosystem as an example for demonstrating the calculations, it is disconcerting to have that one ecosystem repeatedly mentioned from the beginning of the abstract, throughout the paper. Also, while I appreciate that the authors intend this paper to be brief and to the point, the paper would be better suited for a broad audience if the citations were more representative of the many scientists world-wide who are concerned with estimating forest ecosystem budgets.

Both reviewers note that the science here is not new. Reviewer 2 wonders about its appropriateness for the journal Ecosystems. On the other hand, Reviewer 1 feels that this is an important topic and that the paper can be important in getting uncertainty estimates made more often. I tend to agree with this latter point, but I believe that the paper needs more work to make it better balanced. I don’t think that the authors should try to expand this to cover many sites besides Hubbard Brook. It’s brevity is a strong point. However, the citations should be expanded and the Introductory sections should be revised to downplay its Hubbard Brook orientation.

The introduction has been revised to better explain the problems associated with uncertainty estimates in ecosystems. The examples of budgets in which we have been involved have been removed (e.g., Friedland et al. 1991,Yanai 1992, Likens et al. 1998, Johnson et al. 2008). We had cited only our own work because we did not want to accuse other authors of failing to account for uncertainty in their budgets (as we have). There may be many scientists world-wide constructing forest ecosystem budgets, but very few (we found only one) have propagated the uncertainty in their biomass estimates when calculating nutrient pools. This point is made more clearly in the revised Introduction (copied below under Reviewer 2's first comment). The first mention of Hubbard Brook is now in the description of the objectives, where we take it as our demonstration example.

I would like to see a bit of discussion concerning what concepts underlie the difference in the approach to calculating the error of the mean by the authors and the alternative method by Chave et al. (2006) (lines 234-237).

We decided to use a more exact error term, since this is the model our readers should follow if they can. We improved the section called "Representing Model Error", which is now called "Advice on Selecting Error Terms." It includes both the error term we used (Equation 5) and the approximation used by Chave's group (Equation 4), which we have also used in the past.

REVIEWERS' COMMENTS
Reviewer: 1
Comments to the Author(s)
General Comments

This manuscript covers a very important topic in any ecosystem analysis that requires the synthesis of various empirical models and data. The authors are correct in pointing out that the time has come for these estimates to be accompanied by estimates of uncertainty. This is not only necessary to provide some basis for comparison, but also to allow one to have some sense of how reliable the synthesis was.

While I support the idea of a paper such as this being published, I found the current manuscript fluctuates from general to the specific in a way that is a bit confusing. The introduction would best be delivered at the general level, yet there is a great deal of specific information about the Hubbard Brook data sets and models. While examples help, this example is covered in great detail later, so it probably should not appear in the introduction other than to point out it is used as an example. There is also a problem when the Monte Carlo method is presented on pages 5-6. It starts very general, but then gets into the specifics of the Hubbard Brook analysis before the case study is presented. Again it would be best to stick to general issues and concepts until the actual example is given. If the materials were sorted into general versus case study items it would be far easier to follow and repetition of material would be reduced.

Specific Comments(page/line)

2/36 I believe it should be standard operating procedure. Otherwise it is impossible to just how well the budget synthesis worked.

The reviewer is commenting on the concluding sentence of the Abstract: "The analysis of uncertainty in complex calculations does not have to be difficult and should be attempted more often."
Yes, the purpose of this paper is to help change the culture of ecosystem science. We believe that it should be unacceptable to publish an ecosystem budget without uncertainty estimates. The revised sentence reads, "The analysis of uncertainty in complex calculations does not have to be difficult and should be standard practice in constructing ecosystem budgets."

3/44 to 63 This takes a very Hubbard Brook view of the world in the introduction. This needs to be more general in nature. As written this should largely be covered under the specific case study.

The first two paragraphs of the Introduction have been replaced, in part to better describe the multiple sources of variation addressed in our analysis and to better point out the sources commonly overlooked (this text is provided under Reviewer 2's first comment). Hubbard Brook is now introduced only when we come to the objectives and our case study.

4/69 since CV is expressed as a percent, I am not sure 0.3% CV is a lot of variation.

In this case, we did not use %, so 0.3 = 30%. We changed "0.3" to "30%" here to be consistent with our reporting of CVs in the rest of the paper.

