Nitrogen stable isotopes indicate differences in nitrogen cycling between two contrasting Jamaican montane forests

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Abstract

Background and Aims The aim of this study is to enhance our knowledge of nitrogen (N) cycling and N acquisition in tropical montane forests through analysis of stable N isotopes ($\delta^{15}N$).

Methods Leaves from eight common tree species, leaf litter, soils from three depths and roots were sampled from two contrasting montane forest types in Jamaica (mull ridge and mor ridge) and were analysed for $\delta^{15}N$.

Results All foliar $\delta^{15}N$ values were negative and varied among the tree species but were significantly more negative in the mor ridge forest (by about 2 ‰). $\delta^{15}N$ of soils and roots were also more negative in mor ridge forests by about 3 ‰. Foliar $\delta^{15}N$ values were closer to that of soil ammonium than soil nitrate suggesting that trees in these forests may have a preference for ammonium; this may explain the high losses of nitrate from similar tropical montane forests. There was no correlation between the rankings of foliar $\delta^{15}N$ in the two forest types suggesting a changing uptake ratio of different N forms between forest types.

Conclusions These results indicate that N is found at low concentrations in this ecosystem and that there is a tighter N cycle in the mor ridge forest, confirmed by reduced nitrogen availability and lower rates of nitrification. Overall, soil or root $\delta^{15}N$ values are more useful in assessing ecosystem N cycling patterns as different tree species showed differences in foliar $\delta^{15}N$ between the two forest types.

Running head Nitrogen isotopes in tropical montane forests

Key words Jamaica; tropical montane forest; nitrogen cycling; nitrogen isotopes; soils

Introduction

Understanding ecosystem nutrient cycling is important as soil nutrient availability has a strong effect on plant and microbial growth, performance and community composition which, in turn, will affect ecosystem productivity and other biogeochemical processes. Understanding nutrient cycling is also important from the point of view of community ecology, as variation in nutrients in both space and time may lead to species co-existence through partitioning of various aspects of this essential resource (*e.g.* McKane *et al.* 2002). The nature of nutrient limitation in tropical rain forests is still being debated, but a simple interpretation of current evidence suggests that lowland forests on old and highly weathered soils are more limited by phosphorus whereas montane forests are more limited by the supply of nitrogen (N) due to slower N mineralisation rates in the cooler climates (Tanner *et al.* 1998). Furthermore, with increased N deposition to tropical forests which is predicted to increase in the future, we need to improve our understanding of tropical forest N cycling (Ortiz-Zayas *et al.* 2006; Phoenix *et al.* 2007; Hietz *et al.* 2011)

The N cycle is complex and below-ground controls on N cycling can be difficult to study due to the hidden nature of the soil environment. Stable isotopes are becoming increasingly important in studies of N cycling as they provide a time-integrated measure of ecosystem processes (reviewed in Högberg 1997; Robinson 2001; Evans 2007; Makarov 2009). For example, they can identify sources, infer processes, estimate rates, determine inputs, and constrain models (Sulzman 2007). As N cycles among different compartments of the ecosystem, many processes can cause isotopic discrimination: therefore examination of stable N isotope values can be used to shed extra light on ecosystem N cycling. N isotope values of ecosystem compartments can be influenced by multiple factors including i) uptake of differing N sources and forms with different isotopic signatures (partly mediated by rooting depth), ii) fixation of atmospheric N, iii) fertilisation, iv) isotopic discrimination during uptake of N both by the plant and by symbiotic micro-organisms, v) internal physiological fractionation (associated with uptake, translocation and loss of N), vi) changes in N demand, and vii) losses of nitrogen *via* denitrification and volatilisation (Shearer & Kohl 1986; Erskine *et al.* 1998; Michelsen *et al.* 1996; Högberg *et al.* 1996; Nadehoffer *et al.* 1996; Högberg; 1997; Evans 2001, 2007; Robinson 2001; Kohzu *et al.* 2003; Hobbie *et al.* 2005; Houlton *et al.* 2006, 2007; Craine *et al.* 2009; Houlton & Bai 2009; Makarov 2009).

The use of N isotopes may be especially helpful in tropical regions where we have less information on N cycling and research is often conducted at remote locations with less well developed infrastructure to support complex research capacity. For example, in a comparison of soil and foliar $\delta^{15}N$ values, Martinelli *et al.* (1999) showed that tropical lowland forest leaves and soils were more enriched in ^{15}N relative to temperate forests. Because of the loss of isotopically lighter N from the ecosystem *via* the fractionating pathways of nitrification and denitrification, it was suggested that the N cycle was more 'closed' in temperate forests and that gaseous losses of N from tropical lowland forests were higher. This was confirmed by Houlton *et al.* (2006) who used an isotopic approach to show high losses of nitrous oxide from tropical forests in Hawai'i.

In this study, we attempt to ascertain differences in the N cycle in two contrasting tropical montane forest types in the Blue Mountains of Jamaica; these are the 'mor ridge' and 'mull ridge' of Tanner (1977). The advantage to using this study system is that potential differences in N cycling due to climate (*e.g.* latitude, altitude, rainfall, and temperature) can be excluded as the two forest types are within close proximity (< 1 km) to one another with minimal differences in climatic characteristics (Tanner 1980). We aim to examine variation in plant $\delta^{15}N$ values and relate this to soil $\delta^{15}N$ values, N availability, and rooting depth in the two forest types.

The reason for the formation of the mor ridge forest is not clear but is probably related to the chance aggregation of trees with more recalcitrant litter leading to the formation of the deep, acidic humus layer and consequently a positive feedback processes that amplified the development of this forest type. Previous work (Tanner 1977) suggested a slower rate of N mineralisation in this forest type and there may therefore be less nitrate available leading to the hypothesis that the mor ridge forest has a more 'closed' N cycle and therefore $\delta^{15}N$ values of both soils and plant material would be more negative. The goal of this study is to determine whether $\delta^{15}N$ values can be used as successful indicators of different rates of N cycling in these differing montane forest types.

