

Impact of Oak and Pine Canopy Cover on Soil Biochemical and Microbial Indicators of Binsar Wildlife Sanctuary in the Western Himalaya, India

Arun Kumar¹, R.D. Singh², A.K. Patra³, S.K. Sahu⁴ and Maninder Singh¹

¹Department of Agronomy, School of Agriculture, Lovely Professional University, Phagwara-144411, India.

²Division of Soil Science and Agricultural Chemistry, Indian Agricultural Research Institute, New Delhi-110012, India.

³Director, Indian Institute of Soil Science, Bhopal, India.

⁴Department of Environmental Science, Sambalpur University, Sambalpur 768001, India.

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Binsar Wildlife Sanctuary is an ecological confined area having high diversity of plants and animals. Oak and pine are the dominant species in this sanctuary. Since soil biological indicators are highly sensitive, which reflect the impact of land use systems in short time, soils under oak (*Quercusleucotrichophora*) and pine (*Pinusroxburghii*) canopy cover were investigated for enzyme activities such as dehydrogenase, acid phosphatase, alkaline phosphatase, nitrate reductase, MBC, OC and abundance of bacteria, fungi and actinomycetes during 2008-2010. The mean DHA, acid phosphatase, alkaline phosphatase, nitrate reductase activities recorded highest values in oak $9.3 \mu\text{g TPF g}^{-1} \text{soil h}^{-1}$, $452 \mu\text{g PNP g}^{-1} \text{soil h}^{-1}$, $234.3 \mu\text{g PNP g}^{-1} \text{soil h}^{-1}$, 1.3 mg kg^{-1} respectively as compared to the pine canopy cover soil $5.3 \mu\text{g TPF g}^{-1} \text{soil h}^{-1}$, $186 \mu\text{g PNP g}^{-1} \text{soil h}^{-1}$, $118 \mu\text{g PNP g}^{-1} \text{soil h}^{-1}$, 0.6 mg kg^{-1} respectively. The MBC values were from 460.2 mg kg^{-1} in pine canopy to 596.7 mg kg^{-1} in oak forest. Similarly the values of OC were also recorded significantly high in oak forest. From this study it is concluded that the canopy of oak forest is much more effective to improve the biological indicators in soil.

Keywords: Oak Canopy, Pine Canopy, Indian Himalayas, Soil Enzymes, Microbial Biomass Carbon, Organic Carbon.

Binsar Wildlife Sanctuary (BWS) is the conserved and protected hill area spread over 45.59 km^2 and situated at an altitude varying 900 to 2500 mts with an average height of 2412 mts. Because of minimum anthropogenic intervention, the natural vegetation and wild life are in harmony and self sustained natural ecosystem. Forest soils receive litter fall continuously and consequently constant litter decomposition. Recycling of nutrients

is mediated by organic matter decomposition by soil microorganisms (Couteaux et al., 1995). Constant process of litter decomposition is regulated by litter composition and associated with climatic factors as temperature and rainfall. Soil enzymes are sensitive indicators of microbial activity and soil quality which respond quickly to environmental changes (Gianfreda et al., 2005; Salgado et al., 2010; Mijangos et al., 2006; Bandick and Dick, 1999; Sinsabaugh et al., 2009; Acosta-Martínez and Tabatabai, 2000). The microbial activities reflect the sum of all physical chemical and biological factors regulating the decomposition and transformation of nutrients. Tree species have

* To whom all correspondence should be addressed.
E-mail: rdsingh55iari@gmail.com

significant impact on the microbial properties of the soil (Phillips & Fahe, 2006; Meng et al., 2012; Ushio et al., 2009; Chodak & Niklinska, 2010) and this plant soil association regulates biogeochemical process (Kulmatiski et al., 2008). BWS hill forest on is an ideal site to examine the effects of plant species on soil microbial enzymes and microbial biomass as the BWS forest of Indian Himalayan is undisturbed ecosystem and thus organic matter specific to a tree species (e.g., in terms of nutrient concentration and litter composition) accumulates beneath the tree, and it can influence the soil microbial enzymes. Forest biogeochemical cycles are shaped by effects of dominant tree species on soils, but the underlying mechanisms and the extent of impacts are important area of ecosystem research (Mueller et al., 2011). Oak (*Quercus incana*) and Pine (*Pinus roxburghii*) are the most important plantation tree species in Binsar wild life tiger reserve hill in terms of area, yield and other forest resources uses.

