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Natural abundance of ¹⁵N in soils along forest-to-pasture chronosequences in the western Brazilian Amazon Basin

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Abstract We examined the natural abundance of ¹⁵N in soil profiles along two chronosequences in the western Brazilian Amazon Basin state of Rondônia, to investigate possible mechanisms for changes to soil nitrogen sources and transformations that occur as a result of land use. One chronosequence consisted of forest and 3-, 5- and 20-year-old pasture, the other of forest and 8- and 20year-old pasture. The δ^{15} N values of surface soil and soil to 1 m depth in the native forest ranged from 9.8 to 13.6% and were higher than reported for temperate forest soils. Fractionation associated with nitrification and denitrification and selective losses of ¹⁵N-depleted nitrate, could potentially result in a strong enrichment of nitrogen in soil organic matter over the time scale of soil development in highly weathered tropical soils. Pasture surface soils were 1-3‰, depleted in ¹⁵N compared with forest soils. Lower δ^{15} N values in 20-year-old pastures is consistent with greater cumulative inputs of ¹⁵N-depleted atmospheric-derived nitrogen, fixed by free-living bacteria associated with planted pasture grasses in older pastures, or differential plant utilization of soil inorganic N pools with different δ^{15} N values. The pattern of δ^{15} N values following conversion of forest to agricultural use differs from the pattern in the temperate zone, where pasture or cultivated soils are typically more enriched in ¹⁵N than the forest soils from which they were derived.

Key words Stable isotopes \cdot Nitrogen \cdot Fixation Forest \cdot Rondônia

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Introduction

The natural variation of 15 N in soil reflects both soil nitrogen (N) sources and fractionation of the soil N pool during N transformations. In most ecosystems, N in soil organic matter (SOM) is enriched in 15 N compared with atmospheric N₂ (Delwiche and Steyn 1970; Shearer et al. 1978; Karamanos et al. 1981). Fractionation during nitrification and denitrification alter natural soil 15 N, resulting in higher 15 N abundance deeper in soil profiles or in more thoroughly decomposed SOM (Nadelhoffer and Fry 1988). This pattern is reflected in soils under temperate forest (Riga et al. 1971; Nadelhoffer and Fry 1988) and pasture (Steele et al. 1981; Ledgard et al. 1984).

Because the ¹⁵N abundance of N from SOM and the atmosphere are different, plants that take up N derived from the decay of SOM have an ¹⁵N abundance which is higher than plants that derive their N uptake from both soil and atmospheric N sources. The natural abundance of ¹⁵N can be used as an indicator of the importance of N fixation to soil N stocks (Virginia and Delwiche 1982; Virginia et al. 1988; Shearer and Kohl 1989). Natural ¹⁵N abundance can also be used as an indicator of changes in N transformations and soil N sources that occur as a result of changes in land use. Several studies have examined land use changes from natural forest or grassland vegetation to cropland in the temperate zone (Mariotti et al. 1980; Karamanos et al. 1981).

Conversion of tropical forest to pasture is the most widespread land use change occurring in the Amazon Basin (Fearnside 1987). Pasture creation results in changes to soil inorganic N pools, net mineralization and nitrification rates (Luizão et al. 1992; Piccolo et al. 1994) and total soil N stocks (unpublished data). Pasture creation in the Amazon Basin may also change the sources of N to soil by the establishment of grasses known to be associated with free-living N fixing bacteria (Boddey and Dobereiner 1988). There is currently no information on how these changes in N transformations and potential soil N sources affect soil ¹⁵N values. This

paper reports on the natural abundance of ¹⁵N in total N in soil profiles from forests and pastures created from cleared forest in the Brazilian Amazon Basin. These data are used to suggest possible mechanisms for changes to soil N transformations and soil N sources that occur as a result of land use change.

