## APPENDIX- 1

### Composition of Media

#### Nutrient Agar

<table>
<thead>
<tr>
<th>Composition per litre:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar</td>
</tr>
<tr>
<td>Pancreatic digest of gelatin</td>
</tr>
<tr>
<td>Beef extract</td>
</tr>
</tbody>
</table>

Final pH 6.8 ± 0.2 at 25°C

#### Nutrient Broth

<table>
<thead>
<tr>
<th>Composition per litre:</th>
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</thead>
<tbody>
<tr>
<td>Peptone</td>
</tr>
<tr>
<td>NaCl</td>
</tr>
<tr>
<td>Yeast extract</td>
</tr>
<tr>
<td>Beef extract</td>
</tr>
</tbody>
</table>

pH 7.4 ± 0.2 at 25°C

#### Skim Milk Agar

<table>
<thead>
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<th>Composition per litre:</th>
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</thead>
<tbody>
<tr>
<td>Agar</td>
</tr>
<tr>
<td>Skim milk</td>
</tr>
</tbody>
</table>
### Carboxy Methyl Cellulose Agar

**Composition per litre:**

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<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
<tr>
<td>NH$_4$H$_2$PO$_4$</td>
<td>1 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.2 g</td>
</tr>
<tr>
<td>MgSO$_4$ • 7H$_2$O</td>
<td>1 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1 g</td>
</tr>
<tr>
<td>Carboxymethyl cellulose</td>
<td>26 g</td>
</tr>
<tr>
<td>Agar</td>
<td>3 g</td>
</tr>
</tbody>
</table>

### Starch Agar

**Composition per litre:**

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<tr>
<th>Ingredient</th>
<th>Quantity</th>
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</thead>
<tbody>
<tr>
<td>Starch, soluble</td>
<td>20.0 g</td>
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<tr>
<td>Agar</td>
<td>10.0 g</td>
</tr>
<tr>
<td>NaNO3</td>
<td>2.5 g</td>
</tr>
<tr>
<td>K2HPO4</td>
<td>1.0 g</td>
</tr>
<tr>
<td>MgSO$_4$•7H$_2$O</td>
<td>0.6 g</td>
</tr>
<tr>
<td>CaCl$_2$•2H$_2$O</td>
<td>0.1 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.1 g</td>
</tr>
<tr>
<td>FeCl$_3$</td>
<td>1 mg</td>
</tr>
</tbody>
</table>

pH 7.2 ± 0.2 at 25°C
Simmons Citrate Agar

**Composition per litre:**

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<tr>
<th>Ingredient</th>
<th>Amount</th>
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<tbody>
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<td>1.0g</td>
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<tr>
<td>K₂HPO₄</td>
<td>1.0g</td>
</tr>
<tr>
<td>NaCl</td>
<td>5.0g</td>
</tr>
<tr>
<td>Na₃C₆H₅O₇</td>
<td>2.0g</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>0.2g</td>
</tr>
<tr>
<td>Agar</td>
<td>2.0g</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.08g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
</tbody>
</table>

Tributyrin agar

**Composition per litre:**

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<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic digest of animal tissue</td>
<td>5.0g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.0</td>
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<tr>
<td>Agar</td>
<td>15.0g</td>
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<tr>
<td>Distilled water</td>
<td>1000 ml</td>
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</table>

pH 7.2 ± 0.2 at 25°C
Mannitol Motility Agar

**Composition per litre:**

- Mannitol: 26g
- NaCl: 15g
- Agar: 4g
- Distilled water: 1000 ml

pH 7.2 ± 0.2 at 25°C

**Gram’s stain**

**Solution-A**
- Crystal Violet: 2gm
- Ethyl alcohol (95%): 20ml

**Solution-B**
- Ammonium oxalate: 0.8gm
- Distilled Water: 80ml

Solution A and B mixed

**Spore stain**

- Malachite green: 5gm
- Distilled water: 100ml
APPENDIX-II

Nucleotide Sequence and the table showing the most similarity of the sequence of the highly potential litter degrading bacteria

>Bacillus firmus K3
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NCBI Accession No: K3

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> *Bacillus cereus* K4

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> Solibacillus silvestris K2
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List of Publications


**Paper submitted to Journal**


**Conference Abstracts/Proceedings**


Bacillus anthracis strain ba1 16S ribosomal RNA gene, partial sequence

Nucleotide

GenBank

Bacillus anthracis strain ba1 16S ribosomal RNA gene, partial sequence

FASTA

LOCUS   MG548582    1416 bp DNA linear BCT 17-JAN-2018
DEFINITION Bacillus anthracis strain ba1 16S ribosomal RNA gene, partial sequence.
ACCESSION MG548582
VERSION   MG548582.1
KEYWORDS
SOURCE Bacillus anthracis
ORGANISM Bacillus anthracis
           Bacteriia; Firmicutes; Bacillii; Bacillales; Bacillaceae; Bacillus; Bacillus cereus group.
REFERENCE 1 (bases 1 to 1416)
AUTHORS Krishna,M.P. and Mohan,M.
TITLE Direct Submission
JOURNAL Submitted (17-NOV-2017) School of Environmental Sciences, Mahatma Gandhi University, PD Hills, Kottayam, Kerala 686 560, India
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//
Bacillus cereus strain K4 16S ribosomal RNA gene, partial sequence

GenBank: MF692694.1

FASTA  Graphics

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AUTHORS    Krishna, M.P. and Mohan, M.
TITLES     Bacteria isolated from forest litter
JOURNAL    Unpublished
REFERENCE  2 (bases 1 to 1416)
AUTHORS    Krishna, M.P. and Mohan, M.
TITLES     Direct Submission
JOURNAL    Submitted (14-SEP-2017) School of Environmental Sciences, Mahatma Gandhi University, FG Hills, Kottayam, Kerala 686 560, India
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//
Bacillus firmus strain K3 16S ribosomal RNA gene, partial sequence

GenBank: MF962923.1
Fasta

To: LOCUS MF962923 1416 bp DNA linear BCT 16-JAN-2018
DEFINITION Bacillus firmus strain K3 16S ribosomal RNA gene, partial sequence.
ACCESSION MF962923
VERSION MF962923.1
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ORGANISM Bacillus firmus
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  AUTHORS Krishna,M.P. and Mohan,M.
  TITLE Bacteria isolated from forest litter
  JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1416)
  AUTHORS Krishna,M.P. and Mohan,M.
  TITLE Direct Submission
  JOURNAL Submitted (14-SEP-2017) School of Environmental Sciences, Mahatma Gandhi University, P.O. Hills, Kottayam, Kerala 686 560, India
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GenBank: MF943223.1

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Solibacillus silvestris strain K2 16S ribosomal RNA gene, partial sequence

GenBank: MF962922.1

FASTA  Graphica

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Evaluation of Phytoconstituents of *Syzygium arnottianum* Leaves

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Available Online:25th October, 2017

**ABSTRACT**

Aim: The present study is carried out to explore the major phytoconstituents and functional groups present in the methanolic extract of the leaves of *Syzygium arnottianum* using FTIR and GC-MS. Methods: For the identification of the phytochemical constituents, Perkin – Elmer GC Clarus 500 system (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perkin Elmer Turboboras 5.2 spectrometer with an Elite – 5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30 m x 0.25 μm DF of capillary column is used. The molecular weight and structure of the compounds were determined by analysis of the mass spectrum of GC-MS using the database of National Institute Standard and Technology (NIST) library data. For the analysis of functional groups, ATR- FTIR spectroscope (Shimadzu IR Prestige-21) is used. Results: The GC-MS analysis of the methanolic extract of *S. arnottianum* revealed the presence of 11 bioactive compounds with valuable biological activities. The FTIR analysis indicated the presence of alcohol, alkane, alkene, alkyl halide, alkyne, amine, aromatic, carbonyl, ether, acid, aldehyde, anhydride and ester. **Conclusion:** The phytochemical profile of the plant *S. arnottianum* leaf extract indicates the presence of various bioactive compounds which can be utilised further for medicinal purposes.

