

## Article

# Fine Root and Soil Nitrogen Dynamics during Stand Development Following Shifting Agriculture in Northeast India

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Received: 26 October 2020; Accepted: 20 November 2020; Published: 24 November 2020



**Abstract:** Nitrogen (N) dynamics during changes in land use patterns in tropical forests may profoundly affect fine root dynamics and nutrient cycling processes. Variations in fine root biomass and soil N dynamics were assessed in developing stands of increasing ages following shifting agriculture in Mizoram, Northeast India, and comparisons were made with a natural forest stand. Concentrations of soil available N ( $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ ) and the proportion of  $\text{NH}_4\text{-N}$  in total available N increased with stand age. The N-mineralization rate also increased with stand age whilst the proportion of nitrification relative to ammonification declined during succession. Fine root biomass and N-mineralization increased, and available N decreased during the monsoon season while this pattern was reversed during the winter season. A greater proportion of fine roots were  $<0.5$  mm diameter in the younger sites, and turnover of fine roots was more rapid in the developing stands compared to the natural forest. Fine root biomass was correlated positively with N-mineralization rate and soil water content. Thus, it can be concluded that the fine root growth was aided by rapid N-mineralization, and both fine root growth and N-mineralization increase as stands redevelop following shifting cultivation disturbance.

**Keywords:** fine roots; forest fallow; N-mineralization; nitrification; nitrogen availability

## 1. Introduction

Landscape transformations are rapidly occurring in tropical regions due to anthropogenic activities that alter ecosystem structure and functioning including nutrient cycling and other below-ground processes [1–3]. Slash-and-burn and the subsequent shifting agriculture is a common land-use in many parts of the tropics [4], including in Mizoram in Northeast India [5,6]. During the shifting cultivation process, forests are cut down and burned, and crops are grown for one or a few years depending on the soil nutrient status, after which the land is left fallow and allowed to restore natural vegetation and soil nutrients. The sustainability of this land-use depends upon the successful replenishment of nutrient stocks during the fallow phase. During forest burning, many of the nutrients within the biomass are returned to the soil as ash; however, large amounts of topsoil are lost through runoff on the steep slopes due to torrential monsoon rains following slash burning [6]. Biomass burning further leads to particulate and volatilization losses of both nitrogen (N) and phosphorus (P), although the extent of this loss appears to depend upon soil type [7,8].

Nitrogen mineralization plays a major role in plant N availability [9] and critically affects ecosystem productivity; indeed, N is the most important limiting nutrient in many plant

communities [10,11]. In particular, tropical forests recovering from disturbance, as well as tropical montane forests, show conservative N cycling processes [12–14]. During subsequent stand development, there is a recovery of soil N, but it remains limiting for some years [14]. However, soil N cycling is a complex process, and little attention has been paid to an in-depth understanding of soil N dynamics during stand development following shifting cultivation.

Fine roots constitute only a small proportion of the total biomass in a forest ecosystem, but they play a crucial role in nutrient dynamics by efficient absorption of nutrients and enrichment of the soil with organic matter and nutrients through their senescence [3,15,16]. Fine root biomass depends on a range of factors such as soil moisture, nutrient availability, climatic conditions and stand characteristics [17–19]. Following shifting cultivation, root biomass declines [20], and it is important to assess the recovery of below-ground biomass as it is linked to the recovery of soil nutrients and above-ground biomass. A general pattern shows an increase in root biomass with stand age [21] with variations across sites due to local factors [22]. Fine roots are the foremost component of the plants which are in contact with the soil during stand development; because of soil N limitation in younger stands, the functioning of these roots is critical in affecting stand development. For example, fine roots have been reported to have a key effect on soil carbon (C) and nutrient restoration in developing stands following severe soil disturbance in Northeast India [3,23]. Therefore, the mechanisms controlling fine root dynamics, and their linkages with soil N availability, need to be studied to better understand forest C and nutrient dynamics, particularly following forest disturbances such as shifting cultivation.

Consequently, the main objectives of this study were to (1) assess seasonal changes in soil available N and rates of N-mineralization, (2) quantify changes in fine root biomass and growth dynamics and (3) understand the relationship between fine root growth and soil nutrients during stand development following shifting agriculture in forests in Northeast India. Specifically, we hypothesize that soil N will exhibit seasonal changes as well as being influenced by forest stand development and that these will interact to influence root biomass and growth dynamics.

## 2. Materials and Methods

### 2.1. Study Sites

The study was conducted in the Mamit district of Mizoram, India, with an elevation range of 970 to 1275 m a.m.s.l. (23°42' N; 92°37' E). Different aged stands developing following shifting agriculture in Ailawng village (i.e., 3, 5, 15 and 40 years) and a protected natural forest (>100 years) in Reiek village were selected. All stands were within 5 km of one another and were similar with respect to topography, aspect, slope and soil type (Inceptisols); they were all 0.5 to 1 ha in size. The climate of the area is typically monsoonal with distinct seasons: cold and dry winter (December–February), warm premonsoon (March–June), humid monsoon period (July–September) and cool postmonsoon (October–November). The mean annual rainfall is ca. 2350 mm. The forest vegetation falls under three major categories: tropical wet evergreen forest, tropical semi-evergreen forest and subtropical pine forest [24].

### 2.2. Vegetation Sampling

In all the stands, vegetation was sampled by five randomly placed quadrats of 10 m × 10 m for woody species of ≥10 cm circumference at breast height (1.3 m), as there were many small stems particularly in the younger stands, and ten quadrats of 1 m × 1 m for herbaceous vegetation. Density and basal area of each species were determined following methods proposed by Misra [25].

### 2.3. Soil Sampling and Analysis

Soil samples from 0 to 30 cm depth were collected (using a cylindrical soil corer having an inner diameter of 4.2 cm) at bimonthly intervals between December 2014 and December 2015 from three random locations within each of the five quadrats at each site and were subsequently composited

to provide a single replicate per quadrat. Each composited field-moist sample was then divided into two parts: one part was transported to the laboratory for the determination of available N, total C and N, soil moisture content, pH and texture, and the other part was incubated in situ for the estimation of N-mineralization rates. Soil bulk density was measured using a metallic tube of known inner volume and recording the dry weight (105 °C for 48 h) of soil within. Total C and N were determined by using a Heraeus CHNSO elemental analyzer (Hanau, Germany). Soil temperature was recorded bimonthly using a soil thermometer at a depth of ca. 12 cm. Soil texture was determined by Bouyoucos hydrometer method [26], and pH was determined using a combined glass electrode in a 1:2.5 suspension of soil:water. Soil moisture content was determined gravimetrically by oven-drying the samples at 105 °C for 48 h to constant weight.

