

Research Article

## 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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### Abstract

The present study aims to understand microbial succession and dynamics of dry matter, carbon and nitrogen during the decomposition of leaf litters of *Tephrosia candida* (Roxb.) DC. and *Oryza sativa* L. in two fallow lands (FL) of 3 years (FL-3) and 8 years (FL-8) following shifting cultivation in Mizoram. A total of 64 microorganisms were isolated from two leaf litters by serial dilution method, out of which 13 microbes were identified as decomposers as they exhibited a positive response towards the enzyme activity. Among these 13 microorganisms, 4 (SKT 02, SKT 05, SKT 09 and SKT 020) were bacteria (*Streptobacillus sp.* and *Bacillus sp.*), 5 (SKT 033, SKT 034, SKT 035, SKT 040 and SKT045) were fungi (*Microsporium sp.*, *Rhizopus sp.* and *Aspergillus sp.*) and 4 (SKT 030, SKT 052, SKT 053 and SKT 060) were actinomycetes (*Streptomyces sp.*). *T. candida* leaf litter possessed low initial Carbon/Nitrogen (8.77) and Lignin/Nitrogen ratio (2.29) and considered a high-quality resource exhibiting higher decomposition rate. Mass loss of carbon and nitrogen (~ 40-80%) was maximum during the initial two months, which slowed down in the later period of decomposition. It was concluded that the number of bacteria, fungi and actinomycetes regulate organic matter and nutrient dynamics in the soil through various enzymatic actions on high- and low-quality litters. The combination of *O. sativa* and *T. candida* litter is recommended to manage soil fertility in shifting cultivation of Mizoram, Northeast India.

**Keywords:** Carbon- Nitrogen dynamics, Decay rate, Decomposition, Fertility, Microorganisms

### 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

concentration is high and the C:N ratio is low (Cornwell *et al.*, 2008).

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 decompo-

sition (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,

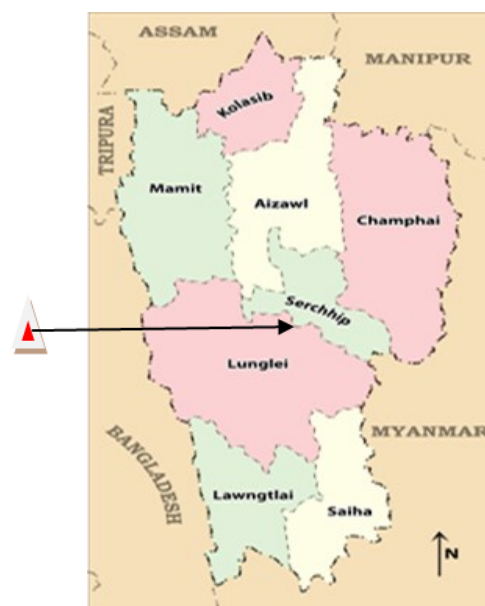


Fig. 1. Mizoram map showing the study area.

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  $10^{-6}$  (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 morpho-

logical and microscopical characteristics (like aerial mycelia, colony color, spore morphology etc.) were taken into consideration.

#### 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:

$$RDR (g g^{-1} day^{-1}) = \ln (W_t - W_0) / (t_1 - t_0) \quad \dots\dots Eq.1$$

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

$$t_{50} = 0.693/k \text{ and for 95\% mass loss as } t_{95} = 3/k.$$

## 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

**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



045 and SKT 052) as they produced vigorous effervescence 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 diameter) on skim milk agar plate around colonies (Fig.2.B). On the other hand, no lipase activity was noticed 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 colony diameter was between 0.5cm-1.5cm. However, fungal 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 morphology and microscopic view (Fig. 2 C and D), 13 isolated 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 bacterial strains were also isolated by Debi & Prakash (2017) from rhizospheric soil of northeast India. Previous enzymatic studies on these two bacterial genera

(*Streptobacillus sp.* and *Bacillus sp.*) displayed positive activity towards cellulase (Sethi *et al.*, 2013 and Rajagopal *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 present study found 3 different genera of fungi namely- *Microsporium 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 activities that further breakdown the recalcitrant compounds of leaf litters and results in decomposition. Actinomycetes are highly distributed in different habitats and ecosystems (Ghorbani-Nasrabadi *et al.*, 2013). A previous study reported salt tolerant activity in *Streptomyces sp.* obtained from shifting cultivated area of Mizoram (Momin and Tripathi, 2019). Therefore, the presence 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).

