Eurasian Journal of Forest Science

Volume 8 Issue 2 June 2020

ISSN: 2147-7493

Eurasian Journal of Forest Science

ISSN: 2147 - 7493

Journal Cover Design
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Bolu forests, Turkey 2019 Ufuk COŞGUN

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Journal Web Page

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Eurasian Journal of Forest Science

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Eurasian Journal of Forest Science is a member of ULAKBIM DergiPark and is listed in the TR-DİZİN of TUBITAK and indexed in Index Copernicus.

ISSN: 2147 - 7493

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DOI: 10.3195/ejejfs.695673

Eurasian Journal of Forest Science

2020 8(2): 109-122

http://dergipark.gov.tr/ejejfs

Seasonal variation of microbial activity in soil and forest floor under three different fir plantations

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Abstract

Microbial activity is one of the important processes for biochemical cycles in soil and forest floor of ecosystems. Because, some of the carbon dioxide and nutrients needed by plants are released during the microbial activity. In this study, the relationships between environmental factors (moisture, temperature, pH, electric conductivity, C, N, Na, Ca, Mg, K, P) and seasonal variations of microbial respiration, microbial biomass-C and metabolic quotient (qCO2) in the forest floor and soil (0-5cm) under three adjacent fir plantation plots (Abies nordmanniana ssp. bornmuelleriana Mattf. (Ab), Abies cilicica Carr. (Ac) and Abies nordmanniana ssp. nordmanniana Mattf (An)) are investigated in Atatürk Arboretum, located in Istanbul-Turkey. A bimonthly sampling (from May-2012 to March-2013) was carried out by collecting 54 samples for each soil and forest floor samples within each species. According to the results, soil microbial respiration (SMR) has a significantly lower value in A_b plot. Although SMR and soil microbial biomass-C (SMBC) were correlated with moisture and temperature in A_n plot, they were correlated with nutrients in the other plots. In general, an increase in soil respiration rates was observed in autumn and early spring. Forest floor microbial respiration (FFMR), microbial biomass-C (FFMBC) and metabolic quotent (qCO_2) did not differ among the plots. The measured FFMR, FFMBC and qCO_2 parameters were lower in autumn than spring. Forest floor microbial parameters were thought to be drived by the variation of nutrients quantities. As a result, the microbial processes in both soil and forest floor were changed with the effect of different factors, although there was no clear difference among the plots.

Keywords: Microbial respiration, Microbial biomass-C, qCO_2, Abies

Introduction

Microorganism activities are one of the most important ecosystem components evaluated as an indicator of soil quality protection and sustainability (Kara and Bolat, 2008; Bolat *et al.*, 2015; Oyedele *et al.*, 2015). Microbial biomass and microbial respiration are considered as an index to evaluate soil microbial activity and health (Schoenholtz *et al.*, 2000; Mariani *et al.*, 2006).

Soil microbial biomass plays an essential role in soil fertility and nutrient retention in terrestrial ecosystems (Allen and Schlesinger, 2004) and serves as a resource of nutrients available for plant development. For instance, soil microbial biomass variations reflect immobilization levels–mineralization of nutrients (carbon and nitrogen) (Yang *et al.*, 2010). Similarly, Oktay and Tecimen (2016) indicated that nitrogen mineralization in summer was close to zero and this fact could happen due to microbial immobilization. Microbial biomass is a viable component of soil organic matter, accounting for 1-5% of the total organic matter content (Jiang *et al.*, 2009) and faster response to alterations in soil than soil organic matter (SOM) (Brookes *et al.*, 2008; Araujo *et al.*, 2010; Haripal and

Sahoo, 2014). The chemical and physical properties of the soil can both directly and indirectly affect the distribution and structure of the decomposer community. Many studies have investigated the relationships among microbial community structure, activity and environmental factors such as moisture, temperature, pH, nitrogen, carbon and nutrients (Na, Ca, P, Mg, S, etc.) (Diaz-Ravina *et al.*, 1993; Priess and Fölster, 2001; Nsabimana *et al.*, 2004; Pietri and Brookes, 2008; Pei *et al.*, 2016; Soong *et al.*, 2018; Wang *et al.*, 2018). However, the precise and detailed mechanism of how the microbial community structure or activities respond to changes in environmental parameters is still uncertain (Singh, 2018).

Vegetation can directly influence the microbial activity and microbial biomass through the impact on microclimate and the amount and quality of the forest floor. Relatedly, Prescott and Grayston (2013) stated that vegetation characteristics affect the microbial activity and microbial community structure.

Due to the release of carbon dioxide and nutrients during the process (Glassman *et al.*, 2018), litter decomposition is an important process for the nutrient cycle in soils of forest ecosystems. Litter decomposition rate is affected by abiotic (litter chemistry, nutrient presence, etc.) and biotic (soil microorganisms, fauna, etc.) factors (Wang *et al.*, 2018). For example, low nutrient concentration in the forest floor can reduce microbial respiration and biomass (Priess and Fölster, 2001). In addition, Prescott and Grayston (2013) emphasized that examining the relationships between cations in the forest floor and the microbial community can be instructive in understanding the mechanisms underlying different nutrient cycles and microbial community structures under different tree species.

The Abies family (Pinaceae) is represented by two species and six subspecies in Turkey (Çakır, 2018). *Abies nordmanniana* ssp. *bornmuelleriana* Mattf., *Abies nordmanniana* ssp *nordmanniana* Mattf. and *Abies cilicica* Carr. are the most common abies species in Turkey. These species constitute to 670390 ha of entire forest area in Turkey (OGM, 2013) and are found in different regions such as Marmara, Mediterranean and Black Sea. In the sense of this study, it is crucial to state that these tree species were planted in Atatürk Arboretum in 1960. It is determined that the stands have the same site characteristics such as soil type, climate etc. (Çakır, 2018).

The aim of the study is to investigate the seasonal changes of microbial parameters (microbial respiration and microbial biomass carbon) in forest floor and soil under three fir species in the same site and their relationship with various environmental factors (moisture, temperature, pH, electric conductivity, C, N, Na, Ca, Mg, K, P).

Material and Methods

Research Area

The study area, Atatürk Arboretum, is located in Belgrad Forest in Istanbul, Turkey (41°09′48″–41°10′55″ N and 28°57′27″–28°59′2 7″ E, 140 m asl.). According to the long-term data from the Bahçeköy Meteorology Station, the average annual temperature and precipitation is 13.0 °C and 1121 mm, respectively. Belgrad Forest has a maritime climate with moderate water deficiency in summers. The soil in the research area is Luvisol (WRB) and soil texture is loamy. The vegetative period lasts for 7.5 months (230 days) on average (Çakır, 2018).

Field data

Sampling was carried out in three adjacent fir plantation plots (*Abies nordmanniana* ssp. *bornmuelleriana* Mattf., *Abies cilicica* Carr. and *Abies nordmanniana* ssp *nordmanniana* Mattf).

Sampling was performed at the central 40 × 40 m plot within each plot to minimize the negative edge effects. The sample plots had homogeneity of abiotic environmental conditions (the aspect, slope, elevation and soil type). Sampling was carried out by collecting both 54 samples in soil and forest floor within each species (3 species × 3 cores × 6 dates = 54 cores) on each sampling date (a bimonthly from May-2012 to March-2013). Soil samples were taken from the upper 5 cm of soil layer with steel soil cores with a 100 cm^3 volume. The forest floor organic matter was collected from 0.25 m^2 area. Three replicated samples (soil and forest floor) were taken systematically and composited on each plot.

Chemical Measurements

Soil samples were oven-dried at 105 ˚C and entire roots and materials larger than 2 mm were removed by use of 2 mm sieve. Soil acidity (pH) and electrical conductivity (EC) were measured from 1:2.5 and 1:5 ratios of soil to deionized water soil slurry, respectively. Exchangeable cations (K, Ca, Mg, Na) in 10 g soil samples were extracted with ammonium acetate solution (1 N, pH = 7). Exchangeable P in 2 g samples were extracted with sodium bicarbonate solution (0.5 M, pH = 8.5). The concentrations of the individual cations were determined by ICP/OES (Perkin Elmer Optima DV7000) (Akburak *et al.*, 2018).

The dry mass of forest floor samples was determined at 65 °C for 48 h. Samples were digested with concentrated HNO_3 (% 65) and H_2O_2 (% 37) in a microwave oven (Berghoff Speedwave). The total concentration of elements (P, K, Ca, Mg, Na) were measured by ICP/OES (Perkin Elmer Optima DV7000). The carbon (C) and nitrogen (N) contents of soil and forest floor were determined with dry combustion method, using a LECO Truspec CN-2000 analyzer (Çakır and Akburak, 2017).