**Keywords:** FT-IR, GC-MS, plant extract, Western Ghats and medicine.

**INTRODUCTION**

Phytochemical constituents are responsible for medicinal property of plant species. Plants are capable of producing a vast variety of secondary metabolites, which are low-molecular weight organic compounds, usually with unique and complex structures. Most of the herbal medicines and their derivatives were often prepared from crude plant extracts, which consist of a complex mixture of different phytochemical constituents. The chemical features of these constituents vary greatly among different species. The cell walls of different plant species have a variety of physically different polysaccharides and proteins. On the basis of the involvement in plant metabolism, phytochemicals are classified into two groups such as primary and secondary metabolites. Primary metabolites include carbohydrates, amino acids, proteins and chlorophylls whereas secondary metabolites include alkaloids, saponins, steroids, flavonoids, tannins and so on. The phytochemical constituents play a major part in the identification of crude drugs. *Syzygium arnottianum* (Wright Walp.) belongs to the family Myrtaceae (Fig.1) and the synonym is *Syzygium densiflorum*. It is endemic to southern Western Ghats and is coming under the category ‘vulnerable species’. It is a tall tree with grey bark and simple and opposite leaves. The tree has been used as food and source of wood. Essential oil of leaves of *Syzygium arnottianum* showed high anti-oxidant capacity. The fruit has been used for the treatment of diabetes mellitus from earlier time itself. Trace elements present in this tree make a good daily supplement for people suffering from bone and anaemic disorders. The potential phytochemical compounds such as tannins, saponoids, flavonoids, alkaloids, quinine, cardiac glycosides, terpenoids, phenols and carbohydrates were present in the leaves of *S. arnottianum*. Identification and determination of bioactive compounds can be done with the help of FT-IR and GC-MS technologies. GC-MS is a unique method to recognise the bioactive components of long chain branched hydrocarbons, alcohols, acids, ester etc. The functional groups in plant samples can be identified by FT-IR Spectroscopy. The present study evaluated the functional groups and phytocomponents present in methanol extract of *S. arnottianum* leaf with the help of FT-IR and GC-MS.

**MATERIALS AND METHODS**

**Collection and processing of plant material**

The leaves of *S. arnottianum* were collected from Pampadum Shola National Park, Kerala and identified with taxonomic keys. Freshly fallen leaves of the plant were collected and thoroughly washed with distilled water. The leaves were then cut into small pieces and shade dried. The dried leaves were crushed into powder and preserved in air sealed polythene cover.

**Preparation of plant extract**

Soxlet apparatus was employed to extract the dried leaves using methanol as a solvent. 20gm of plant material was extracted in 250ml of methanol, filtered and the extracts were concentrated using rotary evaporator at 100 rpm for...
FT-IR Analysis

The results showed that the leaves of *S. arnottianum* contains the foremost phytochemical constituents include alkaloids, proteins, phenols, tannins, carbohydrates, saponins, glycosides, flavonoids, diterpenes and phytosterols (Table 1).

The various phytochemical compounds detected are known to have beneficial importance in medical sciences. The secondary metabolites like phenolics and flavonoids from the plants are considered to be powerful free radical scavengers due to their inherent capacity to transform the body’s response to allergies and virus; and they indicated their anti-allergic, anti-inflammatory, anti-microbial, anti-oxidant and anti-cancerous activities. Flavonoids, a group of poly phenolic compounds, also can be used as an anti-inflammatory agent as it affects the radical scavenging, inhibition of hydrolytic and oxidative enzymes.

Phenolic compounds perform as a cell supportive material; as they form an important part in the cell wall structure by polymeric phenolics. Large quantity of phenolic content in any plant specifies its use in the treatment of inflammatory diseases and wound healing. Hence, pharmacologists are generally searching plants with high phenolic content. *S. arnottianum* leaves were already used indigenously for the treatment of inflammatory diseases. Tannins are complex compounds of non-carbohydrate residue which have astringent, anti-inflammatory, anti diarrheal, antioxidant and antimicrobial activities like antiviral, antibacterial, antifungal and anti-tumorous activities. It was also reported that certain tannins were able to prevent HIV replication selectively and was also used as diuretic. Hence, the leaves of *S. arnottianum* can also consider for such treatments as it exhibit these properties. The presence of saponins enhances the utilisation of *S. arnottianum* leaves for the treatment of hypercholesterolemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory, weight loss etc.

The GC-MS chromatogram was represented in fig. 2 and the constituents obtained from GC-MS analysis are given in table 2.

The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula (Table 2). Eleven phytochemical compounds are present in the leaves of *S. arnottianum*. These compounds are: 4-Aminopyrimidine, 21 and 4-Hydroxyphenylaceticacid (RT=20.567), hexyl ester, 4-Hydroxy-5-methyl-2-pentanediyl bis-Propanedinitrile (RT=26.341), 1,3,5,7-cyclooctatetraene)-Cyclopentane (RT=26.341), 1,1’-[3-(2-cyclopentylethyl)-1,5-pentanediyl] bis-Propanedinitrile (RT=26.341), Cyclohexane (RT=26.341), 1,2,4,5-tetraethyl-2-Thiopheneacetic acid (RT=26.410), Oct-3-en-2-yl ester, Cyclopentanone (RT=26.410, 1,2,3-eta.-2-butenyl) etra.8-1,3,5,7-cyclooctatetraene)-Cyclopentane. The identified compounds have several biological properties. The results obtained from GC-MS analysis shows that most of the phytochemical groups in the leaves of *S. arnottianum* have pharmaceutical activities and some of are discussed here. 4-Aminopyrimidine has been used as a drug for curing multiple sclerosis. This is used as a hair growth stimulant and potent convulsant. Oxazole have anti-cancerous, anti-viral, anti-diabetic and antibiotic activity. Cyclopentanone, another compound obtained, has anti-inflammatory effects.
been used as an intermediate for the production of medicines and perfumes. The structure of the various phytochemicals (Fig. 3) which contribute to the medicinal activity of the plant methanol extract of *S. arnottianum*.

FTIR spectroscopy is used to be an essential and sensitive technique for finding out the bio-molecular composition. The results of FTIR spectroscopic analysis in the methanol extract of leaf litter of *Syzygium arnottianum* have revealed the presence of numerous chemical compounds (Fig. 4). The peak formation in the FTIR spectrum represents the functional groups (Table 3). The absorption at 3363.86 cm\(^{-1}\) assigned to O-H of alcohols and phenol groups. The band at 3307.92 cm\(^{-1}\), 3292.49 cm\(^{-1}\), 3263.56 cm\(^{-1}\), 3203.76 cm\(^{-1}\), 1627.92 cm\(^{-1}\), 1614.42 cm\(^{-1}\) is due to the N-H stretching present in the extract. The band at 2918.30 cm\(^{-1}\), 2850.79 cm\(^{-1}\) is due to C-H stretching of methylene asym. /sym. The IR fingerprints of protein are featured by a set of absorption regions represented as the amide region and the C-H region. The band at 1379.10 cm\(^{-1}\) showed aromatic C-H in plane bend.
Table 3: FTIR Frequency range and functional groups present in the leaf extracts of *Syzygium arnottianum*.

