

# Litter Decomposition and Microbial Biomass in Temperate Forests in Northwestern Turkey

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## Abstract

The purpose of this study was to investigate the influence of microbial biomass on the litter decomposition in relation to litter quality, litterfall, canopy leaf area and soil characteristics. The mean values for litterfall were 4245, 3510 and 2935 kg ha<sup>-1</sup> year<sup>-1</sup> for beech, fir-beech and fir stands, respectively. In the research area, beech stand has higher litterfall than fir, this may be attributed to high allocation of biomass to leaves, thus, makes them fall as litter more easily. One-year mass loss of litter decreased in this order: fir (23.6%) > fir-beech (17.2%) > beech (13.16%). Annual decay constant was significantly lower for beech (k=0.159) than fir litter (k=0.254), reflecting differences in nitrogen and lignin content between litter types (beech, 0.63% and 36.15%; fir, 1.40% and 28.10%). Fir litters have greater microbial biomass C content during the sampling period compared with beech and fir-beech litters. Microbial communities in fir litter were energetically more efficient (had a lower  $qCO_2$ ) with a higher  $C_{mic}/C_{org}$  compared to those in beech leaf litter. The results of this study indicate that admixed fir needles tended to speed up decomposition of beech foliage in these types of forest ecosystems.

**Keywords:** Litterfall, leaf area index, litter decomposition, microbial biomass C

## 1. Introduction

The litterfall is related to primary production in terrestrial ecosystems and it is a principal pathway for return of organic matter, nutrients and energy from the vegetation to the soils in forest ecosystems (Maguire, 1994). A large proportion of the nutrients taken up during the growing season are returned to the soil through litterfall and are then progressively released during decomposition (Attiwill and Adams, 1993). Several studies have shown conifer forests have higher

litter production than deciduous forests in the northern hemisphere (Millar, 1974). On the contrary, more recent studies have shown that at a local scale the litterfall in broadleaf forests can be significantly higher than in coniferous stands, although their nature is evergreen (Kavvadias *et al.*, 2001).

On a global basis, precipitation and temperature are the most important climatic factors that control

litterfall production. Leaf litter production is low at high latitudes where short growing seasons limit plant growth; it increases towards the equator where plant growth can happen throughout the entire year. However, in a local region with similar temperature and rainfall, the variation in the litterfall is attributed to differences in physiology and ecology of tree species, physiographic factors (i.e., slope and aspect) and site productivity (Barnes *et al.*, 1998).

The forest litter regulates most of the functional processes occurring in the forest ecosystem: it insulates the soil surface from extremes of temperature and moisture content, it protects the mineral soil from the raindrop impact and erosion forces and it improves water infiltration rates (Gallardo *et al.*, 1998). On the other hand, the decomposition of litter is essential for ecological processes, which controls nutrient and carbon cycling and primary productivity and contributions to the maintenance of soil fertility in terrestrial ecosystems. The general conclusion has been that the litter decomposition rate is influenced by a number of interacting biotic and abiotic factors. Decomposition rate and nutrient release of plant litters are mainly controlled by environmental conditions, composition and activities of soil organisms, and chemical quality of the substrate (Cox *et al.*, 2001; Berger and Berger, 2012).

Soil microorganisms are integral parts of forest ecosystems and play a critical role in the decomposition of organic matter and the immobilization and mineralization of nutrients (Kennedy and Papendick, 1995). Soil microbial biomass acts as an important ecological indicator and is responsible for the decomposition and mineralization of plant and animal residues in the soil (Marinari *et al.*, 2006). Therefore, knowledge of soil microorganisms' effects on litter decomposition is fundamental for a sustainable forest management.

Litter quality is most often related to the chemical characteristics of the litter, for example lignin/N and C/N ratios or lignin content. By accepting the hypothesis

that the lignin/N and C/N ratios are correlated, it shows that litters with a low decomposition rate have a higher lignin/N or C/N ratios than litters with high rate of decomposition (Muys, 1995). The rate of nutrient cycling can also be reduced by tannins, which amount up to 20% of the plant dry weight (Kraus *et al.*, 2003). Initial litter N and P contents are often positively correlated with early decay rates (Berg, 2000).