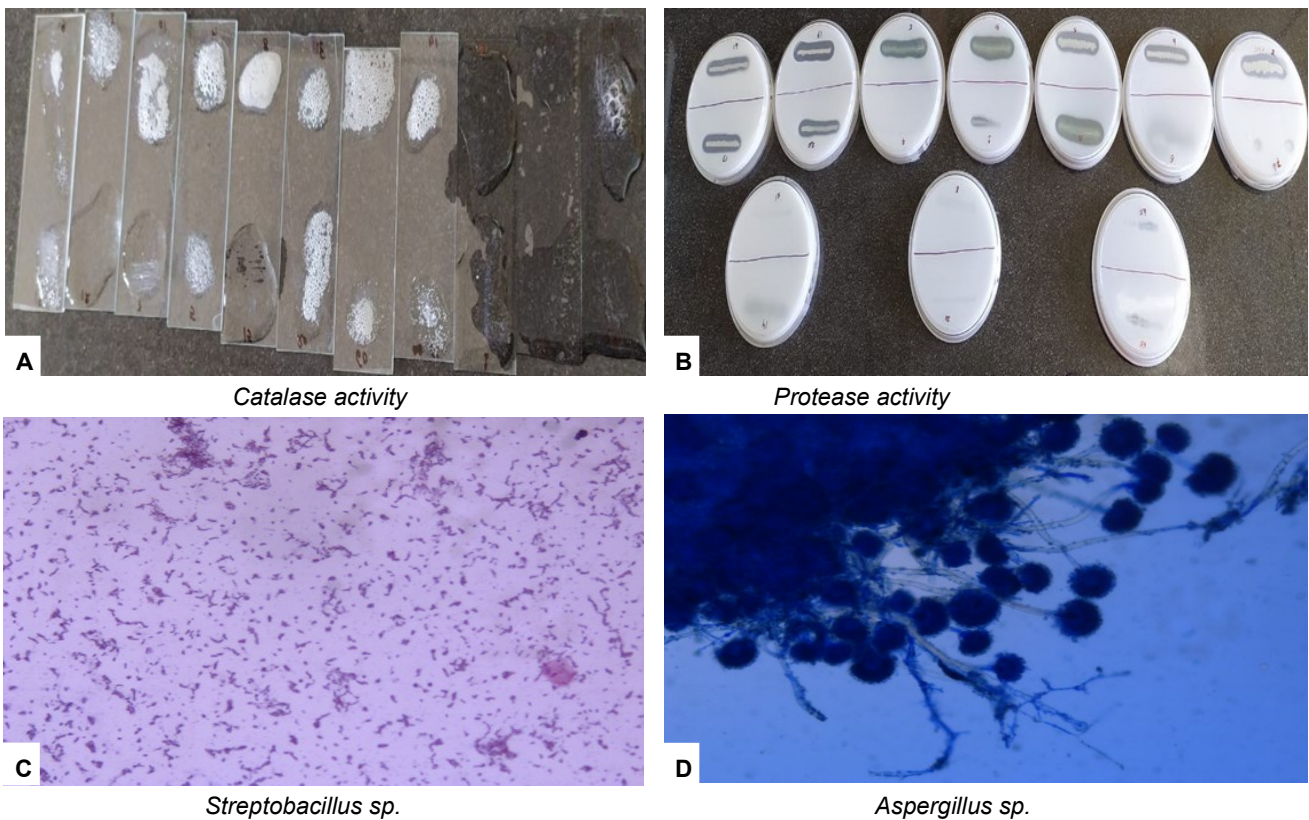


Fig. 2. Showing enzyme activities (A-B) and microscopic view (C-D) of isolates.

**Table 2.** Morphological characterization of 13 isolated microbial strains of Mizoram. AM and SM-representatives of Aerial Mycelium and Substrate Mycelium.