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The band at 1571.99 cm⁻¹, 1537.27 cm⁻¹, 1517.98 cm⁻¹, 1433.11 cm⁻¹ showed alkanyl C=O stretch. The band at 1728.22 cm⁻¹ showed C=O stretching vibration of the peptide group means that some carbonyl compounds existed in the leaves of *S. arnottianum*. The band at 1317.38 cm⁻¹ showed C-O stretching vibration of the carbonyl and ether group. The band at 1317.38 cm⁻¹ showed C-F stretching present in the extract. The band at 1031.92 cm⁻¹ showed the stretching vibration of C-O. The band at 696.30 cm⁻¹ showed the presence of carbohydrates present in the leaves. Bands in 1220 cm⁻¹ represents C-O stretching of phenolics and asymmetric C-O stretching of esters. The major components are identified based on the fingerprint characters of the peak positions, shapes and intensities. Based on the analysis of functional groups, it can be proved that carboxylic acids, aldehydes, aromatics, alkenes, phenols or tertiary alcohols, alkanes, aliphatic bromo compounds and alkynes might be accountable for numerous pharmaceutical properties of *S. arnottianum*. The FT-IR spectrum at 1101.28-1152.10 cm⁻¹ is because of the vibration stretching for (C-H) bond of aromatic compound which contains carboxylic acids, aldehydes, aromatics, alkenes, phenols, alkanes, aliphatic bromo compounds, alkynes, carbonyl and ether group. Alkenes are used as anaesthetics. Carboxylic acids help in maintaining the cell membrane and control nutrient use along with metabolism. Phenol is used as an antiseptic and is also used as a preservative in some vaccines. Alkynes have antifungal and antitumor activities. Phenol spray is used medically to help sore throat. The peak at 2923.95-2926.37 cm⁻¹ is allocated to the C-H stretching means that some alkane compounds were present in rare medicinal plants. Hence, the FT-IR results showed that the leaves of *S. arnottianum* have antiseptic, anaesthetic, antimicrobial and antitumor activities. The functional groups appeared in FT-IR was related to qualitative phytochemical screening of methanol extracts and these studies pave a method for active separation of different phytochemical compounds with the help of GC-MS. The plants species can be used for antifungal agents as it is confirmed that phenolic compounds from natural resources have antifungal activity. Carboxylic acids, present in various plant metabolites, were linked with numerous antimicrobial and antifungal activities. Hence the leaf extract of the studied species can be used as antibacterial agent. Every organism is physiologically controlled by enzymes and hormones, which are basically proteins. The leaves...
of *S. arnottianum* contain rich content of proteins as observed in this study. Hence the present study substantiates the use of leaves as food supplement during the past.

**CONCLUSION**

The present study concluded that the leaves of *S. arnottianum* have numerous medicinal properties and isolation of individual phytochemical constituents may proceed to find out novel drugs.

**ACKNOWLEDGEMENT**

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**REFERENCES**


Litter decomposition in forest ecosystems: a review

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Abstract Litter decomposition in terrestrial ecosystems has a major role in the biogeochemical cycling of elements in the environment. Climatic features, like temperature, rainfall, humidity, and seasonal variations affect the rate of litter decomposition. This review attempts to understand the litter decomposition process in tropical forest ecosystems. It also reviews the influence of various factors on litter degradation and techniques used for assessing leaf litter decomposition. It is observed that very few studies were conducted on litter decomposition in forest ecosystems, such as tropical and temperate forests. Hence, comprehensive studies on litter degradation have to be undertaken in order to understand the turnover rate of nutrients and other elements in these sensitive ecosystems.

Keywords Biogeochemistry · Plant · Bacteria · Fungi · Respiration · Carbon

1 Introduction

Litter fall in terrestrial ecosystems signifies a crucial pathway for nutrient return to the soil. Leaf tissue can account for more than 70% of above ground litter fall in forests, and the rest is composed of stems, small twigs and propagative structures (Robertson and Paul 1999). “Litter mass loss” or “decay” is the sum of carbon dioxide (CO₂) release and discharge of compounds, which contains both carbon compounds and nutrients (Brady and Weil 2010). Litter decomposition proceeds through numerous mechanisms, especially heterotrophic consumption of organic composites in litter (Bezkorovainaya 2005). Rainwater leaching and the activities of small insects do not lead straight to CO₂ release to the atmosphere, even though they support litter decomposition. The CO₂ released through microbial decomposition can add more than 20% to soil surface CO₂ efflux, which is known as soil respiration. In advance, nitrogen (N), phosphorus (P) and calcium (Ca) released from plant litter through decomposition are accessible for plants and microbial uptake. This review summarises the role of microbes and plants in the litter degradation process and also the importance of nutrient cycling and the mineralisation process (Ball 1997).

2 Litter

Ecologically the term litter has two meanings: the layer of dead plant material present on the soil surface or dead plant material that is detached from a living plant. The litter strata can be different from the mineral layer but this is not true for the layer comprising of identifiable plant materials and the layer encompassing merely amorphous organic material (Anderson and Ingram 1983). There is no benchmark for the beginning of decomposition of litter that is detached from the living plant. A dead branch in the crown of a tree may have decomposed to its partial live weight before it drops to the ground, and the heartwood of a tree may die and decompose wholly before the tree falls (Bremer et al. 1991). The presence of a large amount of litter on the forest floor has a significant influence on forest ecosystem dynamics (Olsen 1963).
3 Litter decomposition

Litter decomposition plays a vital part in the nutrient budget of a forest ecosystem, where flora is influenced most significantly by nutrient recycling from plant litter (Vesterdal 1999; Wedderburn and Carter 1999). Litter decomposition encompasses ample breakdown of organic matter into CO$_2$ and nutrients via physical, biological and chemical pathways (Aerts 1997). It returns carbon, as CO$_2$, into the atmosphere through the heterotrophic respiration of soil microorganisms and animals (Chandrasekhara 1997; Schimel 1995; Wachendorf et al. 1997).

Slow decomposition rates result in the building up of organic matter and nutrient stocks in soil; however, fast decomposition rates help to meet plant intake requirements (Isaac and Nair 2005). Climatic features, such as temperature, rainfall and seasonal variations, may influence the existence of microbes and other soil fauna that significantly affect the rate of decomposition. The litter diversity also influences the activity of soil communities and processes during decomposition (Chapman and Koch 2007). The ecosystem significance of a variety of soil organisms is poorly understood, except for earthworms, termites and ants (Jones et al. 1994; Anderson 1995). A schematic representation of litter degradation is shown in Fig. 1.

4 Factors affecting litter decomposition

Litter decomposition consists of two simultaneous processes: (a) the associated mineralisation and humification of lignin, cellulose and other compounds through a series of actions by microorganisms and (b) the leaching of soluble compounds into the soil whose carbon and nitrogen are gradually mineralised (Anderson 1988). These methods depend on abiotic factors like temperature, humidity and biotic features, such as chemical composition of litter and soil organisms (Aber and Melillo 1982). Hence, the physico-chemical environment, litter quality and the composition of the decomposer community are the three leading features regulating litter decomposition (Berg et al. 1993; Couteaux et al. 1995; Cadish and Giller 1997; Bohlen et al. 1997; Dechaime et al. 2005).