Results from previous studies have shown that litter quality and abiotic factors affects litter decomposition rate in Turkish forests (Sariyildiz *et al.*, 2005). These studies have demonstrated that lower litter quality and adverse environmental conditions can retard decay in adjacent, less recalcitrant litters. However, the impacts of microbial biomass on the litter decomposition process in the region were not studied. Therefore, we carried out this present study to investigate the influence of microbial biomass on the litter decomposition rate as well as the effects of litter quality, litterfall, canopy leaf area and soil characteristics.

In this study, our objectives were (i) to determine differences in litterfall and leaf area index (LAI) among Oriental beech (*Fagus orientalis* Lipsky.), Bornmüllerian Fir (*Abies nordmanniana* ssp. *Bornmülleriana* Mattf.) and mixed stands with similar site conditions, (ii) to investigate differences in litter decomposition rate, and (iii) to determine how microbial biomass correlates with litter decomposition rate.

## 2. Material and Methods

### 2.1. Study sites

The study area was located approximately 20 km northeast of Bartın province (latitude 41°39'- 41°42' N and longitude 32°30'-32°40' E) in three undisturbed forested sites. The oriental beech site included even-aged stands derived from natural reforestation. The fir and mixed fir-beech sites were composed of uneven-aged stands mature enough for harvest.

Tree density was 337 stems/ha in the pure oriental beech stand, 378 stems/ha in the pure fir stand, and 340 stems/ha in the mixed fir-beech stand at the time of the investigation. Elevation is approximately 800 m, with a north aspect. The region occupies a temperate zone of an oceanic climate. The area has mean annual temperature of 8.9 °C and mean annual precipitation of 1,394 mm and about 46% of rainfall occurs in the growing season between May and October. The maximum and minimum mean annual temperatures are 0.4 °C in January and 18.2 °C in July, respectively. Humus type is moder on sandstone and limestone is deep, slightly acidic, and heavily weathered ultisol. Soil samples were collected in a completely randomized design. Soil cores were taken from 30 points in each stand. The litter layer was removed before soil samples were taken at a depth of 5 cm. Table 1 presents a detailed description of soil properties of the stands.

### 2.2. Litterfall

Litter collection, monthly, took place on December 2008 to September 2010. For litterfall measurements, five 0.33 m<sup>2</sup> polyethylene traps were placed randomly in each stand. Each trap consisted of 65cm diameter punched-container suspended from a wire hoop and held 1m above the ground by three metal poles. Litter samples were then dried at 70 °C for 48 h and weighed. Monthly and annual litterfall amounts were estimated from the monthly collected litterfall amount in the five traps on each stand.

### 2.3. Leaf area index

A Canon EOS 3 SLR film camera with a Sigma 8-mm fisheye lens was used to take 30 hemispherical photographs from each stand. The photographs were taken in the absence of direct sun radiation using Konica 400 ASA film. The negatives were scanned using a Minolta Dimage Scan Elite II scanner and analyzed with Hemisfer 1.41 software (Schleppi *et al.*, 2007).

### 2.4. Litter decomposition

Litter bags (20 x 20 cm) made of 0.5-mm mesh plastic netting were filled with 3 g (dry mass equivalent) of litter and fixed to the ground with metal pegs. Samples were also taken to determine a correction factor to calculate the initial oven dry mass of the material at 70 °C. The number of litter-bags used for 21- months experiment (December 2008 to September 2010) were 54 litter bags (3 stands x 6 removal dates x 3 replicates = 54 bags).

### 2.5. Litter analyses

The litter samples were air-dried in the laboratory and then oven-dried at 70 °C for 24 h. The oven-dried litters were slightly crushed by hand. All samples were then stored in plastic bags at 4 °C until they required for chemical analyses. Litter subsamples were also taken and stored at 4 °C before microbial analyses were conducted.

The stored leaf and needle litters were oven-dried at 70 °C, and then ground in a laboratory mill to a mesh fraction less than 1mm. The ground litters were then analyzed for organic carbon, total nitrogen, ADF (acid detergent fiber), and lignin. Organic C was determined by wet oxidation. Total N was determined by Kjeldahl digestion method (Rowell, 1994). Acid detergent fiber (ADF),  $\alpha$ -cellulose and lignin were determined using an ADF-sulphuric lignin method by Rowland and Roberts (1994). Organic analyses were carried out in triplicate.