4/82 Harmon et al 2004 Ecosystems 7:498-512 also did this

We said "Harmon et al. (2007) applied Monte Carlo simulations to calculate uncertainty in estimates of net primary production for several ecosystems." However, closer inspection of the 2004 paper and consultation with Mark Harmon reveals that parameter uncertainty was not included in their treatment of the Wind River study. Only the oak savannah study reported in the Harmon et al. 2007 chapter did this. The sentence now reads, "Monte Carlo propagation of uncertainty in tree biomass equations has been applied to tropical forests (Chave et al. 2004), temperate hardwood forests (Fahey et al. 2005), temperate conifer plantations (Sicard et al. 2006) and oak woodlands (Harmon et al. 2007)." We decided not to cite any studies that have used Monte Carlo for other purposes.

9/177 is the error presented as a percent of the mean? Or as a fraction of the total? I think the former, but this should be made completely clear.

We said, "expressed as the standard deviation divided by the mean (the coefficient of variation)" and the values are given in percent, which is common for a CV, as pointed out by the reviewer, above. There should not be any possible confusion now, as we replaced our earlier use of a fractional a CV with a percent (described above).

9/193 and it would be even higher if one had to use equations developed at other sites, which is commonly done by most making biomass estimates. One has to address the issue of which of the various equations is "correct".

That is an important point, relevant to the section headed "Sources of Error Not Included in this Example." We added this sentence: "Regression equations are commonly used at sites other than those at which they were developed, which introduces error not described by the uncertainty in the regression model (Harmon et al. 2007)."

The sentence was: "Equations from other sources would likely contribute more uncertainty to the N content of vegetation." Here, the point we wanted to make was that Whittaker's error is unusually low. We revised the sentence to make this meaning clear. "Most other allometric equations would contribute more uncertainty to the nutrient content of vegetation than our example shows."

Figure 3. I was not sure this was actually needed.

We eliminated this figure, which is a screen shot of the Excel lookup table. It shows that the error terms are sampled independently of the tree inventory. However, we make this point in the text, in the flowchart, and in the excel worksheets.

Reviewer: 2
Comments to the Author(s)
The manuscript presents a Monte Carlo methodology to estimate uncertainty in ecosystem budget calculations. This methodology is commonly known and rather frequently used. The manuscript provides little new on the methodology or its application in ecosystem budget calculations. However, the manuscript gives a rather concrete example and detailed instructions on applying the method in practice. In addition, the authors make a point that estimates of ecosystem budgets are all too rarely accompanied by uncertainty estimates, and they emphasize the importance of such estimates. Their example can encourage other researchers to express error in their results, and it may help in making the error estimates a standard part of results reported in this field of research. I fully agree with the authors on these points and acknowledge the value of demonstrating a clear example. Still, I am not sure if a manuscript like this is suitable for the journal. I think it is up to the Editors to decide.

Generally, the text of the manuscript is inaccurate at places and the manuscript would be more informative if the results were presented in more detail. Some sentences of the text provide very basic level information that is already known by potential readers of the paper. Some of my detailed comments that follow deal with these issues.

Detailed comments

Line 41: It would be clarifying if the authors described sources of uncertainty their analysis covered, e.g. model error, sampling error and measurement error.

This is an excellent suggestion. The initial focus of the paper was on the parameter uncertainties, and the measurement error and interplot variation were added later. Our revision better introduces the multiple sources of variation addressed in our analysis, makes it more clear that a complete uncertainty analysis has not yet been accomplished, and removed the references to our own examples of ecosystem budgets deficient in this respect.

The new Introduction begins with this paragraph, replacing two paragraphs that generated complaints about the undue focus on Hubbard Brook examples.

"There are many sources of uncertainty in nutrient budgets for forested ecosystems. Some sources of uncertainty are well understood and commonly reported, such as the variability reflected in replicate plots. For systems of small stature, such as grasslands or tundra, ecosystem nutrient stocks can be assessed independently on multiple plots, and reporting the variation across plots is sufficient to describe the uncertainty in the estimates. Forest nutrient budgets, however, require the use of allometric equations to estimate the biomass of tree components. The uncertainty in these equations should be included in estimates of uncertainty in nutrient budgets, along with the uncertainty in nutrient concentrations of tissues and the measurement and sampling error. To our knowledge, the uncertainty in all these components has never been propagated through a calculation of nutrient contents of a forest."

We also added this sentence to the Objectives: "We include measurement uncertainty and inter-plot variation to demonstrate how they should be represented and to allow all these sources of uncertainty to be compared."

L 26 to 28: It remains unclear what the percentages refer to.

The reviewer noted a problem with the presentation of our results in the Abstract. When we said, "The root mean squared error in the allometric equations contributed 3% uncertainty in the aboveground biomass of trees," this could have meant 3% of the total uncertainty. We added an explicit definition of our units for uncertainty, consistent with our definition in the results section. "The total N content of trees was estimated at 847 kg ha^{-1} with an uncertainty of 8%, expressed as the standard deviation divided by the mean (the coefficient of variation)." The remaining sentences are consistent with this reading.