Material and methods

Study sites

Field collections were made along two forest-to-pasture chronosequences in the western Brazilian Amazon state of Rondônia. One chronosequence was located at Fazenda (Ranch) Nova Vida, 50 km south east of Ariquemes, 472 km along BR-364 (10° 30' S, 62° 30' W). The Nova Vida chronosequence consisted of a forest tract and 3-, 5- and 20-year-old pasture. A second chronosequence was located at Fazenda Benjamin, in the Município of Ouro Preto do Oeste (10° 44' 30'' S, 62° 13' 30'' W), approximately 100 km south east of Nova Vida. It consisted of a forest tract and 8- and 20-year-old pasture.

Soils along both chronosequences were classified as Tropudults (Podzólicos Vermelho Amarelo distróficos in Brazilian classification). Forest vegetation of both chronosequences was typical of the open, humid tropical forests with large numbers of palms that dominate most of Rondônia. Approximately three or four large trees per hectare were removed by logging in each forest tract. At Fazenda Nova Vida, the 5- and 20- year-old pastures were dominated by *Brachiaria brizantha*, which made up >95% of the plant cover. The 3-year-old pasture was dominated by a mixture of *Panicum maximum*, *B. brizantha* and palms. *B. brizantha* and *P. maximum* are the most widely planted forages in the western Amazon region. No mechanical agricultural practices or chemical fertilizers were used on any of the pastures.

The climate of central Rondônia is humid tropical, with a dry season from May to September. Annual rainfall is approximately 2.2 m (Bastos and Diniz 1982). Mean annual maximum and minimum temperatures range from 24.4°C to 25.6°C and from 18.8°C to 20.3°C, respectively. Mean annual relative humidity is 89% (Bastos and Diniz 1982).

Field sampling

Soil samples were collected from each site along the chronosequences in the following way. One soil pit (approximately 0.8×1.5 m $\times 1.5$ m deep) was dug at each location. Soil was collected at Nova Vida during the dry season in July 1992 at depths of 0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm, 30-40 cm, 40-50 cm, 80-90 cm, and 120-130 cm in the pit. At Ouro Preto, soil was collected during the beginning of the wet season in September and October 1990 from soil pits at depths of 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm, 40-50 cm, 70-80 cm and 90-100 cm. Replicate samples to 30 cm depths were collected at all sites, from two points 25 m out in opposite directions from the large soil pit.

Samples of leaf litter and green leaf tissues of the dominant plants in forest and pasture sites along the Nova Vida chronosequence were collected in June and July 1992. To provide additional samples of green tissues of *P. maximum*, which is widely planted in Rondônia but not well represented along our Nova Vida chronosequence, we also collected samples from two additional sites, comprising 9- and 14-year-old pastures. We prepared one composite green tissue and one composite litter sample for each site. Each composite consisted of material collected over an approximately 0.25 ha area. Sample preparation and analysis

Litter samples were washed with de-ionized water to remove soil. All soil, plant and litter samples were oven dried (60°C) to constant mass, then ground to a fine powder for analysis of carbon and nitrogen, and ¹⁵N. The ¹⁵N was measured on an automated Finnigan MAT delta S mass spectrometer (Fry et al. 1992). The ¹⁵N abundance is expressed as delta values (‰), or deviations from standard reference materials.

 δ^{15} N=[($R_{sample}/R_{standard}$) - 1]×1000, where R=¹⁵N/¹⁴N and standard N in air (¹⁵N_{air}/¹⁴N_{air})=0.0036765.

Soil pH was determined in water (2.5:1). Elemental carbon and nitrogen of litter were determined on a Perkin Elmer 2400 analyzer. Carbon in plant tissues was determined on a Carmograph 12A analyzer. N in green plant tissues was determined by Kjeldahl digestion.

Comparison of mean δ^{15} N values were compared with Bonferroni t-tests using the GLM procedure of SAS.