Isolate code	Nature of colony			Color of colony		Days of growth	Media	Identified microbe	Litter type
	Forms	Elevation	Margin	AM	SM				
SKT 02	Irregular	Umbonate	Undulate	Dark cream, rough, colony with 0.8cm in dia.	Light cream	1 day	NA	<i>Streptobacillus</i> sp. (Gram -ve)	<i>T.candida</i>
SKT 05	Irregular	Raised	Undulate	Light cream, rough, colony with 0.5cm in dia.	Light cream	1 day	NA	<i>Streptobacillus</i> sp. (Gram -ve)	<i>T.candida</i> and <i>O.sativa</i>
SKT 09	Circular	Flat	Undulate	Dark cream, rough, colony with 1.4cm in dia.	Cream	1 day	NA	<i>Streptobacillus</i> sp. (Gram -ve)	<i>T.candida</i> + <i>O.sativa</i>
SKT 020	Irregular	Raised	Undulate	Dark cream, smooth, colony with 1cm in dia.	Cream	1 day	NA	<i>Bacillus</i> sp. (Gram +ve)	<i>O.sativa</i>
SKT 033	Circular	Raised	Entire	Greenish grey, rough, colony with 0.1cm in dia.	White	5 days	PDA	<i>Microsporium</i> sp.	<i>T.candida</i>
SKT 034	Circular	Umbonate	Filliform	White, wrinkled, colony with 2cm in dia.	White	5days	PDA	<i>Rhizopus</i> sp.	<i>T.candida</i>
SKT 035	Circular	Umbonate	Filliform	White margin and light yellow in center, wrinkled, colony with 1.3cm in dia.	White	5 days	PDA	<i>Rhizopus</i> sp.	<i>T.candida</i> + <i>O.sativa</i> and <i>O.sativa</i>
SKT 040	Circular	Undulate	Entire	White, Rough, colony with 2cm in dia.	Cream	7 days	PDA	<i>Aspergillus</i> sp.	<i>T.candida</i>
SKT 045	Irregular	Umbonate	Curled	White, rough, colony with 1.5cm in dia.	White	2 days	PDA	<i>Aspergillus</i> sp.	<i>T.candida</i> + <i>O.sativa</i>
SKT 030	Irregular	Raised	Curled	White, rough, colony with 0.3cm in dia.	Light cream	3 days	SCA	<i>Streptomyces</i> sp. (Gram +ve)	<i>T.candida</i> + <i>O.sativa</i>
SKT 052	Irregular	Flat	Undulate	Light grey, powdery, colony with 0.2cm in dia.	Light cream	2 days	SCA	<i>Streptomyces</i> sp. (Gram +ve)	<i>O.sativa</i>
SKT 053	Irregular	Flat	Undulate	White, powdery, colony with 0.3cm in dia.	Light orange	2 days	SCA	<i>Streptomyces</i> sp. (Gram +ve)	<i>O.sativa</i>
SKT 060	Irregular	Flat	Undulate	Light grey, powdery, colony with 0.5cm in dia.	Black	9 days	SCA	<i>Streptomyces</i> sp. (Gram +ve)	<i>T.candida</i> + <i>O.sativa</i>

**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 ( <i>k</i> )	<i>t</i> <sub>50</sub> (days)	<i>t</i> <sub>95</sub> (days)
3 years	<i>T.candida</i>	9.33	0.006001	115	500
	<i>O.sativa</i>	12.97	0.00587	118	511
	<i>T.candida + O.sativa</i>	11.83	0.005933	116	505
8 years	<i>T.candida</i>	0	0.006116	113	491
	<i>O.sativa</i>	0	0.00608	114	494
	<i>T.candida + O.sativa</i>	7.17	0.006074	114	493

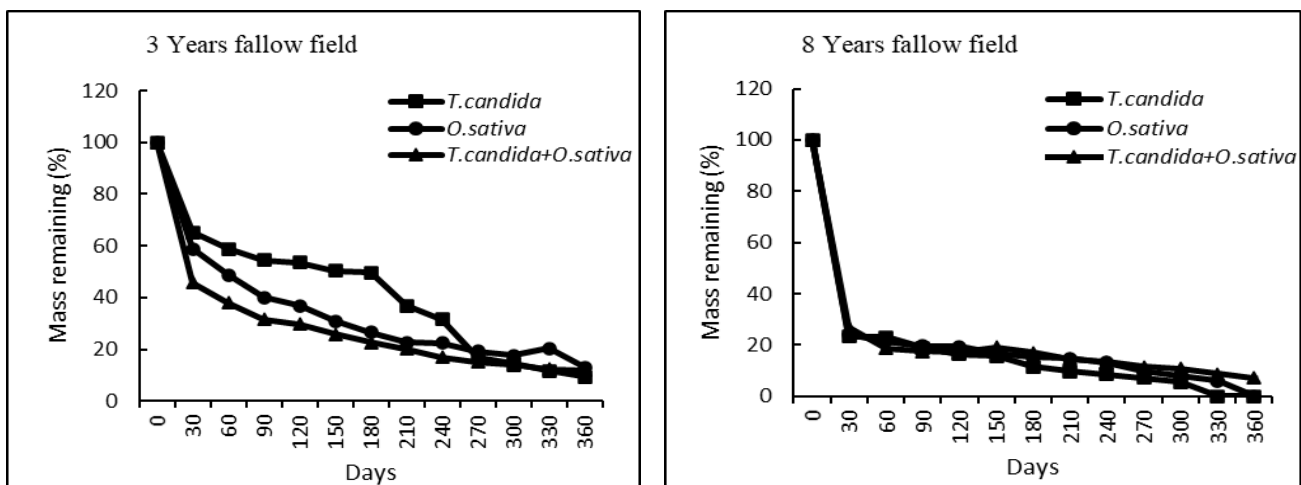
**Table 4.** Initial chemical composition of different leaf litters.

