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Leaf Litter Decomposition and Nutrient Release Patterns of Chirpine, Cupressus Plantation, and Oak Dominated Forest

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Abstract

Litter decomposition study on Oak (Quercus griffithii), Cupressus (Cupressus sp.) and Chirpine (Pinus roxburghii) was carried out at Talo geog, Punakha Dzongkhag. The objectives of this study were to examine the rate of leaf litter decomposition (mass loss) and nutrient release pattern under three forest types using the litter bag technique. Two replicate plots (20 x 20 m) were established in each forest type and in each replicate 24 litter bags were laid on the forest floor. Samples were drawn at the interval of 4, 8, 12, 16, 20, 24, 28, 32 weeks and analysed to determine the mass of dry matter remaining and, carbon and nitrogen contents. Highly significant differences (p < .01) were observed in mass loss and nutrient released among different leaf litters. Oak litters lost the maximum mass and nutrients compared to the other two litters. Carbon to Nitrogen (C:N) ratio and nitrogen immobilisation of the three litter types were in the order of Chirpine > Cupressus > Oak. Oak litters with high initial nitrogen content and low C:N ratio decomposed faster than Cupressus and Chirpine needles. The immobilisation nature and high C:N ratio of Chirpine and Cupressus litters indicate their poor litter quality. On the contrary, the fast decomposing Oak litters with high N and low C:N ratio readily releases the nutrients to enrich soil fertility. Study of nutrient dynamics of litters in different forests is important for a greater understanding of the nutrient cycling in the forest ecosystems in the country.

Keywords: C:N ratio, litter bag, litter decomposition, nutrient immobilisation

Introduction

Decomposition refers to both physical and chemical breakdown of leaf litter and mineralisation of nutrients (Baker *et al.*, 2001). It is regulated by a number of abiotic and biotic factors. These factors include soil organisms (e.g. microorganisms, microfauna, mesofauna and macrofauna); litter quality denoted by the inorganic nutrients and organic compounds; physico -chemical properties of soil-litter system, and climate (Swarnalatha and Reddy, 2011).

Litter decomposition plays a vital role in nutrients cycle and carbon (C) fluxes of the terrestrial ecosystem (Berglund, 2012), thus forming an important part of all life cycles both in the terrestrial and aquatic environments (Abugre *et al.*, 2011). Plant detritus is a major component of soil organic matter (SOM) and serves as a main source of energy and nutrients for plants and soil micro-organisms (Odum and De la Cruz, 1963). As such, the micro- and macroinvertebrate, bacterial, and fungal communities depend on SOM for food and energy (Baker et al., 2001). The litters including upper decomposed materials and its production in natural forests as well as in plantation have one of the most important aspects of nutrient cycling in the ecosystem and it is essential for the maintenance of its process (Bhalawe et al., 2013). Litter supplies nutrients to plants and carbon to heterotrophic soil microorganisms (Bothwell et al., 2014). Decomposition is influenced by the type of soil microbes, litter quality, and abiotic environmental conditions (Swift et al., 1979). The soil microbes play an important role in biogeochemical transformation of organic matter and improving the soil fertility. Leaf litter is major part of the total litter fall, providing key nutrient pool (Moore et al., 2006). Litter de-

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composition is responsible for release of soil nutrients and sequestration of carbon through formation of organo-mineral complexes in the soil (Berg and McClaugherty, 2008). The amounts of humus in forest floor express the carbon that comes from litter decomposition (Prescott, 2005). Isaac and Nair (2005) reported that litter quality can be determined by its rate of decay which is influenced by both biotic and abiotic factors.

Around the world, lots of studies have been carried out on litter decomposition particularly in tropical, subtropical (Xu *et al.*, 2011), and temperate forest (Jacob *et al.*, 2010). However, only few studies are done in Bhutan in terms of litter decomposition (Chhetri *et al.*, 2012; Ahmed *et al.*, 2015) which is crucial to understand the nutrient cycling in the forest ecosystems. The objectives of this research were to study the





mass loss and nutrient release patterns of Oak, Cupressus and Chirpine needles under three forest types mainly Oak dominated, Cupressus, and Chirpine at Tala *geog* under Punakha *Dzongkhag*.

Materials and Method

The study area

The present study was conducted in three different forest types: Oak dominated forest (*Quercus griffithii* Hook.f. & Thomson *ex* Miq.) associated with *Castonopsis* spp. and *Myrica* sp., Cupressus plantation (*Cupressus* sp.) and Chirpine (*Pinus roxburghii* Sarg.) forest at Talo geog, Punakha Dzongkhag. The Oak forest and Cupressus plantation fall within the Mangizingkha community forest, while Chirpine forest falls in the Puensum Community forest under Talo geog (Figure 1). The study area spans at an altitude of about 1620 to 2600 m above sea level. The rainfall ranged from 1.50 to 142.30 mm with highest rainfall received during August month and minimum in February month. The mean temperature ranged from 13.69 to 26.98°C (Figure 2). The study commenced from August 1st 2014 to March 31st 2015 for a period of 8 months.

