Nitrogen Release Dynamics of Erythrina Abyssinica and Erythrina Brueci Litters as Influenced by Polyphenol, Lignin and Nitrogen Contents

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Abstract
Litter mineralization is a crucial process in providing nutrients through decomposition to plants. The processes depend on the chemical composition of the litter and soil properties as well. Not only the initial chemical composition of litter such as lignin, ADF, cellulose and total polyphenol content controls decomposition of the litter but also the ratios of lignin, ADF, cellulose and total polyphenol have a role in the processes. To measure the effect of chemical composition of Erythrina abyssinica and Erythrina brucei incorporated into Luvisol, an incubation pot experiment, in complete randomized design in replication, were conducted. The nutrient release dynamics was measured in terms of their capacity to release ammonium and nitrate in the mineralization process. The samples of Erythrina abyssinica and Erythrina brucei were ground and incorporated with Luvisol in pots. Erythrina abyssinica has an average of 4.05%, 9.7% and 2.04% total nitrogen, lignin and total polyphenol content respectively. Erythrina brucei has also an average of 3.05 %, 12.63 % and 1.05 % content of Total Nitrogen, lignin and total polyphenol. Treated and control Samples were collected and analyzed on weekly basis to determine the amount of ammonium and nitrate released. The result showed that the lignin and total polyphenol content significantly, positively correlated with the release of ammonium, while the nitrate showed significant negative correlations with the release of ammonium. From the experiment, the Erythrina abyssinica with lower content of lignin and high in TN has released the nutrients faster where as Erythrina brucei with high lignin and low total polyphenol content released slowly. In general, these leguminous trees released ammonium and nitrate easily because of their high total nitrogen content and low lignin, ADF, cellulose and total polyphenol content. They attained their half-life within 2–3 weeks. Erythrina abyssinica and Erythrina brucei shows fast mineralization; as a result they can be used for fast-term correction of crop nutrient demand. Furthermore, intensive field experiment on the effect of Erythrina abyssinica and Erythrina brucei biomass on yields and capacity to amend soil quality shall be studied in depth.

Keywords: Incubation, Lignin, Luvisol, Total Polyphenol, Nitrate.

1.0 INTRODUCTION
Incorporation of agroforestry legumes trees to the soil is an important management practice to enhance the fertility of the soil. Plant litter mineralization is biological disintegration of litter by microorganisms and transfer of organic and mineral compounds to the soil (Loranger et al., 2002). Studies on Ethiopian soil and forest revealed that the very wide range of climate, topography, parent material and microorganisms’ and the local conditions of the specific areas have assisted in development of different soil types as well as over 6,600 higher plant species including indigenous leguminous and non-leguminous trees (Mesfin, 1998; MoA, 2000; Mohammed, 2003; Bekele, 2007). According to MoA (2000), Luvisol is one of the soil types estimated to cover 64,063.5 km² or 5.8% of the country. In the future agricultural system, application of organic farming systems is important in order to lessen impacts of climate change, decrease soil erosion (Reganold, et al., 1987), improve biodiversity (Hole, et al., 2005), and enhance soil fertility (Watson, et al., 2002).

Organic farming incorporates researched outputs with ecology and cultural agricultural practices based on naturally occurring microbial processes (Shi, 2013). Among the potentially valuable plant species used for organic farming, Erythrina abyssinica and Erythrina brucei, are leguminous trees with immense use. Erythrina abyssinian fixes Nitrogen in the roots infected by Rhizobia nodulate (Legesse, 2002) while Erythrina brucei fixes Nitrogen in the leaves with leaf symbiotic N-fixing characteristics (Legesse, 2002, Orwa, et al., 2009). The application of these organic nutrient resources can also be by directly incorporation in to the soil and allow it to decompose and release nutrients. Nevertheless, the initial lignin, total carbon, total nitrogen, cellulose, polyphenol and phosphorus, potassium concentrations and ratios of carbon/nitrogen (C/N), lignin/N, lignocellulose/N, and lignin: N governs litter decomposition rates.