Methods

Study sites The study sites are found at around 1600 m a.s.l. along John Crow ridge in the western Blue Mountains of Jamaica at 18° 05' N, 76° 39' W and have been described extensively by Tanner (1977). There is no comprehensive data on climatic conditions but

annual rainfall is estimated at 2500 mm with an annual temperatures range from around 11˚C to 20 ˚C (Shreve 1914; Tanner 1980). Whilst the remaining forests in the Blue Mountains are generally found on very steep terrain, the sites chosen for this study were situated along the Grand Ridge and, as such, were relatively flat. Two contrasting forest types were chosen for the study: 'mull ridge' which has trees up to 13-15 m tall (Soil pH: 3.6-4.0; Loss-on-ignition: 25-75 %) was compared with 'mor ridge' which is a much more stunted forest formation with trees of up to 5-7 m tall found over an acidic, highly organic soil (Soil pH: 3.0; Loss-on-ignition: *c.* 95 %) (Tanner 1977; Stewart 2000; F. Q. Brearley unpublished data).

Foliar samples In July-August 2006, foliar samples of mature leaves were collected from the two forest types. Samples were collected from mature, sunlit leaves of eight tree species from each forest type (three or six samples per species in each forest type) using a long-handled pruner; these eight species accounted for *c.* 75-80 % of total basal area in each forest type (Tanner 1977). The species were: *Alchornea latifolia* (Euphorbiaceae), *Clethra occidentalis* (Clethraceae), *Clusia havetioides* (Clusiaceae; mor ridge only), *Cyrilla racemiflora* (Cyrillaceae), *Hedyosmum arborescens* (Chloranthaceae; mull ridge only), *Lyonia octandra* (Ericaceae), *Pittosporum undulatum* (Pittosporaceae), *Podocarpus urbanii* (Podocarpaceae) and *Schefflera sciadophyllum* (Araliaceae). Each sample was a composite of a number of leaves (dependent upon leaf size). The leaves were dried at 50 ° C (Brearley 2009) and the petioles and mid-rib removed. The leaves were then finely ground (Yellowline A10, IKA-Werke, Staufen, Germany), sealed in air-tight plastic vials, and sent to the University of Washington, USA for isotope analyses (see '*Isotope analyses*' below).

Soil and litter samples Six soil samples were collected from depths of 0-10, 10-20 and 20-30 cm using a metal corer in July-August 2006 from the two forest types (hereafter, 'bulk soils'). Bulked leaf litter samples were taken from an area of approximately 100 cm^2 immediately adjacent to the holes made by the corer. Bulk soils and litter were dried at 50 ° C, finely ground, sealed in air-tight plastic vials, and sent for isotope analyses as above.

Soil N availability In May-June 2008 and again in February 2010, five samples of 15 to 20 g of fresh soil (from 0-10 cm depth only) were collected from the two forest types and shaken within 8 hours of collection with 100 ml of 1 M KCl (1 minute of shaking by hand at *c.* 100 rpm every ten minutes six times) and left to stand overnight (14-16 h) before being

filtered (filter papers were pre-washed in 1 M KCl and rinsed in deionised water before use) and stored in 30 ml vials (with 1 ml of 1 % HCl added). Ammonium and nitrate were then determined on a Dionex ICS-2000 ion chromatography system with a CG16 guard column and CS16 separation column for ammonium and an AG18 guard column and AS18 separation column for nitrate (Dionex (UK), Camberley, Surrey, UK). To determine soil moisture content, fresh soils were placed in plastic bags to keep them moist and then dried at 105°C for 24 hours; soil weights were therefore adjusted accordingly. Nitrogen mineralisation and nitrification rates were assessed by incubating three or four *c.* 100 g portions of soil (on which the original ammonium and nitrate concentrations had been determined as above) in plastic bags immediately below the surface litter layer for 10 days (in May-June 2008) after which ammonium and nitrate concentrations were determined again. Mineralisation and nitrification rates were calculated as the difference in mineral N concentrations between the end and beginning of the incubations. PRS™ probes (WesternAg Innovations, Saskatoon, Saskatchewan, Canada) were inserted in the soil for 49 days in April-May 2009 (each of the eight samples consisted of two anion and two cation probes which were combined for analysis, except for one sample of a single probe of each type). After being taken out of the soil extraneous material was removed; they were then washed thoroughly in distilled water and shipped to Canada for analysis.

Soil ammonium and nitrate $\delta^{15}N$ values Ammonium and nitrate were captured on acidified filter papers using a modified diffusion methodology (Brooks *et al.* 1989; Claudia Schütz, pers. comm.) in July-August 2006 and again in May-June 2008. Twenty g of fresh soil (from 0-10 cm depth only) was shaken with 100 ml 1 M KCl (1 minute of shaking every ten minutes six times) and left to stand overnight (14-16 h) before being filtered into 300 ml glass jars. Fifty mg of MgO (pre-heated at 600°C for 4 hrs) was added to the KCl extract to convert NH_{4+} to NH_3 which was captured on acidified [10 µl of KH_2SO_4 (7 % H_2SO_4, 22 % KSO_4) pipetted on to the paper] squares of 1 cm^2 Whatman GF/C filter paper elevated above the solution on stainless steel wire. The KCl extract was then incubated at ambient temperature (mean: 18.0 ° C, range: 15.3-23.3 ° C) for 4 days and swirled gently daily. Filter papers were then removed and dried by placing them in a sealed plastic container containing silica gel. New filter papers were placed on the steel wires in the glass jars and NO_{3-} remaining in the solution was converted to NH_{4+} and subsequently to NH_3 by adding 0.04 g of Devarda's alloy. After incubation for a further 3 days, the filter papers were removed and dried as above. Filter papers were sealed in plastic vials and sent for

isotope analyses. Any filter papers which had a N content greater than one standard deviation from the mean N recovery expected as analysed by KCl extraction and ion chromatography (see above) were excluded from the subsequent statistical analyses; each forest type x N-species combination therefore had between four and seven replicates remaining.