#### 2.4. Determinations of Available Nitrogen and N-Mineralization Rate

Field-moist soils sieved through a 2-mm mesh were used for the analysis of mineral N, i.e., nitrate ( $\text{NO}_3\text{-N}$ ) and ammonium ( $\text{NH}_4\text{-N}$ ). Soil samples were analyzed immediately after collection; if not, they were kept in a deep freezer (−15 °C) for analysis within 24 h. Nitrate was measured by the phenol disulfonic acid method using 1 N  $\text{CuSO}_4$  as the soil extractant (soil:solution ratio of 1:5) [27], and  $\text{NH}_4\text{-N}$  was estimated by the phenate method using 2 M KCl as the soil extractant (soil:solution ratio of 1:5) [28]. The N-mineralization rate was measured in situ by the buried bag technique [29] whereby three soil samples (each about 150 g) enclosed in polythene bags were buried at 0 to 30 cm depth in each stand and incubated for a period of one month; in order to avoid N immobilization during the incubation, plant parts were carefully removed. Nitrate and  $\text{NH}_4\text{-N}$  were determined initially (at time zero) and after recovery of the buried bags; the increase in  $\text{NH}_4\text{-N}$  was considered as ammonification, the increase in  $\text{NO}_3\text{-N}$  was considered as nitrification, and their sum was considered as net N-mineralization.

#### 2.5. Sampling and Analysis of Fine Roots

Fine roots were sampled using cuboid soil core monoliths (10 cm × 10 cm × 30 cm deep); five randomly located cores within the quadrats were collected at bimonthly intervals (December 2014 to December 2015). The core samples were stored at −15 °C before being washed over a multiple sieve system. Live and dead roots were separated on the basis of cohesion between the cortex and periderm and color: dead roots were dark and spongy while live roots were brown and firm [30]. The fine roots were categorized into two diameter classes (<0.5 mm and 0.5 to 2.0 mm) and oven-dried at 80 °C for 24 h to constant mass. Net fine root production was estimated as the sum of all the apparent biomass increments for each size class over the course of one year [30,31], and fine root turnover was calculated as the ratio of annual net production to annual mean biomass [3].

#### 2.6. Statistical Analysis

One-way analysis of variance (ANOVA) was used to determine the influence of stand age on ecological parameters, whereas two-way ANOVA was used to determine the effects of season and stand age on soil water content, available N and N-mineralization rates and fine root mass and production with Tukey's honest significant difference tests performed for comparisons of mean values (significance at  $p < 0.05$ ). Correlations (Pearson's) between soil water content and N availability and fine root biomass were conducted within stands. Statistical analyses were carried out using SPSS 16.0 (IBM, Chicago, IL, USA).

### 3. Results

#### 3.1. Vegetation

Species composition varied with the age of the developing stands (Supplementary Table S1). The density and basal area of woody species was an order of magnitude greater in the older stands

than the younger stands reaching a maximum in the natural forest; by contrast, the density of ground vegetation was reduced in the older stands by about one-third (Table 1).

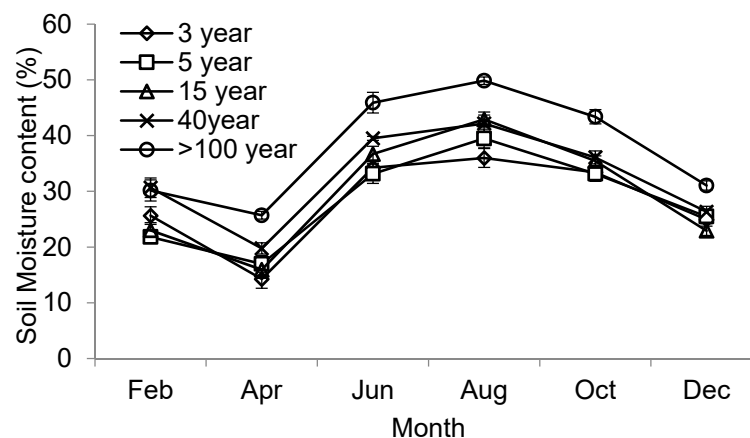
**Table 1.** Stand structural characteristics (mean  $\pm$  SE) of developing stands following shifting agriculture and a natural forest (NF) in Mizoram, Northeast India.

|                                                    | Stand Age (Years)           |                             |                             |                             |                             |
|----------------------------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                                                    | 3                           | 5                           | 15                          | 40                          | NF                          |
| Tree density (no. ha <sup>-1</sup> )               | 399 $\pm$ 36 <sup>a</sup>   | 933 $\pm$ 88 <sup>b</sup>   | 1690 $\pm$ 124 <sup>c</sup> | 2290 $\pm$ 169 <sup>d</sup> | 4290 $\pm$ 281 <sup>e</sup> |
| Tree basal area (m <sup>2</sup> ha <sup>-1</sup> ) | 2.1 $\pm$ 0.5 <sup>a</sup>  | 6.9 $\pm$ 0.8 <sup>b</sup>  | 12.3 $\pm$ 1.3 <sup>c</sup> | 41.4 $\pm$ 4.8 <sup>d</sup> | 64.7 $\pm$ 6.2 <sup>e</sup> |
| Herb density (no. m <sup>-2</sup> )                | 39.1 $\pm$ 3.4 <sup>b</sup> | 35.4 $\pm$ 3.6 <sup>b</sup> | 33.9 $\pm$ 4.2 <sup>b</sup> | 28.2 $\pm$ 2.9 <sup>a</sup> | 27.6 $\pm$ 3.1 <sup>a</sup> |

Different letters within a row indicate significant differences with  $p < 0.05$ .

### 3.2. Soil Physicochemical Properties

The soils of all study sites were loamy sand in texture (except the 3-year-old stand that was sandy loam) and acidic in reaction (pH 4.5 to 4.8). Soil bulk density was less in the older stands while soil moisture content was greater and showed clear seasonal variation (Figure 1). Soil C and N were not significantly different between the young stands (<40 years) but were significantly greater in the natural forest (Table 2). Soil pH and temperature did not vary between stands (Table 2).



**Figure 1.** Seasonal variation in soil moisture content (Stand age:  $F = 4.60$ ,  $p < 0.05$ ; Season:  $F = 7.60$ ,  $p < 0.001$ ) of developing stands following shifting agriculture and a natural forest (>100 years) in Mizoram, Northeast India. All values are mean  $\pm$  SE.

