Microbial succession and changes in carbon and nitrogen during decomposition of leaf litters of *Tephrosia candida* (Roxb.) DC. and *Oryza sativa* L. under shifting cultivation in Mizoram, northeast India

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Research Article

**INTRODUCTION**

Mizoram is one of the seven states of Northeast India with undulating topography dominated by tribal populations. The majority of the people are involved in shifting cultivation or *jhum* for their livelihood. Previously, this practice of cultivation was fairly productive due to recuperated soil fertility through plant regeneration under prolonged fallow periods (20-30 years). However, in recent years the length of the fallow period decreased due to increasing population that lead to a decrease in soil fertility and crop productivity (Grogan *et al.*, 2012), and raised concern among the poor farmers for their livelihood. Thus, there is a need to revitalize this agri-cultural system to be ecologically beneficial by understanding the decomposition dynamics of locally accessible litters. Litter decomposition affects nutrient cycle and carbon storage in the ecosystem (Hobbie *et al.*, 2012), which is highly governed by litter substrate quality and microbial community composition during litter decomposition (Swift *et al.*, 1979). Lignin/N and C/N ratios of litters have been widely used to predict decay rate in different ecosystems (Taylor *et al.*, 1989, Tripathi *et al* 1992a & b, Wapongnunsang *et al* 2017, Lalnunzira and Tripathi 2018). The litter N concentration and soil N availability also have important impacts on litter decomposition (Li *et al.*, 2017). Generally, decomposition rates are greater when the initial N
Soil inhabits a diverse group of microorganisms (mainly bacteria, fungi, and actinomycetes) responsible for the decomposition of organic matter (Manpoong et al., 2020). The biodegradation of organic matter occurs by the concentrated action of various microorganisms which produce a series of enzymes that contribute to the decomposition process (Pérez et al., 2002, Lalnunzira and Tripathi 2018). During decomposition, litter rapidly loses easily soluble compounds, including starch, amino acids and sugar, due to leaching, microbial activity and soil fauna attack (Singh and Tripathi, 2020). The bacterial population is most dominant, whereas actinomycetes are one-tenth in number, but the fungal population is dominant in uninterrupted soil (Hoorman, 2010). Decomposing litters revealed a successional shift from fast-growing to specialized bacteria and fungi (Herzog et al., 2019). Bacteria play an important role in the early stage of decomposition, and fungi and actinomycetes in the later stages of decomposition (Singh and Tripathi, 2020). Fungi are considered to be the major contributors to decomposition due to their ability to produce specific enzymes and the possibility to access new substrates through their hyphae (Bani et al., 2018). Actinomycetes have been reported to promote a slow breakdown of humates (Abbott and Murphy, 2003). Leaf litter accounted for approximately 81% of annual plant litter production (Mason, 1977; Scarascia-Mugnozza et al., 2000). Inputs of litter from the plants play an important role in the soil microorganisms and fertility levels under shifting cultivation areas as they are the main source of nutrients to the crop plants through the microbial transformation of litters (Momin et al., 2021 and Hauchhum and Tripathi, 2019). Therefore, the quality and the quantity of litter inputs from various plants significantly affect the interaction of the microbial communities on the litter and ultimately affect the ability of the soil to provide available nutrients to the crop plants (Lalnunzira and Tripathi, 2018). Tephrosia candida, a bushy species, forms dense vegetation cover and thus produces high biomass, and additionally, add N to the soil through fixation (Nguyen and Thai, 1993 and Wapongnungsang et al., 2017). The species have been reported to be widely used in the rehabilitation of degraded land through fixing atmospheric nitrogen, controlling soil erosion and increasing the levels of soil nutrients by adding organic matter and nutrients through the process of decomposition (Wapongnungsang et al., 2017). Oryza sativa L is the major crop plant under shifting cultivation in NE region and after the harvesting of rice, the whole plant parts are left on the ground to decompose and add nutrients for the second-year cropping. The O. sativa litter release 15.4% to 38.4% of total organic carbon in soil through decomposition (Villegas-Pangga et al., 2000). Keeping this in mind, this paper aims to provide comprehensive information on microbial changes during the decomposing of T. candida and O. sativa leaf litter and the successive changes in C and N release from these litters to the soil under shifting cultivation field in Mizoram, northeast India.