The objective of the present study was to assess various enzyme activities, microbial biomass carbon and population of bacteria, fungi and actinomycetes in soils beneath the litter of oak and pine canopy in the BWS hill forest and also to find out the temporal variations. The information generated is expected to be useful to understand the soil-microbes interaction of mountain forest ecosystems with dominant species such in pine and oak.

MATERIALS AND METHODS

Study site

The selected hill forests for the study are located in the Binsar region (29.63° N, 79.33° E) of Uttarakhand, India. Oak (*Quercus leucotrichophora*) and Pine (*Pinus roxburghii*) canopy are the prime tree species in this region and were selected for the study. Of this, pine canopy are located at the 1800 amsl while the oak canopy were located at 2300 m above mean sea level. The climate is sub temperate, characterized by moderate summer (May–June), extreme winter (Dec–Jan) and general dryness, except during the southwest monsoon season (June–Sept).

Soil and weather characteristics

The parent material of these soils consists of mica, schist, slates, sand stone, calcium deficient

granite and seynite rocks (Singh et al., 2000). Some of the soil properties at the time of first sampling (July 2008) are presented in Table 1. Genetically these soils come under climatogenic podsolized grey-brown forest soils. Soil under oak and pine were having acidic soil reaction. The pH values were 5.7 (Oak forest) and 6.1 (Pine forest). The EC values were oak forest (0.11 dS m⁻¹) and pine forest (0.18 dS m⁻¹). During the two sampling periods (summer and winter), the temperature and rainfall conditions varied. During July, the temperature 30.1°C (max) 20.9°C (min), and in January, 20.3°C (max) and -0.3°C (min). Rainfall also varied much between the two sampling months: 137.5 mm in July (summer) and 16.5 mm in January (winter).

Soil sampling

The soil samples of two different depths (0-15cm, 15-30cm) were collected from the oak and pine canopy after removing the litter in July 2008, 2009 and 2010. Eight to ten composite soil samples were collected from each site for each depth. For making one composite sample, soil cores of these samples were pooled. Pseudo-replicated approach of sampling was followed in this study. Such sampling technique has also been adopted by other workers (Patra et al. 2005, Patra et al. 2006). The field moist soil samples were stored in refrigerator at temperature less than 4°C for preserving the biological activities till the analysis were over in 7-10 days. All chemical results are mean of triplicate analysis and expressed on the oven dry weight basis. Soil moisture was determined after drying at 105°C for 24 h.

Soil analyses

Soil dehydrogenase activity was determined using the method of Klein et al., (1971). Acid and alkaline phosphatase activities were determined by the method of Tabatabaiani and Bremner (1969). Nitrate reductase activity was determined by the method of Fu and Tabatabaiani (1989, Patra et al. 2006). MBC was determined by fumigation extraction method given by Horwath and Paul (1994). The organic carbon was estimated following the procedure of Walkley and Black. (1934). Enumeration of bacteria, fungi and actinomycetes was done following the procedure as described by Chhonkar et al. (2007).

Statistical analysis

Three-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) for

comparison of means were performed using software SAS 9.1.3. PAST3.x software is used for PCA analysis. Unless otherwise stated, the level of significance referred to in the results is $P < 0.05$.

RESULTS AND DISCUSSION

Soil Enzymes

Soil Dehydrogenase Activity

Highest value of dehydrogenase activity was observed in case of undisturbed natural oak canopy cover soil while pine canopy cover showed significantly lowest value (Table 2). During the years (2008-2010) the DHA activity of oak forest soil were 10.7, 12.7 and 9.9 $\mu\text{g TPF g}^{-1}\text{ soil h}^{-1}$ and Pine forest soil were 6.4, 7.2 and 6 $\mu\text{g TPF g}^{-1}\text{ soil h}^{-1}$ respectively. However in lower depth the DHA activity of oak canopy soil were 7.7, 8.1 and 7.1 $\mu\text{g TPF g}^{-1}\text{ soil h}^{-1}$ and for pine canopy soil the values are 4, 4, and 3.9 $\mu\text{g TPF g}^{-1}\text{ soil h}^{-1}$ for subsequent three years (2008-2010). The DHA activity was 40 % and 47 % lesser at 0-15 and 15-30 cm in pine canopy soil as compared to oak forest mean values respectively. In all the years the DHA activity was higher in oak canopy covered soil. The yearly fluctuation in DHA activity is least in both oak and pine soils. After considering the depth and year factor, the oak canopy soil found to maintain highest dehydrogenase activity irrespective of the year and depth differences.