Field methods

The litter decomposition rate was determined using the litter bag technique (20×20 cm with 2 mm mesh size). At the end of July 2014, leaf litters were collected from each forest floor and

air dried for one week to make their weight constant. A subsample of the leaf litters was analysed for carbon (C) and nitrogen (N) content as initial nutrient contents. The C content was determined by Loss on Ignition method and N content by the Kjeldahl method. Leaves were carefully separated from other leaf species. In each litter bag, 20 g of leaf litter was weighed and placed inside the litter bag. In the field, two replicate plots (20 x 20 m) were established under each forest type to lay out the litter bags. In each 20 x 20 m plot, 24 litter bags were ran-

domly placed on the surface of forest floor on 1^{st} August 2014. There were 144 litter bags in total. The litter bags were tethered with nylon thread to wooden peg to reduce movement and also to ensure a suitable contact with the organic soil layer. Three litter bags were randomly retrieved from each study plot at the interval of 4, 8, 12, 16, 20, 24, 28, and 32 weeks. Extraneous materials including roots, soil, and mosses were carefully brushed off from the litter bag. To remove soil particles adhered to the litters, the litter bags were lightly washed with running tap water and sun dried for three days. Then they were transferred to paper envelopes and oven dried at 70°C for 48 hours. Dry weight was de-



Figure 2: Mean temperature and rainfall from August, 2014 to March, 2015 in the study area

termined and a sub-sample was ground and analysed for total N and Carbon content. Dry weight remaining (%) was calculated by using the following formula:

$$\mathsf{D}_{\mathsf{r}}(\%) = \frac{wt}{wi} \times 100$$

where D_r is dry weight remaining, *wt* is oven dry weight at time "t" and *wi* is initial oven dry weight.

Remaining C and N content

Remaining C and N contents after a given week on forest floor was calculated by the following formula:

Remaining (%) =
$$\frac{L_t C_t}{L_o C_o} \times 100$$

where L_t is the mass of dry matter after time 't', L_0 is the initial mass of dry matter, C_t is the concentration of C or N of the leaf litters after time 't', and C_0 is the initial concentration of C or N of the leaf litters (Alhamd *et al.*, 2004).

Data analysis

The data and graphical presentations were computed in Microsoft Excel. One way ANO-VA was done with Bonferroni test (SPSS ver-

Table 1: Initial Carbon and Nitrogen contents

Forest types	Concentration (%)		
	С	Ν	C:N ratio
Oak litter	85.49	1.83	46.72
Cupressus	59.52	0.91	65.41
Chirpine	48.96	0.65	75.32

sion 16.0) used to compare the mean differences in mass loss and nutrient release from leaf litter at p < .05 significance level.

Results and Discussion

Initial chemical properties of leaf litters Both C and N concentrations were different among Cupressus, Chirpine, and Oak leaf litters. Oak litter had high carbon content

(85.49%) compared to Cupressus (59.52%) and Chirpine (48.96%). Similarly, Oak litters had high N content (1.83%) followed by Cupressus (0.91%) and the least in Chirpine (0.65%). Chirpine needles had higher C:N ratio with 75.32%, followed by Cupressus with 65.41% and Oak litter with 46.72% (Table 1). The N concentration of Oak and Chirpine litters at time zero was higher in this study than the results reported by Chhetri et al. (2012), this could be due to the different location of the forests from where the leaf litters were collected besides other factors. Similarly, initial N concentration of Cupressus was higher than result reported by Pandey and Singh (1982) who reported N content of 0.83%. However, Silveira et al. (2011) reported high nutrient where Oak litter had 49% carbon and 7.8% nitrogen; Pine needles had 50.8% carbon and 4.7% nitrogen. Their C:N ratio for Oak litter was 62 and pine needle was 108.

Dry matter remaining (mass loss)

The remaining mass of the three litter types at a given time is presented in Figure 3. As expected, the mass loss was observed in all the three litter types in the order of Oak > Cupressus > Chirpine needles. Oak litters decomposed

significantly faster (p < .05) than Cupressus and Chirpine needles. However, significant difference in mass loss was not observed between Cupressus and Chirpine needles (p > .05). At the end of the experiment, the total litter mass remaining was

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54.31% for Oak, 74.15% for Cupressus, and 78.57% for Chirpine. The rate of mass loss in all the three species was higher between week 0 and week 16 than between week 20 and week 32. More specifically, after week 4 (August) and week 8 (September), Oak litters lost the maximum mass of 15.76% and 9.09% respectively compared to the other two species with mass loss of less than 8%. As expected, mass loss started to decline after week 12 (October) till week 24 (January) where minimum mass loss was observed in all the three species with 0.72% for Oak, 0.58% for Cupressus, and 0.27% for Chirpine. This was followed by slight increase from week 28 till the end of the experiment (Figure 3).