MATERIALS AND METHODS

Site description

The present study was carried out in Tanhril (Fig. 1), Mizoram on shifting cultivation fields i.e., on two fallow ages of 3 years (FL-3; 23°39´55´´N, 92°31´48´´-92°33´24´´E) and 8 years (FL-8; 23°43´55´´-23°44´48´´N, 92°38´43´´-92°40´4´´E). Tanhril is located about 15km away from Aizawl city. The temperature of the area is sub-tropical (20-30°C in summer and 11-21°C in winter) with considerable seasonal variations. The total annual rainfall ranged ~2250-3200 mm from 2017-2020 and relative humidity was over 70% throughout the year (Anonymus, 2020).

Experimental design

T. candida and O. sativa leaf litters were collected from the field, air dried and 10g of each litter type were enclosed in nylon net bags measuring 20cm x 20cm with 2mm mesh size. Leaf litter bags of T. candida, O. sativa alone and in combinations (T. candida + O. sativa in equal proportions) were made. A total of 50 litter bags of each litter type were placed randomly on soil of two fallow lands (FL-3 and FL-8). Every month four litter bags of each litter type were retrieved for one year so a total 12 recoveries were made. One bag was used for microbial isolation and rest three bags were cleaned.
leaf litters were dried and powdered for determining nutrient changes in litters during decomposition.

Isolation of microbes
Microbes were isolated from the decomposing litters within 24 hours of collection by serial dilution plate method. 1g of crushed litter sample was dissolved in 10ml of 0.9% NaCl solution and serially diluted in saline solution up to 10<sup>6</sup> (Martin, 1950). An aliquot of 0.1ml of each dilution was taken and transferred to a separate Nutrient Agar (NA) plate, Potato Dextrose Agar (PDA) plate and Starch Casein Agar (SCA) plate and spread with L-spreader carefully which was then incubated at 37°C, 24°C and 28°C for 24 hours, 48 hours and 4 weeks for bacteria, fungi and actinomycetes, respectively.

Determination of microbial enzyme activity
Five enzyme tests of isolated microbes were carried out during the study. Catalase, cellulase, amylase, protease and lipase test (Kumar et al., 2015) were done with all three microbial types. Microbes showing positive enzyme activity were highly responsible for decomposition.

Characterization of microorganisms
13 microbial isolates (SKT 02, SKT 05, SKT 09, SKT 020, SKT 033, SKT 034, SKT 035, SKT 040, SKT 045, SKT 030, SKT 052, SKT 053, SKT 060) showing positive enzyme activity were purified. Purified isolates of bacteria and actinomycetes were identified up to genus level according to Bergey’s Manual of Determinative Bacteriology (Bergey and Holt, 2000) and fungi according to Gilman’s Manual of Soil Fungi (Gilman 1957). The isolation and identification were made through micro-morphological observation and enzyme activity, respectively. Visual observation included both morphological and microscopical characteristics (like aerial mycelia, colony color, spore morphology etc.) were taken into consideration.

Table 1. In vitro screening of enzyme activity of isolates.

| Isolate Code | Catalase | Cellulase | Amylase | Protease | Lipase |
|--------------|----------|-----------|---------|----------|--------|
| SKT 02       | +        | +         | +       | +++      | -      |
| SKT 05       | +        | +         | +       | +++      | -      |
| SKT 09       | ++       | +         | +       | +++      | -      |
| SKT 020      | +        | +         | +       | +        | -      |
| SKT 033      | +++      | +         | +       | -        | -      |
| SKT 034      | +++      | +         | -       | +        | -      |
| SKT 035      | +        | +         | +       | +        | -      |
| SKT 040      | +++      | -         | +       | +        | -      |
| SKT 045      | +++      | +         | +       | +        | -      |
| SKT 030      | +        | -         | +       | +        | -      |
| SKT 052      | +++      | +         | -       | ++       | -      |
| SKT 053      | +        | +         | -       | +        | -      |
| SKT 060      | +        | +         | +       | +        | -      |

- -, +, ++, +++ indicative of nil, low, medium and high activity

Analysis of carbon, nitrogen and lignin
Dried and powdered litter materials were used for analysis. The carbon content of the litter was estimated by loss on ignition method (Mcbrayer and Cromack, 1980), total Nitrogen by Micro Kjeldahl methods (Jackson, 1973) and lignin by acid detergent fiber method (Van Soest and Wine, 1968).