Results

The $\delta^{15}N$ values of forest soils ranged from +9.8 to +13.6% (Fig. 1). The surface (0–10 cm) soil of the forest samples of both chronosequences was slightly depleted (approximately 1–2%) compared with deeper (>10 cm) layers (Fig. 1). The surface soil of pastures in both chronosequences had lower $\delta^{15}N$ values than forest soils, and there was a general trend toward lower $\delta^{15}N$ values

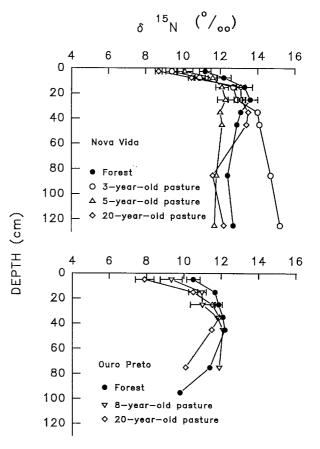


Fig. 1 The δ^{15} N values of soil in profiles from chronosequences consisting of forest and pastures of different ages from Nova Vida and Ouro Preto, Rondônia, in the western Brazilian Amazon Basin. Error bars represent ± 1 SE

| Table 1 Percentage carbon, percentage nitrogen and δ^{15} N of | green |
|--|-------|
| plant leaves and litter samples at Fazenda Nova Vida | • |

| | Percentage | | |
|---------------------------|------------|------|-----------------|
| | С | N | δ^{15} N |
| Nova Vida chronosequence | | | |
| Forest | | | |
| Leaves (mixed species) | 37.08 | 1.43 | +8.0 |
| Litter | 39.40 | 1.90 | +9.2 |
| Three-year-old pasture | | | |
| Leaves | | | |
| Brachiaria brizantha | 22.27 | 0.88 | +5.5 |
| Panicum maximum | 25.19 | 1.10 | +6.0 |
| Mixed litter | 36.35 | 0.72 | +8.3 |
| Five-year-old pasture | | | |
| Leaves (B. brizantha) | 24.10 | 1.02 | +4.0 |
| Mixed litter | 38.28 | 0.23 | +3.4 |
| Twenty-year-old pasture | | | |
| Leaves (B. brizantha) | 28.42 | 1.17 | +4.0 |
| Mixed litter | 40.10 | 0.27 | +3.2 |
| Additional plant samples | | | |
| Nine-year-old pasture | | | |
| Leaves (P. maximum) | 26.79 | 1.77 | +6.3 |
| Fourteen-year-old pasture | | | |
| Leaves (P. maximum) | 36.25 | 1.24 | +5.0 |

in older pastures. In the Nova Vida chronosequence, the oldest (20-year-old) pasture was more depleted in ¹⁵N at the surface (0–5 cm and 5–10 cm depth) than the forest (P<.05; Fig. 1). The Nova Vida 3- and 5-year-old pastures also showed a small (approximately 1–2%) shift compared with the forest (Fig. 1). The δ^{15} N values at 0–5 cm and 5–10 cm were not statistically different among pasture ages. Below 10 cm at Nova Vida, pasture soil δ^{15} N values did not differ from the original forest.

In the Ouro Preto chronosequence, 0-10 cm and 10-20 cm surface soil also had more depleted δ^{15} N values in the older pasture than in the forest (*P*<.05; Fig. 1). This chronosequence had the same pattern of lower δ^{15} N values in the older pasture, but the difference between pasture ages was not significant. Pasture and forest ¹⁵N values did not differ at the 20–30 cm depth. At all locations, spatial variation of ¹⁵N values within individual forest or pasture sites was low, with standard errors generally less than 1% (Fig. 1).

All sites in both chronosequences showed a pattern of increasing ¹⁵N values up to depths of 30 cm (Fig. 1). At greater depths, the δ^{15} N profiles of all sites were similar, with the exception of 3-year-old pasture at Nova Vida, which showed very high values of up to +15‰ (Fig. 1).