Root biomass and depth distribution Root biomass (of live and dead roots up to 1 mm diameter) was estimated using standard techniques (Brearley 2011) from the same three depths as bulk soil samples were taken in both July-August 2006 and May-June 2008. The exponential decrease in root biomass was modelled using the equation of Gale & Grigal (1987):

$y = 1 - \beta^d$

where y is the cumulative fraction of root biomass to a depth of d cm; with low β values representing a steady decline with depth and high β values representing a more rapid decline with depth.

Isotope analyses Around 1-2 mg of sample (or the whole of a filter paper) was weighed accurately into a tin capsule and the $\delta^{15}N$ (and % N) value of all the samples were determined using a Thermo Finnegan Delta Plus XP isotope ratio mass spectrometer (Thermo Finnegan, Bremen, Germany) coupled to a Costech ECS 4010 elemental analyser (Costech Analytical, Valencia, CA, USA) at the University of Washington, USA. The results are expressed in δ notation whereby $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$ when R is the ratio of $^{15}N/^{14}N$. Precision of duplicate analyses of standard samples was better than 0.12 ‰ in all cases. Comparison to reference material of NIST peach or laboratory spinach leaves had a mean difference of 0.12 ‰.

Results

Whilst there was a significant difference between forest types in mean foliar $\delta^{15}N$ values, with the trees from the mor ridge forest showing more negative mean foliar $\delta^{15}N$ values of -4.52 ‰ compared with -2.98 ‰ ($F_{1,61}$ = 24.9, $p < 0.001$; Figure 1), there was also considerable variation in the foliar $\delta^{15}N$ values of the seven species sampled from both forest types ($F_{6,61}$ = 8.22, $p < 0.001$; Figure 1), and a strong interaction between species and forest type ($F_{6,61}$ = 2.98, $p = 0.013$; Figure 1). As an example, the difference in foliar $\delta^{15}N$ values in *Pittosporum undulatum* between the two forest types was about 3.9 ‰ whereas *Cyrilla racemiflora* had a minimal difference between the forest types of only 0.1 ‰ (*n.b.*

Hedyosmum arborescens and *Clusia havetiodes* were excluded from the above analyses as leaves were only collected from one forest type; if they are included, the mean values become -4.62 ‰ and -2.65 ‰ for mor and mull ridge forests). Whilst there was a significant effect of forest type when all species were combined, these were only actually significant for *Pittosporum undulatum* and *Podocarpus urbanii* after a Tukey's test ($p < 0.05$). There was no correlation between the species rankings of $\delta^{15}N$ values in the two forest types ($r_s = 0.39$, $p = 0.34$).

There were significant positive correlations between foliar $\delta^{15}N$ and % N in both forest types (Mor: $r = 0.56$ $p < 0.001$; Mull: $r = 0.34$, $p = 0.024$; Figure 2a). Similarly, there were positive correlations between foliar $\delta^{15}N$ and 10 year diameter (at 1.3 m) growth rates between 1994 and 2004 (E. V. J Tanner, unpublished data) for both forest types (Mor: $r = 0.36$, $p = 0.43$; Mull: $r = 0.62$, $p = 0.14$; Figure 2b) although these were not statistically significant (*Pittosporum undulatum* was removed from this analysis as no individuals of this species were present in 1994).

Bulk soils (including the litter layer) were significantly more depleted in ^{15}N in the mor ridge forests (by about 3 ‰) at all depths ($F_{1,34} = 56.7$, $p < 0.001$; Figure 3); this was significant at all depths following a Tukey's test ($p < 0.05$) The bulk soil became increasingly enriched in ^{15}N with depth in both forest types ($F_{3,34} = 50.5$, $p < 0.001$; Figure 3) with the degree of enrichment being similar in both forest types indicated by the lack of a significant interaction term in the ANOVA ($F_{3,34} = 0.54$, $p = 0.66$; Figure 3). In addition, bulked root samples similarly had lower $\delta^{15}N$ values in the mor ridge forest ($t_{12} = 7.68$, $p < 0.001$; Figure 3). There was a negative correlation between bulk soil $\delta^{15}N$ and % N in both forest types (Mor: $r = -0.85$, $p < 0.001$; Mull: $r = -0.65$, $p = 0.009$).

Ammonium was found at greater concentrations, although not always significantly, in the mor ridge forest soil than the mull ridge forest soil across all methods and studies (Table 1). In contrast, depending upon the method used, values for nitrate varied from being slightly greater in mor ridge soil (KCl extractions in 2008) to being fifteen-fold greater in mull ridge soil (PRS^{TM} probes; Table 1). The nitrate:ammonium ratio was always greater (although never significantly so) in the mull ridge forest soil (Table 1). Nitrogen mineralisation and nitrification were greater in the mull ridge soils (Table 1). Using the data on soil bulk density from Tanner (1977) of 0.1 and 0.45 g cm^{-3} for mor and mull ridge forest, the

amounts of both ammonium and nitrate were greater in the mull ridge forest soil when expressed on an area basis (data not shown) and mineralisation and nitrification were both over an order of magnitude greater in the mull ridge forest soil (Table 1).