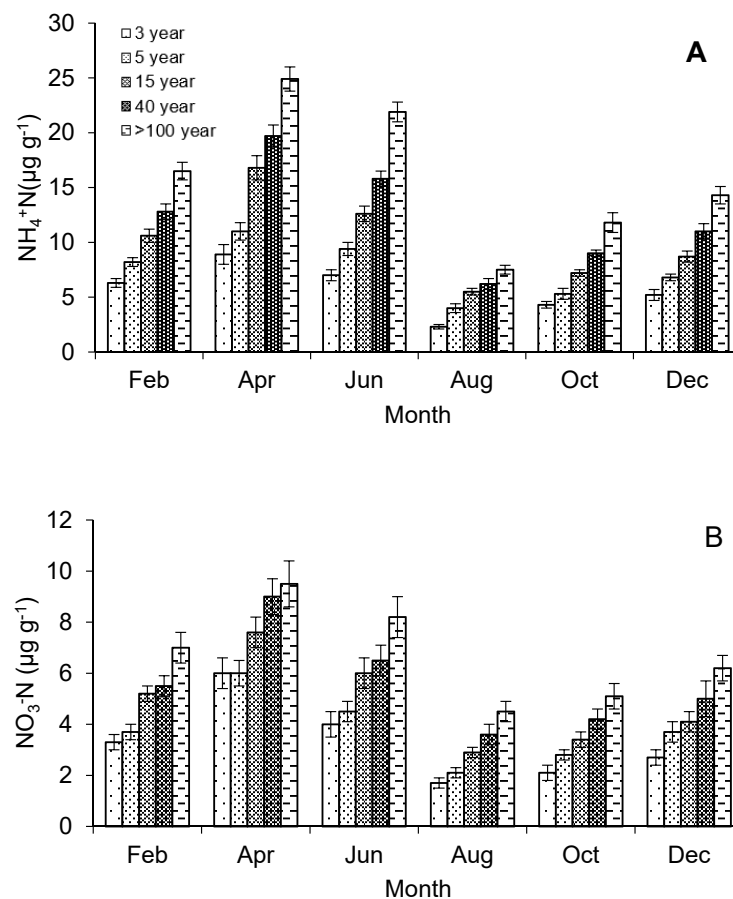
**Table 2.** Soil (0–30 cm depth) physicochemical characteristics (mean  $\pm$  SE) in developing stands following shifting agriculture and a natural forest (NF) in Mizoram, Northeast India.

|                                    | Stand Age (Years)            |                              |                              |                              |                              |
|------------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
|                                    | 3                            | 5                            | 15                           | 40                           | NF                           |
| Soil temperature (°C) *            | 20.6 $\pm$ 1.1 <sup>a</sup>  | 20.4 $\pm$ 0.9 <sup>a</sup>  | 19.8 $\pm$ 1.2 <sup>a</sup>  | 19.1 $\pm$ 0.7 <sup>a</sup>  | 18.7 $\pm$ 1.0 <sup>a</sup>  |
| Bulk density (g cm <sup>-3</sup> ) | 0.97 $\pm$ 0.03 <sup>a</sup> | 0.92 $\pm$ 0.03 <sup>b</sup> | 0.80 $\pm$ 0.02 <sup>c</sup> | 0.80 $\pm$ 0.05 <sup>c</sup> | 0.69 $\pm$ 0.05 <sup>d</sup> |
| Textural class                     | Sandy<br>Loam                | Loamy<br>Sand                | Loamy<br>Sand                | Loamy<br>sand                | Loamy<br>Sand                |
| Moisture content (%) *             | 29.7 $\pm$ 1.1 <sup>a</sup>  | 30.1 $\pm$ 1.6 <sup>a</sup>  | 30.4 $\pm$ 0.8 <sup>a</sup>  | 31.8 $\pm$ 1.2 <sup>a</sup>  | 41.3 $\pm$ 1.2 <sup>b</sup>  |
| pH in water                        | 4.6 $\pm$ 0.2 <sup>a</sup>   | 4.8 $\pm$ 0.4 <sup>a</sup>   | 4.8 $\pm$ 0.4 <sup>a</sup>   | 4.7 $\pm$ 0.1 <sup>a</sup>   | 4.5 $\pm$ 0.2 <sup>a</sup>   |
| C (mg g <sup>-1</sup> ) *          | 23.4 $\pm$ 0.3 <sup>a</sup>  | 28.2 $\pm$ 0.3 <sup>a</sup>  | 26.5 $\pm$ 0.3 <sup>a</sup>  | 27.3 $\pm$ 0.3 <sup>a</sup>  | 44.1 $\pm$ 0.4 <sup>b</sup>  |
| N (mg g <sup>-1</sup> ) *          | 2.3 $\pm$ 0.02 <sup>a</sup>  | 2.4 $\pm$ 0.02 <sup>a</sup>  | 2.0 $\pm$ 0.03 <sup>a</sup>  | 2.2 $\pm$ 0.02 <sup>a</sup>  | 3.3 $\pm$ 0.3 <sup>b</sup>   |
| C:N ratio                          | 10.2 $\pm$ 0.6 <sup>a</sup>  | 11.8 $\pm$ 1.3 <sup>a</sup>  | 13.2 $\pm$ 0.9 <sup>a</sup>  | 12.4 $\pm$ 1.1 <sup>a</sup>  | 13.4 $\pm$ 0.9 <sup>a</sup>  |

\* Mean of bimonthly samples across the year (n = 30: 6 time points  $\times$  5 replicates). Different letters within a row indicate significant differences with  $p < 0.05$ .

### 3.3. Available N Concentrations

Soil mineral N concentrations were lowest in the 3-year-old stand and highest in the natural forest. The difference was around 3-fold and 2-fold for  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ , respectively, between the 3-year-old stand and the natural forest (Table 3). Seasonal variations were also observed in the mineral N concentrations in all stands; maximum values were recorded in April (premonsoon season) and minimum values were recorded in August (monsoon season) (Figure 2). In all stands,  $\text{NH}_4\text{-N}$  was the dominant form of N, but the  $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$  ratio was greater in the older stands (Table 3).



**Figure 2.** Seasonal variation in available (A)  $\text{NH}_4\text{-N}$  (Stand age:  $F = 38.8$ ,  $p < 0.001$ ; Season:  $F = 50.1$ ,  $p < 0.001$ ) and (B)  $\text{NO}_3\text{-N}$  (Stand age:  $F = 90.6$ ,  $p < 0.001$ ; Season  $F = 114$ ,  $p < 0.001$ ) in soils of developing stands following shifting agriculture and a natural forest (>100 years) in Mizoram, Northeast India. All values are mean  $\pm$  SE.