The δ^{15} N value of green leaves from forest trees (+8.0‰) was greater than the δ^{15} N values of pasture grasses (4.0–6.3‰; Table 1). The mean ¹⁵N values of *P. maximum* collected from Nova Vida (+5.7‰) was slightly greater than of *B. brizantha* (+4.5‰). Litter samples showed the same pattern of higher values for forest (+9.2‰) and 3-year-old pasture (8.3‰) compared with 5-year-old pasture (3.4‰) and 20-year-old pasture (+3.2‰).

Soil pH was higher in pasture than forest in both chronosequences (Fig. 2). Highest soil pH occurred in

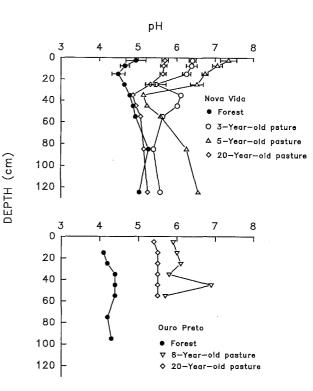


Fig. 2 Soil pH in profiles from forest and pastures along chronosequences at Nova Vida and Ouro Preto, Rondônia. Error bars represent ± 1 SE

the younger pastures of each sequence (3- and 5 year-old pastures at Nova Vida, 8-year-old pasture at Ouro Preto). Changes in soil pH were greatest at the soil surface (Fig. 2).

Discussion

Native forest

The δ^{15} N values of surface soil in native tropical forest in Rondônia were similar to values reported from scattered Brazilian forest locations, but higher than δ^{15} N values reported for a variety of temperate forests. Yoneyama et al. (1993) in Brazil found a soil δ^{15} N value at depths of 0-5 cm of 7.4% in a secondary tropical forest near Manaus, and 10.9% from a forest in Pará. In the temperate zone, Fry (1991) found $\delta^{15}N$ values from 0 to +8% in surface soils across a range of North American forests. In Californian forest soils, Broadbent et al. (1980) found δ^{15} N ranged from -0.4 to +3.8%. Nadelhoffer and Fry (1988) reported a value of +2.6% at 0–10 cm depth in Wisconsin soils. Mariotti et al. (1980) reported similar values for European forest soils. Slightly higher values, averaging +8.5%, were reported for surface soils from 13 widely scattered U.S. forest sites by Shearer et al. (1978). At depth, the tropical forest soils we examined also exhibited greater δ^{15} N values than known temperate forest soils (Shearer et al. 1978; Nadelhoffer and Fry 1988).

We observed the same general pattern of increasing $\delta^{15}N$ with depth that is widespread in temperate forest soils (Shearer et al. 1978). Two processes generally contribute to this pattern: (1) inputs to the soil surface of leaf litter that is typically depleted in ¹⁵N relative to soil (Mariotti et al. 1980; Nadelhoffer and Fry 1988), and (2) a fractionation during decomposition of litter and soil humus that results in the production of isotopically light ammonium and the gradual enrichment of residual soil organic matter (Turner et al. 1983; Melillo et al. 1989; Nadelhoffer and Fry 1994).

The $\delta^{15}N$ of organic matter in the top several centimeters of forest soil is more similar to plant litter than deeper soil layers, because a large proportion of the organic matter near the soil surface has been recently formed from decomposition of litter inputs (Nadelhoffer and Fry 1988). Downward movement of organic matter, smaller organic matter particle size and lower C:N ratio all contribute to increasing enrichment of soil $\delta^{15}N$ with depth (Nadelhoffer and Fry 1994). Our finding of a 2–3‰ depletion of $\delta^{15}N$ in green leaves and leaf litter compared with forest surface soil is consistent with this general pattern.