Initial statistical tests suggested that year of collection (2006 *vs.* 2008) did not have an effect on $\delta^{15}N$ of soil ammonium and nitrate ($p > 0.50$) and hence both years were considered together in subsequent analyses. Ammonium in the upper soil layers (0-10 cm) was significantly more enriched in ^{15}N in comparison to nitrate in both forest types ($F_{1,21} = 36.7$, $p < 0.001$; Table 2) and both ammonium and nitrate in the mor ridge forest soils were depleted in ^{15}N relative to the mull ridge forest soils ($F_{1,21} = 4.00$, $p = 0.058$; Table 2). In a separate analysis in which $\delta^{15}N$ of ammonium, nitrate and bulk soil (bulk soil data in Figure 3) were compared, in both forests' soils, $\delta^{15}N$ of ammonium was not significantly different to $\delta^{15}N$ of bulk soil (Tukey's test, $p > 0.40$) in contrast to nitrate which was significantly depleted in ^{15}N when compared to bulk soil (Tukey's test, $p < 0.001$).

The root biomass of live and dead roots up to 1 cm diameter was approximately the same in the upper 10 cm of soil in both forest types but there was a more rapid decline in root biomass with depth in the mull ridge forests (Figure 4) indicated by significantly lower β values in the mull ridge forest soil ($t_{16} = 2.34$ $p = 0.032$).

Discussion

Foliar $\delta^{15}N$ and comparisons with other studies

The values for foliar $\delta^{15}N$ were, in general, very low (Figure 1) when compared to, for example, tropical lowland forests in French Guiana where no leaf sample had a $\delta^{15}N$ value of less than -0.6 ‰ (Roggy *et al.* 1999) or in Brazil (Ometto *et al.* 2006) where the mean value was 5.8 ‰ and the lowest value 0.9 ‰. The values were more similar to those from Hawai'ian forests with an overall of mean of -5.1 ‰ (Vitousek *et al.* 1989). Indeed, the foliar $\delta^{15}N$ values are comparable to, if not lower than, many samples from temperate forests which are considered to be more N limited than tropical forests where the mean value for foliage in temperate forests in the compilation of Martinelli *et al.* (1999) was -2.8 ‰.

If we calculate a weighed foliar $\delta^{15}N$ average for each forest type [weighted by species basal area; data in Tanner (1977)] then the values are -2.94 ‰ for mor ridge forest and -2.36 ‰

for mull ridge forest. For the mor ridge forest, this is less than the value for the bulked leaf litter, suggesting some isotopic discrimination related to foliar N re-absorption in contrast to studies by Garten (1993) and Kolb & Evans (2002) who found little evidence for isotopic discrimination during this process.

Foliage was depleted in ^{15}N relative to the soil as anticipated, due to physiological (or mycorrhizal mediated) fractionation during N uptake (Michelsen *et al.* 1996; Evans 2001; Robinson 2001; Amundsen *et al.* 2003). The difference between surface bulk soil (0-10 cm) samples and foliage was 3-4 ‰ as predicted by Amundsen *et al.* (2003) and Houlton *et al.* (2007). When the data was compared with the global database of Craine *et al.* (2009), the values were about 3 ‰ lower than might be expected as predicted by climate, foliar N concentration and type of mycorrhizal association (J. M. Craine, pers. comm.). Although we might expect, given the hypothesis of N-limitation in these forests, complete uptake of available N and hence little fractionation.

Mycorrhizas and root symbioses There were no differences in foliar $\delta^{15}N$ in relation to symbiotic micro-organisms within the roots of the various species. Whilst the mycorrhizal associations of the species found in this montane forest are not known for certain, we can make informed guesses that most of the species will form arbuscular mycorrhizas (AM). *Podocarpus urbanii*, in addition, has root nodules (F. Q. Brearley, pers. obs.), but, as found by other studies (Baylis *et al.* 1963; Russell *et al.* 2002), these actually form a housing for AM fungi and do not appear to be providing atmospheric N to this species as its foliar $\delta^{15}N$ values were significantly less than 0 ‰ and fell well within the values for the other species studied. The AM status of the nodules of *P. urbanii* has been confirmed by molecular detection of AM fungi using the primers of Helgason *et al.* (1998) (F. Q. Brearley, unpubl. data). *Lyonia octandra* is expected to have ericoid mycorrhizas and all other species to have AM but there was no difference in foliar $\delta^{15}N$ between them: perhaps due to maximal N uptake and, hence, minimal isotopic fractionation. Elbers (1996, in Hafkenscheid 2000) showed a greater soil fungal hyphal length in the mor ridge forest soil and, therefore, a greater reliance on mycorrhizal fungi to supply N in the mor ridge forest may be leading to more depleted foliar $\delta^{15}N$ values (Brearley *et al.* 2003; Hobbie *et al.* 2005).

Rooting depth To examine whether the differing $\delta^{15}N$ values might be related to rooting depth of the trees, soil cores were taken from the two forest types and roots extracted.

The more rapid decline in root mass with depth in the mull ridge forests (Figure 4) suggested that trees in the mor ridge will be taking soil N from, on average, a slightly greater depth than in the mull ridge. This is corroborated by the results of Stewart (2000) who noted that root growth into ingrowth cores in the mor ridge soils was evenly distributed along the 15.5 cm deep cores, but was mostly in the top third of the core in the mull ridge soils. However, this process would lead to ^{15}N-enriched foliage which is not what is seen, suggesting that other factors are cancelling out any potential changes in δ^{15}N values due to rooting depth. A more detailed study of root patterns on a species-by-species basis would be helpful to provide more information on patterns of N uptake in these forests.