L 44 to 47: "Uptake of a nutrient" is not a sum of the three factors but a sum of the nutrient mass in the factors.

This sentence was improved but then removed in the revision of the Introduction. "Calculating the uncertainty in nutrient uptake is even more daunting, as it requires error propagation though many fluxes, including leaf production, root turnover, leaching losses, and perennial tissue production."

L 64: I suggest adding "in principle" after "any calculation".

Good suggestion. We put this modifier at the beginning of the sentence to give it more emphasis. The revised sentence is, "In principle, the uncertainty associated with any calculation can be derived analytically from the reported uncertainty in the components (Taylor 1996, Lo 2005)." This is an important improvement because we argue that the analytical approach cannot help us "in practice"—see the next sentence with the next suggestion, below.

L 67: I suggest adding "in practice" to the sentence.

The revised sentence is "In practice, however, analytical error propagation is problematic in situations where the calculations are difficult to represent mathematically or the coefficients of variation are high (>30%) (Harmon et al. 2007).

L 68: I do not understand why a high degree of variation makes the analytical calculations impossible. This requires explanation.

We added this sentence: "Gaussian error propagation uses partial derivatives to estimate errors associated with changes in parameters, but the slope is an inaccurate approximation of a non-linear effect especially if the uncertainties are large."

L 77 to 82: As far as I see, only references to the work of US scientists given. Relevant work has been carried out also by Finnish researchers Peltoniemi et al. and Monni et al.. I suggest referring also to work carried out on other continents.

Chave is French and his work was conducted in the neotropics. We found another paper by French researchers conducted in France, which is very relevant because they used Monte Carlo to propagate uncertainty in their allometric estimates of forest nutrient content. We added this reference: "Monte Carlo propagation of uncertainty in tree biomass equations has been applied to tropical forests (Chave et al. 2004), temperate hardwood forests (Fahey et al. 2005), temperate conifer plantations (Sicard et al. 2006), and oak woodlands (Harmon et al. 2007)." We did not cite the work by Peltoniemi, Monni, and colleagues because although they use Monte Carlo approaches in estimates involving forest ecosystems, they do not propagate uncertainty in tree allometry within an ecosystem. For the same reason, we also cut this reference, which helps reduce the number of references pertaining to North America: "For example, ecosystem carbon balances characterized using the eddy covariance method have used Monte Carlo approaches to quantify data uncertainties (Richardson and Hollinger 2007)."

L 93 to 110: I suggest adding reference to some basic literature on the Monte Carlo method.

We added a reference to Press et al. (1986) in the paragraph describing the Monte Carlo approach.

L 121 to 122 and 126 to 127: The same sentence appears twice in the same paragraph.

Thank you for pointing this out. We cut the first sentence: "All residual errors are assumed to be normally distributed with a mean of zero and standard deviations σ_{Hi}, σ_{Bi}, and σ_{Ni}."

Was the assumption on the normal distribution critical to the results?

We did not test any other distributions. We added a phrase to this paragraph to better justify our use of the normal distribution. "The propagation of error does not require that the variables be normally distributed. We used a normal distribution of error in this illustration, consistent with the assumptions of Whittaker's regression models (Whittaker et al. 1974). If a distribution is known to be non-normal, the actual distribution should be used in the random resampling procedure."

L 143: Sampling error must have been dependent on the number of plots. I think this should be accounted for when presenting the results.

The number of plots is accounted for in the uncertainty calculation, obviously. Perhaps the reviewer is suggesting that we vary the number of plots to illustrate the importance of this variable? See below.

L 181 to 183: Why is the sum of the individual error sources greater than the uncertainty with all errors combined?

We provided an explanation, which we think other readers will also find useful. "The sum of the individual sources of error, reported in units of coefficient of variation or kg N/ha (Table 1), is much greater than the uncertainty from the Monte Carlo simulations with all errors combined. The variance of a sum is the sum of the variances (Taylor 1996); the sum of standard deviations is not meaningful. In this case, squaring the standard deviations of the errors of individual sources and summing them approximates the square root of the standard deviation of the errors of combined sources."

L 184 to 185: I think it would be very informative to show the effect of increasing the number of plots.