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

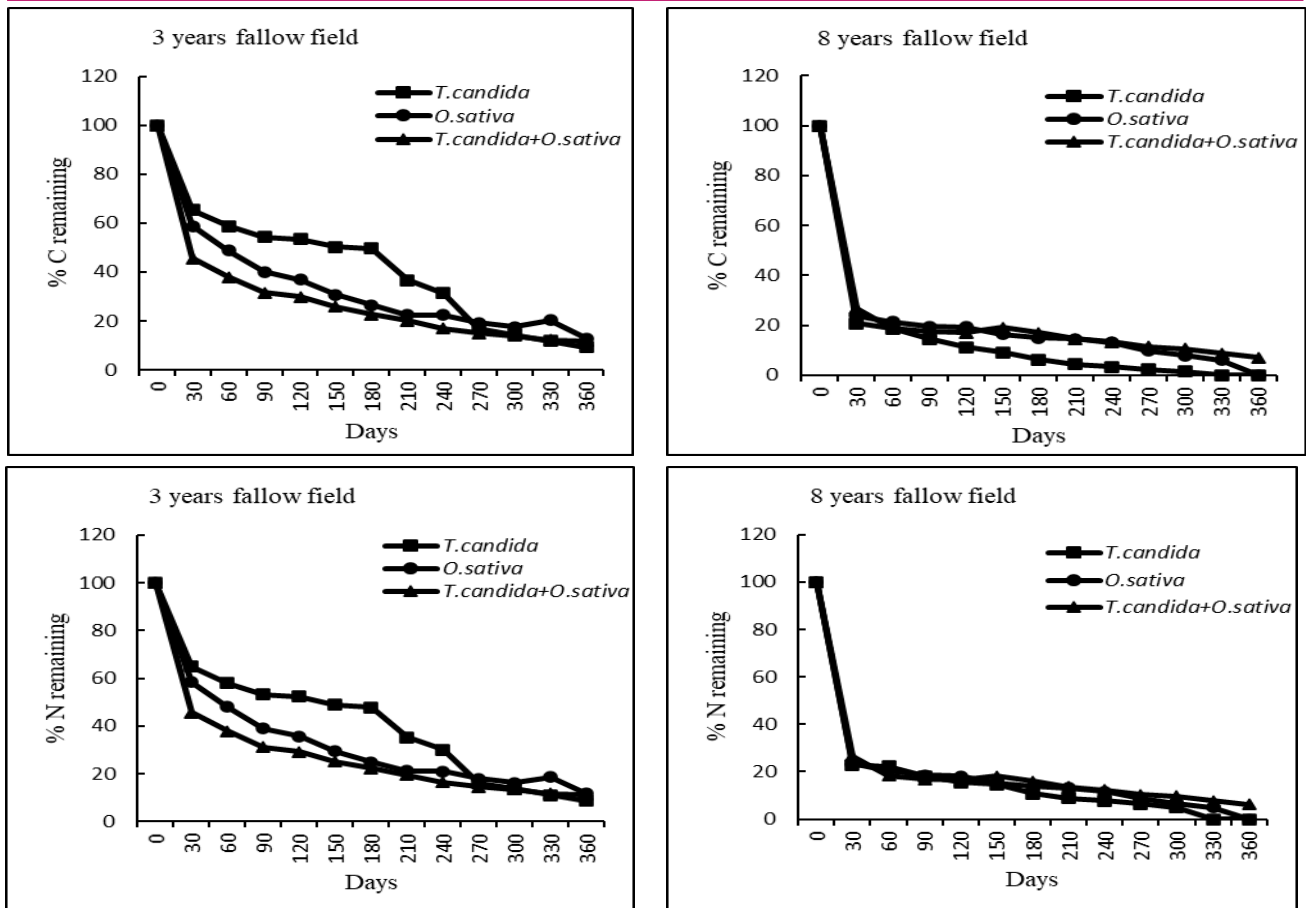
Values are means ± SE (n=4)

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*= 0.006074 – 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*<sub>50</sub>) and 95% (*t*<sub>95</sub>) 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



**Fig.3.** Mass remaining of different leaf litters in two fallow lands.



**Fig. 4.** Carbon(C) and Nitrogen(N) release pattern from leaf litters of *T.candida*, *O.sativa* and *T.candida+O.sativa* in 3 years and 8 years fallow land.

(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 enzy-

matic 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, North-east 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.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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