

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 #2 Received and published: 19 November 2012

Actually PN has been frequently ignored in studies on N biogeochemistry and it is good if the importance of PN on N biogeochemistry of the forest ecosystems. In this paper, the authors revealed some correlations between $d15\text{N}$ of surface soils and slope angles, and discuss the importance of PN (or soil erosion) that can shape the $d15\text{N}$ of soils.

We were pleased that the referee understood the novel contribution of our study in Taiwan where we consider loss of particulate N, an understudied aspect of N cycling in mountain ecosystems as the referee suggests. Finding that $\delta^{15}\text{N}$ of plants and soils in Taiwan were significantly correlated with slope angle, we have sought to explain the isotopic variability by adapting an ecosystem N isotope model (Brenner et al., 2001) and seeking plausible, process-based explanations of the trends. As **R1** highlighted, we extend the modelling framework to consider how particulate N export impacts the soil $\delta^{15}\text{N}$ and show it can explain the variability in our data and the negative correlation between $\delta^{15}\text{N}$ and slope.

Questions are

R2.1. *The $d15\text{N}$ signature observed here can be attributed to PN loss? 2. The dataset looks quite small. Is the dataset enough to discuss ecosystem N loss?*

I think the authors discussed too much with too small dataset – with no direct information of PN on $d15\text{N}$ of surface soils in different ecosystems

Identical to our reply to Referee 1, we observed significant correlations between topographic slope and $\delta^{15}\text{N}$ values of plants and soils growing in Taiwan ($P = 0.003$ and 0.025 , respectively Tables 1 and 2), which as **R1** highlights, are also evident in other ecosystems (Fig. 6). However, both reviewers commented on the size of the dataset used (see also **R1.1**). To address this issue, we assess how the size of a dataset may impact its ability to record of environment controls on $\delta^{15}\text{N}$. For this purpose, we use a global compilation of leaf $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ measurements from organic matter collected from 24 geographic localities. Repeats from single sites give us confidence that the $\delta^{15}\text{N}$ 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 randomly sampled 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ from that study uses a similar number of localities and $\delta^{15}\text{N}$ measurements ($n=29$) as our study ($n=24$). In addition, we note that significant correlations between plant $\delta^{13}\text{C}$ and elevation (Körner et al., 1988) in a global dataset ($n=147$) are preserved in sub-samples similar in size to our study ($n<30$). Coupled to this, the rigorous analysis herein confirms that we have enough data to assess the dominant controls on $\delta^{15}\text{N}$ values of mountain forest in Taiwan, and that the reviewers' 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 $\delta^{15}\text{N}$ values of plants and bulk soil and environmental variables in a global completion. They showed that the statistical link between $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$, 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 $\delta^{15}\text{N}$." RM Section 5.3

R2.2. *Different slope angle can be simply linked to PN loss? Lots of other mechanisms that can be affected by different slope angles should be considered.*

There are lots of possible mechanisms that can explain the relationship between slope angles and $d15\text{N}$ of soils (see the latest review on soil $d15\text{N}$ by Hobbie and Hogberg (2012) New Phytologist. How can the authors consider that PN loss (or erosion) is the most important factor shaping the $d15\text{N}$ trend observed in this paper? It seems to me that MAT would be more important (From Table 1) as Amundson et al. (2003) considered.

In line with the comments of **R1** (see **R1.2**), these remarks relate to the suggestion that particulate N loss is important, but may be one process amongst many to explain the trends in the data. In the submitted manuscript we examined correlations between plant and soil $\delta^{15}\text{N}$ and environmental variables, with elevation (a very good proxy for MAT) sampled systematically, and MAP and slope angle sampled randomly. Our analysis yields statistically significant correlations between topographic slope and $\delta^{15}\text{N}$ values of plants and soils growing in Taiwan (Tables 1 and 2), which as **R1** highlights, were evident in other ecosystems (Fig. 6). We then seek to provide a mechanistic explanation for these trends, considering variability in N inputs and N loss pathways (Section 5.2). In fact, this is entirely consistent with one of the major conclusions of the paper **R2** refers us to:

“Climate correlates poorly with soil $\delta^{15}\text{N}$; climate may primarily influence $\delta^{15}\text{N}$ patterns in soils and plants by determining the primary loss mechanisms...” Abstract from Hobbie and Hogberg, 2012, *New Phytol.*

In our assessment of the processes that can explain our dataset, first, we considered N inputs and explained that their flux and their isotopic composition are an important term in the isotopic mass balance of the soil (see Fig. 1). 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 was a poor predictor of deposition rates. Therefore, it is difficult to invoke how input rate (I_{ex}) should vary systematically with slope. **R1** also pointed us toward literature where N inputs associated with mycorrhizal fungi symbionts can cause variability in $\delta^{15}\text{N}$. 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). **R1** agrees that this is a “*common-sense case*” which can explain the first order variability in the dataset. While the role of inputs may remain uncertain, we have provided a 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 $\delta^{15}\text{N}$. 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 (please see reply to R1.2 for more detail of the modifications).

Regarding other N loss pathways that may explain the data, this was recognised explicitly in our modelling approach which considers 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 (e.g. Brookshire et al., 2012a; Ohte, 2012), and these are likely to increase with slope. We recognise the uncertainty in the behaviour of fractionating N losses in our model, considering a scenario 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $1 \times 10^{-3} \text{ yr}^{-1}$, while k_E increases from 0 yr^{-1} to $1 \times 10^{-3} \text{ yr}^{-1}$ (i.e. ‘ k_{ex} variable’). This predicts a negative reciprocal relationship between k_E and ecosystem $\delta^{15}\text{N}$ (Fig. 5). A reciprocal trend between $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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.”

R2.3. *Surface soil (0-10cm) can be a representative parameter of whole forest ecosystem? Page 12599 Line 6 The soil from 0 to 10cm can be representative for the N status in each ecosystem?*

Our sampling approach is identical to that reported elsewhere (e.g. Amundson et al., 2003) where homogenising soil over 0-10cm depth provides a ‘bulk’ soil $\delta^{15}\text{N}$ value that integrates micro-scale variability in $\delta^{15}\text{N}$ values (Baisden et al., 2002a). This provides the best measure of $\delta^{15}\text{N}$ values in the sampled ecosystem. In addition, we report:

“...duplicate and triplicate samples collected at two of the sites (Tables S1 and S3) were indistinguishable within the analytical uncertainty of 0.4 ‰, with means of $6.2 \pm 0.3\text{‰}$ ($n = 2$) and $4.5 \pm 0.3\text{‰}$ ($n = 3$), indicating that measured soil $\delta^{15}\text{N}$ values can be taken as representative site averages.” (pg12601 ML14-18)

R2.4. *Page 19596 Line 7 Is it appropriate to cite Fig. 1 in this sentence??*

No. We agree with the referee and have removed this referral from the revised manuscript.

R2.5. *Page 12599 Line 3- Where did the authors collect the soil samples in a mountain? Slope position of the sampling point would be important if slope angle is the important parameter controlling $\delta^{15}\text{N}$. Microtopography can also affect the $\delta^{15}\text{N}$ of soils and should be considered when soils are sampled...*

The mountain slopes of Taiwan are convex and we sampled from sites at the mid-point of hillslope sections that are not in a state of net deposition. We have added text in Section 2 ‘Study area and site characteristics’, to make this clear. Micro-topography may be important on slopes $< 10^\circ$ (one site out of 24 in this study) but above this angle gravity acts to make sites highly prone to loss of material.

R2.6. *Fig. 2 I think that it is easier for the readers to understand the distribution of $\delta^{15}\text{N}$ data if the number of data is set as y axis. Why probability density (because the number of data is not so large)?*

We have followed convention in the geosciences literature by normalising sample numbers to probability density and reporting the $\delta^{15}\text{N}$ values on the x-axis (e.g. Kao et al., 2000; Hilton et al., 2010). We have clearly labelled the legend with the size of the dataset (Fig. 3). We feel this provides a very clear, graphical representation of the distribution of $\delta^{15}\text{N}$ values in the soils and plants. In emerging fields at the boundary of two communities,

like *Biogeosciences*, conventions are not so well established and we would be happy to conform to the Editor's suggestions on the matter.