Dehydrogenase activity reflects the total range of oxidative activity of soil microflora and is a good indicator of microbial activity (Defrieri et al., 2011; Dick et al., 1996; Sebiomo et al., 2011; Quilchano & Marañón 2002). Generally the forest ecosystem holds high substrate availability due to the continuous organic matter decomposition and may cause higher DHA activity by soil microbes. Litter composition seems to be the important factor affecting the microbial population as well as diversity. This reflects the greater biological activity in the system and stabilization of extracellular enzymes through complexation with humic substances (Colvan et al., 2001). Result recorded shows significance of moisture in soil as higher DHA activity were recorded (12.7 for oak and 7.2 TPF $\text{g}^{-1}\text{ soil}^{-1}$ for pine) in year 2009 just after few days of rainfall, also suggest us the ideal soil conditions. At surface soil the litters are under different stages of decomposition with

Table 1. Some soil properties of oak and pine soil. Each value is an average of three analyses

Land use and cropping systems	Location	Description	pH	EC (dS m^{-1})	Sand (%)	Silt (%)	Clay (%)	N (%)	Avail. K (mg kg^{-1})	Respiration (O_2 $\text{g}^{-1}\text{ h}^{-1}$)
Oak (<i>Quercusincana</i>) forest	Corbet national park, Binsar 29°39'N 79°07'E. Altitude 2400 m amsl	Natural vegetation predominantly under oak trees.	5.7	0.11	55	29	16	0.24	377.8	12.1
Pine (<i>Pinusroxburghii</i>) forest	Corbet national park, Binsar 29°39'N 79°07'E. Altitude 2000 m amsl	Natural vegetation predominantly under Pine trees.	5.8	0.18	46	18	36	0.15	298.4	8.3

higher DHA activity where as in depth may be due to lack of nutrient and aerobic conditions the DHA activity decreases. Faster rates of litter and roots decomposition is mediated by the microorganisms, in turn microbial population and community structure is determined by the quality of the litter and the root of the tree species. Our study reveal higher DHA activity in oak forest soil than pine forest soil suggest the quality of the litter produced by each tree species and also higher availability the organic matter increases higher decomposition as reported in studies by Pandey et al., (2005) and Arunachalam et al., (1998).

Acid Phosphatase

Highest acid phosphatase activity was observed in case of oak canopy cover soil than the pine cover soil (Table 2). The three subsequent year average acid phosphatase activity in oak canopy cover surface soil was $642 \mu\text{g PNP g}^{-1} \text{soil h}^{-1}$ and $272.6 \mu\text{g PNP g}^{-1} \text{soil h}^{-1}$ for pine canopy cover surface soil. In comparing all the years the surface mean acid phosphatase activity was 57% more in oak canopy cover soil than pine canopy cover soil. Similarly at lower depth (15-30cm) 61 % more acid

phosphatase values were observed in oak soil than pine soil

Phosphorus (P) is often a limiting nutrient for plant growth in tropical and subtropical forests. (Huang et al., 2011). Acid phosphatase can be affected by changes of different related factors such as plant species (Ushio et al., 2010) and litter quality (Conn and Dighton, 2000) as plant roots are the major producer of acid phosphatase (Speir and Cowling, 1991). Higher acid phosphatase activity in the oak and pine were observed and is influenced by the effect of soil pH. Acidic nature of the soil influences and increases the acid phosphatase as reported by several workers (Wang et al., 2006, Sarapatka et al., 2004 and Dick et al., 2000).