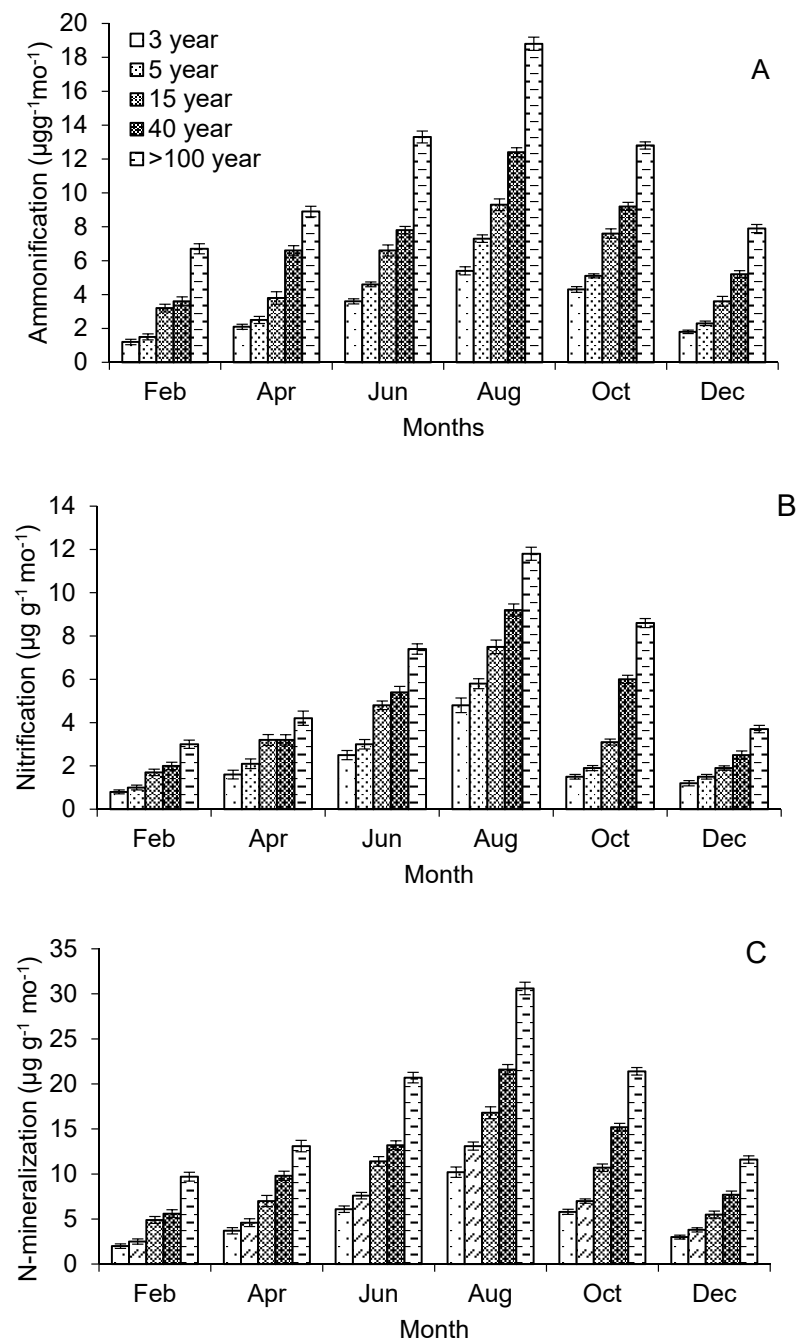
**Table 3.** Available N and N-mineralization rates (mean  $\pm$  SE) in soils of developing stands following shifting agriculture and a natural forest (NF) in Mizoram, Northeast India.

|                                                              | Stand Age (Years) |                     |                    |                    |                  |
|--------------------------------------------------------------|-------------------|---------------------|--------------------|--------------------|------------------|
|                                                              | 3                 | 5                   | 15                 | 40                 | NF               |
| $\text{NH}_4\text{-N}$ ( $\mu\text{g g}^{-1}$ ) *            | $5.7 \pm 0.5^a$   | $7.4 \pm 0.6^{ab}$  | $10.2 \pm 0.6^b$   | $12.4 \pm 0.5^b$   | $16.1 \pm 0.8^c$ |
| $\text{NO}_3\text{-N}$ ( $\mu\text{g g}^{-1}$ ) *            | $3.3 \pm 0.4^a$   | $3.8 \pm 0.3^{ab}$  | $4.8 \pm 0.4^{ab}$ | $5.6 \pm 0.5^{bc}$ | $6.7 \pm 0.6^c$  |
| $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$                  | $1.7 \pm 0.1^a$   | $1.9 \pm 0.05^{ab}$ | $2.1 \pm 0.04^b$   | $2.2 \pm 0.1^b$    | $2.4 \pm 0.1^c$  |
| Ammonification ( $\mu\text{g g}^{-1} \text{ month}^{-1}$ ) * | $3.1 \pm 0.1^a$   | $3.9 \pm 0.1^a$     | $5.7 \pm 0.3^{ab}$ | $7.5 \pm 0.2^b$    | $11.4 \pm 0.3^c$ |
| Nitrification ( $\mu\text{g g}^{-1} \text{ month}^{-1}$ ) *  | $2.1 \pm 0.2^a$   | $2.6 \pm 0.1^a$     | $3.7 \pm 0.2^a$    | $4.7 \pm 0.3^{ab}$ | $6.5 \pm 0.3^b$  |
| N-mineralization ( $\mu\text{g g}^{-1} \text{ month}^{-1}$ ) | $5.2 \pm 0.3^a$   | $6.5 \pm 0.2^a$     | $9.4 \pm 0.5^b$    | $12.2 \pm 0.5^b$   | $17.9 \pm 0.6^c$ |
| Ammonification: nitrification                                | $1.5 \pm 0.2^a$   | $1.5 \pm 0.2^a$     | $1.5 \pm 0.2^a$    | $1.6 \pm 0.1^a$    | $1.8 \pm 0.1^a$  |

\* Mean of bimonthly samples across the year ( $n = 30$ ; 6 time points  $\times$  5 replicates). Different letters within a row indicate significant differences with  $p < 0.05$ .

### 3.4. Nitrification and N-Mineralization Rate

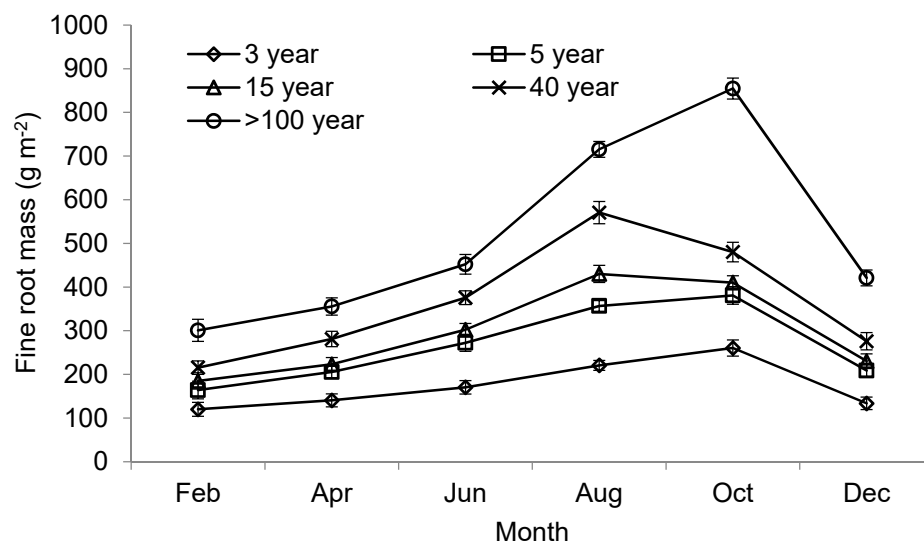
Nitrogen mineralization rates (both ammonification and nitrification) were lowest in the 3-year-old stand and highest in natural forest, varying about 3-fold (Table 3). Mineralization also exhibited a distinct seasonal pattern in all the stands; the maximum rates were reached in August (monsoon season), after which mineralization declined and reached minimum rates in February (winter season) (Figure 3). The ammonification:nitrification ratio ranged between 1.5 and 1.8 (Table 3), indicating the dominance of ammonification over nitrification in all stands.