Ammonium/nitrate preference In agreement with our study (Table 2), it has often been shown that soil nitrate is depleted in ^{15}N relative to ammonium (Garten 1993; Koba *et al.* 1998; Miller & Bowman 2002; Schimann *et al.* 2008; Cheng *et al.* 2010) and, therefore, species with a preference for nitrate over ammonium (within a given soil type) would have lower δ^{15}N values. In ecosystems where there was not expected to be a significant loss of nitrate, species taking up proportionally more nitrate had more negative δ^{15}N values relative to those taking up ammonium (Miller & Bowman 2002). Indeed, 64 % of the variation in N isotope ratios between the co-existing alpine grassland species was explained by their ammonium:nitrate uptake ratios in the study of Miller & Bowman (2002). It is acknowledged that there may be temporal changes in δ^{15}N values for ammonium and nitrate but the lack of significant differences between our two sampling dates suggests a consistency of this general pattern in the montane forest soils studied here. Interestingly, it appears that, although nitrate is being produced in these forest soils, and in some cases is the dominant form of inorganic nitrogen [Table 1; although note that the time between sample collection and extraction may have increased nitrate values in the soils; see Arnold *et al.* (2008)], most trees are using nitrogen with an isotopic value closer to that of ammonium. This might explain the somewhat counter-intuitive results of Brookshire *et al.* (2012) who found high losses of nitrate from montane forests that are generally thought to be N-limited as various aspects of their ecosystem productivity respond to additions of nitrogen fertiliser (as urea) (Adamek *et al.* 2011; Tanner *et al.* 1990).

In addition, leaves in the mor ridge were closer to the bulk soil δ^{15}N (Figure 1, Table 2) suggesting greater ammonium uptake as it has been shown that soil ammonium is

isotopically more similar to bulk soil N than is soil nitrate, both in this study (Table 2) and by Koba *et al.* (1998). To test the relative importance of nitrate nutrition, relative to ammonium nutrition, in these Jamaican forests it would be helpful to examine leaf nitrate reductase activity (*e.g.* Michelsen *et al.* 1996; Nadelhoffer *et al.* 1996; Miller & Bowman 2002) or conduct a soil ^{15}N labelling experiment.

A key question arising from this study is why there was a large change in the species rankings of foliar $\delta^{15}N$ values between the two forest types? There was a consistent change in soil $\delta^{15}N$ values between forest types and thus this would have affected all species equally. We consider it most likely that a change in competitive interactions between the species in the different forest types – either by changing the depth at which they foraged for soil resources or, perhaps more likely, changing uptake of the different forms of N available in the soil, led to this change in rankings. Houlton *et al.* (2007) showed how a number of tropical Hawai'ian plants changed preference for differing N forms with increasing rainfall suggesting a strong flexibility in N usage in these species. The flexibility of N use strategies in the Jamaican plants remains to be ascertained experimentally but our isotope results do suggest some flexibility of N form preference on differing soil types and that this flexibility differed between species. Interestingly, the species with the greatest apparent flexibility was *Pittosporum undulatum* which may be a contributing factor to its successful invasion in this area (Goodland & Healey 1996; Bellingham *et al.* 2005).

Soil $\delta^{15}N$ and comparisons with other studies

Bulk soil $\delta^{15}N$ values were also low (Figure 3) and, especially for the mor ridge forest, were some of the lowest recorded. Indeed, they are similar to sites which have very young soils with minimal N content such as soils developing on glacier forefronts (Hobbie *et al.* 2005) or young lava flows (Vitousek *et al.* 1989). Brearley *et al.* (2011) have shown lowland tropical soil $\delta^{15}N$ values of around 2 ‰ to 6 ‰ across a range of sites and values in Brazilian soils were around 8 ‰ in surface horizons, falling to around 11 ‰ at 50 cm depth (Ometto *et al.* 2006). Our Jamaican $\delta^{15}N$ values were more similar to, although still lower than, those from other montane tropical forests at 1500 m in Ecuador (2.3 ‰; Arnold *et al.* 2009) and 1700 m in Borneo (0.3 ‰; Kitayama & Iwamoto 2001). The most similar values were from ridge soils at 2000 m altitude in Ecuador of -1.3 ‰ (Wolf *et al.* 2011).

There was considerable enrichment of ^{15}N with depth in both forests (Figure 3) as seen in other studies (Nadelhoffer & Fry 1988, 1994; Koba *et al.* 1998; Boeckx *et al.* 2005; Cheng *et al.* 2010), this being attributed to processes occurring during N mineralisation which favour ^{14}N as a substrate over ^{15}N, thereby leaving (microbially) ^{15}N enriched products in the soil which will form organic matter over time (Nadelhoffer & Fry 1994; Högberg 1997). During organic matter formation and stabilisation, the accumulation of ^{15}N-enriched compounds from decay products will lead to the enrichment of soil ^{15}N over time. Enrichment of soil ^{15}N is also achieved by the input of ^{15}N-depleted foliage to upper soil layers (Nadelhoffer & Fry 1988) and preferential mineralisation of ^{15}N-depleted compounds.

$\delta^{15}N$ and N cycling rates

It has been suggested that soil and root $\delta^{15}N$ values can be used as an indicator of rates of N cycling between sites (Martinelli *et al.* 1999; Templer *et al.* 2007). The soils and roots were significantly more depleted in ^{15}N in the Jamaican mor ridge forests (by about 3 ‰) at all depths (Figure 3) confirming slower rates of N mineralisation and nitrification (Table 1) and a more 'closed' N cycle. This includes the litter layer and therefore suggests that there is more N available in the mull ridge forest as losses of N from systems with excess N are more likely to be fractionating (nitrate leaching and denitrification) compared to more N-limited systems where losses may be minimal and/or by non fractionating pathways (*e.g.* loss of dissolved organic nitrogen: Perakis & Hedin 2002). Various studies have shown a positive correlation between nitrification rates and plant $\delta^{15}N$ (Garten 1993; Garten & Van Miegroet 1994; Pardo *et al.* 2006; Templer *et al.* 2007; Cheng *et al.* 2010) in broad agreement with our study where the mull ridge forests had greater nitrification (and N mineralisation) rates (Table 1) and less negative foliar $\delta^{15}N$ values (Figure 1). However, it should be noted that there were differences in the absolute values for mineral N values and transformations obtained by the different studies in Table 1. There are a number of reasons for this that could include the time taken to process samples between collection and extraction (Arnold *et al.* 2008), increased N deposition rates over the 30-year period (not quantified) and whether the incubations were conducted *in situ* or in warmer lowland climates (as in Tanner's 1977 study). In addition, there may be some issues associated with the use of PRS™ probes including severing of roots leading to less competition for nitrate, nitrification occurring on the probes or uptake of ammonium from the probes.