Alkaline Phosphatase

Alkaline phosphatase activity was also found to vary significantly among oak and pine canopy cover soils. During 2008-2010 the Alkaline phosphatase activity of oak forest surface soil were 232.4, 334.8 and 314 $\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$ and Pine forest soil were 171, 210 and 112.5 $\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$, respectively. However in lower depth the alkaline phosphatase activity of oak canopy soil

Table 2. Impact of Canopy cover on Dehydrogenase (DHA- $\mu\text{g TPF g}^{-1} \text{soil h}^{-1}$), Acid phosphatase (AcP- $\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$), Alkaline Phosphatase (AlkP- $\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$), Nitrate reductase (NR- mg kg^{-1}), Microbial Biomass Carbon (MBC- mg kg^{-1}), Organic Carbon (OC-Percentage)at different depths under different land use systems in central Himalayan region. MSE –Mean Standard Error

Year	At Depth 0-15cm					At Depth 15-30				
	2008	2009	Oak Canopy 2010	Mean	MSE	2008	2009	Pine Canopy 2010	Mean	MSE
DHA	10.7	12.7	9.9	11.1	0.33	6.8	7.2	6.04	6.7	0.12
Acid P	563.6	725	637.3	642.0	20.48	238.3	286.8	292.7	272.6	10.20
Alk P	232.4	334.8	314	293.7	5.39	171	210.1	112.5	164.5	7.90
Nit R	1.5	2	1.7	1.7	0.13	0.7	0.9	0.7	0.8	0.06
MBC	732	834	731.5	765.8	22.67	633	630.6	587.3	617.0	11.38
OC	3.4	3.5	3.1	3.3	0.14	1.3	1.4	1.1	1.3	0.05
DHA	7.7	8.1	7.1	7.6	0.15	4	4	3.91	4.0	0.07
Acid P	212	304.2	270.1	262.1	9.42	89	115.1	95.7	99.9	5.20
Alk P	181.3	198.7	147.8	175.9	4.56	84.6	73	56.8	71.5	2.47
Nit R	1.1	1	0.9	1.0	0.05	0.4	0.5	0.3	0.4	0.05
MBC	437.7	425.5	419.7	427.6	18.31	225.2	344.5	341.1	303.6	11.48
OC	2.1	1.6	1.1	1.6	0.16	0.5	0.4	0.3	0.4	0.03

were 181.3, 198.7 and 147.8 $\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$ and for pine canopy soil the values are 84.6, 73, and 56.8 $\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$ for subsequent three years (2008-2010). Over all alkaline phosphatase activity found was 50 % lesser in pine canopy soil as compared to oak system mean value (0-15 and 15-30).

Nitrate reductase

Nitrate reductase activity follows the same trend as mean values of oak canopy cover surface soil NRA activity recorded is higher (1.7 mg kg^{-1}) than pine canopy cover (0.8 mg kg^{-1}) and similarly at lower depth (15-30cm) oak soil exhibited higher value (1 mg kg^{-1}) than pine soils (0.4 mg kg^{-1}). Plant litter quality have significant impact on nitrate reductase activity (Barford and Lajtha 1992) and thus can be used as indicator to access the litter quality impact on the soil. The ecology process of dissimilatory nitrogen reduction is not well understood Tiedje et al., (1982). Nitrate reductase activity is generally altered by nutrient status of the soil as reported by Ramana et al., (2008) and Poobathiraj et al., (2012). The rate of nitrate reduction and soil organic carbon are closely

related as revealed by many studies (Rashid and Schaefer 1987).

Microbial Biomass

Microbial Biomass Carbon

Higher MBC was observed in case of undisturbed natural oak canopy cover soil while pine canopy cover showed significantly lower MBC (Table 2). During the years (2008-2010) the mean surface microbial biomass carbon status of oak forest soil was 765.8 mg kg^{-1} and pine forest soil was 617 mg kg^{-1} respectively. However in lower depth the DHA activity of oak canopy soil were 419.7 $\text{mg kg}^{-1} \text{soil h}^{-1}$ and for pine canopy soil the value is 341.1 mg kg^{-1} . MBC status was found to be 45 % and 27 % lesser at lower depth (0-15cm) in comparison with surface soil (0-15cm) of oak and pine canopy cover soil respectively.