One of the major decomposition retarding chemical composition of litter is lignin. It represents nearly 30% of the carbon sequestered in plant materials annually (Boerjan et al., 2003). Lignin is a complex organic polymer used in the support of tissues of vascular plants and some algae as well as in formation of cell walls (Martone, et al., 2009; Lebo, et al., 2001).

The second mineralization retarding chemical constituents are Phenolic compounds. These compounds constituting up to 60% of plant dry mass (Cates and Rhoades, 1997). These consist of more than one aromatic
ring, bearing one or more hydroxyl functional groups. Once integrated into the soil, it controls the belowground processes, including soil organic matter decomposition and nutrient cycling (Rovira and Vallejo, 2002; Toberman, et al., 2010). As outlined by Zhang et al., (2008) the litter chemicals compositions and their ratio are strong predictors of litter decay, which accounts for over 73% of the variation in litter decomposition rates. At large, the reset parameters that can affect the degradation of litter depends on environmental factors, season and soil types and properties, such as soil pH, temperature, humidity, oxygen, bulk density and particle size.

In order to manage and predict the nutrients released from organic residues for crop uptake there is need to understand the N mineralization dynamics of the litter in relation to their major chemical composition under specific soil type. Leaf litters of different plant species has diverse nutrient release patterns, related to quality, season, and environmental factors (Arunachalam et al., 2003, Abiven et al., 2005).

In this study in order to comprehend the effects of litter quality on N release or the dynamics by which *Erythrina abyssinian* and *Erythrina brucei* mineralization rate is affected by chemical constituents such as lignin, cellulose, polyphenol, and TN content were studied through incubation. The experiment was designed to evaluate and correlate the release of $\text{NH}_4^+$ and $\text{NO}_3^-$ in Luvisol with chemical constituents.

Moreover, the study investigation the influence of different sites on the chemical composition (N, lignin and polyphenol) of the present leguminous tree species under investigation.

### 2.0 MATERIALS AND METHODS

#### 2.1 Description of the Sampling Sites

Sidama and Wolaita zones found in southern part of Ethiopian. Sidama covers 6972.1 km$^2$ and lies between 6°14' to 7°18' N and 37°92' to 39°19'' E. It has an elevation ranging from 502 to 3000 m.a.s.l. The annual mean temperature of the zone ranges between 10.5 to 27.1 °C and the annual mean rainfall ranges from 801 to 1600 mm (HMD, 2015). Wolaita covers an area of 4471.3 km$^2$. The zone lies on an elevation ranging from 1200 to 2950 m.a.s.l. with annual average temperature of 15.0 °C to 28 °C. The area has a bimodal rainfall pattern, with an average annual rainfall of 1300 to 2000 mm distributed over 8 to 9 months (HMD, 2015).

#### 2.2 Leaf and Soil sampling

Based on soil colour, collection of samples of *Erythrina abyssinian* and *Erythrina brucei* from ten different randomly selected but geo-referenced locations of Sidama and Wolaita zones of southern Ethiopia were done. (Tables 1 and 2). Representative, surface soil samples was collected from Dystric Luvisol.

<table>
<thead>
<tr>
<th>Site</th>
<th>N E</th>
<th>masl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aleta Wondo</td>
<td>06º 35’48.2'' 038º 25’36.6''</td>
<td>1941</td>
</tr>
<tr>
<td>Aleta Wondo</td>
<td>06º 34’53.2'' 038º 26’39.3''</td>
<td>2234</td>
</tr>
<tr>
<td>Aleta Wondo</td>
<td>06º 34’ 56.7'' 038º 23’53.7 ''</td>
<td>2483</td>
</tr>
<tr>
<td>Titecha</td>
<td>06º 33’ 28.2'' 038º 31’28.5 ''</td>
<td>2686</td>
</tr>
<tr>
<td>Hula</td>
<td>06º 29’26.1’’ 038º 30’45.3’’</td>
<td>2767</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site</th>
<th>N E</th>
<th>masl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delbo Atwero</td>
<td>06º 54’ 34.2’’ 37º 49’ 04.0’’</td>
<td>2236</td>
</tr>
<tr>
<td>Doga</td>
<td>06º 58’ 26’’ 37º 52’ 25.7’’</td>
<td>1975</td>
</tr>
<tr>
<td>Gacheno</td>
<td>07º 02’ 37.7’’ 37º 55’ 33’’</td>
<td>1884</td>
</tr>
<tr>
<td>Kokote</td>
<td>06º 52’ 37.7’’ 37º 35’ 33’’</td>
<td>2154</td>
</tr>
<tr>
<td>Shone</td>
<td>07º 09’ 34.3’’ 37º 57’ 25.5’’</td>
<td>1996</td>
</tr>
</tbody>
</table>