It would be very interesting to compare the $\delta^{15}N$ values in this study with those of Tanner (1977) to assess if N cycling patterns may have changed in a manner similar to Hietz *et al.* (2011) who showed an increase in foliar $\delta^{15}N$ values in lowland forests in Panama suggesting increased N deposition over a *c.* 40 year time period. We may well expect to see similar patterns in these Jamaican forests although this has not been directly measured.

A number of authors (Högberg 1997; Pardo *et al.* 2006; Templer *et al.* 2007) consider below-ground (*i.e.* root) $\delta^{15}N$ values to be a better indicator of the relative rates of N cycling which concurs with our study, as we found considerable variation in foliar $\delta^{15}N$ values between species (*c.* 4 ‰; Figure 1), most likely due to internal physiological fractionation processes which may hide any soil-based variation between sites.

Conclusions

We have shown how $\delta^{15}N$ values of soils and roots are more negative in mor ridge tropical montane forests of Jamaica suggesting a tighter N cycle and hence this element is suggested to be more limiting in this forest type. $\delta^{15}N$ values varied between tree species, and the rankings changed between soil types, indicating that the use of foliar $\delta^{15}N$ values are less helpful in assessing N limitation due to the different responses of species to the two soil types and the relative uptake of different N forms (which appeared to be a preference for ammonium in most cases). The physiological processes underlying this inter-specific variation require further study but are likely to be due to differential preferences/uptake for ammonium or nitrate. It will be valuable to assess how the N cycle is altered in these tropical montane forests in the future with increasing N deposition.

Acknowledgements

I am indebted to Ed Tanner (University of Cambridge) for his continued support and encouragement of this research. Many thanks to Ben Harlow (University of Washington) for isotope analysis; thanks also to Kurt McLaren and Jane Cohen (University of the West Indies) for advice and logistical support, Raymond Ramdon (Ministry of Agriculture, Jamaica) for permission to use Cinchona Botanic Gardens as a base, the staff of the gardens for their assistance, Claudia Schütz (Technischen Universität München) for advising on isotopic analysis of ammonium and nitrate, Shauna-Lee Chai (University of Cambridge) for placing and collecting the PRS™ probes, and Steve Hoon (Manchester Metropolitan University) for help in modelling root biomass. This study was financially supported by the British Ecological Society, Dalton Research Institute

(Manchester Metropolitan University), Percy Sladen Memorial Fund, Trinity College Dublin Association and Trust, and WesternAg Innovations.

445

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Figure 1 Foliar $\delta^{15}N$ values for nine tree species in two contrasting forest types in the Blue Mountains of Jamaica. All values are mean ± standard error.

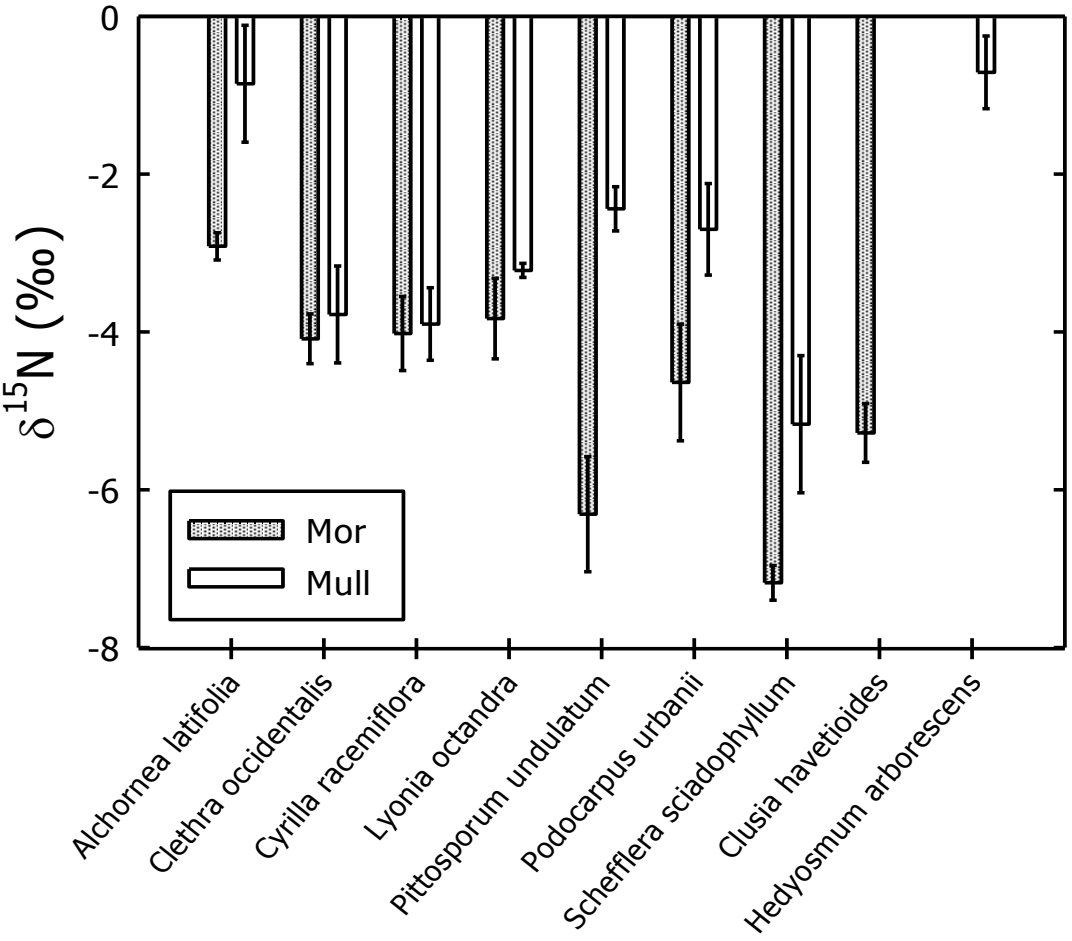

Figure 2 Positive correlations between foliar $\delta^{15}N$ and (**a**) leaf foliar N and (**b**) tree absolute growth rates (AGR) between 1994 and 2004 in two contrasting forest types in the Blue Mountains of Jamaica.