**Figure 3.** Seasonal variation in (A) ammonification (Stand age:  $F = 61.8$ ,  $p < 0.001$ ; Season:  $F = 35.6$ ,  $p < 0.001$ ), (B) nitrification (Stand age:  $F = 21.6$ ,  $p < 0.001$ ; Season:  $F = 16.0$ ,  $p < 0.001$ ) and (C) N-mineralization (Stand age:  $F = 127$ ,  $p < 0.001$ ; Season:  $F = 101$ ,  $p < 0.001$ ) rates in soils of developing stands following shifting agriculture and a natural forest (>100 years) in Mizoram, Northeast India. All values are mean  $\pm$  SE.

### 3.5. Fine Root Biomass

Fine root biomass and necromass of different size classes varied with stand age. The total fine root mass (i.e., biomass + necromass, <2 mm) increased from  $174.3 \pm 15.1$  (SE)  $\text{g m}^{-2}$  in the 3-year-old stand to  $516.6 \pm 21.2$   $\text{g m}^{-2}$  in the natural forest; biomass (i.e., live roots) consisted of about 60% of the total fine root mass. The proportion of very fine (<0.5 mm) roots to the total root (<2 mm) biomass was about one-third in the youngest stand to one-fifth to one-quarter the older stands. Seasonal variation was shown in the fine root mass in all the stands: maximum fine root mass was recorded during August or October (monsoon or postmonsoon season), and the minimum fine root mass was recorded in February (winter season) (Figure 4). Fine root production and turnover were also faster in older stands (Table 4). In all the developing stands and natural forest, fine root biomass was negatively correlated with the total available N but positively, and significantly (for the 5-, 15- and 40-year-old stands), correlated with N-mineralization rates. Soil moisture in all the stands was positively correlated with fine root biomass but not significantly (Table 5).



**Figure 4.** Seasonal variation in fine root mass (<2 mm) in developing stands following shifting agriculture and a natural forest (>100 years) in Mizoram, Northeast India (Stand age:  $F = 28.1$ ,  $p < 0.001$ ; Season:  $F = 19.6$ ,  $p < 0.001$ ). All values are mean  $\pm$  SE.

**Table 4.** Fine root mass of different size classes, annual fine root mass, fine root production and root turnover (all values are mean  $\pm$  SE) in developing stands following shifting agriculture and a natural forest in Mizoram, Northeast India.

| Fine Root Parameter                                |               | Stand Age (Years)  |                       |                    |                    |                    |
|----------------------------------------------------|---------------|--------------------|-----------------------|--------------------|--------------------|--------------------|
|                                                    | Diameter (mm) | 3                  | 5                     | 15                 | 40                 | >100               |
| Biomass ( $\text{g m}^{-2}$ )                      | <0.5          | $34.4 \pm 3.0^a$   | $49.0 \pm 6.6^b$      | $38.5 \pm 11.0^a$  | $45.0 \pm 14.1^b$  | $87.1 \pm 12.6^c$  |
|                                                    | 0.5–2         | $68.3 \pm 13.3^a$  | $101.9 \pm 10.8^a$    | $134.9 \pm 12.8^b$ | $169.8 \pm 14.5^b$ | $242.4 \pm 20.3^c$ |
| Necromass ( $\text{g m}^{-2}$ )                    | <0.5          | $40.9 \pm 4.4^a$   | $64.8 \pm 7.8^b$      | $56.1 \pm 9.88^b$  | $84.7 \pm 13.3^c$  | $103.9 \pm 11.4^d$ |
|                                                    | 0.5–2         | $30.7 \pm 5.7^a$   | $49.0 \pm 8.2^a$      | $67.3 \pm 10.9^b$  | $66.9 \pm 9.7^b$   | $83.2 \pm 6.8^c$   |
| Total mass ( $\text{g m}^{-2}$ )                   | <2            | $174.3 \pm 15.1^a$ | $264.7 \pm 16.4^a$    | $296.8 \pm 16.8^a$ | $366.4 \pm 19.4^b$ | $516.6 \pm 21.2^b$ |
| Very fine (<0.5 mm):                               | NA            |                    |                       |                    |                    |                    |
| total (<2 mm) biomass ratio                        |               | $0.33 \pm 0.03^b$  | $0.32 \pm 0.03^b$     | $0.22 \pm 0.02^a$  | $0.21 \pm 0.02^a$  | $0.26 \pm 0.01^a$  |
| Production ( $\text{g m}^{-2} \text{ year}^{-1}$ ) | <2            | $140.4 \pm 7.2^a$  | $216.7 \pm 12.5^{ab}$ | $244.9 \pm 14.7^b$ | $355.1 \pm 24.1^c$ | $553.8 \pm 27.2^c$ |
| Turnover ( $\text{year}^{-1}$ )                    | <2            | $0.81 \pm 0.07^a$  | $0.82 \pm 0.06^a$     | $0.83 \pm 0.11^a$  | $0.97 \pm 0.13^b$  | $1.07 \pm 0.09^b$  |

Different letters within a row indicate significant differences with  $p < 0.05$ .