Microbial parameters measurement

To measure microbial respiration (MR), soil samples (20 g oven-dry soil equivalent) and forest floor samples (5 g oven-dry mass equivalent) were incubated for 7 days at 25 °C in 500 ml vessels and 10 ml of sodium hydroxide (1 M) was placed in the media to absorb the respired CO_2-C. The released CO_2–C was determined by adding $BaCl_2$ and later titrating with 1 M hydrochloric acid in the end of every 7 days (Alef and Nannipieri, 1995). Microbial biomass-C (MBC) was determined by the substrate-induced respiration (SIR) method. SIR was acquired by adding 60 mg glucose to soil samples (20 g oven-dry soil equivalent) . The released CO_2 was trapped in 0.05 M sodium hydroxide for 4-h incubation at 25 °C and measured by titration (Alef and Nannipieri, 1995). The metabolic quotient, qCO_2, was calculated by dividing microbial respiration with microbial biomass carbon (qCO_2 = MR / MBC) (Anderson and Domsch, 1986).

Statistical analysis

All data were tested for normal distribution using the Kolmogorov-Smirnov test. The data that did not accord with normal distribution were transformed with Box-Cox transformation. The significance of differences among tree species was tested by one-way analysis of variance (ANOVA) followed by Duncan test ($p < 0.05$). In addition, correlations between microbial parameters and environmental variables were determined using the simple Pearson correlation coefficient. The statistical analyzes were performed in SPSS 21.0 package program.

Results

Soil

Soil N, Mg and K ratio were significantly lower in A_b plot compared to other fir plots. However, P was significantly higher in A_b plot. Soil C and C/N ratios in A_c plot were significantly higher ($p < 0.05$) than the other two plots. Soil pH was significantly higher ($p < 0.05$) in A_n plot.

The respiration rate ranged from 0.18 - 1.04 µg CO_2-C g^- h^- in the A_b, 0.36 - 0.72 µg CO_2-C g^- h^- in A_c plot, and 0.47 - 1.01 µg CO_2-C g^- h^- in A_n plot (Figure 1). SMR in the A_n plot was significantly and 1.46 times higher in the A_b plot, 1.33 times higher in the A_c plot ($p < 0.05$). Although SMBC and $SqCO_2$ were at the lowest level in the A_b plot, there were not statistical differences among the plots (Table 1).

Table 1: Chemical, physical and microbial properties of soil in the study plot

	A. bornmuelleriana	***A. cilicica***	***A. nordmanniana***
	Mean ± Std.Er.	**Mean ± Std. Er**	**Mean ± Std. Er**
SMR (µg CO_2-C g^- h^-)	0.49 ± 0.07 a	0.54 ± 0.04 b	0.72 ± 0.06 b
SMBC (mg C g^- h^-)	0.27 ± 0.03 a	0.32 ± 0.04 a	0.31 ± 0.03 a
$SqCO_2$ (µg CO_2-C mg C h^-)	1.84 ± 0.22 a	1.95 ± 0.24 a	2.40 ± 0.22 a
SN* (%)	0.17 ± 0.01 b	0.19 ± 0.01 a	0.18 ± 0.01 ab
SC* (%)	5.28 ± 0.55 b	6.91 ± 0.44 a	4.65 ± 0.38 b
SCN	29.57 ± 1.55 b	35.37 ± 1.15 a	25.33 ± 1.31 c
SM* (%)	28.75 ± 2.81 a	34.96 ± 3.07 a	31.59 ± 3.10 a
ST* (^{0}C)	16.08 ± 1.24 a	16.69 ± 1.29 a	16.02 ± 1.26 a
pH*	5.15 ± 0.08 a	5.08 ± 0.08 a	5.58 ± 0.03 b
EC* (µS cm^-)	178.73 ± 19.58 a	185.52 ± 13.90 a	198.41 ± 11.58 a
Ca (mg kg^-)	1177.21 ± 66.45 a	1337.27 ± 52.83 a	1357.92 ± 70.06 a
Na (mg kg^-)	17.40 ± 1.50 a	24.41 ± 2.10 b	15.36 ± 1.40 a
K (mg kg^-)	88.93 ± 7.90 b	124.63 ± 9.24 a	111.47 ± 8.16 a
Mg (mg kg^-)	118.95 ± 7.52 b	237.32 ± 30.07 a	198.13 ± 22.61 a
P (mg kg^-)	47.62 ± 5.49 a	42.16 ± 3.10 a	31.43 ± 1.601 b

SMR : Soil microbial respiration, SMBC: Soil microbial biomass-Carbon, $SqCO_2$: Soil metabolic quotient, SN: Soil nitrogen, SC: Soil carbon, SCN: Carbon/nitrogen, SM: Soil moisture, ST: Soil temperature, pH: Soil acidity, EC: Soil electric conductivity, Ca: Calcium, Na: Sodium, K: Potassium, Mg: Magnesium, P: Phosphorous.
Means with different letters in the same line are different ($P < 0.05$).
"*" From Çakır (2018)

While SMR showed temporarily a parallel change among plots, an increase were observed for the respiration rates in autumn and early spring in all plots (Figure 1a). SMBC temporarily decreased from spring to autumn and reached the highest level in March in all plots (Figure 1b). $SqCO_2$ showed a parallel change among all plots and reached its highest level in September (Figure 1c). Additionally, a high level of change for both SMR and $SqCO_2$ was observed in A_n plots (Figure 1a, c).

A positive relationship was found between SMR and SMBC, SqCO2, N, P, EC, SM (soil moisture) in the A_b plots, with $SqCO_2$ ST (soil temperature) in the A_n plots. Whereas, there was only a significant positive relationship with Na and K in the A_c plots ($p < 0.05$). Additionally, a negative relationship was determined with SM in the A_n plot and with SCN in the A_c plot (Table 2).

In terms of SMBC, there was a positive relationship with EC and P in A_b plots, while the only significant one was between with K in A_c plots and with SM in A_n plots. However, while there was no negative relationship in A_b plots, SMBC showed negative relation with $SqCO_2$ and C in A_c plot and with SqCO2, SM, EC and Ca in A_n plot (Table 2).

In terms of SqCO2, while there was no significant relationship in Ac plot, linear correlations were determined with N and SM (Ab plots) and ST and EC (An plot). In addition, An plot had a negative relationship with SqCO2 and SM (Table 2).