Calculation and statistical analysis
The relative decomposition rate (RDR) was calculated by using formula:

\[
\text{RDR} (\text{g g}^{-1} \text{ day}^{-1}) = \frac{\ln (W_t/W_0)}{(t_1 - t_0)} \\
\text{Eq.1}
\]

Where \( W_0 \) = mass of litter present at time \( t_0 \), \( W_t \) = mass of litter at time \( t_1 \), and \( t_1 - t_0 \) = sampling interval (days). The daily instantaneous decayed constant (k) of litter was calculated through the negative exponential decay model of Olson (1963):

\[
W_t/W_0 = \exp(-kt)
\]

Where \( W_0 \) = initial mass of the litter, and \( W_t \) = mass remaining after time \( t \). As suggested by Olson (1963), the time required for 50% mass loss was calculated as \( t_{50} = 0.693/k \) and for 95% mass loss as \( t_{95} = 3/k \).

RESULTS AND DISCUSSION
A total 64 microbes were isolated from three different decomposing litters (T. candida, O. sativa and T. candida + O. sativa) on two fallow lands (FL-3 and FL-8) following shifting cultivation in Mizoram. Among them, 13 microbes showed positive enzyme activity (Table 1). The highest positive response to catalase activity was recorded in strains (SKT 033, SKT034, SKT 040, SKT...
045 and SKT 052) as they produced vigorous efferves-
cence on the addition of 3% hydrogen peroxide
(Fig.2.A). However, a negative response was noted
against cellulase activity in strains (SKT 040 and SKT
030), and against amylase activity in strains (SKT 034,
SKT 052 and SKT 053) and protease activity in SKT
033. SKT 02, SKT 05 and SKT 09 were found to have
more protease activity than other strains as they were
 capable of producing a large clear zone (1.5 cm in di-
ameter) on skim milk agar plate around colonies
(Fig.2.B). On the other hand, no lipase activity was no-
ticed on these 13 strains. Out of 13 strains, 31% were
bacteria, 38%fungi and 31% were actinomycetes. On
NA plate, all the bacterial colonies were able to grow
well in 24 hours. Their colony nature was maximum
irregular in shape, dark to light cream in color and colo-
ny diameter was between 0.5cm-1.5cm. However, fun-
gal colonies took 5-7 days to grow on PDA plate, some
of the colonies showed colored pigment with 0.1cm-
2cm colony diameter and actinomycetal colonies took 2
-9 days to grow in SCA plate and maximum colonies
were powdery in nature. According to the colony mor-
phology and microscopic view (Fig. 2 C and D), 13 iso-
lated strains were identified up to genus level (Table 2).
In the present study, most of the bacterial genus was
Streptobacillus sp. followed by Bacillus sp. Similar bac-
terial strains were also isolated by Debi & Prakash
(2017) from rhizospheric soil of northeast India. Previ-
ous enzymatic studies on these two bacterial genera
(Streptobacillus sp. and Bacillus sp.) displayed positive
activity towards cellulase (Sethi et al., 2013 and Ra-
jagopal et al., 2007), catalase (Babiker et al., 2017 and
Eisenberg et al., 2015) and protease (Pant et al., 2015
and Eisenberg et al., 2015) test. These enzymes are
active during the course of decomposition process.
Some soil fungi are responsible for the decomposition
of plant litters and releasing nutrients to the soil for
plant growth (Raaijmakers et al., 2009), because they
are responsible for decomposing cellulose and lignin
through enzymatic actions (Hoorman, 2011). The pre-
sent study found 3 different genera of fungi namely-
Microsporum sp., Rhizopus sp. and Aspergillus sp. from
three different litter types (T. candida, O. sativa and
T. candida + O. sativa). Parallel fungal findings were also
recorded by Giudice et al. (2012) and Debi & Prakash
(2017) from the soil, showing proteolytic enzyme activi-
ties that further breakdown the recalcitrant compounds
of leaf litters and results in decomposition. Actinomy-
cetes are highly distributed in different habitats and
ecosystems (Ghorbani-Nasrabadi et al., 2013). A previ-
ous study reported salt tolerant activity in Streptomy-
cetes sp. obtained from shifting cultivated area of Mizo-
ram (Momin and Tripathi, 2019). Therefore, the pres-
ence of this species as decomposing actinomycetal
strain indicated the salt tolerance ability in the shifting
cultivation. Additionally, actinomycetes are also known
to have cellulose-degrading properties (Eida et al.,