**Table 5.** Correlations between fine root biomass (<2 mm) and soil properties in soils of developing stands following shifting agriculture and a natural forest (NF) in Mizoram, Northeast India. All values are Pearson's correlation coefficients with significance shown as \* =  $p < 0.05$  and \*\* =  $p < 0.01$ .

| Stand Age<br>(Years) | Soil Property |                 |               |                |                  |
|----------------------|---------------|-----------------|---------------|----------------|------------------|
|                      | Soil Moisture | Total Mineral N | Nitrification | Ammonification | N-Mineralization |
| 3                    | 0.535         | −0.619          | 0.487         | 0.882 *        | 0.727            |
| 5                    | 0.732         | −0.683          | 0.616         | 0.908 *        | 0.803 *          |
| 15                   | 0.729         | −0.665          | 0.739         | 0.972 **       | 0.898 *          |
| 40                   | 0.499         | −0.643          | 0.978 **      | 0.977 **       | 0.981 **         |
| NF                   | 0.689         | −0.705          | 0.827 *       | 0.727          | 0.773            |

#### 4. Discussion

In a variety of forest ecosystems, fine roots contribute significantly (20% to 77%) to organic matter input [32], and therefore they constitute a significant pathway for the input of C and N in the soil. Total fine root mass in this study was comparable to that found in other reports from tropical forests of India [3,33–35] and increased with stand age, in agreement with other studies in boreal [36] and temperate [37] forests. The variation in fine root biomass among the developing stands and natural forest can be attributed to differing soil nutrient and moisture content, species composition and stand above-ground biomass and tree density [18]. Marked seasonality of fine roots in the present study is related to the changing temperature and precipitation patterns during the year and has been reported in tropical as well as temperate forests [36,38,39]. The greater proportion of very fine roots (<0.5 mm) in younger stands likely represents a strategy to produce such roots rapidly following disturbance to enhance foraging for resources within the greatest possible soil volume from a nutrient-limited condition and, in turn, return organic matter to the soil to enhance plant growth.

There are several studies showing declines in fine root biomass in tropical forests with increases in soil N [22,40,41], although reports of experimental inorganic N additions in forest stands have given mixed results [42] and depend, at least partly, on the dominant species in the stand [43]. Whilst fine root biomass was negatively correlated with available N, there were strong positive correlations with the N-mineralization rate. The positive correlation of N-mineralization with fine roots is in contrast with the reports of Aber et al. [44] for 13 forest stands of Wisconsin and Massachusetts (northern United States) where the root biomass was negatively correlated with nitrification. The relationship between fine roots and N-mineralization in our stands could be mediated by water availability, but N is more important than water overall in this tropical forest with an annual precipitation of more than 2000 mm. We hypothesize that fine roots in our study experienced N limitation and thus positively responded to N-mineralization to acquire more available N to enhance the productivity of developing stands, whereas they were negatively correlated under saturated conditions as in Aber et al. [44]. The negative correlation of fine root biomass with N availability indicates that plants take up greater amounts of N for rapid growth during the rainy season, consequently depleting the soil mineral N. In contrast, available N increased due to reduced fine root biomass and N uptake by the plants during the dry period. Reduced N uptake may also be attributed to the occurrence of deciduous species in all the stands that will be dormant during dry periods. Mineralized N is either immobilized in microbial biomass or accumulated in the soil, resulting in a greater mineral N pool during the premonsoon in comparison to the monsoon season [45]. Roy and Singh [46] found a positive relationship between fine root biomass and N-mineralization rates in a dry tropical forest of northern India, concurring with this study. Production and turnover of fine roots were lowest in the youngest stand and highest in the natural forest. This can be attributed to the change in soil properties, such as increased available N, and altered species composition and biomass with stand age [23,47–49]. The presence of a greater proportion of very fine roots in the young stand is an efficient strategy to enhance the absorption of available nutrients through the exploration of large soil volumes and enables stand recovery following

disturbance [50]. Slower turnover of fine roots in the young stands was counter to expectations but could be a nutrient conservation mechanism where nutrients are in shorter supply.

The recovery of the N cycle with increasing stand age following disturbance showed a pattern similar to that seen in many other tropical forests [14,51,52], albeit not all [53], with increases in soil mineral N concentrations along with increased N-mineralization rates during succession. The increasing dominance in  $\text{NH}_4\text{-N}$  over  $\text{NO}_3\text{-N}$  with older stand age reflects a change in N cycling through the loss or rapid uptake of  $\text{NO}_3\text{-N}$  in initial successional stages followed by accumulation of  $\text{NH}_4\text{-N}$  in later successional stages leading to a “tighter” N cycle. Whilst both nitrification and ammonification increased during succession, disturbance often results in a decline in the  $\text{NH}_4\text{-N}:\text{NO}_3\text{-N}$  ratio, and this ratio then increases as the ecosystem approaches maturity [54,55]. Early work by Rice and Pancholy [54] suggested that nitrifying bacteria are inhibited under climax conditions; hence,  $\text{NH}_4\text{-N}$  is not rapidly oxidized to nitrate as occurs in the earlier successional stages. Other reasons for changes in the balance between nitrification and ammonification could include competition for limited nutrient resources, availability of substrate, reduced populations of nitrifying bacteria and edaphic conditions including acidic pH unfavorable to autotrophic nitrifiers [56]. Robertson and Vitousek [57] reported that the number of ammonia-oxidizing bacteria was less in mature forest soils than the number found in earlier stages along a secondary sere in North America, and it would therefore be valuable to assess changes in the microbial community involved in the nitrogen cycle using molecular tools in future work [58].

## 5. Conclusions

In conclusion, young forest stands recovering from disturbance due to shifting cultivation had low soil mineral N pools as well as N-mineralization rates. These then increased, along with fine root biomass and production, facilitated by increases in plant biomass. Increased rates of N-mineralization and the mineral N pools were found in older recovering forests, as well as greater fine root mass, whose growth was correlated with N-mineralization. Furthermore, seasonal dynamics in soil N cycling were correlated with fine root dynamics influencing temporal changes on a yearly basis. Longer fallow periods facilitate the restoration of these disturbed forests through biogeochemical recovery of the ecological processes therein.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/1999-4907/11/12/1236/s1>, Table S1: Tree species composition ( $\geq 10$  cm circumference at breast height; 1.3 m) in a natural forest and developing stands following shifting agriculture in Mizoram, Northeast India.

**Author Contributions:** Conception/design, D.S. and S.K.T., acquisition, analysis and interpretation of data: D.S., F.Q.B. and S.K.T.; drafted/revised text, D.S., F.Q.B. and S.K.T. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Council of Scientific and Industrial Research, Government of India, grant number 8/1322/12.

**Acknowledgments:** Financial assistance from the Council of Scientific and Industrial Research, Government of India, is thankfully acknowledged.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Tripathi, S.K.; Kushwaha, C.P.; Singh, K.P. Tropical forest and savanna ecosystems show differential impact of N and P additions on soil organic matter and aggregate structure. *Glob. Chang. Biol.* **2008**, *14*, 2572–2581. [CrossRef]
2. Tripathi, S.K.; Singh, K.P.; Singh, P.K. Temporal changes in spatial pattern of fine-root mass and nutrient concentrations in Indian bamboo savanna. *Appl. Veg. Sci.* **1999**, *2*, 229–238. [CrossRef]
3. Lalnunzira, C.; Brearley, F.Q.; Tripathi, S.K. Root growth dynamics during recovery of tropical mountain forest in North-east India. *J. Mt. Sci.* **2019**, *16*, 2335–2347. [CrossRef]
4. Cairns, M.F. *Shifting Cultivation and Environmental Change*; Routledge: Abingdon, UK, 2015.