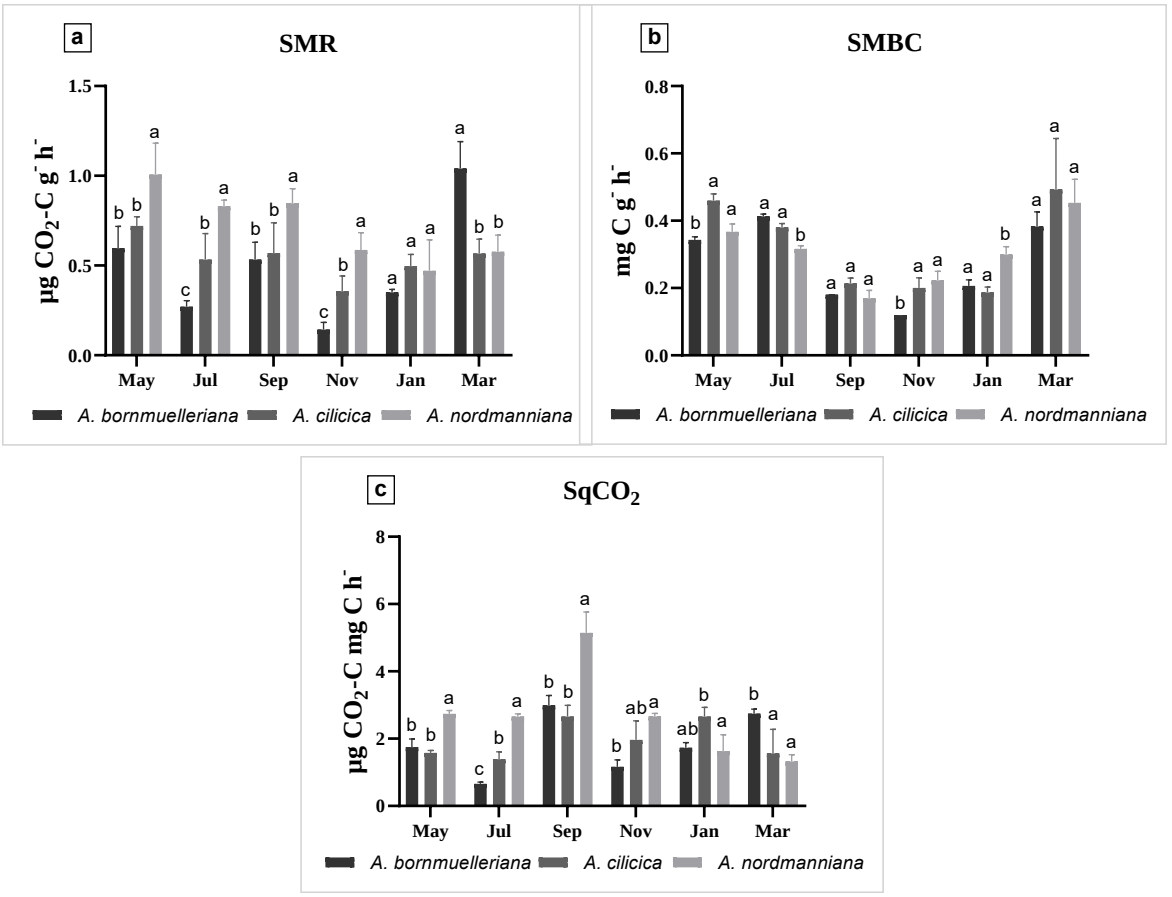

Figure 1: Seasonal variations of soil microbial parameter. (a: SMR; Soil microbial respiration, b: SMBC; Soil Microbial Biomass-C, c: qCO2; metabolic quotient).

Forest floor

In terms of FFMR, FFMBC, and $FFqCO_2$, no significant differences were encountered among the plots. The mass of the forest floor (FF) in A_n was significantly higher than the A_b and A_c ($p < 0.05$) although in A_n, the FFC was significantly lower than the other plots (Table 3). K concentrations in A_b plot were significantly different from A_c and A_n plots.

FFMR in all plots showed a decline in the autumn period (Figure 2a). FFMBC temporarily decreased from spring to autumn (Figure 2b). In addition, $FFqCO_2$ showed a parallel change among all plots, reaching its highest level in November (Figure 2c).

According to relationships between the variables, the amount of FFMR in the A_b plot showed a linear correlation with FFMBC, $FFqCO_2$ and Ca, and a negative correlation with Na. While there was a positive relationship with FFMBC and FM, a negative correlation was observed with N, C, P in the An plot. While it showed a positive relationship with FFMBC, FFM and a negative correlation with N, C, P in the A_n plot was observed. In the A_c plot, FFMR had a positive correlation with FFMBC and FFqCO2, and a negative relationship with P (Table 4).

In terms of FFMBC, there was no linear correlations in the A_b and A_c plots, while there was a linear correlation with K in the A_n. However, there were negative correlations with Na in A_b plot, with P in A_c plot and with $FFqCO_2$, Ca in A_n plots (Table 4).

In respect of $FFqCO_2$, there was a positive correlation with Ca and a negative correlation with K in the A_b plot. While it showed a negative correlation with FFN and Mg in the A_c plot and CN, C and K in the A_n plot. Also, there was a linear correlation between $FFqCO_2$ and FF in the A_n plot (Table 4).

Table 2: Pearson correlation coefficients among the soil variables in the study

A. bornmuelleriana	**SMR**	**SMBC**	**SqCO2**	**SN**	**SC**	**SCN**	**SM**	**ST**	**pH**	**EC**	**Ca**	**Na**	**K**	**Mg**
SMBC	***0.48***													
SqCO2	***0.73***	-0.22												
SN	***0.59***	0.29	***0.48***											
SC	0.40	0.21	0.33	***0.87***										
SCN	0.24	0.15	0.17	***0.68***	***0.94***									
SM	***0.56***	-0.14	***0.67***	0.30	0.31	0.23								
ST	-0.21	0.31	-0.39	0.04	0.03	0.08	***-0.72***							
pH	-0.06	-0.13	0.05	-0.11	-0.08	-0.02	0.12	-0.18						
EC	***0.49***	***0.47***	0.24	***0.68***	***0.71***	***0.67***	0.17	0.16	0.28					
Ca	-0.12	0.07	-0.15	0.15	***0.45***	***0.62***	-0.04	0.20	0.07	***0.47***				
Na	0.24	0.44	-0.02	***0.50***	***0.48***	0.42	-0.10	0.43	-0.08	***0.72***	***0.44***			
K	0.36	0.30	0.21	***0.70***	***0.51***	0.35	-0.03	0.12	-0.06	***0.63***	0.19	***0.74***		
Mg	0.18	0.27	0.07	***0.47***	***0.57***	***0.63***	-0.14	0.33	0.01	***0.72***	***0.79***	***0.65***	***0.60***	
P	***0.58***	***0.49***	0.24	***0.63***	***0.67***	***0.61***	0.40	-0.07	-0.33	***0.52***	0.29	0.42	0.30	0.36
A. cilicica	**SMR**	**SMBC**	**SqCO2**	**SN**	**SC**	**SCN**	**SM**	**ST**	**pH**	**EC**	**Ca**	**Na**	**K**	**Mg**
SMBC	0.39													
SqCO2	0.22	***-0.81***												
SN	-0.15	-0.37	0.33											
SC	-0.34	-0.42	0.25	***0.93***										
SCN	***-0.46***	-0.40	0.14	***0.77***	***0.95***									
SM	0.03	-0.11	0.14	-0.42	***-0.46***	***-0.46***								
ST	0.21	0.29	-0.19	0.16	0.11	0.07	***-0.82***							
pH	0.12	0.40	-0.32	0.34	0.26	0.18	0.03	0.04						
EC	-0.22	-0.29	0.19	***0.72***	***0.65***	***0.51***	-0.30	0.16	0.31					
Ca	-0.18	-0.15	0.08	0.27	0.30	0.30	-0.03	-0.10	0.10	0.21				
Na	***0.47***	0.26	0.01	0.25	0.16	0.12	***-0.46***	***0.49***	-0.01	0.15	-0.05			
K	***0.53***	***0.44***	-0.11	0.24	0.06	-0.04	-0.19	0.26	0.35	-0.05	-0.27	***0.67***		
Mg	0.32	0.12	0.07	0.40	0.37	0.34	-0.29	0.15	0.23	-0.03	-0.06	***0.68***	***0.76***	
P	-0.15	-0.16	0.11	0.40	0.31	0.20	-0.16	-0.03	-0.06	0.39	***0.53***	-0.01	-0.13	-0.20
A. nordmanniana	**SMR**	**SMBC**	**SqCO2**	**SN**	**SC**	**SCN**	**SM**	**ST**	**pH**	**EC**	**Ca**	**Na**	**K**	**Mg**
SMBC	-0.01													
SqCO2	***0.64***	***-0.76***												
SN	0.16	0.02	0.08											
SC	0.22	-0.32	0.34	***0.66***										
SCN	0.22	-0.38	0.38	0.40	***0.94***									
SM	***-0.50***	***0.44***	***-0.65***	0.00	-0.38	***-0.50***								
ST	***0.72***	-0.29	***0.69***	0.03	0.36	***0.45***	***-0.90***							
pH	-0.13	0.11	-0.20	0.07	0.15	0.14	0.23	-0.21						
EC	0.04	***-0.60***	***0.44***	0.14	0.35	0.32	-0.26	0.24	***0.44***					
Ca	-0.27	***-0.57***	0.22	-0.02	0.36	***0.44***	-0.15	0.04	0.08	***0.51***				
Na	0.24	-0.10	0.21	0.14	0.34	0.33	-0.07	0.24	***0.48***	***0.59***	0.23			
K	0.27	-0.30	0.37	0.26	***0.47***	0.41	-0.24	0.40	0.40	***0.73***	0.26	***0.73***		
Mg	0.25	-0.36	0.39	***0.52***	***0.65***	***0.53***	-0.09	0.25	***0.44***	***0.63***	0.40	***0.62***	***0.81***	
P	0.42	0.20	0.13	0.23	0.38	0.38	-0.08	0.23	0.02	-0.06	-0.24	0.22	0.17	0.10

SMR : Soil microbial respiration, SMBC: Soil microbial biomass-Carbon, $SqCO_2$: Soil metabolic quotient, SN: Soil nitrogen, SC: Soil carbon, SCN: Carbon/nitrogen, SM: Soil moisture, ST: Soil temperature, pH: Soil acidity, EC: Soil electric conductivity, Ca: Calcium, Na: Sodium, K: Potassium, Mg: Magnesium, P: Phosphorous. Significant differences ($P \leq 0.05$) are marked with bold and italic

Table 3: Chemical, physical and microbial properties of the forest floor in the study plot