2012).
| Isolate code | Nature of colony | Color of colony | Days of growth | Media | Identified microbe | Litter type |
|--------------|------------------|----------------|----------------|-------|-------------------|-------------|
| SKT 02       | Irregular        | Undulate       | Dark cream, rough, colony with 0.8 cm in dia. | Light cream | 1 day | NA | Streptobacillus sp. (Gram -ve) | T. candida |
| SKT 05       | Irregular        | Undulate       | Light cream, rough, colony with 0.5 cm in dia. | Light cream | 1 day | NA | Streptobacillus sp. (Gram -ve) | T. candida and O. sativa |
| SKT 09       | Circular         | Undulate       | Dark cream, rough, colony with 1.4 cm in dia. | Cream | 1 day | NA | Streptobacillus sp. (Gram -ve) | T. candida |
| SKT 020      | Irregular        | Undulate       | Dark cream, smooth, colony with 1 cm in dia. | Cream | 1 day | NA | Bacillus sp. (Gram +ve) | O. sativa |
| SKT 033      | Circular         | Entire         | Greenish grey, rough, colony with 0.1 cm in dia. | White | 5 days | PDA | Microsporum sp. | T. candida |
| SKT 034      | Circular         | Umbonate Filiform | White, wrinkled, colony with 2 cm in dia. | White | 5 days | PDA | Rhizopus sp. | T. candida |
| SKT 035      | Circular         | Umbonate Filiform | White margin and light yellow in center, wrinkled, colony with 1.3 cm in dia. | White | 5 days | PDA | Rhizopus sp. | T. candida and O. sativa |
| SKT 040      | Circular         | Undulate Entire | White, Rough, colony with 2 cm in dia. | Cream | 7 days | PDA | Aspergillus sp. | T. candida |
| SKT 045      | Irregular        | Umbonate Curled | White, rough, colony with 1.5 cm in dia. | White | 2 days | PDA | Aspergillus sp. | T. candida and O. sativa |
| SKT 030      | Irregular        | Curled         | White, rough, colony with 0.3 cm in dia. | Light cream | 3 days | SCA | Streptomyces sp. (Gram +ve) | T. candida and O. sativa |
| SKT 052      | Irregular        | Undulate       | Light grey, powdery, colony with 0.2 cm in dia. | Light cream | 2 days | SCA | Streptomyces sp. (Gram +ve) | O. sativa |
| SKT 053      | Irregular        | Undulate       | White, powdery, colony with 0.3 cm in dia. | Light orange | 2 days | SCA | Streptomyces sp. (Gram +ve) | O. sativa |
| SKT 060      | Irregular        | Undulate       | Light grey, powdery, colony with 0.5 cm in dia. | Black | 9 days | SCA | Streptomyces sp. (Gram +ve) | T. candida and O. sativa |
The decomposition rate of leaf litters of *T. candida*, *O. sativa* and *T. candida* + *O. sativa* showed a similar pattern in both fallow fields (FL-3 and FL-8). The rate of decomposition was faster in FL-8 (k= 0.006001 – 0.006116) as compared to FL-3 (k= 0.00587 – 0.006001). Mass loss (~80%) was more rapid for *T. candida* in the initial months. Mass loss was faster in FL-8 as compared to FL-3. In general, the mass loss was considerably high during the initial months than the later months. The significant differences in mass loss among litter types were not clear in FL-8, but the differences in the mass were evident in the later stages of different litter types (Fig. 3). Similar findings were reported in litter decomposition of two species of *Tephrosia* sp. in Malawi, Africa (Munthali et al., 2015). Rapid mass loss in the initial months may be related to the release of higher concentrations of liable substances in the initial period of decomposition (Wang et al., 2004). The daily instantaneous decay constant (k), number of days required for 50% (t_{50}) and 95% (t_{95}) decomposition of all leaf litter types were in order: *T. candida* > *T. candida* + *O. sativa* > *O. sativa* (Table 3). Variations in the initial concentrations of C, N and Lignin as reflected by C/N and Lignin/N ratio (Table 4) are important determinants of decomposition in the litter (Tripathi and Singh 1992 a & b; Wapondnungsang et al., 2017; Lalnunzira and Tripathi, 2018). The C release from the leaf litters followed a pattern similar to that of mass loss with rapid mass loss at the beginning, which slowed down in the latter part of decomposition (Fig. 4). N mass loss was also rapid during the initial months. Higher initial N content and lower C/N ratio in leaf litters