Litter microbial biomass C ( $C_{mic}$ ) was estimated by extracting 3-g oven-dry equivalents of field-moist litter samples in 0.5 M K<sub>2</sub>SO<sub>4</sub> (1/33 w/v) by the chloroform-fumigation-extraction method described by Vance *et al.* (1987).  $C_{mic}$  was calculated from the difference in extractable organic C between fumigated and unfumigated litter samples as follows: biomass C = 2.64 EC, where EC refers to the difference in extractable organic C between the fumigated and unfumigated treatments; 2.64 is the proportionality factor for biomass C released by fumigation extraction (Vance *et al.*, 1987).

**Table 1.** Comparison of soil physical and chemical characteristics under beech, fir and fir-beech mixed stands

Soil characteristics	Beech	Fir	Fir -Beech
Sand (%)	44.96 ( $\pm 8.03$ ) <sup>a</sup>	42.36( $\pm 7.43$ ) <sup>a</sup>	42.03 ( $\pm 14.50$ ) <sup>a</sup>
Silt (%)	20.55 ( $\pm 5.01$ ) <sup>a</sup>	19.04 ( $\pm 5.97$ ) <sup>a</sup>	22.94 ( $\pm 5.28$ ) <sup>b</sup>
Clay (%)	34.48 ( $\pm 5.40$ ) <sup>a</sup>	38.58 ( $\pm 6.30$ ) <sup>a</sup>	35.01 ( $\pm 10.50$ ) <sup>a</sup>
Soil texture	Loamy clay	Loamy clay	Loamy clay
pH in water	5.62 ( $\pm 0.90$ ) <sup>a</sup>	6.66( $\pm 0.79$ ) <sup>b</sup>	5.84 ( $\pm 0.97$ ) <sup>a</sup>
Organic carbon (%)	4.29 ( $\pm 1.70$ ) <sup>a</sup>	6.09 ( $\pm 1.38$ ) <sup>b</sup>	4.51 ( $\pm 1.90$ ) <sup>b</sup>
Total nitrogen (%)	0.24 ( $\pm 0.12$ ) <sup>a</sup>	0.31 ( $\pm 0.09$ ) <sup>a</sup>	0.27 ( $\pm 0.15$ ) <sup>a</sup>
C/N ratio	17.32( $\pm 1.90$ ) <sup>a</sup>	20.08.32( $\pm 3.53$ ) <sup>b</sup>	17.37( $\pm 2.57$ ) <sup>a</sup>

\* Values are the mean of 30 samples, SD is in brackets. Values in the same row followed by the different letter indicate significant ( $p < 0.05$ ) differences between means (Independent-samples t test)

Basal respiration was determined by placing 50-g sediment samples into 50 mL beakers and incubating them in the dark at 25 °C in 1-L airtight, sealed jars along with 25 mL 0.05 M NaOH. After 7 days, the generated CO<sub>2</sub> was measured by titration of excess NaOH with 0.05 M HCl (Alef, 1995). The metabolic quotient ( $q\text{CO}_2$ ) was calculated as the basal respiration rate ( $\mu\text{g CO}_2\text{-C h}^{-1}$ ) mg<sup>-1</sup> of microbial biomass C.

## 2.6. Soil analyses

Soil samples were air-dried, ground, and sieved (<2 mm). Soil particle size distribution was determined using the hydrometer method. Soil pH in a 1/2.5 soil/water suspension was determined using a pH meter. Electrical conductivity (EC) in a 1/5 soil/water suspension was determined using an electrical conductivity meter. The total organic C content was estimated using potassium dichromate oxidation, and the total N content was estimated using Kjeldahl digestion (Rowell, 1994).

## 2.7. Data analyses

The percentage of dry mass remaining in the litter bags (%RM) was calculated from the weight of litter (W<sub>t</sub>) at each sample period (t) and the initial mass (W<sub>0</sub>) using the following formula: %RM = (W<sub>t</sub>/W<sub>0</sub>) x 100.