	A. bornmuelleriana	***A. cilicica***	***A. nordmanniana***
	Mean ± Std. Er	**Mean ± Std. Er**	**Mean ± Std. Er**
FFMR (µg CO_2-C g^{-} h^{-})	4.18 ± 0.64 a	4.26 ± 0.62 a	4.70 ± 0.66 a
FFMBC (mg C g^{-} h^{-})	2.40 ± 0.28 a	1.96 ± 0.24 a	2.37 ± 0.31 a
FFqCO_2 (µg CO_2-C mg C h^{-})	1.99 ± 0.36 a	2.22 ± 0.27 a	2.39 ± 0.36 a
FFN* (%)	1.58 ± 0.02 a	1.19 ± 0.03 a	1.39 ± 0.03 a
FFC* (%)	42.96 ± 0.46 a	41.92 ± 0.88 a	37.09 ± 1.19 b
FFCN*	27.32 ± 0.43 ab	28.26 ± 0.83 a	26.45 ± 0.82 b
FF* (g m^{-2})	1027.02 ± 49.97 a	1005.04 ± 67.28 a	1452.86 ± 84.61 b
FFM (%)	44.93 ± 2.12 a	41.27 ± 2.98 a	46.97 ± 2.04 a
Ca (mg kg^{-})	14326.67 ± 369.46 a	14650.83 ± 567.98 a	14272.50 ± 498.38 a
Na (mg kg^{-})	54.08 ± 5.26 a	68.23 ± 8.30 a	52.57 ± 4.46 a
K (mg kg^{-})	922.75 ± 42.78 a	1081.77 ± 34.37 b	1072.517 ± 50.21 b
Mg (mg kg^{-})	1193.82 ± 46.69 a	1291.25 ± 30.11 a	1339.583 ± 42.89 a
P (mg kg^{-})	475.67 ± 8.08 a	462.04 ± 12.92 a	479.192 ± 9.46 a

FFMR : Forest floor microbial respiration, FFMBC: Forest floor microbial biomass-Carbon, FFqCO_2: Forest floor metabolic quotient, FFN: Forest floor nitrogen, FFC: Forest floor carbon, FFCN: Forest floor Carbon/nitrogen, FF: Forest floor amount, FFM: Forest floor moisture, Ca: Calcium, Na: Sodium, K: Potassium, Mg: Magnesium, P: Phosphorous. Means with different letters in the same line are different (P < 0.05).
"*" from Çakır (2018)

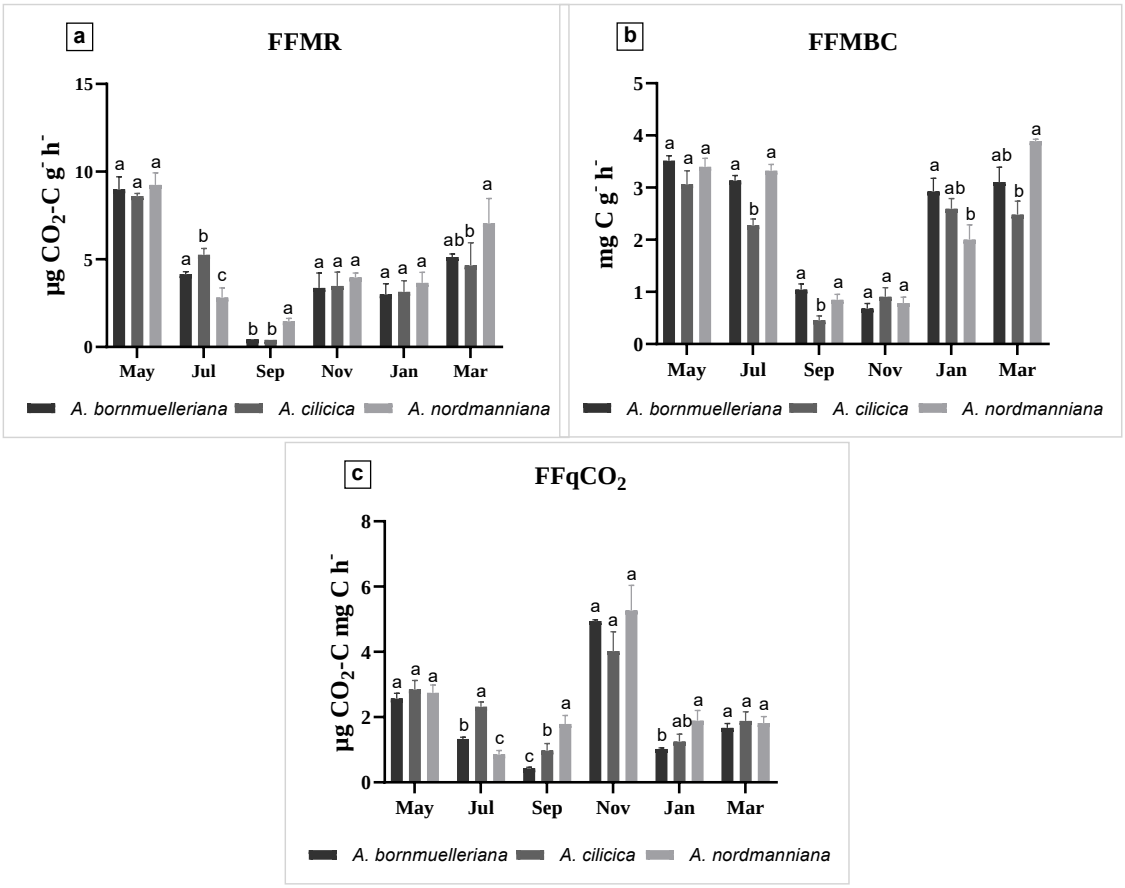

Figure 2: Seasonal variations of forest floor microbial parameters. (a: FFMR, Forest floor microbial respiration, b: FFMBC: Forest Floor Microbial Biomass-C, c: FFqCO2: Forest Floor metabolic quotient)

Table 4: Pearson correlation coefficients among the forest floor variables in the study

A. bornmuelleriana	**FFMR**	**FFMBC**	**FFqCO2**	**FFN**	**FFC**	**FFCN**	**FF**	**FFM**	**Ca**	**Na**	**K**	**Mg**
FFMBC	***0.72***											
FFqCO2	***0.63***	-0.02										
FFN	0.17	0.01	0.16									
FFC	0.11	0.14	0.06	0.32								
FFCN	-0.09	0.09	-0.12	***-0.75***	0.38							
FF	0.24	-0.08	0.26	0.34	0.03	-0.34						
FFM	0.23	0.39	-0.13	-0.14	-0.42	-0.15	0.04					
Ca	***0.50***	0.19	***0.48***	-0.19	0.06	0.22	0.22	-0.25				
Na	***-0.69***	***-0.63***	-0.30	-0.21	-0.16	0.11	-0.25	-0.15	-0.08			
K	-0.29	0.37	***-0.72***	-0.10	-0.05	0.07	-0.36	0.35	***-0.52***	0.11		
Mg	0.04	0.15	-0.18	0.31	-0.28	***-0.49***	0.11	0.32	-0.22	0.02	***0.49***	
P	0.38	0.15	0.19	-0.21	-0.05	0.20	0.07	-0.17	***0.63***	0.14	-0.35	-0.03
A. cilicica	**FFMR**	**FFMBC**	**FFqCO2**	**FFN**	**FFC**	**FFCN**	**FF**	**FFM**	**Ca**	**Na**	**K**	**Mg**
FFMBC	***0.76***											
FFqCO2	***0.46***	-0.08										
FFN	-0.23	0.09	***-0.45***									
FFC	0.07	0.04	-0.22	-0.18								
FFCN	0.15	-0.02	0.06	***-0.71***	***0.82***							
FF	-0.23	-0.31	0.29	0.04	***-0.72***	***-0.57***						
FFM	0.06	0.27	-0.14	***0.50***	-0.39	***-0.56***	0.25					
Ca	0.07	-0.06	-0.20	0.26	0.30	0.05	-0.12	-0.01				
Na	-0.20	-0.09	-0.37	0.28	0.19	-0.03	0.06	-0.02	***0.75***			
K	-0.19	-0.19	-0.37	0.05	0.25	0.16	-0.10	-0.21	***0.71***	***0.87***		
Mg	-0.22	0.07	***-0.46***	***0.66***	-0.03	-0.36	-0.26	0.34	0.13	0.09	-0.03	
P	***-0.50***	***-0.50***	-0.38	***0.48***	0.09	-0.19	-0.10	-0.03	***0.70***	***0.54***	***0.55***	***0.48***
A. nordmanniana	**FFMR**	**FFMBC**	**FFqCO2**	**FFN**	**FFC**	**FFCN**	**FF**	**FFM**	**Ca**	**Na**	**K**	**Mg**
FFMBC	***0.62***											
FFqCO2	0.36	***-0.50***										
FFN	***-0.47***	-0.27	-0.24									
FFC	***-0.50***	0.06	***-0.60***	***0.45***								
FFCN	-0.26	0.26	***-0.53***	-0.14	***0.81***							
FF	0.24	-0.25	***0.59***	***-0.57***	***-0.61***	-0.37						
FFM	***0.46***	0.11	0.35	-0.27	***-0.43***	-0.24	0.02					
Ca	-0.36	***-0.48***	0.26	0.00	0.18	0.25	0.29	-0.18				
Na	0.02	-0.15	0.15	0.23	0.12	-0.03	-0.18	***0.52***	0.02			
K	0.11	***0.43***	***-0.46***	0.14	-0.02	-0.17	-0.27	-0.31	***-0.81***	-0.41		
Mg	0.39	0.21	0.19	***-0.52***	-0.41	-0.17	0.27	-0.18	-0.42	***-0.53***	***0.43***	
P	***-0.56***	-0.26	-0.27	0.39	***0.47***	0.28	-0.04	***-0.59***	***0.66***	-0.07	-0.22	***-0.57***