That's a great idea. We did that, and added this sentence to the paper: "We investigated the effect of sampling intensity on uncertainty by selecting different numbers of plots (Table 1). With only 5 plots, the uncertainty in N contents of the ecosystem was 15%. With 30 to 60 plots, it was 7%. Adding more plots cannot reduce the uncertainty below that contributed by the other sources, which was 7%. Plot sampling error alone is only 3% with 60 plots." The results are also presented in the new Table 1, described below.

L 194 to 210: I suggest adding a table or a figure of the contribution of the various error sources to the total uncertainty. Then, it would be much easier to compare the contribution of the different error sources.

We added Table 1, which adds detail to the results, because it includes the contributions of error to uncertainty in each of the tissue types. Thank you for the suggestion.

L 203 to 204: I do not understand the statement. If the biomass equations give uncertain biomass estimates for each site, I think it makes it difficult to observe a difference between sites.

This is a very important question. We had said, "The uncertainty in the biomass equations might not be very important when comparing ecosystems, as the errors would apply equally to multiple sites." We recognize that this is not an easy concept. We created a new section to better address the issue of common vs. independent error sampling, and eliminated the paragraph that contained this sentence. (previously at the end of the results of the N budget calculations). The new section is called "Advice on Applying Error Terms." It follows the section on selecting error terms.

"As a general rule, errors should be generated to simulate the measurement and analytical procedures. In this sense, every Monte Carlo iteration is a like a resampling of the study. For example, measurement uncertainty applies independently for each measurement. In our case study, we randomly sampled the measurement uncertainty in DBH for every tree in our sample. The errors are as likely to be positive as negative, and they tend to cancel out. In contrast, at each iteration we simulated a single set of allometric equations and a single set of N-concentration parameters and applied them to all the trees to estimate N_T.

"It is possible to select the right form of error but to apply it incorrectly. A common mistake is to apply parameter uncertainty independently for each observation in the data set. To calculate uncertainty in the ecosystem total, we are interested not in the variation from tree to tree, but the possible inaccuracy of the equation describing the average tree. For example, consider the equation for the mass of the branches of a sugar maple tree, which has high uncertainty. If this equation is inaccurate, this error applies equally to every sugar maple tree in the sample. For this reason, we sample the error terms in the table of parameters in Excel, not in the list of trees. Each tree is calculated with the same sample of the error term (or sample of the parameter, in the case of nitrogen concentration), until the next iteration of the Monte Carlo.

"The same argument applies when comparing ecosystem totals across multiple plots or sites. It is important to apply the parameter uncertainty simultaneously for all observations at each iteration of the Monte Carlo. Using the same example as before, if Whittaker's equation is in error about the branches of sugar maple trees, it is equally so for all the trees in the population. This source of error does not contribute as much to the uncertainty in detecting differences between plots or sites as it does to the uncertainty in the mean.

"Our case study illustrates the sampling of multiple plots. We used the same parameters, sampled with error, at each iteration of the Monte Carlo, and at each iteration we estimated the ecosystem total as a random sample based on the mean and standard error of the plot totals. To compare two sites each with multiple plots (not illustrated in this paper), we would compute the t statistic for the site difference at each iteration of the Monte Carlo, and report the proportion of all iterations with a significant t. To compare more than two sites, the proportion of significant results of analysis of variance would be reported for many iterations. A confidence of 95% in the difference across sites would be indicated if more than 95% of the Monte Carlo iterations produced a significant difference.

"Designing a flowchart (Figure 1) can help to plan the sequence of calculations. Using a programming language would make the structure of the calculations more explicit than in Excel. The implementation of a Monte Carlo calculation is not difficult; conceptualizing the approach to take is more challenging."

L 207 to 210: I do think it would be possible for a reader to calculate the uncertainty based on the information given. In addition, I do not think the last sentence of the paragraph is necessary.

The text in question was this: "The first N budget for Hubbard Brook (Bormann et al. 1977) reported a rate of N fixation of 14.2 kg/ha/yr, which was calculated as the sum of streamwater export and living and dead biomass increments minus atmospheric inputs, assuming no change in the mineral soil. Estimating the uncertainty associated with this 14.2 kg/ha/yr is left as an exercise for the reader." The uncertainty in the measurement of the mineral soil pools is thousands of kg N/ha, but converting this uncertainty into a rate of change is difficult. The last sentence, therefore, was meant to allude to the very large likely uncertainty and also to the fact that the exercise is not trivial. This paragraph has been eliminated, and replaced by a section titled "Advice on Applying Error Terms," which is copied above.

L 216 to 220: I do not think it is necessary to describe the different between std error and std deviation on the pages of this journal.