#### 2.3 Selected Soil Physical Analysis

For the soil particle size analysis hydrometer method Bouyoucos (1951) was used. The bulk density of the sample was determined from sample collected using core ring sampler. The plant available soil water holding capacity was determined after determining the Field capacity and Permanent Wilting Point of the Luvisol as described by Hillel D., (1980).

#### 2.4 Selected Soil Chemical Analyses

Soil pH and electrical conductivity were measured using soil: water (1:2.5) as described by Reeuwijk, (2002). Soil Organic carbon content and Available P were determined using Walkley-Black (1934) chromic acid wet oxidation method and Olsen and Sommer (1982) based on alkaline extraction by 0.5 Normal NaHCO$_3$ Methods respectively. The total N content in the soil was determined according to Reeuwijk, (2002). Mineral N (NH$_4^+$ and NO$_3^-$) was extracted at a ratio of 1:4 (soil: 2M KCl) and determined according to Keeney and Nelson (1982).
2.5 Determination of Lignin and Cellulose via Acid Detergent Fiber (ADF)
Klasson method in Ref. Browning (1967) was selected for the analysis of lignin and cellulose via ADF. ADF is prepared from ONS material by boiling with sulphuric acid solution of cetyltrimethyl ammonium bromide (CTAB) under controlled condition. The CTAB dissolves nearly all nitrogenous constituents, and the acid hydrolyses the starch to residue containing lignin, cellulose and ash Clancy and Wilson, (1966). Cellulose is destroyed by 72% H2SO4; lignin is then determined by weight-loss upon ashing.

2.6 Incubation of Soil Samples
From the composited soil two hundred gram for each pot was weighed and mixed with the plant material followed by fully homogenizing with 0.127 g and 0.169 g of Erythrina abyssinica and Erythrina brucei respectively in complete random design in replication (Figure 1). Then watering to field capacity was made every day or two until the end of the experiment. Every week from each treatment and control were sampled and analyzed on weekly bases to determine the amount of ammonium and nitrate released.

![Figure 1: partial view of the Incubation Experiment set up in greenhouse](image)

2.7 Statistical Analyses
The data obtained from the laboratory analyses of litters and mineralization were subjected to analysis of variance (ANOVA) using statistical analysis software. The least significant difference (LSD) was worked to separate means at p ≤ 0.05 using Duncan Multiple Range Test. Simple correlation analysis (at p ≤ 0.05) was carried out to measure release of nutrients (Ammonium and Nitrate) in soil.

3.0 RESULTS AND DISCUSSION
3.1 Phsico-chemical property of Soil used for mineralization
The soil particle size analysis of experiment soil sample was found to be clayey. The critical bulk density value for agricultural use according to Hillel (1980) is 1.4 g cm⁻³, implying that there is no excessive compaction and restriction to root development. The bulk density of the Luvisol was 1.23 g cm⁻³. As described by Werner (1997) the soils in this range possesses good porosity for aerobic microorganisms’ activities. According to Landon, (1996) rating the OC and TN contents of the soil fall in the “very low” and “low” categorized respectively. The soil is also rated as “low” in Available p; according to Havlin, et al., (2010) rating. Since the soil is at low pH, it possesses Phosphorus fixation as well (Table 3).