FFMR : Forest floor microbial respiration, FFMBC: Forest floor microbial biomass-Carbon, $FFqCO_2$: Forest floor metabolic quotient, FFN: Forest floor nitrogen, FFC: Forest floor carbon, FFCN: Forest floor Carbon/nitrogen, FF: Forest floor amount, FFM: Forest floor moisture, Fe: Iron, Ca: Calcium, Na: Sodium, K: Potassium, Zn: Zinc, Cu: copper, Mg: Magnesium, Mn: Manganese, P: Phosphorous. Significant differences ($P \leq 0.05$) are marked with bold and italic

Discussion

Soil

Soil basal respiration provides an estimate of microbial activity (Bolat *et al.*, 2015). The SMR rate obtained in the study sites ranged from 0.18 to 1.04 µg CO_2-C g^{-} h^{-}. These results are similar to those obtained with other studies (Hofman *et al.*, 2004; Bolat, 2014; Bolat *et al.*, 2015). SMR in A_b plot was significantly lower than other species. This may be due to the low amount of N, SM and SMBC in this plot compared to other fir species in the study site. Previous studies indicated that change of microbial respiration have relation with factors such as mineralized N, moisture and MBC (Priess and Fölster, 2001; Nsabimana *et al.*, 2004).

Generally, a temporary increase in respiration rates was observed in fall and spring across all plots. As it was pointed out in Bolat *et al.* (2015), this increase might be a consequence of a promoting microbial

activity which was triggered by higher soil temperature and moisture in these seasons. In addition, there were significant differences among the related species in July and November. Baldrian (2017) pointed out that seasonal variation is the most important driving force of microbial change with the soil temperature. When the interactions between SMR and variables are examined, it was seen that ST has a strong effect on SMR in the A_n plot, while the presence of nutrients (N, Na, K and P) in the A_b and A_c plots were effective on SMR. As the results demonstrated in many studies, the microbial respiration is related to available nutrients (Cheng *et al.*, 2013; Li *et al.*, 2015; Spohn, 2015; Wang *et al.*, 2018) and microbial biomass (Mariani *et al.*, 2006; Bolat *et al.*, 2015; Qu *et al.*, 2018). Likewise, Spohn (2015) found that respiration have linear correlation with soil temperature. Wang *et al.* (2003) suggested that soil respiration under favorable temperature and moisture conditions was principally determined by substrate supply rather than by the pool size of MBC. As a result, it can be stated that different environmental factors have different effects on microbial process among plots.

The mean SMBC values were not showed significant differences among the plots. This may be due to the fact that soil properties (such as ST, SM and pH) that affect SMBC do not differ between the plots. However, in terms of seasonal variations, SMBC decreased from spring to autumn and reached its highest level in all plots in March. Similar to the results of this study, Qu *et al.* (2018) stated that MBC was showed an increase early in the growing season (May) and then gradually decrease (July). This seasonal change may have two possible causes. The first one is the seasonal change in soil moisture and temperature. Second, the reduction in soil microbial biomass may be the result of mineralization of nutrients as stated by Jia *et al.* (2005). Soil microbial biomass is strongly affected by multiple factors such as local abiotic conditions and plant traits (Pei *et al.*, 2016). Considering the relationship between SMBC and environmental variables, there is a positive relationship with EC and P in the A_b plot, while there is a positive relationship only with K in the A_c plot, with SM and a negative relationship with EC and Ca in the A_n plot. Previous studies showed that the microbial biomass is strongly dependent on soil properties such as exchangeable Ca, and pH (Wolters and Joergensen, 1991; Agnelli *et al.*, 2001). While the relationships between MBC and C, N were determined in the studies (Park *et al.*, 2002; Mariani *et al.*, 2006; Pei *et al.*, 2016), no significant relationships were detected in presented study. Similar to current study results,Tan *et al.* (2008), Pei *et al.* (2016), Docherty *et al.* (2015) and Cheng *et al.* (2013) stated that moisture and nutrients have significant relationships with SMBC. The results showed that although SMBC under different tree species did not differ, soil microbial biomass are drived by different factors.

The qCO_2 is a measure that varies according to the state of the microbial biomass, the availability of nutrients, and various abiotic factors (Wardle and Ghani, 1995; Gonçalves *et al.*, 2009). qCO_2 showed a parallel change between plots and reached its highest level in September. This situation can be explained as in this period, with moisture and temperature changes (low moisture and high temperature) as it was stated by Yuan and Yue (2012). In addition, $SqCO_2$ has generally changed at high levels in A_n plot. Increase of qCO_2 was interpreted as a microbial response to adverse environmental stresses that was observed when the soil conditions were unfavorable (Wardle and Ghani, 1995). $SqCO_2$ had a positive relationship with N and SM in the A_b plots, had a negative relationship with SMBC in the A_c plot and had a positive relationship with ST and EC, and had a negative relationship with SM in the A_n plot. These results show that moisture is the limiting factor in A_n plot and microbial biomass plays a role as the limiting factor in A_c plots.

Forest floor

In this study, FFMR ranged from 0.41 to 9.24 µg CO_2-C g^{-} h^{-}. These values are lower than the values found for fir forests by Bolat *et al.* (2015). Although the a mean FFMR values did not differ significantly among the species, forest floor properties (FFC, FFCN, FF and K) differed among plots. FFMR in all

plots was observed as it decreased in the autumn period and an increase afterwards. This can be explained by the change in the amounts of FMBC. In relation to this, when the relationships among the variables were evaluated, it showed a linear correlation between FFMR and FFMBC in all plots. In addition, FFMR had a positive correlation with Ca in the A_b plot, a positive relationship with FM and a negative relationship with N, C and P in the A_n plot. Also, it has shown the only negative relationship with P in the A_c plot. Wardle (1992) stated that microbial activity may also be limited by the availability of N or P. Contrary to the presented study results, Allen and Schlesinger (2004) found a linear correlation between FFMR and C, N, P. As a result, nutrient status may also affect the activity of the microflora.

Microbial biomass mediates the conversion of biogenic nutrients between inorganic and organic components (Wu *et al.*, 2000). FFMR values were similar and there was no difference between the plots in terms of average FFMBC amounts. It had decreased from spring to autumn in terms of the temporal change. This variation can be caused by changes in temperature and humidity. Similar to the study results, Butenschoen *et al.* (2011) found that the litter microbial biomass decreases with increasing temperature. In addition, it is possible to say that the mobilization and immobilization status of nutrients can be temporally effective. As a matter of fact, when the relationships between FFMBC and variables are evaluated, there was no positive relationship in the A_b and A_c plot while there was a positive relationship with K in the A_n plot. On the contrary, the results showed a negative relationship with Na in A_b plot, with P in A_c plot and with Ca in A_n plot. This observation shows that different nutrients may have a limiting effect on a variation of forest floor microbial biomass among the plots.

The mean values of $FFqCO_2$ were not showed a significant difference among the plots like the other microbial parameters and it reached the highest level in November. This can be explained by the mobilization process of nutrients. Because the $FFqCO_2$ showed a negative relationship with K in the A_b plot, with P in the A_c plot, and with Ca in the A_n plot. This result supports the view that the limiting factors mentioned above were caused by different nutrients.