In the present study, oak and pine canopy cover greatly affected soil MBC (Table 2). Our results suggest the increase in organic matter enhances the proliferation of microbes in soil in agreement with earlier studies (Haynes, 1999, 2000, Wu et al., 2004). The higher MBC values observed in the undisturbed oak forest similar as in



Fig. 1. (a) Location of the study area 29.63° N, 79.33° E- Binsar wildlife sanctuary (b) Sampling site of pine (indicated as red triangle) and oak (indicated in blue triangle). (c) 3D view of Binsar Zero Point (8000 ft) - Dense Oak canopy site

conformity with Omay (1997), however the MBC values are lower in pine soil can be attributed to the relatively continuous and more organic matter deposition *via* leaf litter (Omay1997). Dead root cells tend to increase nutrient availability are higher in oak forest soil than in pine forest soil which in

turns increase the microbial activity in oak forest (Srivastava 2009; Kang et al., 2009)

Bacteria, Fungi and Actinomycetes

Enumeration of bacteria population reveal higher bacterial inhabitants in oak canopy soil than the pine canopy soil (Table 3). The mean bacterial,

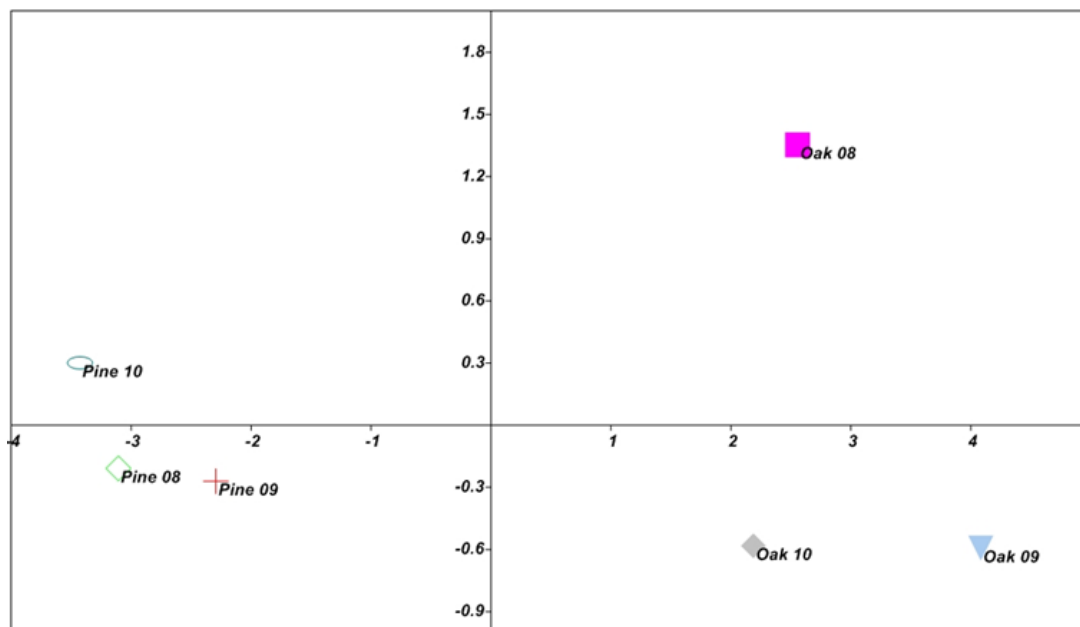


Fig. 2. Ordination of Oak and Pine forest soils sites in function of sampling periods (July-2008,2009 and 2010), in the space defined by the PC1 and PC2 axis of the PCA analysis carried out with Soil enzymes (Dehydrogenase, Acid Phosphatase, Alkaline Phosphatase and Nitrate Reductase, Microbial biomass carbon and Organic carbon). Dashed circles indicate the groups formed in the ordination of the soil biological properties and soil sampling areas in function of sampling periods

Table 3. Impact of Canopy cover on Bacteria (CFU x 10⁷), fungi (CFU x 10⁵) and Actinomycetes (CFU x 10⁶) population at different depths under different land use systems in central Himalayan region, MSE –Mean Standard Error