R2.7. *Page 12600 Line 15- The difference in $d15N$ between grass and pine should be clarified. The authors mentioned that the difference between two species was "non-systematic" when they used the averaged values, I think this is not appropriate. The differences are (from Table S2) Site 6: -2.5 vs $-3.0 = +0.5$ Site 10: -0.5 vs $-2.2 = +1.7$ Site 14: -3.7 vs $-2.2 = -1.5$ Site 23: 1.6 vs $3.8 = -2.2$ Large differences in $d15N$ for each site..... So, I am not convinced that inter-species variability in $d15N$ was minor (Line 17-).*

The analysis by the referee supports our interpretation that the offset between plant and soil was non-systematic – i.e. both positive and negative, and this is the most important observation. In the revised version, we have removed the statement that suggested that inter-species variability in $\delta^{15}N$ was minor as we agree with the referee's comment. The most important observation is that:

“When the two species were combined ($n = 23$) the mean $\delta^{15}N = -0.9 \pm 0.5\%$ and the negative correlation between $\delta^{15}N$ and slope angle was strengthened ($P = 0.003$), remaining the only statistical link to a site attribute (Table 2).” (pg12600 ML20)

R2.8. *Page 12600 Line 20. $d15N$ data from two species can be combined simply??? I think weighted-average (based on biomass or basal area, for instance) should be applied because the biomass of grass would be much smaller than pine.*

The most important observation is that when both species are considered together, the only significant environmental control on $\delta^{15}N$ values is slope (Table 2). The referee suggests that we could improve this analysis by weighting the datasets based on their biomass contribution. However, we do not see the benefit of doing so for two reasons: 1) the plant species show no systematic difference between $\delta^{15}N$ values (see previous comment); and 2) it will not change the output of the statistical analysis. Given that our data on C3 plants already describe a statistically significant correlation between $\delta^{15}N$ values and slope ($P = 0.006$, see Section 4.1 ‘Vegetation’), a weighted-average won't change our conclusion that the negative correlation between $\delta^{15}N$ and slope angle is the only statistical link to a site attribute. In addition, we do not have data on C3 versus C4 biomass distribution in Taiwan and the assumptions necessary to make this weighting would undermine its potential benefit.

R2.9. *Page 12600 Line 25 In Result section, I found some sentences that would be in Discussion section, and this sentence is one example.*

When observed results are consistent with published measurements and datasets it seems preferable to refer to this in the results. In the case here, the trend between $\delta^{13}C$ and elevation has been observed in the literature for >20 years and it seems inappropriate to refer to this in the discussion. In other places in the results we briefly compare our $\delta^{15}N$ values to others made in Taiwan, showing they are broadly consistent. The results are the best place to make these statements, otherwise the discussion would lose its focus on the interrogation of new findings in the dataset.

R2.10. *Page 12601 Line 3 I am not familiar with $14C$ but the normalization with $d13C = -25$ permil can be applied to the samples with C3 and C4 mixed soil???*

The correction of ^{14}C measurements for isotopic fractionation is done using the measured $\delta^{13}C$, normalised to -25% . So the measured $\delta^{13}C$ of the soil (Table S3) is explicitly taken into account during the fractionation correction and C3-C4 mixtures fully considered. We refer the referee to Section 2 of Stuiver, M., & H. A. Polach (1977), Reporting of ^{14}C data, *Radiocarbon*, **19**, 355-363, and the useful web resource <http://www.c14dating.com/frac.html>.

R2.11. *5.1 Lots of data from outside of this paper, together with many assumptions should be incorporated in calculations.*

Section 5.1 focuses on explaining the observed negative trend between soil ^{14}C age and C/N in the context of rates of N loss from the ecosystem (Table 1) and is focused on N cycling in the mountainous topography of Taiwan. We fully reference all studies of N inputs and outputs from Taiwanese forest for which we are aware (Kao and Liu 2000, Kao et al., 2004, Huang et al., 2012 being the most relevant) and the source of our modelling approach and assumptions (Brenner et al., 2001). In the final paragraph of the Section, we refer to wider literature on this topic as we discuss the implications of the model outputs. Therefore, we are unsure what data and assumptions from outside this paper the reviewer refers to.