Conclusion

In the presented study, SMBC, $SqCO_2$ values did not show a significant difference among the three different fir species, and SMR has the lowest value in *Abies nordmanniana* ssp. *bornmuelleriana* Mattf. plot. While SMR and SMBC were associated with moisture and temperature conditions in the *Abies nordmanniana* ssp *nordmanniana* Mattf plot, they had interactions with nutrients (N, Na, K and P) in other plots. Microbial parameters in forest floor did not differ between plots. Temporarily, a decrease in microbial parameters have been detected from spring to autumn in plots. Forest floor microbial parameters prominently changed under the influence of nutrients (Ca, N, C, P and K). As a result, in this short-term study, although there was no clear difference between the plots, the microbial processes in both soil and forest floor changed with the effect of different factors. These results will contribute to understanding the relationships between microbial processes and biochemical cycles in plantations. In future studies, considering the long-term measurements of these interrelations, including microbial community structure and enzyme activities, will also provide a clearer evaluation opportunity in plantations constituted different species.

Acknowledgements

This work was supported by the Scientific Research Projects Coordination Unit of Istanbul University-Cerrahpasa, Project number: BYP-2017-23685. It was presented as a oral presentation titled “Doğu Karadeniz ve Uludağ Göknarı Meşcereleri Altındaki Topraklarda Bazı Mikrobiyal Parametrelerin Mevsimsel Değişimi”, and its abstract (Turkish) was published in the abstracts book of the Congress “22. Ulusal Biyoloji Kongresi ” in Eskisehir, Turkey, 23-27 June 2014. I thank to Res. Assis. Alper Gün ÖZTURNA for his support with English language editing.

References

Agnelli, A., Ugolini, F., Corti, G., Pietramellara, G. (2001). Microbial biomass-C and basal respiration of fine earth and highly altered rock fragments of two forest soils. Soil Biology and Biochemistry 33, 613-620.

Akburak, S., Son, Y., Makineci, E., Çakir, M. (2018). Impacts of low-intensity prescribed fire on microbial and chemical soil properties in a Quercus frainetto forest. Journal of forestry research 29, 687-696.

Alef, K., Nannipieri, P., 1995. Methods in applied soil microbiology and biochemistry. Academic Press.

Allen, A., Schlesinger, W. (2004). Nutrient limitations to soil microbial biomass and activity in loblolly pine forests. Soil Biology and Biochemistry 36, 581-589.

Anderson, T.H., Domsch, K. (1986). Carbon assimilation and microbial activity in soil. Zeitschrift für Pflanzenernährung und Bodenkunde 149, 457-468.

Araujo, A., Silva, E., Nunes, L., Carneiro, R. (2010). The effect of converting tropical native savanna to Eucalyptus grandis forest on soil microbial biomass. Land degradation & development 21, 540-545.

Baldrian, P. (2017). Microbial activity and the dynamics of ecosystem processes in forest soils. Current opinion in microbiology 37, 128-134.

Bolat, I. (2014). The effect of thinning on microbial biomass C, N and basal respiration in black pine forest soils in Mudurnu, Turkey. European journal of forest research 133, 131-139.

Bolat, İ., Kara, Ö., Tunay, M. (2015). Effects of Seasonal Changes on Microbial Biomass and Respiration of Forest Floor and Topsoil under Bornmullerian Fir Stand. Eurasian Journal of Forest Science 3, 1-13.

Brookes, P., Cayuela, M.L., Contin, M., De Nobili, M., Kemmitt, S., Mondini, C. (2008). The mineralisation of fresh and humified soil organic matter by the soil microbial biomass. Waste Management 28, 716-722.

Butenschoen, O., Scheu, S., Eisenhauer, N. (2011). Interactive effects of warming, soil humidity and plant diversity on litter decomposition and microbial activity. Soil Biology and Biochemistry 43, 1902-1907.

Cheng, F., Peng, X., Zhao, P., Yuan, J., Zhong, C., Cheng, Y., Cui, C., Zhang, S. (2013). Soil microbial biomass, basal respiration and enzyme activity of main forest types in the Qinling Mountains. PLoS One 8.

Çakır, M. (2018). Richness and diversity of litter and soil fauna as affected by differences in three fir species. Bosque 39, 441-447.

Çakır, M., Akburak, S. (2017). Litterfall and nutrients return to soil in pure and mixed stands of oak and beech. İstanbul Üniversitesi Orman Fakültesi Dergisi 67, 185-200.

Diaz-Ravina, M., Acea, M., Carballas, T. (1993). Seasonal fluctuations in microbial populations and available nutrients in forest soils. Biology and Fertility of Soils 16, 205-210.

Docherty, K.M., Borton, H.M., Espinosa, N., Gebhardt, M., Gil-Loaiza, J., Gutknecht, J.L., Maes, P.W., Mott, B.M., Parnell, J.J., Purdy, G. (2015). Key edaphic properties largely explain temporal and geographic variation in soil microbial communities across four biomes. PLoS One 10.

Glassman, S.I., Weihe, C., Li, J., Albright, M.B., Looby, C.I., Martiny, A.C., Treseder, K.K., Allison, S.D., Martiny, J.B. (2018). Decomposition responses to climate depend on microbial community composition. Proceedings of the National Academy of Sciences 115, 11994-11999.

Gonçalves, I., Araújo, A., Carvalho, E., Carneiro, R. (2009). Effect of paclobutrazol on microbial biomass, respiration and cellulose decomposition in soil. European journal of soil biology 45, 235-238.

Haripal, K., Sahoo, S. (2014). Microbial biomass Carbon, Nitrogen, and Phosphorus dynamics along a chronosequence of abandoned tropical agroecosystems. International Journal of Current Microbiology and Applied Sciences 3, 956-970.

Hofman, J., Dušek, L., Klánová, J., Bezchlebová, J., Holoubek, I. (2004). Monitoring microbial biomass and respiration in different soils from the Czech Republic—a summary of results. Environment International 30, 19-30.

Jia, G.-m., Cao, J., Wang, C., Wang, G. (2005). Microbial biomass and nutrients in soil at the different stages of secondary forest succession in Ziwulin, northwest China. Forest Ecology and Management 217, 117-125.

Jiang, J.-P., Xiong, Y.-C., Jiang, H.-M., De-You, Y., Ya-Jie, S., Feng-Min, L. (2009). Soil microbial activity during secondary vegetation succession in semiarid abandoned lands of Loess Plateau. Pedosphere 19, 735-747.

Kara, Ö., Bolat, I. (2008). Soil microbial biomass C and N changes in relation to forest conversion in the Northwestern Turkey. Land Degradation & Development 19, 421-428.

Li, Y., Liu, Y., Wu, S., Niu, L., Tian, Y. (2015). Microbial properties explain temporal variation in soil respiration in a grassland subjected to nitrogen addition. Scientific reports 5, 18496.

Mariani, L., Chang, S.X., Kabzems, R. (2006). Effects of tree harvesting, forest floor removal, and compaction on soil microbial biomass, microbial respiration, and N availability in a boreal aspen forest in British Columbia. Soil Biology and Biochemistry 38, 1734-1744.

Nsabimana, D., Haynes, R., Wallis, F. (2004). Size, activity and catabolic diversity of the soil microbial biomass as affected by land use. Applied Soil Ecology 26, 81-92.

OGM, 2013. Orman Atlası. Orman Genel Müdürlüğü, Ankara.

Oktay, S., Tecimen, B.H. (2016). Nitrogen Mineralization Experiment under Fir Stand with Different Measurement Methods Asian Journal of Agriculture and Food Sciences 4, 157-163.

Oyedele, A.O., Olayungbo, A.A., Denton, O.A., Ogunrewo, O.M., Momodu, F.O. (2015). Assessment of the microbial biomass carbon, nitrogen and phosphorus in relation to physico-chemical properties of Acric Luvisols in Ibadan South West, Nigeria. Journal of Agriculture and Environment for International Development (JAEID) 109, 179-187.

Park, J.-H., Kalbitz, K., Matzner, E. (2002). Resource control on the production of dissolved organic carbon and nitrogen in a deciduous forest floor. Soil Biology and Biochemistry 34, 813-822.

Pei, Z., Eichenberg, D., Bruelheide, H., Kröber, W., Kühn, P., Li, Y., von Oheimb, G., Purschke, O., Scholten, T., Buscot, F. (2016). Soil and tree species traits both shape soil microbial communities during early growth of Chinese subtropical forests. Soil Biology and Biochemistry 96, 180-190.

Pietri, J.A., Brookes, P. (2008). Relationships between soil pH and microbial properties in a UK arable soil. Soil Biology and Biochemistry 40, 1856-1861.

Prescott, C.E., Grayston, S.J. (2013). Tree species influence on microbial communities in litter and soil: current knowledge and research needs. Forest Ecology and Management 309, 19-27.