Temperature can be considered as a prime factor in determining the rates of litter decomposition (Meentemeyer 1978; Hobbie 1996), and decomposition is more sensitive to temperature than the primary production (Lloyd and Taylor 1994; Kirschbaum 2000). Soil microbial activity rises exponentially with soil temperature (Kirschbaum 1995). A few studies have indicated the role of the chemical nature of the litter in decomposition along with climate (Swift et al. 1979; Berg et al. 2000).

Fig. 1 Diagrammatic representation of factors affecting litter degradation (Litter degradation is influenced by various physico-chemical and biological factors. These factors are governed by the climate and type of forests)
Fresh leaf litter is a readily available substrate for soil macro- and microfauna. The litter quality also affects the degradation process, as it generally reduces throughout the decomposition due to the loss of readily accessible carbon and the accumulation of recalcitrant compounds (Dilly and Munch 2001; Rosenbrock et al. 1995). Liu et al. (2010) revealed the influence of the type of leaf litter on the decomposition process and soil microbes (Coleman and Crossley 1996). The major factors that influence litter degradation are diagrammatically represented in Fig. 1.

5 Role of soil properties

Soil physical and chemical characteristics have a significant role in litter decomposition. Among them, texture is the most significant as it stimulates nutrient and water dynamics, porosity, permeability and surface area. The major chemical properties include pH, cation exchange capacity, organic matter content and nutrients (Coleman et al. 1999). The organic matter, which influences the different physico-chemical factors like bulk density, pH, is the major soil property affecting litter decomposition (Cuevas and Medina 1986). The organic matter can also increase the population density of soil macroorganisms, which plays a significant role in litter mixing and decomposition (Akpor et al. 2006). Among the mineral nutrients, soil nitrogen status is deliberated as being the primary regulating factor and has received utmost attention, while phosphorous is usually considered as a limiting nutrient because of the low quantity in circulation in major forests. Calcium, nitrogen and phosphorus are rapidly mineralised in litter (takes several weeks/months), but organic complexes in the soil organic matter pools have much slower turnover times, taking several years or decades (Devī and Yadava 2007). However, while considering an entire decay process, the effects of added nitrogen on the rate of decomposition seem to be irrelevant and can even turn out to be contrary (Fog 1988).

Potassium and magnesium are essential nutrients for higher plants but hardly limit the microbial actions and are easily removed from decomposing litter (Anderson and Ingram 1983). Nutrient cycles in rain forests differ with soil type, climate and topographic locations; hence, the moisture content and temperature are also unavoidable factors in the litter degradation process (Esperschutz et al. 2011).

6 Role of trees and litter quality

The major component of organic material in forest soil results from the vegetation that is deposited on the soil surface as an organic layer (litter) and is partially dispersed into the soil (Klein and Dutrow 2000; Santa Regina and Tarazona 2001).

Plant litter contains various classes of organic compounds. There are four major assemblies of soluble organic material in litter: sugars, phenolics, hydrocarbons and glycerides. The soluble sugars, primarily mono and oligosaccharides are difficult to metabolise. The relative proportions of these compounds differ with the plant part (leaves, stems, roots, bark) and plant species. The plant litter quality is measured by means of chemical composition of nitrogen, phosphorus, potassium and chief cell wall components, such as lignin, cellulose and hemicelluloses that influence the litter decomposition and nutrient release (Swift et al. 1979).

Lignin accounts for about 15–40% of the total litter quantity. In certain extreme cases, litter can have lignin contents as low as 4% or as high as 50%. Lignin, in contrast to cellulose, is an extremely flexible molecule. The structure of lignin differs with the plant species. For example, deciduous species are comprised of fluctuating proportions of syringyl and guaiacyl forms of lignin, while conifers have generally guaiacyl lignin (Esperschutz et al. 2013).

In addition to lignin, the carbohydrates, such as cellulose and hemicelluloses, are the common constituents in plant litter in terms of quantity. Of these, cellulose (10–50% of the litter quantity) is made up of glucose elements linked with β-1-4 bonds that create long chains of molecules organised into fibres. Hemicelluloses are polymers of sugars like glucose, and the amounts of these may differ among litter species (Akpor et al. 2005). The ratios of hemicelluloses to cellulose range from 0.7 to 1.2; upper ratios are frequently perceived in deciduous litter (e.g., beech) and the lower ratios in coniferous litter (e.g., spruce) (Fengel and Wegener 1983).

Litter decomposition rates vary widely among species that decompose in identical ecological situations (Cornelissen 1996; Wardle et al. 1997). These alterations in decomposition are mainly due to differences in litter traits, such as leaf toughness, nitrogen, lignin, polyphenol concentrations, the C/N ratio and lignin/nitrogen ratio (Berg et al. 1993; Cadish and Giller 1997; Perez-Harguindeguy et al. 2000). Among the various traits, nitrogen and lignin content of plant material are the most significant in regulating the rates of decomposition (Millar et al. 1936; Minderman 1968; Fogel and Cromack 1977; Gartner and Cardon 2004; Meentemeyer 1978). On the basis of the close association between litter quality and decomposition, litter traits can be used as forecasters for decay rates between species (Aber et al. 1990) and also serve as important variables in biogeochemical models (Nicolardot et al. 2001).

Litter quality typically reduces throughout decomposition due to the loss of easily attainable carbon and the
accumulation of recalcitrant compounds (Gaudinski et al. 2000). The leaves of coniferous trees decay more slowly than those of deciduous trees, as broad-leaved litter covers more potassium and phosphorus, less lignin and nearly always less ether-soluble sections (Daubemire and Prusso 1963; Gosz et al. 1973; Mikola 1960; Ovington 1954). Alterations among hardwood species remain substantial (Edwards and Heath 1963). The decomposition of teak litter was faster than that of Acacia arabica litter; moreover, leaf litter vanishes much sooner than twigs and branches (Rochow 1974) and litter under forest canopy is softer and disappears more quickly than leaves exposed to sunlight (Giller and Gadisch 1997; Williams and Gray 1974). Deviations in the rate of leaf litter decomposition of the same plants during different seasons at different locations are also observed (Kumar et al. 2012). Studies show that climatic variations could be a major reason for this, as this is known to be the leading factor influencing litter decomposition on a large geographic scale (Meentemeyer 1978; Dyer et al. 1990; Austin and Vitousek 2000).

The rate of decomposition is high in species with extreme ash and nitrogen contents and the lowest C/N ratios and lignin contents. Species showing average ash, nitrogen and lignin contents and a normal C/N ratio appears to decay at a transitional rate. Kucera (1959) reported a progressive correlation between both the rate of decay and ash content of hot-water-soluble materials (Gonzalez and Seastedt 2001).

The concentrations of nutrients vary with the litter species. For instance, leaf litter of the nitrogen fixative genus alder (Alnus) has great actual concentrations of N (often above 3%); in contrast, pine needle litter is nitrogen poor (frequently under 0.4%). Plant species is therefore a prevailing feature in defining the litter value (Gustafson 1943; Berg and McClaugherty 2003).