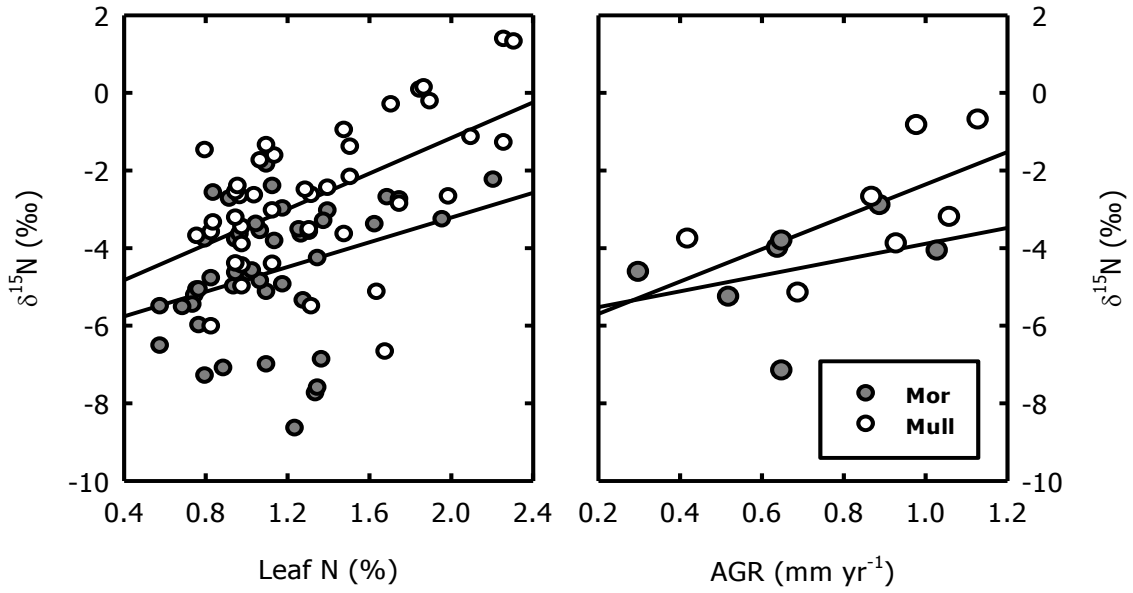

Figure 3 Bulk soil, root, and leaf litter $\delta^{15}N$ values in two contrasting forest types in the Blue Mountains of Jamaica. All values are mean ± standard error.

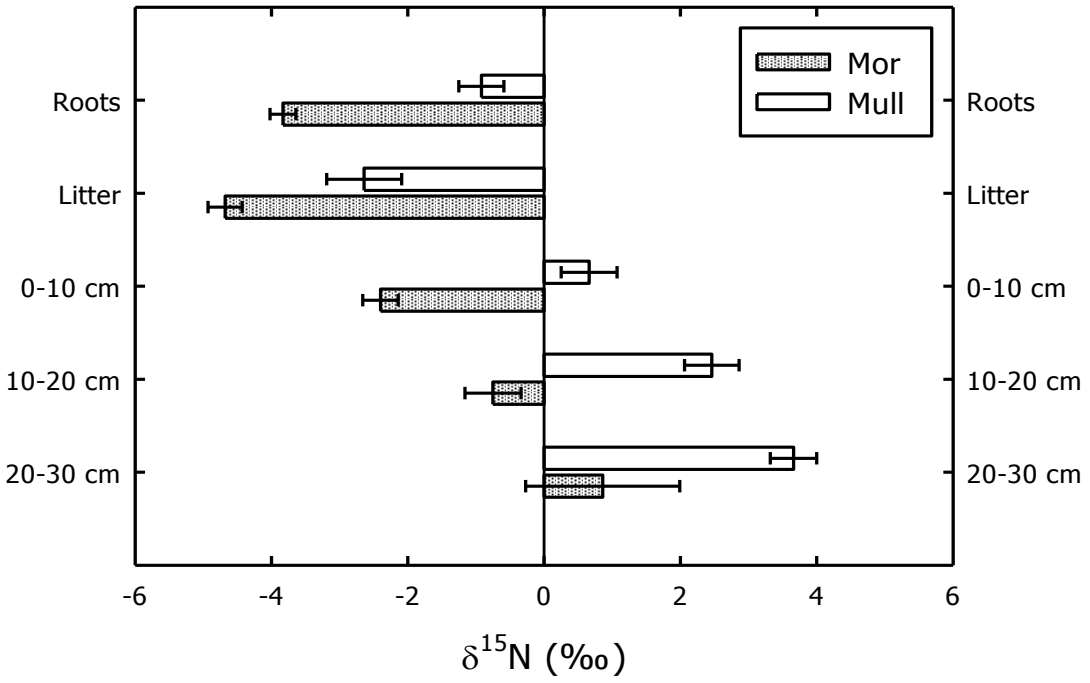

Figure 4 Root biomass (< 1 mm diameter) at thee depths in two contrasting forest types in the Blue Mountains of Jamaica and the corresponding β values describing the exponential decline with depth. All values are mean ± standard error.