Microbial Enumeration	At Depth 0-15							
	Year	Oak canopy soil				Pine canopy soil		
	2008	2009	2010	MSE	2008	2009	2010	
Bacteria	26.2	21.5	18	1.24	8.4	14.6	10.3	0.86
Fungi	5.3	4.5	4.1	0.36	3.8	4.1	2.3	0.14
Actinomycetes	8.6	9.9	11.9	0.84	2.8	4.9	7.8	0.6

Microbial Enumeration	At Depth 15-30							
	Year	Oak canopy soil				Pine canopy soil		
	2008	2009	2010	MSE	2008	2009	2010	
Bacteria	12.5	14.6	11.3	0.5	4.4	8	7	0.3
Fungi	3.7	2.8	2.1	0.3	2.1	1.3	1.3	0.36
Actinomycetes	5.6	7.8	6.6	0.54	2.2	2.2	2.6	0.32

Table 4. Pearson's correlation coefficients among soil Dehydrogenase, Acid phosphatase, Alkaline phosphatase, Nitrate reductase, Microbial biomass carbon and organic carbon from oak and pine canopy soils of Binsar Jim Corbett Tiger Reserve

	DHA	Ac-P	Alk-P	NR	MBC
Ac-P	0.947**				
Alk-P	0.949**	0.928**			
NR	0.975**	0.961**	0.959**		
MBC	0.853**	0.889**	0.846**	0.821**	
OC	0.966**	0.939**	0.925**	0.970**	0.835**

** . Correlation is significant at the 0.01 level (2-tailed).

fungal and actinomycetes density of three years in oak forest surface soil is recorded as 21.9 CFU x 10⁷, 4.6 CFU x 10⁵ and 10.1 CFU x 10⁶, however in pine surface soil the values are 11.1 CFU x 10⁷, 3.4 CFU x 10⁵, 5.2 CFU x 10⁶ respectively. At depth 15-30 of oak forest soil the mean bacterial, fungal and actinomycetes population was reduced by 41% (12.8CFU x 10⁷), 37% (2.9CFU x 10⁵) and 34% (6.7CFU x 10⁶) respectively. Bacterial fungal and actinomycetes population were 40%, 51%, 55% lesser at 15-30cm depth respectively

Correlation and Principal Component Analysis

In general, Pearson correlation values between soil enzymes, microbial biomass carbon and organic carbon variables of oak and pine canopy soil were high (Table 4). The DHA activity was highly and positively correlated with Ac-P (0.94**), Alk-P (0.94**), NR (0.97**), MBC (0.85**), and OC (0.96**). Soil organic carbon was highly correlated with DHA (0.966**), Ac-P (0.939**), Alk-P (0.925**), NR (0.970**), and MBC (0.835**). Our results are in agreement with Nannipieri et al., (2002) and Ginanfreda et al., (2005) that high correlation of soil enzymes, MBC and OC may give an indication of better soil quality and fertility status of a soil with reference to soil microbe interactions. The PCA revealed differences between the systems and variation in the distribution of the indicators representing sampling time (Figure 1). Multivariate analysis of the components is explained by PC1 (94%) and PC2 (4%). PCA was done and obtained an individual correlation between the microbial and biochemical indicators in soil. Soil enzymes (dehydrogenase, acid phosphatase, alkaline phosphatase, nitrate reductase), microbial biomass

carbon and organic carbon indicators were used in PCA for comparative analysis between oak and pine canopy soil and sampling periods (2008, 2009 and 2010) as indicated in the legend of Figure 1.

PC1 values shows that microbial indicators were not much affected substantially by sampling period which may be due to the similar climatic condition mediated biological process (as indicated by similar values in all the years) but two distinguish pattern one for oak and other for pine canopy were observed and shows that the soil enzymes, microbial biomass carbon and organic carbon indicators were distinct to specific plant of canopy which determine the litter quality and decomposition impact on soil. The soil microbial indicators of two soils behaved differently when compared to each other. Our finding also supports the results of Wick et al. (1998) suggesting the use of MBC and OC as indicators to evaluate land use impacts.

CONCLUSION

The oak and pine canopy cover have significant and distinct effects with the type of vegetation on the soil microorganisms under oak and pine canopy. Our study revealed that soil enzyme activities varied significantly and decreased with increasing depth in both the ecosystem. The microbial status of oak forest soil is better than the pine forest suggesting the hard to decompose quality of the litter in the pine forest.

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