7 Role of soil fauna and microbes

The abundance and arrangement of soil fauna and microbial populations are known to affect the rate of litter breakdown at various stages of decomposition (Schaefer and Schauermann 1990; Dilly et al. 2004). Microbial decomposition of organic material on the forest soil has a significant effect on soil carbon and energy flow in the ecosystem. The variety of such soil microes is supposed to be extremely high; however, they are mainly anonymous (Prosser 2002). Species variety of soil fungi is slightly lower than that of bacteria (Bridge and Spooner 2001; Hawksworth 2001), due to their high productivity and fast growth (Hanson et al. 2005). The count of bacterial species is in the order of hundreds to thousands in 1 g of soil, whereas entire species number is more than 2–3 million (Torsvik et al. 1994; Dejonghe et al. 2001; Prescott et al. 2000).

Among the soil microfauna, fungi are the leading decomposer and have more than 75% greater potential to reduce organic matter than other microorganisms (Kjoller and Struwe 1992). Furthermore, their activity will vary seasonally. Besides fungus, litter bacteria are a significant part of the process of organic matter mineralisation and accounts for 25–30% of the total soil microbial biomass (Dilly and Munch 2001; Kurihara and Kikkawa 1986; Persson 1980).

Leaf decomposition by fungi and bacteria tends to be rapid at nutrient-enriched conditions. The involvement of fungi and bacteria in leaf decomposition could react inversely to stress situations (Pascoal and Cassio 2004). Microbes can also be limited by soil moisture. As the temperature rises, soil moisture has a progressively more significant role in retaining high rates of microbial activity (Peterjohn et al. 1994). As a result, the rate of fresh litter decomposition rises with both increasing temperature and precipitation (Meentemeyer 1978).

The growth of microbes, especially fungi, on the litter may initiate decomposition prior to litter fall; however, the growth of decomposers only takes place when the litter reaches the floor. The arrangement of the microbial community that occupies the litter depends on the properties of the litter, soil features and variations of these properties over time (Harmon et al. 1999).

The role of numerous classes of bacteria and fungi in litter degradation was recognised in earlier studies (Table 1) and showed that, under laboratory conditions, forest soil and related microbial communities act as vital variables in litter decomposition process (Frankland 1992; Rosenbrock et al. 1995; Cox et al. 1997, 2001; Prescott 1996; Chadwick et al. 1998). Litter decomposition is also influenced by the quantity and quality of litter input, which is dependent on plant species (Chadwick et al. 1998; Hattenschwiler et al. 2005).

Besides fungi and bacteria, soil biota comprises of both micro- and macroinvertebrates (Heath 1966). Microarthropods, which survive in the litter strata and on the upper layer of the soil, are an essential part of the ecosystems due to their significant role in organic matter decomposition and mineralisation processes, nutrient cycling (Irmler 1982) and pedogenesis. Soil faunal activities mainly help to acclimatise the litter and motivate microbial activity. The labile compounds (e.g., sugars, amino acids) in litter may be absorbed by soil microbes and therefore prone to rapid decay (Hobbie 1996). The labile structural compounds, such as cellulose, are quickly cleaved by exo-enzymes into sugar sub-units, which again are readily absorbed by microbes. In contrast, refractory structural compounds, such as lignin and chitin, are too
large to pass through cell membranes and remain unchanged to extracellular enzymes due to their uneven chemical structure and complex bonding (Horner et al. 1988).

Studies suggested that the presence of fauna on the leaf discs and the leaf tissue consumption was less during winter (Crossley and Hoglund 1962; Madge 1965). Edwards and Heath (1963) noticed that earthworms were able to decompose litter three times faster than minor invertebrates, such as springtails, enchytraeids and larvae (Jenkinson et al. 1994a, b). Various organisms are capable of degrading the more amorphous kind of cellulose (Eriksson et al. 1990). The wood-decay fungus, white-rot basidiomycete (Phanerochaete chrysosporium), has been used for deterioration of lignocellulosic constituents (Tien and Kirk 1984; Higuchi 1993).

Three major hydrolytic enzymes carry out cellulose degradation: endo-1, 4-glucanase shelters the cellulose chain and ruptures the glucosidic relations via a random method. Exo-1, 4-glucanase ruptures either cellobiose or glucose from the non-reducing end of the cellulose chain. Finally 1, 4-glucosidase hydrolyses cellobiose and further water-soluble oligosaccharides, such as triose and tetrose, to glucose. These enzymes are dissimilar in nature and have different specificities (Johnson and Catley 2002). The endo and exoglucanases have a synergistic action that allows them to decompose crystalline and amorphous cellulose. In addition to hydrolytic enzymes, certain cellulolytic entities yield cellobiose dehydrogenase, which is found in a variety of fungi and seems to have a role in lignin and cellulose degradation (Kelly and Beauchamp 1987).

The soft-rot fungus seems to have a cellulose-degrading scheme like that of the white rots. Brown rots have not yet been observed to require the synergistic enzymes that are found in white rots and they do not have the exoglucanase. Highley (1988) found numerous species of brown rots that were able to solubilise microcrystalline cellulose. These fungi simply depolymerise cellulose, without producing

### Table 1 Microorganisms capable of utilising different components of organic matter

<table>
<thead>
<tr>
<th>Components</th>
<th>Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>Alternaria, Aspergillus, Chaetomium, Coprinus, Fomes, Fusarium, Myrothecium, Penicillium, Polyporus, Rhizoctonia, Rhizopus, Trametes, Trichoderma, Trichotheceum, Verticillium, Zygothecium, Achromobacter, Angiococcus, Bacillus, Cellulicola, Cellumonomas, Cellvibrio, Clostridium, Cytophaga, Polyangium, Pseudomonas, Sorangium, Sporocytomyce, Vibrio, Micromonopora, Nocardia, Streptomycyce and Streptosporangium</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>Alternaria, Aspergillus, Fusarium, Rhizopus, Trichothecium, Zygothecium, Chaetomium, Helminthosporium, Penicillium, Polyporus, Coriolus, Fomes, Bacillus, Achromobacter, Pseudomonas, Cytophaga, Sporocytomyce, Lactobacillus, Vibrio and Streptomycyce</td>
</tr>
<tr>
<td>Lignin</td>
<td>Clavaria, Clitocyce, Collybia, Hypholoma, Lepiota, Mycena, Pholiota, Arthrobotrys, Cephalosporium, Humicola, Pseudomonas and Flavobacterium</td>
</tr>
<tr>
<td>Starch</td>
<td>Aspergillus, Fomes, Fusarium, Polyporus, Rhizopus, Achromobacter, Bacillus, Chromobacterium, Clostridium, Cytophaga, Micromonopora, Nocardia and Streptomycyce</td>
</tr>
<tr>
<td>Pectin</td>
<td>Fusarium, Verticillium, Bacillus, Clostridium and Pseudomonas</td>
</tr>
<tr>
<td>Inulin</td>
<td>Penicillium, Aspergillus, Fusarium, Pseudomonas, Flavobacterium, Beneckeia, micrococcus, Cytophaga and Clostridium</td>
</tr>
<tr>
<td>Chitin</td>
<td>Fusarium, Mucor, Mortierella, Trichoderma, Aspergillus, Gliocladium, Penicillium, Thamnidium, Absidia, Cytophaga, Achromobacter, Bacillus, Beneckeia, micrococcus, Pseudomonas, Chromobacterium, Flavobacterium, Nocardia, Streptomycyce and Micromonopora</td>
</tr>
<tr>
<td>Proteins and nucleic acids</td>
<td>Bacillus, Pseudomonas, Clostridium, Serratia and Micrococcus</td>
</tr>
<tr>
<td>Cutin</td>
<td>Penicillium, Rhodotorula, Mortierella, Bacillus and Streptomycyce</td>
</tr>
<tr>
<td>Tannin</td>
<td>Aspergillus and Penicillium</td>
</tr>
<tr>
<td>Humic acid</td>
<td>Penicillium and Polystitus</td>
</tr>
<tr>
<td>Fulvic acid</td>
<td>Poria</td>
</tr>
</tbody>
</table>

Crawford (1981), Jin et al. (1990), Eriksson et al. (1990)
soluble monomers or dimers. Still, no additional enzyme has been found to account for the lost exoglucanase that splits off from the soluble components. Hence, Eriksson et al. (1990) suggested a non-enzymatic mechanism.