All microbially mediated soil N transformations offer opportunities for isotopic fractionation, and typically result in products that are depleted in δ^{15} N relative to substrates (Delwiche and Steyn 1970). The δ^{15} N inorganic N mineralized from SOM is typically less than that of soil N (Feigen et al. 1974; Ledgard et al. 1984). Discrimination against ¹⁵N during nitrification produces nitrate that is depleted in ¹⁵N and residual ammonium enriched in ¹⁵N (Létolle 1980; Herman and Rundel 1989). If soil nitrification rates are high and soil nitrate pools accumulate, additional enrichment can occur as nitrate depleted in ¹⁵N is lost by leaching. Denitrification can further fractionate the soil nitrate pool, resulting in releases of N_2 and N_2O that are depleted in ¹⁵N, and leaving behind enriched residual nitrate (Nadelhoffer and Fry 1994). Nitrate losses occurring either by leaching or denitrification can contribute to the overall enrichment of the residual SOM N pool. Higher substrate pools and incomplete substrate consumption during nitrification and denitrification generally result in greater fractionation than occurs during periods when substrate is limiting and substrate consumption is near 100% (Feigen et al. 1974).

We hypothesize that fractionation associated with nitrification and denitrification and selective losses of ¹⁵Ndepleted nitrate from tropical forest soils, account for the high δ^{15} N values of the Rondônia tropical forest soil relative to temperate forest soils. Nitrification rates in tropical forest soils may be substantial. We have measured net nitrification rates in laboratory incubations of the same Rondônia forest at Nova Vida which are of the order of 1 µg/g dry soil/day during the dry season, and 10 µg/g dry soil/day during the wet season (unpublished data). Net nitrification rates in the range of 0.3–3.5 µg/g dry soil/day have been measured at other tropical forest sites (Luizão et al. 1992; Robertson 1984). Tropical forest soil nitrate pools may also be high, ranging from 3 to more than 20 μ g/g dry soil (Robertson 1984; Matson and Vitousek 1987). Although nitrification can consume all of the available mineralized ammonium (Robertson 1984), nitrification is often incomplete. Piccolo et al. (1994), in laboratory incubations, found that 79% of mineralized N was nitrified during the dry season in the Rondônia forest. The conditions in tropical forest soils that support nitrification and create elevated soil nitrate pools can also accelerate nitrate losses, either through denitrification or leaching (Uhl and Jordan 1984; Robertson and Tiedje 1988). High nitrification rates, elevated soil nitrate pools, incomplete nitrification of mineralized inorganic N and potentially important rates of denitrification in tropical forests all provide conditions for substantial N isotope fractionation.

Patterns of δ^{15} N abundance in soil can suggest relationships between forest N cycling and soil development. In Hawaiian volcanic soils, Vitousek et al. (1989) found low δ^{15} N values on very young (197-year-old) soils with low soil N content, slow N cycling rates, a high reliance in N inputs in precipitation and tight cycling of mineral N. In contrast, δ^{15} N values increased on older (60 000year-old) soils, where reliance on precipitation for meeting plant demands was reduced, and both N cycling rates and N losses were greater (Vitousek et al. 1989). We suggest that in highly weathered tropical forest soils of the Brazilian Amazon Basin, even relatively small losses of nitrate and a small degree of preferential loss of ¹⁴N relative to ¹⁵N could potentially result in a strong enrichment of remaining N in SOM over the time scale of soil development.

The pattern of slightly lower δ^{15} N values at depth (ca. 1 m) in both chronosequences may result from illuvial inputs that make up a large fraction of a relatively small total N pool at that depth. This pattern of lower soil δ^{15} N values in deep soil (0.5–5 m) compared with surface soil have been reported where total soil N pools at depth are low and pools are derived largely from illuvial inputs (Delwiche and Steyn 1970; Karamanos and Rennie 1978).

Effects of forest conversion to pasture

Conversion of forest to pasture induces dramatic changes to vegetation, soil physical and chemical properties and soil carbon and N stocks (Hecht 1982; Veldkamp 1993) which could potentially influence soil N cycling. The large increases of soil pH that we found in pastures compared with forest were similar to those observed after slashing and burning tropical forest for agriculture in other locations (Brinkmann and Nascimento 1973; Hecht 1982). There are also potentially large changes in N cycling rates between forest and pastures. Along the same Nova Vida chronosequence in Rondônia, Piccolo et al. (1994) found higher nitrate than ammonium pools and higher rates of net N mineralization and net nitrification in native forest soils compared with 3-, 9- and 20-yearold pasture soils. In pasture soils, ammonium dominated inorganic N pools and a variable percentage of the mineralized ammonium (27–96%) was nitrified (Piccolo et al. 1994).