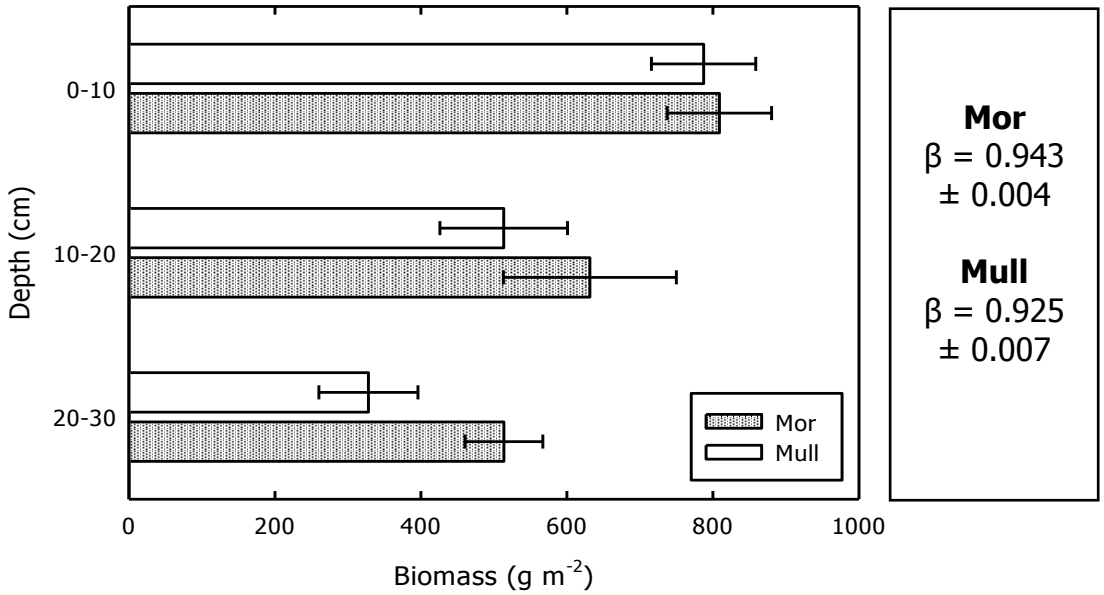

Table 1 Soil nitrogen concentrations and mineralisation rates in two contrasting forest types in the Blue Mountains of Jamaica assessed using KCl extractions of fresh soil and *in-situ* deployment of PRS™ probes. Also included are results from two previous studies for comparative purposes. All values are mean ± standard error.

	Mor	Mull	
KCl extractions (μg g^{-1}) - 2008			
Ammonium	0.79 ± 0.31	0.48 ± 0.09	$t_5 = 0.98, p = 0.37$
Nitrate	0.28 ± 0.25	0.20 ± 0.09	$t_6 = 0.29, p = 0.78$
Nitrate: Ammonium	0.19 ± 0.13	0.48 ± 0.17	$t_{10} = 1.37, p = 0.20$
KCl extractions (μg g^{-1}) - 2010			
Ammonium	0.80 ± 0.15	0.39 ± 0.05	**$t_8 = 2.67$ $p = 0.028$**
Nitrate	0.55 ± 0.31	1.50 ± 0.56	$t_8 = 1.49$ $p = 0.18$
Nitrate: Ammonium	1.07 ± 0.64	4.22 ± 1.55	$t_8 = 1.88$ $p = 0.097$
PRS™ probes (μg 10 cm^{-2} 49 $days^{-1}$)			
Ammonium	15.9 ± 10.0	12.6 ± 5.6	$t_6 = 0.32, p = 0.76$
Nitrate	7.4 ± 0.83	162 ± 49.0	**$t_4 = 3.15, p = 0.035$**
Nitrate: Ammonium	0.98 ± 0.42	26.9 ± 12.0	$t_4 = 2.08, p = 0.11$
Nitrogen mineralisation (μg g^{-1} 10 d^{-1})			
Mineralisation	0.25 ± 0.21	1.34 ± 0.53	$t_5 = 1.67, p = 0.16$
Nitrification	0.01 ± 0.09	1.52 ± 0.35	**$t_5 = 3.61, p = 0.015$**
Nitrogen mineralisation (kg ha^{-1} 10 d^{-1})+			
Mineralisation	0.025 ± 0.021	0.803 ± 0.119	***x***
Nitrification	0.001 ± 0.009	0.912 ± 0.078	***x***
Values from Tanner (1977)			
Ammonium (μg g^{-1})	364	239	-
Nitrate (μg g^{-1})	31	17	-
Nitrate:Ammonium	0.085	0.071	-
Mineralisation (μg g^{-1} 40 d^{-1})	88	160	-
Nitrification (μg g^{-1} 40 d^{-1})	100	136	-
Values from Hafkenscheid (2000)*			
Ammonium (μg g^{-1})	471	235	n.s.
Nitrate (mg g^{-1})	6.2	10.6	n.s.
Nitrate:Ammonium	0.013	0.046	-
Mineralisation (μg g^{-1} d^{-1})	-0.33	8.0	n.s.
Nitrification (μg g^{-1} d^{-1})	0.17	0.38	n.s.

+ n.b. same data as above but converted to different units

* From 'moderately developed' mor ridge forest and 'poorly developed' mull ridge forest

Table 2 Soil ammonium and nitrate $\delta^{15}N$ values (‰) from 0-10 cm depth in two contrasting forest types in the Blue Mountains of Jamaica. Also included are bulk soil $\delta^{15}N$ values (0-10 cm) from Figure 3. All values are mean ± standard error.

	Mor	Mull
Ammonium	-5.50 ± 1.27	-0.26 ± 1.51
Nitrate	-14.39 ± 1.05	-12.71 ± 2.36
Bulk soil	-2.40 ± 0.26	0.66 ± 0.41