Response to reviews of: Hilton, R. G., et al. (2012) Geomorphic control on the ^{15}N of mountain forest, *Biogeosciences Discuss.*, 9, 12593–12626.

Referee comments are provided here in italics with main points raised numbered sequentially, referring to Referee #1 (**R1**) and Referee #2 (**R2**). We refer to the submitted manuscript with page and line numbers as (pgX, ML-X) and to the revised manuscript as (RM).

Anonymous Referee #1 Received and published: 22 October 2012

General comments
This paper deals with the N cycle in terrestrial forest ecosystems, using natural abundance stable nitrogen isotopes to track especially N export from mountainous forests in Taiwan. The paper uses relatively few measurements to inform model calculations that export of leaves can be important in controlling ecosystem N stocks. The leaves have low N isotope values, and export loss of leaves and particulate materials derived from leaves could lead to the observed high N isotope values in residual soil N pools, pools where most ecosystem N resides. The paper is broadly consistent with an early ecosystem N isotope model from the 1970s (Shearer et al. 1974). That model envisions the terrestrial N cycle as turning many times in a largely N-retentive way, with small losses at each turn having 14N-enriched (and 15N depleted) isotopic compositions. Those losses cumulate in higher 15N values observed in soils worldwide. The current study extends this modelling idea to explicitly consider export of particulate materials as the main vector of 14N-enriched export, calling attention to correlations of N isotope values in leaves and soils with slope (Fig. 6), correlations expected when export is strong.

We were pleased that the referee appreciated the novel contribution of our study on loss of particulate N, which is an under constrained aspect of N cycling in mountain ecosystems (see also **R2** opening comments). After finding that δ^{15}N of plants and soils in Taiwan were significantly correlated with slope angle, we sought to explain the isotopic variability by adapting an ecosystem N isotope model (Brenner et al., 2001, similar to Shearer et al., 1974). As the referee highlights, we extend the modelling framework to consider how particulate N export impacts the soil δ^{15}N and show it can explain the variability in our data and the negative correlation between δ^{15}N and slope.

Specific comments: The questions for this manuscript are mostly in the data and concepts
R1.1. *i.e.,* ***first was enough data collected to be convincing****…? The number of samples was relatively few across two transects, but enough to show interesting trends that seemed evident in other systems as well (Figures 3 and 6).*

We observed significant correlations between topographic slope and δ^{15}N values of plants and soils growing in Taiwan ($P = 0.003$ and 0.025, respectively; Tables 1 and 2), which as the referee highlights are also evident in other ecosystems (Fig. 6). However, both reviewers commented on the size of the dataset used (see also **R2.1**). To address this issue, we assess how the size of a dataset may impact its ability to record of environment controls on δ^{15}N. For this purpose, we use a global compilation of leaf δ^{15}N values from Craine (Craine et al., 2009, *New Phytol.*, 183, 980–992). Across the 11,911 samples in that dataset there is a broad, statistically significant ($P < 0.0001$), positive correlation ($r = 0.51$) between δ^{15}N and an environmental site attribute (here mean annual temperature (MAT), but it is not important which environmental variable for the purposes of this exercise). In our paper, we report δ^{15}N measurements from organic matter collected from 24 geographic localities. Repeats from single sites give us confidence that the δ^{15}N values are representative of site conditions (pg12601 ML14-18). We sampled systematically with elevation (a very good proxy for MAT), covering as broad a range of MAT as possible, and so <u>randomly sampled</u> all other environmental variables (MAP, slope) as we explained in the submitted version (pg12598 ML3-4).

We then randomly down-sample the dataset of Craine et al., (2009) to test whether a smaller dataset such as ours retains the trends seen in a larger dataset (and recorded in the wider ecosystem). Randomly selecting 24 sites from the total of 11,911, and repeating this procedure 10,000 times using an automated code in MatLab, we return Pearson's Rank correlation statistics which we can compare to the full dataset. We find that the statistically significant positive correlation between the environmental variable (MAT) and leaf δ^{15}N observed in the global dataset (n=11,911) is preserved at the 95% level in a randomly sampled subset comparable in size to ours (n=24), with mean statistics across all 10,000 iterations of $r = 0.51$ (± 0.21 standard deviation of the mean) and mean $P = 0.048$ (± 0.114 standard deviation of the mean).