Comprehensive studies on *Clostridium cellulolyticum* illustrate that the organism yields at least six dissimilar cellulases, each one with diverse structural and catalytic properties (Klein and Dutrow 2000). Both cellulases and xylanases are held together in a huge arrangement, known as the cellulosome, by a platform protein, as proposed by Eriksson et al. (1990). Earlier, the formation of the cellulosome itself was observed in an anaerobic bacterium *Clostridium thermocellum* (Viljoen et al. 1926).

The degradation of cellulose by bacteria is suggested to be hydrolytic, while the mechanisms seem to be different from those found in fungi. For bacteria, the cellulosolytic enzymes are organised in groups and perform via a collective method (Knapp et al. 1983). There is no additional group of cellulosolytic bacteria, comprising *Cytophaga, Cellulomonas, Pseudomonas* and *Cellvibrio*. It seems that these bacteria have their cellulosolytic enzymes bound to the cell wall and, consequently, an adjacent connection is necessary between the cell and the substrate (Berg et al. 1972; Eriksson et al. 1990; Wiegel and Dykstra 1984). The major bacteria that are capable of utilising cellulose are *Achromobacter, Angiococcus, Bacillus, Cellfalcicula, Cellulomonas, cellvibrio, Clostridium, Cytophaga, Polyangium, Pseudomonas, Sorangium, Sporocytophaga, Micromonospora, Nocardia and Vibrio* (Krivtsov et al. 2005).

Actinomycetes degrade the cellulose in a manner similar to that of fungi and can also degrade the crystalline form. Several strains have the ability to degrade the lignocellulose complex (Wang et al. 1999). Actinomycetes, like *Actinokineosporu, Streptomycetes, Nocardioches, Pseudonocardia, Nocardia* and *Micromonospora*, are capable of decomposing plant litter (Das and Battles 2007). The mode of enzymatic degradation of cellulose and also the lignocellulose complex of actinomycetes is similar to that of fungi (Finlay et al. 2000). The production of cellulases is influenced by cellulose, cellobiose, sorhorese and lactose (Luken et al. 1962). The existence of cellulose seems to be the best stimulation agent, whereas glucose suppresses the production of the cellulase system (Wood 1995). As cellulose is a large and non-soluble molecule, it cannot be absorbed into the microbial cells for a persuading effect to be applied. Currently, the conventional theory is that the entities have a constant, rudimentary level of cellulose on their surface (Mahasneh 2001). Upon connection with cellulose, small quantities of persuading materials are released from the cellulose; these enter into the microbial cell and induce cellulose creation. It is expected a little intra-cellular absorption of a type of compound resembling cellobiose or celiotroide can stimulate the production of cellulose (Martin and Marinissen 1993).

### Table 2 Degradation patterns of major polymers in litter

<table>
<thead>
<tr>
<th>Major polymers</th>
<th>Reaction</th>
<th>Microbes involved</th>
<th>Enzymes involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>Converts into monomers/oligomers of cellulose</td>
<td>Wood-decay fungus (white rot, brown rot and soft rot)</td>
<td>Endo-1,4-glucanase (ruptures glucosidic linkage); Exo-1,4-glucanase (ruptures celllobiose/glucose); 1,4-glucanase (hydrolyse celllobiose to glucose)</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td></td>
<td>Bacillus, Achromobacter, Pseudomonas, Cytophaga, Sporocytophaga, Lactobacillus, Vibrio and Streptomyces</td>
<td>Needs additional composite enzyme systems that are desirable for the hydrolysis of cellulose</td>
</tr>
<tr>
<td>Pectin</td>
<td>Demethylated to pectic acid (polygalacturonate)</td>
<td><em>E. chrysanthemi, E. cartovora, Pseudomonas, Bacillus, Clostridium, Lachospina, Butyrivibrio, and Bacteroides</em></td>
<td>pectin methylesterase, pectatelyase (an endopectatelyase), exopolygalacturonase and oligouronidelyase</td>
</tr>
<tr>
<td>Lignin</td>
<td>Converts lignin into CO₂ and water; Formation of carboxyl and carboxyl group; Softening of the wood by breaking the middle lamella of cell wall</td>
<td>Wood-decay fungus (white rot, brown rot and soft rot)</td>
<td>Mn-peroxidase</td>
</tr>
</tbody>
</table>

Eriksson et al. (1990), Wiegel and Dykstra (1984), Hatakka (2001), D’Souza et al. (1999)

8.2 Hemicelluloses

In wood, the entire absorption of hemicelluloses typically ranges from 20 to 30%. There is variance in the structure and arrangement of hemicelluloses in litters of softwood as compared to hardwood (Wolter et al. 1980). The hemicelluloses are composed of both linear and branched heteropolymers of D-xylose, L-arabinose, D-mannose, D-glucose, D-galactose and D-glucuronic acid and are individually methylated or acetylated (McTiernan et al. 1997).
Degradation of hemicelluloses requires additional composite enzyme systems for the hydrolysis of cellulose. The degradation of such a molecule requires the concentrated action of several hydrolytic enzymes (Eriksson et al. 1990). The major bacterial species involved in the utilisation of hemicellulose are Bacillus, Achromobacter, Pseudomonas, Cytophaga, Sporocytophaga, Lactobacillus, Vibrio and Streptomyces (Mikola 1973).

Major starch-utilising bacteria are Achromobacter, Bacillus, Chromobacterium, Clostridium, Cytophaga, Micromonospora and Nocardia, whereas protein-using bacteria include Bacillus, Pseudomonas, Clostridium, Serratia and Micromonospora.

8.3 Pectin

Pectin is a highly methylated form of poly-1,4-D galacturonic acid. *E. chrysanthemi* and *E. cartovora* are induced to form a complex of enzymes that constitute the degradation of pectin. Initially pectin is demethylated to pectic acid (polygalacturonate) by pectin methyl esterase (Nye 1961). Enzymes, such as pectate lyase (an endo pectate lyase), exo polygalacturonase and oligo uronidelyase, are involved in the degradation of pectin. Enzymes that degrade pectin or poly galacturonic acid are found in *Pseudomonas*, *Bacillus*, *Clostridium*, *Lachospira*, *Butyry vibrio* and *Bacteroides* (Ovington and Madgwick 1957).

8.4 Lignin

Lignin degradation is considered to vary between the three common sets of decomposers: white-rot, soft-rot and brown-rot fungi. The diverse enzymatic mechanisms of lignin degradation are merely defined, except for *Phanerochaete chrysosporium*, which is a white-rot fungus (Rigobelo and Nahas 2004).

9 Lignin degradation by white rot fungi

White-rot fungi have the capability to completely mineralise lignin to CO₂ and water. The outcome, for wood, is that the whole lignocellulosic complex is decayed more or less instantaneously. A bulky cluster of the white rots might even decompose lignin differently to cellulose (Hatakka 2001). The attack of lignin arrangement has long been supposed to start through the elimination of the methoxyl group. An earlier investigation revealed that a mixture of hydroxylation and demethylation is shadowed by an oxidative attack on the aromatic ring (Eriksson et al. 1990).