Fenoglio *et al.* (2006) reported that mass remaining was significantly related to elapsed time, season, leaf types, pollution level, and patch size. In present study, the rapid mass loss observed during rainy and warmer months (August to September) indicates favourable environment for microbial activity necessary to cause decomposition. Chhetri *et al.* (2012) and Pandey and Singh (1982) also reported that maximum mass loss occurred during summer months. Rainfall directly impacts the breakdown of litter in the initial stage of decomposition through leaching of soluble compound (Swarnalatha and Reddy, 2011). Similarly, Pandey and Singh (1982) reported that low mass loss in Cupressus with 72% in 18 months. Usman (2012) also reported higher weight loss in Oak (Banj) where Oak litters completely decomposed after 11 months while Chirpine needles decomposed only 65% after 12 months. Alhamd et al. (2004) reported that loss of mass increased with increasing incubation period and occurrence of two phases in decomposition. The initial mass loss of litter was due to leaching of soluble compound and degradation of recalcitrant biopolymers (Berg and McClaugherty, 2008). In second decomposition phase, primarily lignin and lignified cellulose remain (Alhamd et al. 2004), which slowed down the process. Further, Majila et al. (2005) stated that mass loss of litters continued in postmonsoon period till November. In the present study also, mass loss of litter was observed during winter month but at minimum rate in all litter types. This indicates that decomposition occurred over time affected by precipitation and change of temperature. Li-Xin et al. (2003) reported that temperature and precipitation strongly affected the litter decomposition rate in temperate, subtropical, and tropical forests.



Remaining carbon (% of original) Carbon decreased steadily from initial concentration in all litter types. Highly significant differences were observed in remaining carbon among Oak, Cupressus, and Chirpine needles (p < .01). Oak litters released more carbon compared to other two species during the entire study period. However, there was no significant difference (p > .05) between



Cupressus and Chirpine needles. At the end of week 4, highest carbon loss was observed in Oak litters (17.31%), followed by Cupressus (9.73%), and Chirpine needles (6.23%). In the later stage of the experiment, carbon release was comparatively lower in all the three species, and this may be due to decrease in temperature and precipitation, thus, limiting microbial activities active in decomposition process. The minimum carbon loss was observed during week 24 (January) with 1.09% in Oak, 0.80% in Cupressus, and 0.75% in Chirpine (Figure 4). However, the C remaining showed similar trend to that of mass loss, this could be due to the fact that carbon is the main element predominantly contributing to mass loss (Hirobe et al., 2004). Similarly, Staelens et al. (2011) also found the amount of carbon in decomposing litters decreased at the same rate as the mass loss where carbon was degraded and incorporated by microorganism or respired to carbon-dioxide.

Remaining nitrogen (% of original)

The remaining nitrogen followed similar pattern in all litter types with initial decrease, increase in middle, and decrease in later stage. Highly significant differences were observed in N loss (p < .01) among the three litter types. However, no significant difference in nitrogen loss was observed between Cupressus and Chirpine needles (p > .05). At the end of week 4, Oak litters



lost maximum nitrogen (17.37%) followed by Cupressus (10.50%) and Chirpine (7.66%). At the end of week 8, immobilisation of nitrogen was observed in all species. The Chirpine needles immobilised highest nitrogen with 114.98%, followed by Cupressus with 106.21% and Oak litters with 92.04%. After week 12, release of nitrogen declined till week 24 where minimum nitrogen release was observed with Oak (1.54%) followed by Cupressus (1.09%) and Chirpine (0.33%). Release of nitrogen declined from week 12 probably due to the onset of winter season with low temperature which must have slowed down microbial activities thus reducing decomposition process.