Decomposition constant rate (k) was calculated from the percentage of dry mass remaining using an exponential decay model (Olson, 1963):  $W_t/W_0 = e^{-kt}$ , where  $W_t/W_0$  is the fraction of initial mass remaining at time t (%), and t is the elapsed time (year) and k is the decomposition constant (year<sup>-1</sup>). As suggested by Olson (1963), the time required for 95% mass loss was calculated as  $T_{95}=3/k$ .

Statistical analyses were carried out using SPSS 11.00 package program. The effect of tree species and time on litter microbial biomass was determined by one-way analysis of variance. A 95% confidence limit ( $p < 0.05$ ) was chosen to indicate differences between

samples. Tamhane's T2 Test were calculated when samples were significantly different. Data for physical and chemical characteristics of soil were also subjected to independent sample t-tests to determine significant differences among stand types.

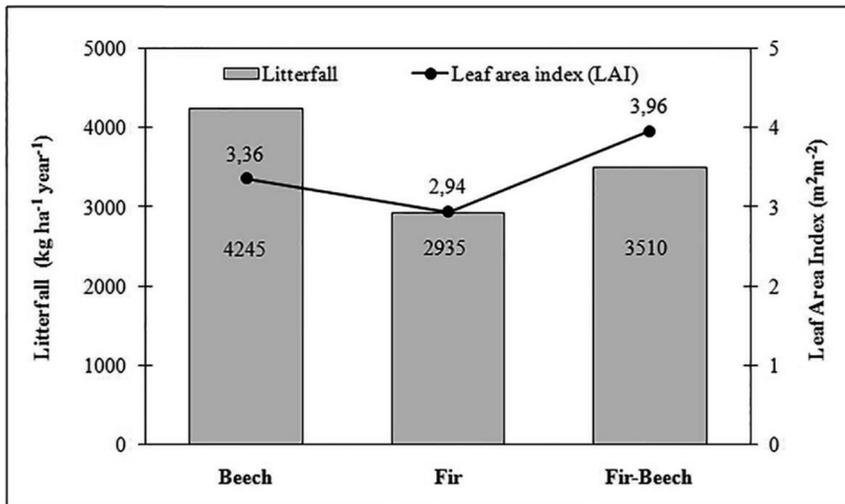
### 3. Results and Discussion

#### 3.1. Litterfall

The mean values for litterfall were 4245, 3510 and 2935 kg ha<sup>-1</sup> year<sup>-1</sup> in the beech, fir-beech and fir stands, respectively (Figure 1). Beech forest has a higher average total litterfall than fir-beech and fir forests. Thus, there is a general tendency to average higher values for broadleaf forests in research area. Litter production at the study sites was quite similar to estimations from coniferous and broadleaves forests in other temperate countries. In the Asian and European temperate zone, total litterfall were 2980 and 3470 kg ha<sup>-1</sup> year<sup>-1</sup> in coniferous forests, and 4340 and 4420

kg ha<sup>-1</sup> year<sup>-1</sup> in broadleaf forests, respectively (Liu *et al.*, 2004). Similarly, annual litterfall varied from 4000 kg ha<sup>-1</sup> in beech stand to 1420 kg ha<sup>-1</sup> in maritime pine stand and decreased in this order: beech>fir>black pine>maritime pine (Kavvadias *et al.*, 2001). In the research area, beech stand has higher litterfall than fir, this may be attributed to high allocation of biomass in leaves, thus, makes them fall as litter more easily.

The leaf area index (LAI) is one of the crucial ecosystem characteristics, because it is a direct measure of the photosynthetically-active surface area which can convert light energy into plant biomass (Barnes *et al.*, 1998). The mean values for leaf area index (LAI) were 3.96, 3.36, and 2.94 m<sup>2</sup>m<sup>-2</sup> in fir-beech, beech and fir stands, respectively. Statistical analyses show that there is a significant difference ( $p < 0.05$ ) among the leaf area indexes of the 3 stand types (Figure 1). Leaf area indexes determined for a wide range of temperate forest ecosystems; they are typically greater than 5.0 m<sup>2</sup>m<sup>-2</sup> during the growing season. However, LAI values found in this study were lower than the ones reported by previous studies.