Lignolytic schemes are species specific and depend on the ecological niche of the fungus (Hatakka 2001). For instance, the white-rot *Ganoderma lucidum* creates Mn-peroxidase in a medium with popular wood; however, it does not in pine (D’Souza et al. 1999). Such interpretations might support the outcome that white-rot fungi are usually found on angiosperm than on gymnosperm woods (Gibbertson 1980).

10 Lignin degradation by brown-rot fungi

Brown-rot fungi mostly decay the cellulose and hemicellulose constituents in wood and have the capability to adapt the lignin molecule (Eriksson et al. 1990). Brown-rot and white-rot fungi have similar decomposition mechanisms whereby hydroxyl radicals are created that attack the wood constituents (Hatakka 2001). It is expected that all brown-rot fungi use a similar mechanism for wood decay. The initiation of the decomposition of lignin and cellulose together seems to be through diffusible minor molecules that can pierce the cell wall. In contrast to white rots, only brown rot is set up to create Mn-peroxidase (Sarah 1996). The radicals made by brown-rot fungi can eradicate methoxyl groups from lignin and yield methanol, leaving the remains of mostly altered lignin (Eriksson et al. 1990) where the presence of phenolic hydroxyl groups is high (Crawford 1981). Carbonyl and carboxyl groups are also produced (Jin et al. 1990). Hence brown-rotted lignin remains more responsive than natural lignin.

11 Lignin degradation by soft-rot fungi

The literature suggests that soft-rot fungi do not decompose lignin; however, it does soften wood by breaking down the middle lamella of the cell wall. Most soft-rot fungi are ascomycetes and deuteromycetes and are most lively in moist wood (Scholle et al. 1992). Crawford (1981) observed that soft-rot fungi remained capable of reducing the lignin content in decomposing wood. Another study showed that soft-rot fungi decompose lignin up to 44% under laboratory conditions (Nilsson et al. 1989). The lignolytic peroxidases of soft-rot fungi do not have the potential to oxidise the softwood lignin, which has a high level of guaiacyl components.

12 Microbial litter decomposition and biogeochemical cycling

The deposition of carbon into the soil is a significant part of carbon cycling in terrestrial ecosystems. The chemical components of the litter are organised and reabsorbed by plant roots, resuming a novel plant nutrient cycling and assuring recurrent situations to the
system (Guo and Sims 1999). The major aspects that govern the organic matter conversion are the quantity and quality of litter material constituents, the physical and chemical environment and the decay entities (Swift et al. 1979). The rate of decomposition and nutrient dynamics of leaf litter are influenced by the arrangement of decomposers in the soil (Swift et al. 1979). The bacterial community, their respiratory action and particular soil chemical complexes designate the transformation development that occurs in soils under exact forest litters (Luizao et al. 1992). Furthermore, edaphic and climatic features affect the action of soil microbial enzymes (Jha et al. 1992). It is expected that the nutrients released during litter decay can account for 67–87% of the annual demand for forest plants (Waring and Schlesinger 1985). The litter decomposition is closely related to microbial activities that modify the litter chemical composition and regulate carbon and nitrogen dynamics in soil. The development of microbes, as well as the subsequent biomass and necromass, considerably changes the chemical features of soil organic matter, as detected in nutrient immobilisation (Simpson et al. 2007). The role of litter decomposition in the biogeochemical cycle is illustrated in Fig. 2.

### 12.1 Carbon cycle

Microbial biomass consists of less than 35% of the total organic carbon in soils (Schlesinger 1997). The decay rate of humus in a natural forest environment is lower than that of an agricultural field. Depending on the substrate value, carbon complexes can be broken down by the enzymatic action of microbes. In forest soils, the decay of leaf litter yields high amounts of dissolved organic carbon compounds (Singh and Gupta 1977). About 5–40% of the whole carbon losses may be due to leaching. On the other hand, a lesser amount of carbon is removed by soil erosion under forest cover. This acts to reduce the decay ratio and total stored carbon in the soil. Johnson and Curtis (2001) revealed that the elimination of saw-log forest tended to increase the quantity of carbon and nitrogen in the soil for a small duration. This is due to the fast assimilation of minor size carbon material into the soil, which facilitates microbial decay of the carbon molecules and discharge of the excess nutrients towards the soil (Swarnalatha and Reddy 2011).

### 12.2 Nitrogen cycle

The adsorbed and complexed nitrogen will be remobilised from the sources by microbes. The mineralised nitrogen is recovered and consumed by plant roots or recycled by the micro-flora when microbes die. Merely 1–3% of the organic nitrogen in soil is mineralised during its development (Bartholomew and Kirkham 1960).

In soils, nitrogen is associated with the soil organic material, which contains about 5% of the total nitrogen (Brady and Weil 2010). This organic nitrogen is not available for plants, so the microorganisms decay the organic matter into smaller particles through the discharge of the biogeochemical cycle.

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**Fig. 2** Role of litter degradation in biogeochemical cycles (the various elements and compounds accumulating in plants return into the environment through litter degradation where these substances leached into the soil and diffused into the atmosphere. Hence, these substances are again getting into the biogeochemical cycle)
of ammonium. The mineralisation of organic nitrogen compounds in natural forest soils is a gradual process and is commonly facilitated by some degree of microbial activity because of the lower availability of organic nitrogen. As such, soil nitrogen cannot be considered as a chief nitrogen pool (Teuben 1991).

The limitations in the degradation of nitrogenous compounds are indicated in previous studies and are: (a) polyphenols, amino acids and additional nitrogenous materials are reduced into small molecules with a comparatively smaller surface available for enzyme action, (b) the physical sorption of humus by clay reduces the dynamic groups of the humus protein that is unreachable to microbial proteases, (c) much of the soil organic material that is placed inside the soil pore spaces is too small to be available for microbes, and (d) in the deadly phases of decomposition, the arrangement of humic molecules is so uneven that there is lower possibility of specific enzymes meeting the particular bonds (Black 1968; Tripathi et al. 2010).

12.2.1 Phosphorous

Phosphorus is next to nitrogen as a regulating nutrient. Like nitrogen, the phosphorous concentration in litter increases during decomposition. The initial concentration is decreased due to leaching. Litter decomposition provides a very small concentration of orthophosphate to plants (Verhoeof and Brussaard 1990). Organic acids formed by microbial decay of plant remains might collect locally to reach concentrations that can increase the accessibility of phosphates to plants. Organic phosphorus generally mineralises gradually, as specified by Mattingly and Williams (1962).

12.2.2 Potassium

The potassium from plant litter does not gather in surface horizons. However, the arrangement and amount of litter decay might affect its reachability to plants more than the influence of the remaining organic matter on the cation exchange capability of the soil. Potassium and magnesium are essential nutrients for higher plants; however, they rarely limit microbial actions and are quickly removed from decaying litter (Anderson and Ingram 1983).

The presence of tree roots running through litter on the forest floor might diminish leaching losses of nutrients via the direct uptake of K, Mg and Ca (Cuevas and Medina 1988; Medina and Cuevas 1989). There is evidence of direct acceptance of potassium from litter through roots (Herrera et al. 1978).