### Table 3. Decomposition rate, daily decay rate (k) and time required for decomposition (t 50%, t 95% mass loss) of leaf litters of *T. candida*, *O. sativa* and *T. candida* + *O. sativa* in different fallow periods.

| Fallow period | Litter type          | Mass remaining (% initial) 365 days | Daily decay rate (k) | t_{50} (days) | t_{95} (days) |
|---------------|----------------------|-----------------------------------|----------------------|---------------|---------------|
| 3 years       | *T. candida*         | 9.33                              | 0.006001             | 115           | 500           |
|               | *O. sativa*          | 12.97                             | 0.00587              | 118           | 511           |
|               | *T. candida* + *O. sativa* | 11.83                       | 0.005933             | 116           | 505           |
| 8 years       | *T. candida*         | 0                                 | 0.006116             | 113           | 491           |
|               | *O. sativa*          | 0                                 | 0.00608              | 114           | 494           |
|               | *T. candida* + *O. sativa* | 7.17                           | 0.006074             | 114           | 493           |

### Table 4. Initial chemical composition of different leaf litters.

| Leaf litter type          | Carbon (%) | Nitrogen (%) | Lignin (%) | C/N ratio | Lignin/N ratio |
|---------------------------|------------|--------------|------------|-----------|----------------|
| *T. candida*              | 36.69±0.01 | 4.14±0.01    | 9.63±0.02  | 8.84      | 2.32           |
| *O. sativa*               | 41.22±0.4  | 1.52±0.01    | 21.89±0.02 | 27.12     | 14.4           |
| *T. candida* + *O. sativa*| 41.55±0.01 | 2.55±0.04    | 18.23±0.02 | 16.29     | 7.15           |

Values are means ± SE (n=4)

Fig. 3. Mass remaining of different leaf litters in two fallow lands.
(Table 4) have been reported to favour the rapid release of N during the initial stages of decomposition (Hoorman, 2010). According to Quideau et al. (2005), leaf litters with higher N content showed a faster decay rate in Chaparral ecosystem in Southern California. The percentage of N mass remaining at the end of the sampling period (i.e. 1 year) for different leaf litters varied from 0-15%, with the lowest in T. candida (FL 3 – 8.72%, FL 8 – 0) followed by T. candida + O. sativa (FL 3 - 11.28 %, FL 8 – 0) and O. sativa (FL 3 – 12%, FL 8 – 0). The high initial C/N ratio in O. sativa may partially be responsible for the slow rate of decomposition and nutrient release in the soil (Bauder, 2000 and Fosu et al., 2007). The higher mass loss in longer fallow than shorter fallow may be due to a higher rate of decomposition that may result from increased microbes and their activity because the higher organic matter present may act as a source of energy for the decomposers, as reported by Wapongnungsang et al., (2017).

Conclusion

This study concluded that different microbes like bacteria, fungi, and actinomycetes present on various high- and low-quality resources showed two or more enzymatic activities responsible for soil organic decomposition and nutrient release. The combination of O. sativa and T. candida litter is recommended for soil fertility management in shifting cultivation of Mizoram, Northeast India. Further, inoculation of isolated microbes can accelerate the process of decomposition and nutrient release in shorter fallow land that may further enhance the productivity of shorter jhum fields.

ACKNOWLEDGEMENTS

The authors are thankful to the Department of Forestry, Mizoram University, Mizoram, for extending the facilities.

Conflict of interest

The authors declare that they have no conflict of interest.

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