This clearly demonstrates that our dataset, which randomly samples environmental variables, is large enough to preserve trends inherent in the wider ecosystem. These findings concur with those of Amundson et al., (2003) who, using regression models fit to a global soil dataset, find significant relationships with dataset sizes of n=85, n=47 and n=29. In fact, the modelled global distribution of soil δ^{15}N from that study uses a similar number of localities and δ^{15}N measurements (n=29) as our study (n=24). In addition, we note that significant correlations between plant δ^{13}C and elevation (Körner et al., 1988) in a global dataset (n=147) are preserved in sub-samples similar in size to our study (n<30). This analysis herein confirms that we have enough data to assess the dominant controls on δ^{15}N values of mountain forest in Taiwan, and that the referees' concerns are unfounded. As a result, in the revised manuscript, referring to Amundson et al., (2003), we have added a new sub-section to the results '4.1 Dataset size' to make the reader aware of the effect of dataset sizes on statistically significant correlations:

> "The number of samples was relatively few across the two transects studied in Taiwan. Amundson et al. (2003) have previously assessed the role of dataset size for the return of significant correlations between δ^{15}N values of plants and bulk soil and environmental variables in a global completion. They showed that the statistical link between δ^{15}N and site conditions (MAP and/or MAT) were preserved both when the number of sites were similar to this study (n<30) and with ~4 times the number of sites studied in Taiwan. These findings are consistent with the results of Körner et al. (1988), who report significant correlations between the isotopic composition of plants and site elevation which are preserved in sample sub-sets with n<30. We are therefore confident that the number of sites in this study can inform us of the first order environmental controls on the measured δ^{15}N of Taiwan plants and soil." RM Section 4.1

We have also added caveats elsewhere in the text, for example in the abstract:

> "Based on our dataset and these observations, we hypothesise that variable physical erosion rates can significantly influence soil δ^{15}N, and suggest particulate nitrogen export is a major, yet under-appreciated, loss term in the nitrogen budget of mountain forest." RM Abstract.

and in Section 5.3:

> "While the Taiwan dataset is relatively small (cf. Craine et al., 2009) and it is therefore difficult to make irrefutable conclusions, the new data highlight a plausible mechanism of N loss that has not been widely considered in the literature (e.g. Brookshire et al., 2012a). Our process-based explanation of the trends in the data should not be unique to Taiwan, but also affect other mountain forest ecosystems around the world. This hypothesis can be tested more widely with additional field data from different biomes and experimental studies of N loss. Here, we seek existing datasets to evaluate the existence of a possible common geomorphic control on δ^{15}N." RM Section 5.3

R1.2. … ***second is the proposed correlative export mechanism the most plausible among other explanations****? Still, little consideration seemed to have been given to sampling along gradients of ecosystem age or succession that may influence these same N isotope patterns (Hobbie et al. 1999).* ***Conceptually, inputs as well as outputs can be important is determining the average N isotope value of ecosystems****, and* ***input values might vary*** *across the mountain ranges where for example fog and condensation might mean more atmospheric N deposition or wetter conditions favouring N fixation. Correlations with slope might be supported in such cases, but the mechanism of N isotope change would not be N export.* ***Or, the export of particulates may be important, but only one mechanism among many,*** *with the multiloss scenario consistent with early thinking by Shearer and Kohl. That early thinking would also indicate that it is the number of times N is recycled, the biological cycling age, rather than the calendar age, that controls N isotope evolution in soils.*

In this study, authors argue that the export of particulate organics accounts for the major N loss term in steep hillslope systems, and because these exports are likely to have low N isotope values, that this is the dominant process controlling N isotope distributions in the remaining soils. Authors show that the isotope difference between plants and soils is fairly constant across sites and slopes at about 4oo/oo, but it is the average of the ecosystem N (plants + soils) that is varying. Other work by Hobbie et al. (1998) give an explanation of these same patterns ***but invoke N inputs*** *as important in setting isotope trends across time and space. The current study does not report N isotope values for inputs, and one wonders if the correlations related to slope are in fact driven by inputs and not losses. In this sense, the paper seems incomplete, especially, could slope conditions be forcing differences in input amounts and isotopes? The answer is uncertain.*

The referee suggests that the δ^{15}N values that we observe may be explained by either: i) variability in N inputs; or ii) another process of N loss other than, or in addition to, the particulate N loss that we have identified. In fact, in our submitted manuscript we had considered both these factors carefully, and assessed their importance. We emphasized that the flux of N inputs and their isotopic composition are an important term in the isotopic mass balance of the soil (see Fig. 1). To explain the negative pattern between δ^{15}N and slope we consider inputs (pg12607 ML-20). We refer to the study of Weathers et al., (2006) who found that while N deposition patterns in mountain topography can be complex, deposition rate can be explained as a function of elevation and canopy height. Slope angle was a poor predictor of deposition rates. Hence, it is not likely that input rate (I_{ex}) varies systematically with slope. The referee also points us toward literature where N inputs associated with mycorrhizal fungi symbionts can cause variability in δ^{15}N. However, there is no clear hypothesis in these studies for why these associations should vary systematically with slope. This is in stark contrast to the strong, observed relationship between geomorphic process rates (i.e. physical erosion from a soil) and slope angle (Roering et al., 2001; Dietrich et al., 2003). As **R1** points out, this is a "*common-sense case*" which can explain the first order variability in the dataset and "*seemed evident in other systems as well*" (Fig. 6). While the role of inputs may remain uncertain, we have provided a sufficient and plausible mechanistic explanation for the trends in the data and find that a mass balance model informed by those processes can explain the variability and values of δ^{15}N.