Priess, J., Fölster, H. (2001). Microbial properties and soil respiration in submontane forests of Venezuelian Guyana: characteristics and response to fertilizer treatments. Soil Biology and Biochemistry 33, 503-509.

Qu, L., Kitaoka, S., Koike, T. (2018). Factors controlling soil microbial respiration during the growing season in a mature larch plantation in Northern Japan. Journal of soils and sediments 18, 661-668.

Schoenholtz, S.H., Van Miegroet, H., Burger, J. (2000). A review of chemical and physical properties as indicators of forest soil quality: challenges and opportunities. Forest ecology and management 138, 335-356.

Singh, J. (2018). Microbes: Key Ecological Drivers in Controlling the Issues Related to Environmental Changes. Annals of Microbiology and Immunology 1, 1009.

Soong, J.L., Marañon-Jimenez, S., Cotrufo, M.F., Boeckx, P., Bodé, S., Guenet, B., Peñuelas, J., Richter, A., Stahl, C., Verbruggen, E. (2018). Soil microbial CNP and respiration responses to organic matter and nutrient additions: Evidence from a tropical soil incubation. Soil Biology and Biochemistry 122, 141-149.

Spohn, M. (2015). Microbial respiration per unit microbial biomass depends on litter layer carbon-to-nitrogen ratio. Biogeosciences 12, 817-823.

Tan, X., Chang, S.X., Kabzems, R. (2008). Soil compaction and forest floor removal reduced microbial biomass and enzyme activities in a boreal aspen forest soil. Biology and Fertility of Soils 44, 471-479.

Wang, Q., Kwak, J.-H., Choi, W.-J., Chang, S.X. (2018). Decomposition of trembling aspen leaf litter under long-term nitrogen and sulfur deposition: effects of litter chemistry and forest floor microbial properties. Forest Ecology and Management 412, 53-61.

Wang, W., Dalal, R., Moody, P., Smith, C. (2003). Relationships of soil respiration to microbial biomass, substrate availability and clay content. Soil Biology and Biochemistry 35, 273-284.

Wardle, D. (1992). A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biological reviews 67, 321-358.

Wardle, D., Ghani. (1995). A critique of the microbial metabolic quotient (qCO2) as a bioindicator of disturbance and ecosystem development. Soil Biology and Biochemistry 27, 1601-1610.

Wolters, V., Joergensen, R. (1991). Microbial carbon turnover in beech forest soils at different stages of acidification. Soil Biology and Biochemistry 23, 897-902.

Wu, J., He, Z.-L., Wei, W.-X., O'donnell, A., Syers, J. (2000). Quantifying microbial biomass phosphorus in acid soils. Biology and Fertility of Soils 32, 500-507.

Yang, K., Zhu, J., Zhang, M., Yan, Q., Sun, O.J. (2010). Soil microbial biomass carbon and nitrogen in forest ecosystems of Northeast China: a comparison between natural secondary forest and larch plantation. Journal of Plant Ecology 3, 175-182.

Yuan, B.-C., Yue, D.-X. (2012). Soil microbial and enzymatic activities across a chronosequence of Chinese pine plantation development on the loess plateau of China. Pedosphere 22, 1-12.

Submitted: 02.03.2020 Accepted: 14.04.2020

DOI: 10.3195/ejejfs.712478

Eurasian Journal of Forest Science

2020 8(2): 123-128

http://dergipark.gov.tr/ejejfs

Abundance of soil microbial communities and plant growth in agroecosystems and forest ecosystems

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Abstract
The objective of this study was to review natural ecosystems and agroecosystems to compare the abundance of soil microbial communities and also plant growth. This study used a random block design method, each soil from both ecosystems is planted with corn and string beans. Each treatment is repeated three times and arranged in random block design. At the end of the study, head height, root display and leaf count were calculated. The results showed that soils from agroecosystems had greater microbial abundance and good plant growth responses. The results showed that agroecosystems are ecosystems that have an abundance of microbial communities that are the most good compared to forest ecosystems. This has an impact on good growth responses in agroecosystems compared to forest ecosystems.
Keywords: Agriculture ecosystem, plant growth promoting agents, natural ecosystem

Introduction

Healthy soils are the capacity of soils to function, within the boundaries of natural and managed ecosystems, to maintain crop productivity, maintain water and air quality, support human welfare, and provide habitat for biodiversity (Doran and Zeiss, 2000; Gugino et al., 2009). Human impacts on soil health largely arise from the need to meet the growing food, fiber and fuel needs of the population. In recent decades significant efforts have been made to increase agricultural productivity through increased fertilization and application of pesticides, increased irrigation, land and crop management, and large-scale land conversion (Tilman et al., 2001).

Recently, awareness and concern have begun to emerge that agricultural intensification is putting tremendous pressure on the capacity of soils to maintain other functions that lead to large-scale

ecosystem degradation and long-term loss of productivity (Foley et al., 2005; Vitousek et al., 2009). For example, the conversion of natural ecosystems into agricultural land has caused huge environmental costs, including desertification, increased greenhouse gas emissions, decreased organic matter in the soil, loss of biodiversity, and changes in biogeochemical and hydrological cycles (Balmford et al., 2005). Modern agriculture thus faces major challenges not only in terms of ensuring global food security by increasing crop yields but also reducing environmental costs especially in the context of a changing environment and increasing competition for land, water and energy (Chen et al., 2014). Therefore, this study aims to examine the natural ecosystems represented by pine forests and agroecosystems to compare the abundance of soil microbial communities and also plant growth. The hypothesis are microbes in forest soils are higher than in agroecosystem soils because forest land is still virgin and there is no additional input from humans

Material and Methods

Site Properties

The experiment was conducted at the pine forest ecosystems Mount Halimun Salak National Park, sub district Pamijahan Bogor district, West Java -6.689177, 106.680534 and Cikabayan Experimental Station, Dramaga Campus of IPB University, Bogor district West Java -6.550665, 106.715212.

Material

The materials used were soil samples from pine forest ecosystems were taken from the Mount Halimun Salak National Park, Bogor, and soils from agroecosystems from the Cikabayan Experimental Station, Dramaga Campus of IPB, Bogor, sweet corn seed varieties of bonanza and peanut varieties of zebras, sterile aquades, Pikovskaya media, Martin jelly and TSA. The equipment used is equipment for analyzing soil properties in the field and laboratories, stationery, and a computer set.

Method

Time and Place of Implementation

The research activities were carried out from September to December 2019 in the Experimental Station and Education Laboratory, Plant Protection Department, Faculty of Agriculture, Dramaga Campus of IPB.

Planting Media Preparation

The planting media used in this study were soil samples with soil depth 0-20 cm latosol type soil, clay soil texture with pH 6, this soil belongs to the type of Mediterranean land with moderate sensitivity to erosion from pine forest ecosystems taken from the Mount Halimun Salak National Park, Bogor, and soil from agroecosystems latosol type soil with soil depth 0-20 cm, clay soil texture with pH 6 from the Cikabayan Experimental Station, Dramaga Campus of IPB, Bogor. Each soil sample is then prepared in a planting tub with a size of 38 x 30 x 15 cm.

Planting of planting material

The planting material used consists of sweet corn seeds of bonanza varieties and peanut varieties of zebra. Each tub is planted with 5 seeds. Then maintained until the age of 4 weeks with treatment in the form of watering and weeding.

Plant growth measurement

At the end of the study it was observed that plant growth included 1) the height of the canopy measured from the ground surface to the highest canopy, 2) the number of leaves, and 3) the length of the roots measured from the base of the stem to the tip of the root.

Population Observation and Enumeration of Soil Microbes

Each soil sample was taken from each treatment as much as 1 g and put in 10 mL of sterile aquades. Then it is diluted serially until dilution 10^{-4}. Soil suspensions at dilutions 10^{-3} and 10^{-4} were taken 0.1 mL and grown on Pikovskaya, Martin jelly, and TSA media by the scatter method. Microbial enumaration is done by calculating the total plate count based on BSN (2006) using the formula:

$$N = \frac{\sum c}{[(1 \times n1) + (0.1 \times n2]x(d)}$$

The items in the formula refer to:
N = Number of product colonies, expressed in CFU per mL
∑c = Number of colonies in all plates was counted
n1 = Number of plates in the first dilution is calculated
n2 = Number of plates in the second dilution is calculated
d = The first dilution is calculated

Data analysis

This study used a randomized block design with 3 replications. The data obtained were processed using SAS program version 9.1. The treatments that showed significant differences were further tested by Duncan's test at 5% level.