The release of nitrogen from the leaf litters notably had three main stages: a) rapid release stage (week 0-4); b) immobilisation stage (week 8); and c) slow but constant release stage (week 12-32) (Figure 5). This corresponds well with the three sequential phases of litter decomposition reported by Staff and Berg (1982) which includes the initial release phase in which leaching predominates, the net gain phase in which nitrogen immobilised by activities of microorganisms and lastly the net loss phase in which an absolute decrease in the nutrient mass of decomposing leaf litter occurs. The unavailability of N in soil leads to stunted growth of plants (Mehta et al., 2014). When N is in short supply compared to C, the former gets immobilised by soil microbes thereby increasing its concentra-

> tion (Salamanca et al., 1998). This could be one of the main reasons for high N concentration in all the litter types during week 8 of this study. The highest N immobilisation observed in Chirpine must be due to the poor litter quality in terms of low initial C and N contents. Although the N concentration might decrease due to its constant release, interplay of the mechanisms of nitrogen fixation, absorption of atmospher-





Figure 5: Remaining nitrogen (% of original) in different leaf litter types at different weeks

ic ammonia, and microbial immobilisation along with the mineralisation might counter balance its concentration (Swarnalatha and Reddy, 2011). The initial fast release of N from the leaf litters can be attributed to leaching of the soluble forms of N, while its slow release in the second phase is probably due to binding of N to lignin and polyphenols in the tissues (Bhalawe *et al.*, 2013). The release of nutrient by litter decomposition is determined by the interaction between litter quality and decomposer as well as interactions with climatic and soil conditions (Ramirez *et al.*, 2014).

Relationship between mass loss, nitrogen content and C:N Ratio

C:N ratio is a good indicator of litter decomposition and its quality (Xing-Jun *et al.*, 2003) and N concentration in leaf litters during decomposition determines microbial activities and influences mineralisation of organic C (Swarnalatha and Reddy, 2011). As decomposition proceeds, the decomposers first utilise the soluble and easily degradable components like sugars, starches, and proteins. As a result, the litter decomposition process is faster in the initial phase than in the later phase. This is because more recalcitrant materials such as lignin, tannins, celluloses, and hemicelluloses with slower rate of decomposition need to be decomposed in the later phase (Loranger *et al.*, 2002). During decomposition, С is used as energy by the decomposers while N assimilated into is cell proteins and othcompounds er (Williams and Gray, 1974). As such, C and N are either continuously mineralised immobilised by or microbes the soil (Couteaux et al., 1995) as shown in the case of N in present study. Leaf litter with high N content tends to decompose faster because N is often the limiting nutrient in the decay

process (Wind, 2013). Further, N exerts influence in the early stage of decomposition due to its influence on physiological adaptation of the decomposers (Salamanca *et al.*, 1998). Silveira *et al.* (2011) reported that higher mass loss and respiration rates occurred in litter with high initial N concentration than litter with low N concentration but this decreases with time.

Salamanca et al. (1998) reported that if the C:N ratio is greater than 30, N is immobilised by microbial decomposers, however, with C:N ratio less than 30, N is mineralised. This could be the reason for exhibiting immobilisation of N in all the three species at week 8 (Figure 5), with highest immobilisation seen in Chirpine with highest C:N ratio compared with Oak and Cupressus. Conn and Dighton (2000) found that both Oak and pine needles immobilised N but the immobilisation was significantly greater in pine than in Oak. Singh and Singh (1987) reported that immobilisation of nutrients due to high C:N ratio was one of the principal strategies through which Chirpine invades other forests and holds the site against possible reinvasion by Oak.

Morphology of litter affects its decomposition (Salah, 2009). The slow mass loss observed in pine needles may be attributed to its hard texture and presence of astringent substances such as polyphenols and gallic acids which make it less favourable for soil microfauna to attack (Heath and Arnold, 1966). It is reported that broad leaf litter decomposes much faster than pine needles because the former contains high concentration of nutrients and polyphenols, and low lignin content (Perry *et al.*, 1987). Cornelissen (1996) reported that leaves of deciduous tree species decompose twice as fast as those of evergreens under controlled conditions. Williams and Grays (1974) stated that leaves of conifer trees usually decompose more slowly than deciduous trees as shown in the present study.

Conclusion

The present study showed that the leaf litter decomposition process varied under different tree species largely due to differences in nutrient content, leaf structure, and leaf composition. The litter mass loss and nutrient release of the three tree species decreased in the order of Oak > Cupressus > Chirpine. The study also revealed that C:N ratio has a significant impact on the decomposition process as it determines the amount of food and energy available for soil microbes. As such, leaf litters with low C:N ratio decomposed faster (e.g. Oak litter) than the leaf litters with high C:N ratio (e.g. Chirpine and Cupressus litters). The immobilisation nature of Cupressus and Chirpine litters indicates longer nutrient retention capacity compared to Oak litter. Since Bhutanese farming system is partly dependent upon forests as the source of plant materials for farmyard manure production, it becomes crucial to understand the nutrient release patterns of leaf litters under different forest types. Additionally, understanding the decomposition process under different forest types is important since it is the main source of soil fertility for plants in forests. Present research did not study the interactions of the litter mixes, therefore, further research is required to understand the nutrient dynamics that result from the interactions of leaf litters in mixes which is crucial in nutrient cycling in the forest ecosystems.

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