We are grateful for the opportunity to clarify the role of N inputs, and have added a paragraph to the start of Section 5.2 to discuss the potential role of N inputs (as we outline above) in more detail:

> "First, if we consider rates of N input by deposition, it is known that spatial patterns can be complicated in mountainous terrain (Weathers et al., 2006). These authors found that elevation and forest canopy height best explained the spatial pattern of N deposition, with slope angle playing a minor role. In addition, we are not aware of any study in which mycorrhizal fungi distribution and N fixation are linked to hillslope angle (Hobbie et al., 1999; Vitousek et al., 2002). Thus, it is difficult to identify a process by which either the rate of N

> deposition or its isotopic composition depends systematically on slope and we still require a mechanistic explanation for the observed in soil and plant δ^{15}N(Fig. 3).
>
> A strong candidate to explain the trends in the data is the loss of PN which operates as a function of slope angle…." RM Section 5.2

Regarding the second point, the referee comments that PN export could be one of many loss terms operating in the ecosystem. This was recognised explicitly in our modelling approach which considers both non-fractionating (PN loss) versus fractionating (e.g. gaseous or dissolved N losses) losses (pg 12608, ML25). We explained that N loss processes other than PN may be controlled by slope angle. For example, we described how water-logging of soils on shallow slopes may increase gaseous N loss by denitrification (e.g. Houlton et al., 2006). This would lead to decreased N loss by fractionating pathways with slope. However, hydrological losses of N are also likely to be important in this forest, and these are likely to increase with slope. We recognise this uncertainty in the behaviour of fractionating N losses in our model, considering scenarios where they are invariant with slope ('k_{ex} variable') and decrease with slope ('k_{ex} constant'). Importantly, both model scenarios require that the relative importance of PN loss increases to produce the variability in soil δ^{15}N that we observe. Therefore, we feel we have already addressed the referee's suggestion that other N loss processes be considered in combination with PN loss. To make this clearer to the reader, we have added text throughout Section 5.2, most notably:

> "We can use the mass balance model to examine how other fractionating N loss processes, k_f (Fig. 1), might vary with topographic slope and impact soil δ^{15}N. … Gaseous loss can occur under anaerobic conditions in water-logged soils (e.g. Houlton et al., 2006) which are more likely on low slopes. This would lead to a decrease of k_f where slopes are steepest. In fact, we model this in the 'k_{ex} constant' scenario described above (Fig. 5), where k_f decreases with increasing slope and PN loss becomes relatively more important. However, increased solute leaching on steep slopes could have the opposite effect on k_f, and high rates of dissolved N loss have been observed in mountain forest elsewhere (Brookshire et al., 2012a; Ohte, 2012). To consider these competing controls on k_f, we also model a scenario where k_f remains constant at $1x10^{-3}$ yr^{-1}, while k_E increases from 0 yr^{-1} to $1x10^{-3}$ yr^{-1} (i.e. 'k_{ex} variable'). This predicts a negative reciprocal relationship between k_E and ecosystem δ^{15}N (Fig. 5). A reciprocal trend between δ^{15}N and slope is also consistent with the soil (r^2= 0.35; $P < 0.0001$) but not the plant data ($r^2 = 0.12$; $P = 0.07$) data. In this case it is also difficult to model the observed variability in δ^{15}N values. The 'k_{ex} constant' scenario describes better the first order pattern in the data (Fig. 3). These findings support the hypothesis of marked heterogeneity in the source of riverine dissolved N from ecosystems (Hedin et al., 2009; Brookshire et al., 2012a) and extend it to PN loss pathways (Fig. 5). It also implies that N loss pathways which fractionate N isotopes may decrease on steep slopes where PN loss dominates export, a geomorphic control on inorganic N that warrants further investigation."