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>pH-H2O</th>
<th>Av.P</th>
<th>TN</th>
<th>OC</th>
<th>C/N</th>
<th>BD</th>
<th>FC</th>
<th>PWP</th>
<th>Sand</th>
<th>clay</th>
<th>Silt</th>
<th>class</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20</td>
<td>4.98</td>
<td>5.32</td>
<td>0.16</td>
<td>1.76</td>
<td>11</td>
<td>1.23</td>
<td>46.20</td>
<td>31.55</td>
<td>14</td>
<td>32</td>
<td>54</td>
<td>clay</td>
</tr>
</tbody>
</table>

3.2 Litter quality rating of E. abyssinica and E. brucei with respect to altitude
In this study, the analyses of variance for Erythrina abyssinica and Erythrina brucei showed significant variation in TN content with altitude and sampling site.

Sangiga and Woomer (2009) indicated that organic nutrients sources with higher than 2.5% TN content to be considered as a high quality organic nutrient sources. Thus, the average TN content of Erythrina abyssinica
(4.05%) and *Erythrina brucei* (3.36%) are categorized with the highest N$_2$ fixing plants (Table 4). The highest TN content mean value for *Erythrina abyssinica* was obtained at 1941 m.a.s.l (5.13%) and the lowest mean value (3.26%) was found at 2234 m.a.s.l (Table 5). Whereas the highest mean value of TN content (3.93%) of *Erythrina brucei* was obtained at 2154 m.a.s.l and the lowest mean value (2.77%) at 1996 (Table 6).

As shown on the table (Table 5 and 6) the variation in TN content of *Erythrina brucei* and *Erythrina abyssinica* can be due to cumulative contribution of micro agro climatic factors: soil fertility, temperature, and microorganism, in addition to the differences in altitude. The existence of different soil orders might have contributed to the difference in percent TN content.

Table 4: Major chemical constituents of *Erythrina abyssinica* and *Erythrina brucei*

<table>
<thead>
<tr>
<th>Plant type</th>
<th>TN (%)</th>
<th>K (%)</th>
<th>P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Erythrina abyssinica</em></td>
<td>4.05$^a$ (3.16-5.16)</td>
<td>2.02$^b$ (1.94-2.08)</td>
<td>0.39$^a$ (0.36-0.43)</td>
</tr>
<tr>
<td><em>Erythrina brucei</em></td>
<td>3.36$^b$ (2.70-3.93)</td>
<td>2.61$^b$ (2.54-2.68)</td>
<td>0.31$^b$ (0.30-0.32)</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>0.70</td>
<td>0.36</td>
<td>0.05</td>
</tr>
<tr>
<td>CV (%)</td>
<td>13.01</td>
<td>6.32</td>
<td>8.01</td>
</tr>
</tbody>
</table>

*Note: Means in a column followed by the same superscript letters are not significantly different.

**Values in brackets shows range**

Table 5: *Erythrina abyssinica* sampling sites and their total nitrogen content

<table>
<thead>
<tr>
<th>Site</th>
<th>Altitude (m.a.s.l)</th>
<th>Erythrina abyssinica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bultima</td>
<td>1941</td>
<td>5.13$^a$ (5.16-5.08)</td>
</tr>
<tr>
<td>Tubito</td>
<td>2234</td>
<td>3.26$^d$ (3.16-3.31)</td>
</tr>
<tr>
<td>Kila</td>
<td>2483</td>
<td>4.21$^b$ (3.85-4.31)</td>
</tr>
<tr>
<td>Aridessa</td>
<td>2686</td>
<td>3.67$^c$ (3.62-3.70)</td>
</tr>
<tr>
<td>Hagereselam</td>
<td>2767</td>
<td>3.98$^b$ (3.85-4.08)</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td></td>
<td>0.29</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>3.97</td>
</tr>
</tbody>
</table>

*Note: Means in a column followed by the same superscript letters are not significantly different.