Loreau (2001) suggested that microbial diversity has a positive influence on nutrient cycling efficiency and ecosystem processes. Among the soil organisms, bacteria and fungi have excellent characteristics of biomass and respiratory metabolic rate and have more involvement in the organic matter decay procedure (Persson 1980). The bacterial community, their respiratory activity and exact soil chemical composites specify the transformation development occurring in soils under specific forest litters (Luizao et al. 1992).

13 C/N ratio of the plant litter and its decomposition

Reports show that leaf litter decomposition can be calculated from the C/N ratio (Melillo et al. 1982). High-quality leaves (nutrient-enriched leaves) will generally decompose more rapidly than low-quality leaves (nutrient-deficient leaves). In general, the decomposition rate is high in species with extreme ash and nitrogen contents and minimum C/N ratios and lignin contents (Singh 1969). Several works showed that the nitrogen concentration of the litter and the C/N ratio is strongly associated with litter decay rates (Berg and Staaf 1981). The concentration of phosphorous and C/P ratios appeared to be good predictors of decay rates (Vitousek et al. 1994). Concentrations of lignin and the lignin/N ratios in plant litter are also good predictors of litter decomposition (Meentemeyer 1978; Melillo et al. 1982). These factors and their effects on litter decomposition depend on soil characteristics and plant species.

14 Various techniques for assessing litter decomposition

14.1 Mass balance technique

Mass balance methods are used to evaluate litter decay in different ecosystems (Olsen 1963; Schlesinger 1997). This method assumes that a constant fraction, k, of the detrital litter quantity decays:

\[ \text{Litter fall} = k(\text{detrital litter mass}) \]

In forest, values for k are larger than 1.0. Environments with slow decomposition rates and low surface litter deposition have k values less than 1. Litter fall is measured by means of litterbags that are unsystematically set apart in the study location (Bubb et al. 1998; Xu and Hirata 2002).

The mass balance method can be used to evaluate litter decay, or to validate model forecasts (Hedin 2000). On the occasion that the forest floor is rapidly aggrading, the technique would over-estimate decay rates. As this technique depends on natural litter fall, this method cannot be used to efficiently explain the role of factors like
temperature and moisture, as is possible with litterbag experiments.

14.2 Litterbag technique

The litterbag method is extensively used to study decay at the soil surface. Fresh leaf litter is placed in litterbags, which are then inserted into the litter layer of the soil and gathered at periodic intermissions so that the remaining quantity can be measured. Mesh size is usually selected as to increase the entry of organisms to the litter, while reducing too much particle loss. Litterbags with different mesh sizes have been used to influence the microbial composition (Crossley and Hoglund 1962). Very small mesh size will not remove certain organisms but will prevent particle loss to mineral soil as well. Fibreglass mesh has been suggested for light concentrated places, as UV light will degrade nylon and other materials (Harmon and Lajtha 1999). Yet, 1–2 mm mesh is most suitable in litterbag studies (Robertson and Paul 1999); mesh size must be more than 2 mm to permit the entry of macrofauna.

The size and content of the litterbags remains a significant constituent of litterbag studies. Litterbags of 20 × 20 cm are common (Robertson and Paul 1999) in different plant populations or where leaves are large and a larger litterbag is suitable.

14.3 Tethered leaves technique

The tethered leaf method is similar to the litterbag method. The specific leaves are tightly packed in packages somewhat located in litterbags. Either a particular leaf or a group of leaves are tied together by means of nylon thread or monofilament fishing line. The line is tied to the leaf petiole for stability; the line is typically attached to a coordination point to facilitate gathering and a tag for recognition.

A “wheel spoke” method, after Vitousek et al. (1994), is frequently used in terrestrial studies, where a group of specific senescent leaves are air-dried, again with their petioles tied to a solitary line. One end remains tied to a recognising tag and the other end to a labelled washer. Numerous sets of threads are tied to every washer in this manner.

Tethered leaf studies remain very relevant in learning the initial phases of decay; therefore, length of study is not equal as that of litterbag methods. As the leaves begin to fragment, this method will over-estimate decay rates as compared to the litterbag method. Studies revealed that litter-feeding invertebrates could attain ready contact with litter in litterbags with mesh sizes as small as 1.5 mm (Scowcroft et al. 2000). Yet, the tethered leaf method permits the leaf intake by macroinvertebrates like crabs and snails, whose contact would otherwise be restricted mesh bags (McKee and Faulkner 2000).

14.4 Cohort layered screen technique

A fourth approach to assess high leaf litter decay is the cohort layered window screen method, or litter sandwich method. By this technique, layers of mesh screen are used to separate consecutive sheets of litter on the forest surface, where the leaf litter decays in situ on the previous litter layer.

The cohort layered screen process is applied to long-term decay studies, normally of three or more years (Binkley 2002). Upon annual litter fall, a new screen window is located above the forest floor. Usually a 1 × 1 m fibreglass or aluminium window screen with a mesh size of 2–3 mm is used. The screen dimension will be determined by the size of the stand tested, and mesh size will differ depending on the exact ecology under study. A fibreglass screen is recommended over aluminium if any chemical or essential properties will be evaluated as well.

Table 3 Comparison of different techniques used for litter decomposition study

<table>
<thead>
<tr>
<th>Methods used for evaluating litter decomposition</th>
<th>Output</th>
<th>Drawbacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass balance technique</td>
<td>Evaluate litter decay, check on model forecasts</td>
<td>This method cannot be used to efficiently explain the role of further features like temperature and moisture as per litterbag experiments</td>
</tr>
<tr>
<td>Litter bag technique</td>
<td>Decay at the soil surface</td>
<td>Large mesh size cause mineral loss to the soil and the entry of macrofauna into the bag</td>
</tr>
<tr>
<td>Tethered leaves technique</td>
<td>Learning the initial phases of decay</td>
<td>It does not used for microbial litter decomposition; because it allows the entry of macrofauna, whose contact would then controlled by mesh bags</td>
</tr>
<tr>
<td>Cohort layered screen technique</td>
<td>Long lasting litter decay studies (more than 3 years)</td>
<td>Fibreglass screen is suggested over aluminium if any chemical or essential properties will be evaluated as well</td>
</tr>
</tbody>
</table>

Karberg et al. (2008)
During the period of study, for every subsequent annual litter fall, an additional screen is positioned straight above the previous screen. Subsamples are gathered, weighed and oven dried. The comparison of different techniques used for litter degradation study shown that the litterbag technique is more appropriate method (Table 3).

15 Conclusion

Litter decomposition is highly significant in the functioning of ecosystems, as it is a major way of recycling of nutrients, especially carbon and nitrogen and other elements in the ecosystem. The degradation rate of plant material and uptake of minerals are in equilibrium in an ecosystem and vary depending on the type of ecosystems. Litter decomposition is a highly complex process that involves a number of physical, chemical and biological factors; however, there is little information about the litter decomposition rate and the role of various factors in different ecosystems. Also it is very difficult to understand the rate of litter degradation as it is influenced by a number of entirely different factors. Researchers are yet to finalise a methodology to detect the rate of litter degradation, which can incorporate all the factors. However, it is significant to study litter degradation in the context of increasing anthropogenic impacts on biogeochemical cycles. This review focuses on various factors that affect the litter degradation and degradation patterns of the various polymers in leaf litter. It also emphasised and discussed various methods for assessing litter degradation. The review found that there are very few studies on litter degradation and element recycling in various ecosystems. Hence, future research must be centralised on the following subject areas: (a) development of a methodology for assessing the rate of litter degradation; (b) litter degradation and climate change; (c) transport pathways of elements during litter degradation, etc.

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