**Figure 1.** Variation in total litterfall and leaf area index (LAI) in pure and mixed type stands. Values represent the means of 5 litter traps for litterfall and 30 hemispherical photographs for LAI.

Most likely this is due to the variations in the controlling factors of LAI, such as climate, soil properties, and site productivity. Similarly, Ellenberg (1996) assumed that *F. sylvatica* may attain LAIs of 8-12 m<sup>2</sup>m<sup>-2</sup> on fertile and base-rich soils, but of only 3-4 m<sup>2</sup>m<sup>-2</sup> on poor and/or acidic ones. Fir stands had the lowest mean LAI values in the research area. This result is consistent with the litterfall amount that has shown fir stands have less LAI than the other stand types. Within a group of stands under climatically similar conditions, increased leaf area index leads to higher annual litterfall.

### 3.2. Litter Decomposition

The litter samples used in the decomposition experiment showed rather similar initial C content. However, leaf litter of Beech had smaller nitrogen content than fir and fir-beech stands (Table 2).

**Table 2.** Initial chemical quality of each litter type (percentage of dry mass,  $n=3$ ).

Litter characteristics	Beech	Fir	Fir -Beech
Organic C (%)	45.71	47.88	47.50
Total N (%)	0.63	1.40	1.06
Lignin (%)	36.15	28.10	32.30
C/N ratio	72.55	34.20	44.80
Lignin/N ratio	57.35	20.05	30.45

Beech leaves had also greater lignin content than other litter types. Therefore, the C/N and Lignin/N ratio were much higher for beech leaves than for fir and fir-beech. Annual mass loss and the time required for 95% mass loss are shown in Table 3. One-year mass loss of litter decreased in this order: fir (23.6%) > fir-beech (17.2%) > beech (13.16%).

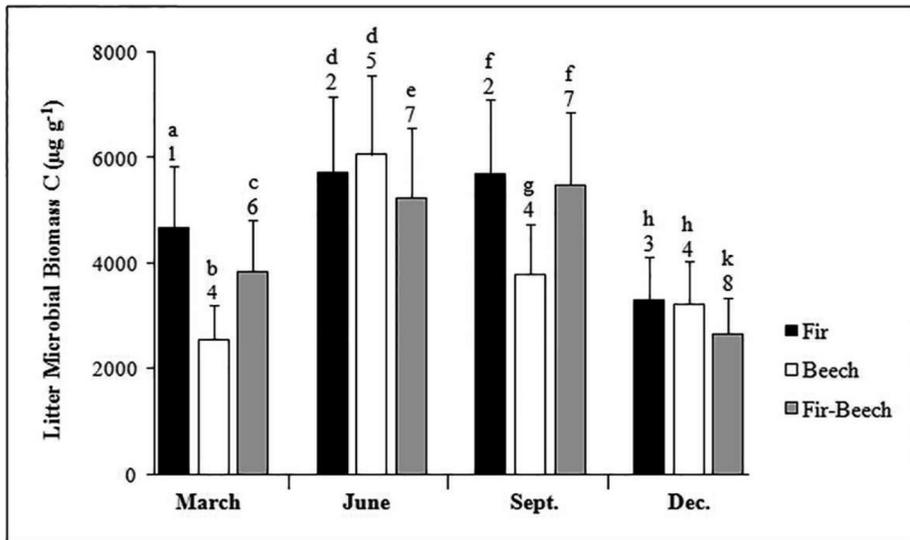
**Table 3.** Annual decay constant (k), mass loss and the time required for 95% mass loss for beech, fir, and fir-beech litters ( $n=18$ ).

Decay parameters	Beech	Fir	Fir-Beech
k (year <sup>-1</sup> )	0.159	0.254	0.184
Mass Loss (%)	13.16	23.60	17.20
T <sub>95</sub> (year)	18.8	11.8	16.3

It appeared that beech litters with a low mass loss have higher C/N and lignin/N ratio than fir litters with high mass loss. The possible differences in litter quality of our stands were sufficient to differentiate the decomposition rate under the same ecological conditions. Numerous studies have shown that the lignin/N ratio can be used as a predictor of organic matter decomposition rate (Kooijman and Martinez-Hernandez, 2009). Beech litter was comprised of large amounts of recalcitrant material, such as lignin, decomposition rate was suppressed in litterbag. Leaf litter decay can be slowed by release of inhibitory compounds such as phenolics and tannins (Prescott *et al.*, 2000). Furthermore, fir litter with high N contents (low C/N ratios) decomposed significantly faster than beech litter with low N contents. This result agrees with those studies which have found a clearly significant and positive relationship between N concentration and litter decomposition (Tripathi *et al.*, 2006).