**Values in brackets shows range**

Table 6: *Erythrina brucei* sampling sites and their total nitrogen content

<table>
<thead>
<tr>
<th>Site</th>
<th>Altitude (m.a.s.l)</th>
<th>Erythrina brucei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delbo Atwero</td>
<td>2236</td>
<td>3.85$^e$ (3.77-3.93)</td>
</tr>
<tr>
<td>Doga</td>
<td>1975</td>
<td>3.38$^b$ (3.31-3.47)</td>
</tr>
<tr>
<td>Gacheno</td>
<td>1884</td>
<td>2.85$^c$ (2.70-3.00)</td>
</tr>
<tr>
<td>Kokate</td>
<td>2154</td>
<td>3.93$^b$ (3.91-3.92)</td>
</tr>
<tr>
<td>Shone</td>
<td>1996</td>
<td>2.77$^b$ (2.70-2.85)</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td></td>
<td>0.17</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>2.71</td>
</tr>
</tbody>
</table>

*Note: Means in a column followed by the same superscript letters are not significantly different.

**Values in brackets shows range**

*Erythrina abyssinica* and *Erythrina brucei* are much better nitrogen fixers as compared to the common organic nutrient sources as referred by Mugendi, et al., (2011) which are *Leucaena diversifolia* 3.9%, *Crotalaria juncea* 3.9%, *Vicia benghalensis* 3.7%, *Mucuna pruriens* 4.0%, *Calliandra calothyrsus* 3.4%, *Crotalaria ochroleuca* 4.5%.

*Erythrina abyssinica* and *Erythrina brucei* had diverse TN content and the data obtained in this study showed that no direct or inverse relationship was found between total nitrogen content and altitude.

3.3 Total polyphenol content

The highest total polyphenol (TPP) content was found in *Erythrina abyssinian* (2.04%) and the lowest in *Erythrina brucei* (1.06%) (Table 7). The study conducted by Palm and Sanchez, (1990) and Anis et al., (2012) also indicated that the primary factors that determines the rate of mineralization and activity of decomposers are the litter quality parameters, especially N, lignin and polyphenol content by inhibiting function of the decomposer organisms by chemically binding to proteins. The study conducted by Grubeˇsi´c et al., (2005) and Srisuda et al., (2008) indicated that the TPP content of pigeon pea leaves and leaves of *P. altissima* were 2.31%, 4.55 % respectively. The TPP contents in different parts of *P holosteum* were: leaves 10.15%, stems 4.13 %, and...
The probable reasons for the difference in mineralization of these spps could be the difference in residue quality, total nitrogen content and microbial activity. The results are also in line with that of Schomberg, et al., (2009) who stated that the decomposition rate of residues is often influenced by temperature, soil moisture, legume quality parameters such as N, polyphenol, and lignin contents and their ratios. regulated by...
environmental factors.

Figure 2: Weekly NH$_4^+$ mineralization of *E. abyssinian* and *E. brucei* in Luvisol

As outlined in Giller and Cadisch, (1997) physical accessibility for microbes may also be an important determinant of decay rate, as was observed by increase in microbial activity or decomposition once litter is ground.

3.5.2 The NO$_3^-$-N release

During the five weeks’ greenhouse mineralization experiment on Luvisol, the experiment revealed that the initial NH$_4^+$ and NO$_3^-$ contents were affected by mineralization and nitrification process. The decomposition of *E. abyssinian* and *E. brucei* had similarities in terms of NO$_3^-$ release pattern and the change in NO$_3^-$ was influenced by both litter types ($r = 0.9243$; $p < 0.0238$), and weeks of incorporation. (Table 9).