R2.12. *Page 12611 Line 4- Lack of significant correlation in Peruvian sites (with whole data) simply suggests that the correlations between slope angles and $d15\text{N}$ is not general. No clear reasons to exclude the sites with slope angles less than 21° .*

There are clear geomorphic reasons to exclude slopes with $\sin\theta < 0.35$ ($\sim 20^\circ$). This is because above this threshold rates of physical erosion by overland flow and mass wasting processes increase with a steeper relationship with slope angle (Roering et al., 2001; Dietrich et al., 2003). We regret that we did not explain this important detail. Below $\sin\theta = 0.35$, erosion rate increases relatively slowly with slope angle. Above this threshold (which relates to thresholds in the activity of erosion processes), soil erosion rates increase rapidly, with a much steeper relationship between slope and erosion rate (Roering et al., 2001).

The result is that PN loss from a mountain forest should be spatially variable with slope angle. If overall erosion rates are lower, it is likely that only parts of the forest where $\sin\theta > 0.35$ will feel the impact of PN loss. In Peru erosion rates are ~ 10 times lower than Taiwan, as we explain in the text (pg12611 ML14-). Therefore, we expect that the relationship between soil $\delta^{15}\text{N}$ and slope would only be present in parts of the landscape where erosion still removed significant amounts of PN. This is what we observe. In Peru, soil and plant $\delta^{15}\text{N}$ values on sites above the threshold of $\sin\theta > 0.35$ have a strong relationship between and slope angle (Fig. 6). Below this threshold, erosion rates are much lower and other N loss processes are likely to dominate the isotopic mass balance. At these sites, the relationship between $\delta^{15}\text{N}$ and slope is not significant as the referee points out (which we clearly state in the manuscript text pg 12611 ML11). We are happy to have the opportunity to modify the text in Section 5.3 and the caption of figure 6 to make this important detail clearer:

“However, on slopes $\sin\theta < 0.35$ the link between soil and plant $\delta^{15}\text{N}$ values and slope is not significant in the Andean forest (Fig. 6). The switch in behaviour is consistent with the threshold behaviour of geomorphic processes (Roering et al., 1999) and the difference in overall erosion rates between these settings. In headwater catchments of the Andes, physical erosion rates have been estimated at $0.2\text{-}0.4\text{ mm yr}^{-1}$ (Safran et al., 2005), $10\text{-}20$ times lower than those of the Central Range, Taiwan (Dadson et al., 2003). Above $\sim 20^\circ$, erosion rate increases more rapidly with slope than below this threshold (Roering et al., 1999, 2001). This means that lower catchment-wide erosion rates are felt most on slopes below this threshold. As a consequence, it is likely that k_E only becomes significant for the N mass balance on the steepest slopes of this forest with the highest erosion rates (when $\sin\theta > 0.35$). On shallower slopes (angle $< 20^\circ$), variability in pathways of fractionating loss (k_f and α_f) or the isotopic expression of N inputs can control $\delta^{15}\text{N}$ values as they are thought to do elsewhere (e.g. Hobbie et al., 1998; Houlton et al., 2006).” RM Section 5.3

“ $\delta^{15}\text{N}$ and slope for soil and plant organic matter from sites in Peru (squares and triangles) and California (diamonds). Lines and shaded region show linear fits to the soil and plant samples from Taiwan (Fig. 3). Below $\sin\theta = 0.35$ ($\sim 20^\circ$), erosion rate increases relatively slowly with slope angle. Above this threshold (which relates to thresholds in the activity of erosion processes), soil erosion rates increase rapidly, with a much steeper relationship

between slope and erosion rate (Roering et al., 1999). For the Peruvian data, filled symbols are those where $\sin\theta > 0.35$ (see Sec. 5.3).” RM Figure caption 6