We removed the following sentences: "It is important to be clear on the difference between the standard deviation and the standard error. The standard deviation is a measure of the variability in the distribution of the sampled population whereas the standard error is a measure of the variability in the distribution of the estimated means of the sampled population (Snedecor and Cochran, 1989). Assuming a normal distribution,

$s_e = \frac{s}{\sqrt{n-1}}$, where s_e is the standard error, s is the sample standard deviation, and n is the number of individuals sampled."

As described above in response to the Associate Editor's questioning our choice of error terms, this section has been revised to better focus on the choice of error terms.

"Selecting the appropriate component error terms for an uncertainty analysis using Monte Carlo simulation depends on the question being asked (Harmon et al. 2007). To describe the variation in the population or the uncertainty in estimates of individuals, the standard deviation is the appropriate term to use. To describe uncertainty in the estimate of the population mean, the standard error of the mean should be used.

"The question we addressed in our example was the uncertainty in the ecosystem total of N in trees, which is the uncertainty in the mean. Therefore, we used the means and standard errors of the N concentrations for the tissues of each species to define the uncertainty in N concentrations (Equation 3). This variation is smaller than the measured variation in tissue concentrations (the standard deviation). This choice is easy to understand in the case of a single parameter.

"For a regression equation, uncertainty is described by the variation around the fitted equation. The standard deviation of the dependent variable based on the regression model, $s_{y\bullet x}$, is calculated as

(4) $$s_{y\bullet x} = \sqrt{\frac{\sum (y_i - \hat{y}_i)^2}{n-2}}$$

where *y* refers to the dependent variable ($\log_{10}$ of tree height or tissue biomass, in our example); y_i is the observed and $\hat{y}_i$ is the predicted value of the i^{th} observation, and *n* is the number of observations used to estimate the regression equation (Snedecor and Cochran 1989 p. 162).

The uncertainty associated with regression predictions also depends on the value of the independent variable *x*, in our case $\log_{10}$ (DBH). The uncertainty described by the standard deviation of the regression, $s_{y\bullet x}$ (Equation 4), describes the error at the mean value of the observations in the regression data set, $\bar{x}$. The uncertainty in predicting *y* increases as values of *x* depart from this mean. Finally, the uncertainty in the regression prediction also depends on the number of observations in the regression data set, *n*. For the error terms in Equations 1 and 2, we used the error appropriate to an estimate of the mean of *y* at a specified value of *x*, s_m (Snedecor and Cochran 1989 p. 164):

(5) $$s_m = s_{y\bullet x}\sqrt{\frac{1}{n} + \frac{(x - \bar{x})^2}{\sum_{i=1}^{n}(x_i - \bar{x})^2}}$$

The uncertainty in predicting the value of *y* for an individual from regression, s_p, is larger, analogous to the standard deviation compared to the standard error (Snedecor and Cochran 1989, p. 166):

(6) $$s_p = s_{y \bullet x} \sqrt{1 + \frac{1}{n} + \frac{(x - \bar{x})^2}{\sum_{i=1}^{n} (x_i - \bar{x})^2}}$$

"The use of Equation 5 or 6 requires the number of observations in the regression, the mean of the *x* observations, and the sum of squared deviations of the *x*, $(x_i - \bar{x})^2$. Because these statistics are not commonly reported, some researchers have chosen to represent the uncertainty in the biomass equations using only the standard deviation of the regression (Equation 4) (Chave et al. 2004, Fahey et al. 2005). This approach results in an overestimate of the uncertainty in the population mean (Equation 5), but an underestimate of the uncertainty in the individual estimates (Equation 6). "

L 246 to 251 and 259 to 260: I think it would be more relevant and interesting if you discussed possible correlations and systematic errors in the variables of this study, at least in addition to the examples you give.

For systematic error, we had: "For example, measurements of precipitation inputs at Hubbard Brook are biased in the spring by pollen inputs to the open-top collectors. Loss of litter mass by decomposition before collection is rarely accounted for in litterfall studies."
Now we say, "For example, we represent minor species using the equations developed for the major species, such as the substitution of sugar maple for red maple (Whittaker et al. 1974)."
For correlations, we retained the example from streams, because we couldn't think of one as good that pertained to our own study. The example for analytical error uses our study: "Analytical procedures are prone to error; there are some values of tissue concentration in the original Hubbard Brook data set (Likens and Bormann 1970) that were not borne out by later measurements (Siccama et al. 1994). Analytical uncertainty is often not reported but is usually small compared to variation across samples (in this case, by tree; Likens and Bormann 1970)."

Thank you again for this opportunity to improve our paper.

Please let me know if you have any questions.

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