5. Grogan, P.; Lalnunmawia, F.; Tripathi, S.K. Shifting cultivation in steeply sloped regions: A review of management options and research priorities for Mizoram state, Northeast India. *Agrofor. Syst.* **2012**, *84*, 163–177. [\[CrossRef\]](#)
6. Tripathi, S.K.; Vanlalfakawma, D.C.; Lalnunmawia, F. Shifting cultivation on steep slopes of Mizoram, India: Impact of policy reforms. In *Shifting Cultivation Policies: Balancing Environmental and Social Sustainability*; Cairns, M., Ed.; CABI Publishing: Wallingford, UK, 2017; pp. 393–413.
7. Kauffman, J.B.; Sanford, R.L.; Cummings, D.L.; Salcedo, I.H.; Sampaio, E.V.S.B. Biomass and nutrient dynamics associated with sash fires in Neotropical dry forests. *Ecology* **1993**, *74*, 140–151. [\[CrossRef\]](#)
8. Filho, A.A.R.; Adams, C.; Manfredini, S.; Aguilar, R.; Neves, W.A. Dynamics of soil chemical properties in shifting cultivation systems in the tropics: A meta-analysis. *Soil Use Manag.* **2015**, *31*, 474–482. [\[CrossRef\]](#)
9. Booth, M.S.; Stark, J.M.; Rastetter, E. Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. *Ecol. Monogr.* **2005**, *75*, 139–157. [\[CrossRef\]](#)
10. Lebauer, D.S.; Treseder, K.K. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* **2008**, *89*, 371–379. [\[CrossRef\]](#)
11. Ostertag, R.; DiManno, N.M. Detecting terrestrial nutrient limitation: A global meta-analysis of foliar nutrient concentrations after fertilization. *Front. Earth Sci.* **2016**, *4*, 23. [\[CrossRef\]](#)
12. Walker, T.; Syers, J. The fate of phosphorus during pedogenesis. *Geoderma* **1976**, *15*, 1–19. [\[CrossRef\]](#)
13. Vitousek, P.M. Litterfall, nutrient cycling, and nutrient limitation in tropical forests. *Ecology* **1984**, *65*, 285–298. [\[CrossRef\]](#)
14. Davidson, E.A.; De Carvalho, C.J.R.; Figueira, A.M.; Ishida, F.Y.; Ometto, J.P.H.B.; Nardoto, G.B.; Sabá, R.T.; Hayashi, S.N.; Leal, E.C.; Vieira, I.C.G.; et al. Recuperation of nitrogen cycling in Amazonian forests following agricultural abandonment. *Nature* **2007**, *447*, 995–998. [\[CrossRef\]](#)
15. Silver, W.L.; Miya, R.K. Global patterns in root decomposition: Comparisons of climate and litter quality effects. *Oecologia* **2001**, *129*, 407–419. [\[CrossRef\]](#)
16. Tripathi, S.K.; Sumida, A.; Ono, K.; Shibata, H.; Uemura, S.; Takahashi, K.; Hara, T. The effects of understorey dwarf bamboo (*Sasa kurilensis*) removal on soil fertility in a *Betula ermanii* forest of northern Japan. *Ecol. Res.* **2005**, *21*, 315–320. [\[CrossRef\]](#)
17. Yuan, Z.Y.; Chen, H.Y.H. Fine root biomass, production, turnover rates, and nutrient contents in boreal forest ecosystems in relation to species, climate, fertility, and stand age: Literature review and meta-analyses. *Crit. Rev. Plant Sci.* **2010**, *29*, 204–221. [\[CrossRef\]](#)
18. Finér, L.; Ohashi, M.; Noguchi, K.; Hirano, Y. Factors causing variation in fine root biomass in forest ecosystems. *For. Ecol. Manag.* **2011**, *261*, 265–277. [\[CrossRef\]](#)
19. Keyes, M.R.; Grier, C.C. Above- and below-ground net production in 40-year-old Douglas-fir stands on low and high productivity sites. *Can. J. For. Res.* **1981**, *11*, 599–605. [\[CrossRef\]](#)
20. Wapongnungsang; Tripathi, S. Fine root growth and soil nutrient dynamics during shifting cultivation in tropical semi-evergreen forests of northeast India. *J. Environ. Biol.* **2019**, *40*, 45–52. [\[CrossRef\]](#)
21. Hertel, D.; Leuschner, C.; Harteveld, M.; Wiens, M. Fine root mass, distribution and regeneration in disturbed primary forests and secondary forests of the moist tropics. In *The Stability of Tropical Rainforest Margins: Linking Ecological, Economic and Social Constraints of Land Use and Conservation*; Tschardtke, T., Leuschner, C., Zeller, M., Guhardja, E., Bidin, A., Eds.; Springer: Berlin, Germany, 2007; pp. 87–106.
22. Brearley, F.Q. Below-ground secondary succession in tropical forests of Borneo. *J. Trop. Ecol.* **2011**, *27*, 413–420. [\[CrossRef\]](#)
23. Lalnunzira, C.; Tripathi, S.K. Leaf and root production, decomposition and carbon and nitrogen fluxes during stand development in tropical moist forests, north-east India. *Soil Res.* **2018**, *56*, 306. [\[CrossRef\]](#)
24. Champion, H.G.; Seth, S.K. *A Revised Survey of the Forest Types of India*; Manager of Publications, Government of India: New Delhi, India, 1968.
25. Misra, R. *Ecology Work Book*; Oxford & IBH Publishing Co.: New Delhi, India, 1968.
26. Bouyoucos, G.J. Directions for making mechanical analyses of soils by the hydrometer method. *Soil Sci.* **1936**, *42*, 225–230. [\[CrossRef\]](#)
27. Jackson, M.L. *Soil Chemical Analysis*; Prentice-Hall Inc.: Englewood Cliffs, NJ, USA, 1958.
28. Wetzel, R.G.; Likens, G.E. *Limnological Analyses*; W. B. Saunders Company: Philadelphia, PA, USA, 1979.
29. Eno, C.F. Nitrate production in the field by incubating the soil in polyethylene bags. *Soil Sci. Soc. Am. J.* **1960**, *24*, 277–279. [\[CrossRef\]](#)