Table 9: Interaction effect of Luvisol, *E. abyssinian*, *E. brucei* (Weeks) incubation on NO$_3^-$ release

<table>
<thead>
<tr>
<th>Week</th>
<th>Control</th>
<th><em>E. abyssinian</em></th>
<th><em>E. brucei</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.100$^e$</td>
<td>1.917$^d$</td>
<td>1.333$^e$</td>
</tr>
<tr>
<td>2</td>
<td>0.950$^g$</td>
<td>2.560$^g$</td>
<td>2.447$^e$</td>
</tr>
<tr>
<td>3</td>
<td>0.943$^g$</td>
<td>3.050$^g$</td>
<td>2.533$^e$</td>
</tr>
<tr>
<td>4</td>
<td>0.950$^g$</td>
<td>3.080$^g$</td>
<td>2.550$^g$</td>
</tr>
<tr>
<td>5</td>
<td>0.950$^g$</td>
<td>2.750$^b$</td>
<td>2.467$^e$</td>
</tr>
</tbody>
</table>

LSD (0.05) 0.0284

CV (%) 1.928

Note: Means in a column followed by the same superscript letters are not significantly different at $p<0.05$

During incubation period, the nitrification of the control was at the lower rate, compared to the amended soil, where the average release of NO$_3^-$ was in the order of *Erythrina abyssinian* > *Erythrina brucei* (figure 3).

Figure 3: Weekly NO$_3^-$ mineralization from *E. abyssinian* and *E. brucei* in Luvisol

Positive correlation ($r = 0.6571$, $p < 0.0283$) was found between period of incubation (weeks) and NO$_3^-$-N...
release in the soil, indicating that there was good association of weeks (incubation) and release of NO$_3^-$-N. Nitrification was significantly and negatively correlated with ammonification ($r = -0.6571$, $p < 0.784$).

The decline in the amount of NO$_3^-$ starting the end of the experiment and latter might be caused by leaching (because of its high solubility) and denitrification. Because of its negative charge, NO$_3^-$ is not retained by the soil's cation exchange site and can be easily lost from the root zone by leaching and inherent microbes potentially accelerating the process of denitrification (Rochette, et al., 2000). Khalil, et al., (2002) who observed that the application of organic residues produced more mineral N in the form of NO$_3^-$ under neutral and slightly alkaline conditions, this study also shows an increase in NO$_3^-$ was depending on the Nitrogen content of the litters. Thus, the increase was in the order of *Erythrina abyssinian* > *Erythrina brucei until* denitrification commence (Figure 4).

![Figure 4: Release of NH$_4^+$ and NO$_3^-$ contents in Luvisol during decomposition](image)

**Error bars represent one standard deviation. Main and interaction effects were significant at** $p < 0.05$

### 4. CONCLUSION

Present results clearly indicate that these species are categorized as the fast decomposing organic materials with highest TN content regardless of the site of sampling. *Erythrina abyssinica* and *Erythrina brucei* had diverse TN content and the data obtained in these study showed that no direct or inverse relationship was found between TN content and altitude but other factors could govern the differences in TN content of the organic sources.

Despite a high-polyphenol concentration the leaves of *Erythrina abyssinian* did not show significantly lower net mineralization. This could be explained similar to Handayanto *et al.* (1997) who stated that the higher net mineralization was probably due to the fact that polyphenols were not very active in binding proteins or inhibiting enzyme activities showing only a minor influence of mineralization.

The faster decomposition and NH$_4^+$ release performance of *Erythrina abyssinian* in the soil can be accounted for its highest TN content, and as outlined by Schomberg, *et al.*, 2009; its low-ADF concentrations and better chemical quality characteristics it could enhance the decomposition. Incorporating *Erythrina abyssinica* and *Erythrina brucei* to Luvisols showed an increase in NH$_4^+$, NO$_3^-$ content of the soil as compared to their respective controls in few weeks. Based on the dynamics of release of NH$_4^+$ and NO$_3^-$ content, the species showed the order: *Erythrina abyssinian* > *Erythrina brucei*.

In general, *Erythrina abyssinian* and *Erythrina brucei* amended soil revealed that NO$_3^-$ release was negatively and significantly correlated with ammonification. During incubation period, the control was at the lower level compared to the amended soil in ammonium and nitrate concentration. Finally, more detailed researches are needed to synchronize and verify laboratory results with field measurements of their effect on crop production on the synchronization of soil nutrient availability and crop demand.

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