### 3.3. Microbial biomass carbon, $C_{mic}/C_{org}$ percentage and Metabolic quotient ( $qCO_2$ )

There was a significant increase in microbial biomass C on the June and September samples relative to other sample dates (Figure 2). The greatest increase occurred in litter microbial biomass C from March to June (3496  $\mu\text{g g}^{-1}$  or 136%) in beech stand. There are several possible reasons for these trends.

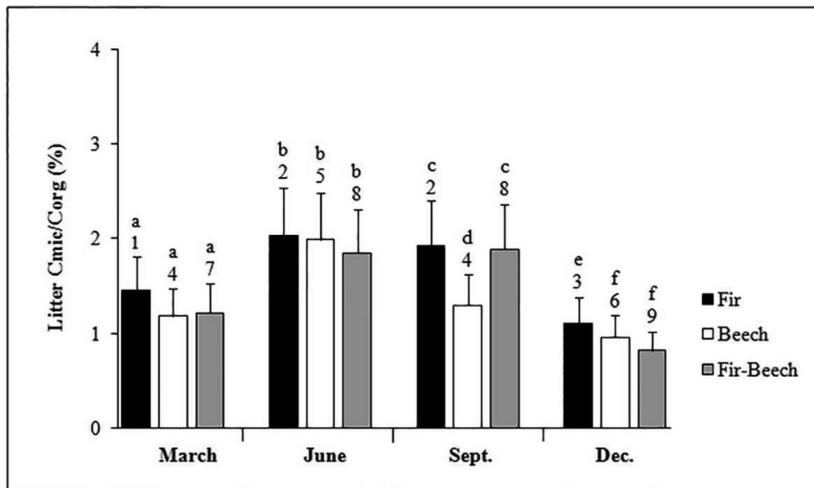


**Figure 2.** Temporal variation in microbial biomass C ( $\mu\text{g C g}^{-1}$ ) in three litter types. Different letters above the bars indicate significant differences among stands within each date. Different numbers represent significant differences among date within each stand (ANOVA followed by Tamhane's T2 Test).

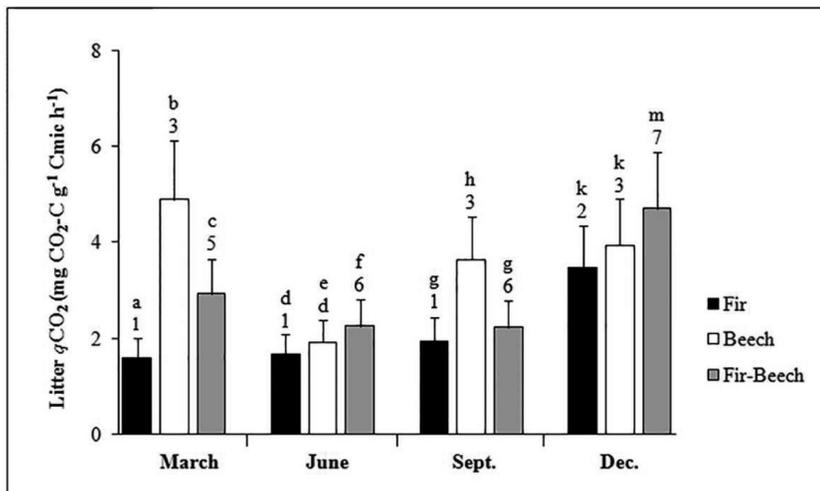
First, the relatively high June and September microbial biomass C of litter in all stands coincided with increase of soil temperature, which was recorded 17.9 and 13.0 °C of mean soil temperature in June and September, respectively. Fluctuations in microbial C appear to be substantial in more temperate region with larger seasonal changes in soil temperature and moisture (Tate *et al.*, 1991). Second, fresh leaf and needle litter layer possibly provided adequate labile organic carbon for microbial growth in this period and therefore microbial biomass increased. Finally, it is likely that there are more root exudates in growing season promoting greater microbial biomass in litter layers. Taken together, these results suggest that soil temperature and physiology of tree species were important factor regulating the seasonal variations of litter microbial biomass C. Similarly, Myers *et al.* (2001) reported that the seasonality of microbial biomass C varies with environmental factors and tree physiology.