30. Persson, H. Root dynamics in a young Scots pine stand in central Sweden. *Oikos* **1978**, *30*, 508. [[CrossRef](#)]
31. McClaugherty, C.; Aber, J.D.; Melillo, J.M. The role of fine roots in the organic matter and nitrogen budgets of two forested ecosystems. *Ecology* **1982**, *63*, 1481–1490. [[CrossRef](#)]
32. Vogt, K.; Grier, C.; Vogt, D. Production, Turnover, and Nutrient Dynamics of Above- and Belowground Detritus of World Forests. *Adv. Ecol. Res.* **1986**, *15*, 303–377. [[CrossRef](#)]
33. Arunachalam, A.; Pandey, H.N.; Tripathi, R.S.; Maithani, K. Biomass and production of fine and coarse roots during regrowth of a disturbed subtropical humid forest in north-east India. *Vegetatio* **1996**, *123*, 73–80. [[CrossRef](#)]
34. Upadhyaya, K.; Pandey, H.N.; Law, P.S.; Tripathi, R.S. Dynamics of fine and coarse roots and nitrogen mineralization in a humid subtropical forest ecosystem of northeast India. *Biol. Fertil. Soils* **2005**, *41*, 144–152. [[CrossRef](#)]
35. Barbhuiya, A.R.; Arunachalam, A.; Pandey, H.N.; Khan, M.L.; Arunachalam, K. Fine root dynamics in undisturbed and disturbed stands of a tropical wet evergreen forest in northeast India. *Trop. Ecol.* **2012**, *53*, 69–79.
36. Yuan, Z.Y.; Chen, H.Y. Fine root dynamics with stand development in the boreal forest. *Funct. Ecol.* **2012**, *26*, 991–998. [[CrossRef](#)]
37. Jagodziński, A.M.; Kałucka, I. Fine root biomass and morphology in an age-sequence of post-agricultural *Pinus sylvestris* L. stands. *Dendrobiology* **2011**, *66*, 71–84.
38. Harris, W.F.; Kinerson, R.S., Jr.; Edwards, N.T. Comparison of belowground biomass of natural deciduous forest and loblolly pine plantations. In *The Belowground Ecosystem: A Synthesis of Plant-Associated Processes*; Marshall, J.K., Ed.; Colorado State University: Fort Collins, CO, USA, 1977; pp. 29–38.
39. Srivastava, S.K.; Singh, K.P.; Upadhyay, R.S. Fine root growth dynamics in teak (*Tectona grandis* Linn. F.). *Can. J. For. Res.* **1986**, *16*, 1360–1364. [[CrossRef](#)]
40. Maycock, C.R.; Congdon, R.A. Fine root biomass and soil N and P in north Queensland rain forests. *Biotropica* **2000**, *32*, 185–190. [[CrossRef](#)]
41. Powers, J.S.; Treseder, K.K.; Lerdau, M.T. Fine roots, arbuscular mycorrhizal hyphae and soil nutrients in four neotropical rain forests: Patterns across large geographic distances. *New Phytol.* **2004**, *165*, 913–921. [[CrossRef](#)]
42. Yuan, Z.Y.; Chen, H.Y.H. A global analysis of fine root production as affected by soil nitrogen and phosphorus. *Proc. Royal Soc. Lond. Ser. B Biol. Sci.* **2012**, *279*, 3796–3802. [[CrossRef](#)]
43. Phillips, R.P.; Fahey, T.J. Fertilization effects on fineroot biomass, rhizosphere microbes and respiratory fluxes in hardwood forest soils. *New Phytol.* **2007**, *176*, 655–664. [[CrossRef](#)]
44. Aber, J.D.; Melillo, J.M.; Nadelhoffer, K.J.; McClaugherty, C.; Pastor, J. Fine root turnover in forest ecosystems in relation to quantity and form of nitrogen availability: A comparison of two methods. *Oecologia* **1985**, *66*, 317–321. [[CrossRef](#)]
45. Singh, J.S.; Raghubanshi, A.S.; Srivastava, S.C. Microbial biomass acts as a source of plant nutrients in dry tropical forest and savanna. *Nature* **1989**, *338*, 499–500. [[CrossRef](#)]
46. Roy, S.; Singh, J. Seasonal and spatial dynamics of plant-available N and P pools and N-mineralization in relation to fine roots in a dry tropical forest habitat. *Soil Biol. Biochem.* **1995**, *27*, 33–40. [[CrossRef](#)]
47. Hertel, D.; Harteveld, M.A.; Leuschner, C. Conversion of a tropical forest into agroforest alters the fine root-related carbon flux to the soil. *Soil Biol. Biochem.* **2009**, *41*, 481–490. [[CrossRef](#)]
48. Singh, S.B.; Mishra, B.P.; Tripathi, S.K. Recovery of plant diversity and soil nutrients during stand development in subtropical forests of Mizoram, Northeast India. *Biodiversitas* **2015**, *16*, 205–212. [[CrossRef](#)]
49. Singha, D.; Tripathi, S.K. Variations in fine root growth during age chronosequence of moist tropical forest following shifting cultivation in Mizoram, northeast India. *Trop. Ecol.* **2017**, *58*, 769–779.
50. Liu, B.; Li, H.; Zhu, B.; Koide, R.T.; Eissenstat, D.M.; Guo, D. Complementarity in nutrient foraging strategies of absorptive fine roots and arbuscular mycorrhizal fungi across 14 co-existing subtropical tree species. *New Phytol.* **2015**, *208*, 125–136. [[CrossRef](#)]
51. Amazonas, N.T.; Martinelli, L.A.; Piccolo, M.D.C.; Rodrigues, R.R. Nitrogen dynamics during ecosystem development in tropical forest restoration. *For. Ecol. Manag.* **2011**, *262*, 1551–1557. [[CrossRef](#)]
52. Myllemngap, W.; Nath, D.; Barik, S.K. Changes in vegetation and nitrogen mineralization during recovery of a montane subtropical broadleaved forest in North-eastern India following anthropogenic disturbance. *Ecol. Res.* **2015**, *31*, 21–38. [[CrossRef](#)]

53. Winbourne, J.B.; Feng, A.; Reynolds, L.; Piotto, D.; Hastings, M.G.; Porder, S. Nitrogen cycling during secondary succession in Atlantic Forest of Bahia, Brazil. *Sci. Rep.* **2018**, *8*, 1–9. [[CrossRef](#)]
54. Rice, E.L.; Pancholy, S.K. Inhibition of nitrification by climax ecosystems. *Am. J. Bot.* **1972**, *59*, 1033–1040. [[CrossRef](#)]
55. Walley, F.; Van Kessel, C.; Pennock, D. Landscape-scale variability of N mineralization in forest soils. *Soil Biol. Biochem.* **1996**, *28*, 383–391. [[CrossRef](#)]
56. Sahrawat, K.L. Factors affecting nitrification in soils. *Commun. Soil Sci. Plant Anal.* **2008**, *39*, 1436–1446. [[CrossRef](#)]
57. Robertson, G.P.; Vitousek, P.M. Nitrification potentials in primary and secondary succession. *Ecology* **1981**, *62*, 376–386. [[CrossRef](#)]
58. Pajares, S.; Bohannon, B.J.M. Ecology of nitrogen fixing, nitrifying, and denitrifying microorganisms in tropical forest soils. *Front. Microbiol.* **2016**, *7*, 1045. [[CrossRef](#)]

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