R1.3. *Also, the modelling emphasizes an overall cumulative loss based partly on anomalously low C/N ratios that may not be realistic (see technical comment below), and generally does not consider recycling in favour of a simpler first order unidirectional loss term. In sum, several pieces of the story seemed lacking and there was no experimental work validating the correlations, but the authors do make a reasonable and common-sense case that loss of PON with low N isotopes will, by difference, help drive up N isotope values in remaining ecosystem N pools.*

The reviewer is correct that we use a model that focuses on the first order losses and does not include multiple pools and recycling terms. In the submitted version, we explain why we make this decision, based on the presence of thin soils in Taiwan, and that our samples are homogenised, bulk samples across ~10cm of depth:

> "A multi-component, multi-pool soil model (Trumbore, 1993; Baisden et al., 2002a,b; Manzoni and Porporato, 2009) is not appropriate here, because the soil C/N and ^{14}C measurements were made on homogenised, bulk surface soil, integrating a range of grain sizes and organic-mineral aggregates. In addition, soils in the forested mountains of Taiwan are thin, with the base of the saprolite typically at <0.8m below surface (Tsai et al., 2001). As such, transport of organic material to deeper horizons can be considered negligible (cf. Yoo et al., 2006). Therefore, we use a single pool soil model, which describes the evolution of bulk soil N as a mass balance of net inputs and outputs (Fig. 1; Brenner et al., 2001)." (pg12602 ML25 – pg12603 ML7).

Experiments would be extremely valuable to test the new observations we have made in Taiwan. However, as **R2** points out, there have been very few studies which consider particulate N export as a significant N loss term in mountain forests (see pg12595 ML19-23). Without this rationale in the literature it is difficult to justify experiments to test how this term impacts the ecosystem. With our new δ^{15}N measurements from Taiwan (combined with our modelling approach and re-evaluation of published data from the Andes and California) which indicate how important particulate N loss could be, we hope the study will provide impetus for future experimental work. We have added these sentiments at the beginning of Section 5.3 in the revised manuscript:

> "The mass balance model demonstrates that the range in plant and soil δ^{15}N in Taiwan can be explained by varying the relative importance of fractionating versus non-fractionating N loss across the mountain landscape (Fig. 5). The negative relationship between soil and plant δ^{15}N values and topographic slope (Fig. 3) is then consistent with an increase in soil erosion and PN loss with increasing slope (Dietrich et al., 2003)….This hypothesis can be tested more widely with additional field data from different biomes and experimental studies of N loss which are outside the scope of the present study. Herein, we seek existing datasets to evaluate the existence of a possible common geomorphic control on δ^{15}N."

For the technical comment relating to C/N values, please see our reply below (**R1.4.**).

Technical comments

R1.4. *There are relatively few (12) soil samples analysed and some have very low C/N ratios, so that 5 of the 12 samples have C/N ratios below 6. These seem anomalously low values for soils that usually have values >10. The low C/N values are used to guide the modelling, introducing the possibility that the modelling is based on poor data. However, the main results of soils with higher N isotope values than plants (Fig. 3) is the general pattern seen in many other studies, and the main subject of the paper. This pattern is the main focus of the modelling.*

The accuracy and precision of our geochemical preparation and analytical methods have been rigorously tested, reported in our work elsewhere (Hilton et al., 2010), and summarised in the submitted manuscript (Section 3.2: Measurement procedures and data analysis). Hilton et al., (2010) report accuracy and precision on %C and %N to better than 10% which are used to calculate the error on C/N. These were reported in Figure 3 and caption when larger than the point size. Therefore, we have confidence that data quality is of the highest standard and the measured C/N values are accurate. Our soil C/N data are also consistent with other measurements from Taiwan, where Kao and Liu (2000) also report soils with C/N values of 6.

The soils with low C/N (<6) all have old ^{14}C ages (Fig. 3). Therefore, rather than being anomalous as the referee states, these soils actually provide a consistent story – aging of organic matter results in a decrease in the C/N. It is this observed relationship between ^{14}C age and C/N (RM Table 1) that we seek to explain. The model of Brenner et al., (2001) which we adapt for this purpose is able to explain the first order pattern in the data well (Fig. 3) and provides estimates of N loss which are consistent with measurements made in Taiwan (as explained in the original manuscript, pg12606, ML7). However, we agree with the reviewer that the main focus of our modelling is to explain the 6‰ variability in soil and plant $\delta^{15}N$ and the output from data in Fig. 3 is not used directly in this part of the model.

Literature Cited

Hobbie, E.A., S.A. Macko and H.H. Shugart. 1999. Patterns in N dynamics and N isotopes during primary succession in Glacier Bay, Alaska. Chemical Geology 152:3-11;

Shearer, G., J. Duffy, K.H. Kohl and B. Commoner. 1974. A steady-state model of isotopic fractionation accompanying nitrogen transformations in soil. Soil Science Society of America, Journal 38:315-322.

We thank the reviewer for pointing us towards these additional relevant papers which are now cited in the revised manuscript.