Fir litters have a greater microbial biomass C content during the sampling period when compared with beech and fir-beech litters, except for June, which was somewhat lower (Figure 2). In support of this finding, the highest microbial biomass C was found in fir litter with high N contents and low C/N ratio (Table 2). These varying trends of litter microbial biomass in the same sampling time could be attributed to differences in litter chemistry of tree species (C/N ratios, lignin, phenolic and tanin). Overall, our study shows higher-quality litter can stimulate microbial biomass C. It is well known that litter quality strongly affects the amount and activity of soil microbial biomass (Kara *et al.*, 2008).

Microbial biomass C ( $C_{\text{mic}}$ )/Soil organic C ( $C_{\text{org}}$ ) percentage generally increased in the June and September samples throughout the study period (Figure 3).



**Figure 3.** Temporal variation in  $C_{mic}/C_{org}$  (%) in three litter types. Different letters above the bars indicate significant differences among stands within each date. Different numbers represent significant differences among date within each stand (ANOVA followed by Tamhane's T2 Test).



**Figure 4.** Temporal variation in metabolic quotient ( $qCO_2$ ) in three litter types. Different letters above the bars indicate significant differences among stands within each date. Different numbers represent significant differences among date within each stand (ANOVA followed by Tamhane's T2 Test).

This increment was probably caused by increasing microbial biomass C in the June and September, rather than decreasing of soil organic C. The mean  $C_{mic}/C_{org}$  (%) was highest in fir litter (1.62%) and relatively low in fir-beech litter (1.44%) and beech litter (1.35%) regardless of sampling date.  $C_{mic}/C_{org}$  (%) reflects the fraction of recalcitrant organic matter, decreasing as the concentration of available organic matter decreases (Brookes, 1995). The low  $C_{mic}/C_{org}$  also reflects a less efficient use of organic substrates by microbial biomass. Our results support the notion that higher  $C_{mic}/C_{org}$  (%) in fir litter is the result of more labile organic substrates maintained in fir litter, allowing a higher microbial biomass C per unit of litter organic C.

The  $qCO_2$  ranged from 1.60 to 3.47 mg  $CO_2$ -C  $g^{-1}$   $C_{mic}$   $h^{-1}$  for fir litter, 1.92 to 4.89 mg  $CO_2$ -C  $g^{-1}$   $C_{mic}$   $h^{-1}$  for beech leaf, and 2.23 to 4.70 mg  $CO_2$ -C  $g^{-1}$   $C_{mic}$   $h^{-1}$  for fir-beech litter (Figure 4). Metabolic quotient (respiration rate per unit microbial C) were significantly lower in the fir litter during the sampling period when compared with beech and fir-beech litters. Metabolic quotient ( $qCO_2$ ) reflects substrate quality, ecosystem development and response stress (Anderson and Domsch, 1993). These results indicate that microbial communities in fir litter are energetically more efficient (lower  $qCO_2$ ) with a corresponding higher  $C_{mic}/C_{org}$  percentage (increased biomass) compared to those in beech leaf.

#### 4. Conclusions

The results of this study emphasize that decomposition rates were affected most strongly by litter type, with N-rich fir litter decomposing faster than N-poor beech litter. Introducing of beech litter into forest floor retards litter decomposition, maintaining lower litter quality, microbial biomass and activity, compared to pure fir stand. Hence, knowing how much fir must be admixed to pure beech stands in order to increase litter decomposition, is of practical relevance for forest management strategies. Such a notion has

to be taken into account for the development of an improved policy for forest management. Furthermore, microbial biomass of litter increased from summer to autumn suggests that there is a seasonal pattern of soil temperature, available C, and plant activity have effects on the rate and controls of litter decomposition in humid ecosystems.

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