The largest effect of pasture creation on soil δ^{15} N values was a depletion in the surface soil of the oldest pastures. The same general pattern of δ^{15} N values increasing with depth resembled the pattern in the forests, and has been found in other pasture soils (Steele et al. 1981; Ledgard et al. 1984). The absence of any consistent differences below 20 cm suggests a shallow active layer for processes that affect δ^{15} N values. Forest soils, 5- and 20year-old pastures from Nova Vida and 8- and 20-year-old pastures from Ouro Preto showed a slight decrease of δ^{15} N at 100 cm compared with 20 cm, while the δ^{15} N value of 3-year-old pasture at Nova Vida continued to increase up to depths greater than 100 cm. We cannot explain the differences of δ^{15} N values at depth among pastures, although other investigators have reported similar decreases of δ^{15} N values deeper in the soil profile, where N concentrations are low and where illuviation is the dominant source of the soil N pool (Delwiche and Steyn 1970; Karamanos and Rennie 1978). Differences in δ^{15} N value at depth among forests and pastures may reflect both the rate of fractionation in the upper soil horizons and rates of N illuviation.

In contrast to the pattern of lower δ^{15} N values in the surface soil of pastures that we found in Rondônia, the δ^{15} N of temperate grassland or cultivated soils is often more enriched than that of the forest soils from which they were derived (Riga et al. 1971; Mariotti et al. 1980). Other studies have observed no or very small changes in total soil δ^{15} N, at a variety of forest and pasture sites, with time of cultivation (Tiessen et al. 1984) or time following pasture improvement (Ledgard et al. 1984). The depleted δ^{15} N of forest surface soils is likely to be the result of heavy and continued inputs of low $\delta^{15}N$ litter (Mariotti et al. 1980; Nadelhoffer and Fry 1988). Soils under forest trees can have lower $\delta^{15}N$ values than those in open areas, presumably as the result of litter decomposition (Shearer and Kohl 1986). Our findings indicated that the reverse occurred in tropical forests and pastures, and that forest plant litter $\delta^{15}N$ was enriched 2–3% relative to pasture plant litter, and that forest surface soil was enriched by a similar amount relative to pasture soil.

Several mechanisms could explain the lower δ^{15} N values in the older pastures relative to the original forest. Nitrogen fixation by free-living bacteria associated with planted grasses in pastures, and greater cumulative atmospheric-derived N input in older pastures, is consistent with this pattern. There is evidence that associations with free-living N fixing bacteria occur in soil of pastures vegetated with the forage grasses widely planted in the Amazon Basin (Boddey and Dobereiner 1988), but there are also indications that fixation does not occur in many locations (Yoneyama et al. 1993). The long-lasting increases in pH caused by burning of forest slash during pasture formation are associated with short-term increases in phosphorus availability (Falesi 1976; Hecht 1982), which may create an environment more conducive to N

fixation in recently formed pastures. The changes in $\delta^{15}N$ values from forest to pasture could also be caused by differences in the $\delta^{15}N$ of inorganic N assimilated by plants along the chronosequence. These could arise from differences in plant rooting depths, differences in the ¹⁵N values of inorganic N pools caused by differential N turnover in forest and pasture, or differential utilization of soil ammonium and nitrate pools. Our data do not allow us to distinguish between these mechanisms.

The results presented here suggest that conversion of forest to pasture is accompanied by important changes in soil N sources, but the factors that control plant N availability in pastures created from converted tropical moist forest are poorly understood. Manipulations of pasture soils and measurement of N fixation rates, and the δ^{15} N values of soil N pools, can provide further insights into the N dynamics and fertility of these increasingly important tropical ecosystems.

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