Results

Abundance of Soil Microbial Communities

Exploration results of soil microbial communities from agroecosystems and forests found bacteria, fungi, and phosphate solubilizing bacteria in each treatment (Table 1). However, the abundance of soil communities tends to be more common in soils from agroecosystems than in pine forest ecosystems. Meanwhile, soil from agroecosystems planted with peanuts showed the greatest abundance of soil microbes, namely bacteria of 2.74 x 105 CFU mL^{-1}, fungi of 1.86 x 104, fungi of CFU mL^{-1}, phosphate solubilizing bacteria of 2.74 x 105 CFU mL^{-1}. Meanwhile, the smallest abundance of microbes is shown by soil from pine forest ecosystem planted with peanuts, with a number of bacteria of 6.27 × 103 CFU mL^{-1}, fungi with too little amount to count, and phosphate solvent bacteria of 4.36 × 104 CFU mL^{-1}.

Table 1. Soil microbial populations in the treatment of agroecosystems and pine forests.

No	Treatments	Bacteria (CFU mL^{-1})	Fungi (CFU mL^{-1})	Phosphate Solvent Bacteria (CFU mL^{-1})
1	T_1E_1	9.04×10^4	TSUD	2.61×10^4
2	T_2E_1	2.74×10^5	1.86×10^4	2.74×10^5
3	T_1E_2	1.97×10^4	6.56×10^3	4.36×10^4
4	T_2E_2	6.27×10^3	TSUD	4.36×10^4

Remarks : T1E1 = corn plants on agroecosystem soils, T2E1 = peanut plants on agroecosystem soils, T1E2 = corn plants on exocytes of pine forests, T2E2 = peanut plants on soils of pine forest ecosystems.

Growth response of corn and peanut plants

Based on plant growth parameters, the best ecosystem for maize and long bean growth is agroecosystem compared to pine forest ecosystem (Table 2). The results of the analysis of variance showed that the two plants growing media from the agroecosystem had a significant influence on the height of the canopy compared to the pine forest ecosystem. The difference in height of the canopy between corn in the agroecosystem and forest ecosystem planting media was 30.75 cm, and the difference in the canopy between the peanuts in the agroecosystem and forest ecosystem planting media was 9.85 cm. Meanwhile, for the root length variation, in the long bean plant based on the analysis of variance, there was no real effect of the use of growing media from both ecosystems.

Table 2. Response of growth of corn and peanuts in planting media from different ecosystems

No	Treatments	Canopy Height	Root Length	Number of Leaves
1	T_1E_1	49.40a	44.76a	4.80c
2	T_2E_1	30.58b	36.87a	15.60a
3	T_1E_2	18.65c	6.93b	3.67c
4	T_2E_2	20.73c	44.33a	10.93b

Remarks : T1E1 = corn plants on agroecosystem soils, T2E1 = peanut plants on agroecosystem soils, T1E2 = corn plants on exocytes of pine forests, T2E2 = peanut plants on soils of pine forest ecosystems. The numbers in the same column followed by the same letter are not significantly different at the 5% test level (Duncan's test).

However, the corn planted in agroecosystem planting media showed a significant effect on increasing root length compared to pine forest ecosystem planting media by selecting root length increase of 37.83 cm. In the parameters of the number of leaves, the only real effect is on the planting of peanuts. The results of the analysis showed that the agroecosystem planting media showed a real effect on the number of peanut leaves compared to the pine forest ecosystem.

Discussion

Soil microbial communities show that their abundance is high in agroecosystems compared to forest ecosystems. Agroecosystem which is an artificial ecosystem occurs practices of soil management, crop rotation, periodic fertilization; and the application of pesticides resulting in temporal and spatial changes in the physical and chemical properties of soils in agricultural systems (Carbonetto et al., 2014). Such agroecosystems represent a rapidly fluctuating environment with highly variable resource gradients and create greater bio-physical and chemical heterogeneity compared to forest ecosystems, thus providing a variety of gaps for microbial growth (Trivedi et al. 2016). Microbial communities in natural systems

may be limited by the availability of nutrients and therefore the addition of fertilizers can allow colonization by new species from regional ponds (Crowther et al., 2014; Figuerola et al., 2015).

In this study, the composition of soil microbes found in abundance in agroecosystem soils correlated with the growth parameters of corn and peanuts in soil media from agro-systems. The presence of microbial communities on the soil has several roles including as biological fertilizer, providing phosphorus to be absorbed by plants, nitrogen fixation, siderophor production, and phytohormone production (Mehmood et al., 2018). The presence of microbes in the form of bacteria from the PGPR group in the soil not only rapidly colonizes the rhizosphere soils and enhances the absorption ability of plant nutrients but also is beneficial in suppressing disease through various mechanisms. One of the nutrients that plants need is phosphorus.

Phosphorus is an important nutrient taken from the soil by plants in the form of phosphate anions (Nautiyal et al., 2000). Because of its highly reactive nature, phosphate anions can be obtained by plants with cation precipitation (Al_3^+, Mg_2^+). The availability and unavailability of phosphorus for plants depends on the quality of the soil because phosphorus is very insoluble in the soil, therefore only a limited amount of phosphorus is available for plants (Yadav and Dadarwal, 1997). It has been documented by many researchers that Hydroxyapatite, di-calcium phosphate, rock phosphate and tricalcium phosphate are transformed into soluble forms by various species of beneficial bacteria present in the soil and these beneficial bacterial species are called phosphate solvent bacteria (PSB) (Chen et al., 2006; Rodríguez and Fraga, 1999). Various mechanisms are followed by PSB to dissolve phosphates that are not available including the release of enzymes and the production of certain acids (Greiner et al., 2001). PSB inoculation not only increases plant growth parameters but also significantly increases overall crop yields (Moura et al., 2001).

Conclusion

The results showed that agroecosystems are ecosystems that have an abundance of microbial communities that are the most good compared to forest ecosystems. This has an impact on good growth responses in agroecosystems compared to forest ecosystems. The limitation in this study are, this study has not been able to identify the type and function of microbes, but this study has implications that can determine the level of soil health through the observed abundance of microbes.

REFERENCES

Balmford, A., Bennun, L., Brink, B. T., Cooper, D., Cote, I. M., Crane, P. (2005). The convention on biological diversity's 2010 target. Science 307, 212–213. doi: 10.1126/science.1106281.

Badan Standar Nasional (BSN). 2006. Cara Uji Mikrobiologi- Bagian 3: Penentuan Angka Lempeng Total (ALT) pada Produk Perikanan. Badan Standar Nasional. Jakarta (Indonesian Language).

Carbonetto, B., Rascovan, N., Álvarez, R., Mentaberry, A., and Vázquez, M. P. (2014). Structure, composition and metagenomic profile of soil microbiomes associated to agricultural land use and tillage systems in Argentine Pampas. PLoS ONE 9:e99949. doi:10.1371/journal.pone.0099949

Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.A., Young, C.C., 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Applied soil ecology 34, 33-41.

Chen, X., Cui, Z., Fan, M., Vitousek, P., Zhao, M., Ma, W., et al. (2014). Producing more grain with lower environmental costs. Nature 514, 486–489. doi: 10.1038/nature13609.

Crowther, T. W., Maynard, D. S., Leff, J. W., Oldfield, E. E., McCulley, R. L., Fierer, N., et al. (2014). Predicting the responsiveness of soil biodiversity to deforestation: a cross-biome study. Glob. Change Biol. 20, 2983–2994. doi:10.1111/gcb.12565

Doran, J. W., and Zeiss, M. R. (2000). Soil health and sustainability: managing the biotic component of soil quality. Appl. Soil Ecol. 15, 3–11. doi: 10.1016/S0929-1393(00)00067-6.

Figuerola, E. L., Guerrero, L. D., Türkowsky, D., Wall, L. G., and Erijman, L. (2015). Crop monoculture rather than agriculture reduces the spatial turnover of soil bacterial communities at a regional scale. Environ. Microbiol. 17, 678–688. doi: 10.1111/1462-2920.12497

Foley, J. A., DeFries, R., Asner, G. P., Barford, C., Bonan, G., Carpenter, S. R., et al. (2005). Global consequences of land use. Science 309,570–574. doi: 10.1126/science.1111772

Greiner, R., Alminger, M.L., Carlsson, N.-G., 2001. Stereospecificity of myo-inositol hexakisphosphate dephosphorylation by a phytate-degrading enzyme of baker's yeast. Journal of agricultural and food chemistry 49, 2228-2233.

Gugino, B. K., Idowu, O. J., Schindelbeck, R. R., van Es, H. M., Wolfe, D. W., Moebius, B. N., et al. (2009). Cornell Soil Health Assessment Training Manual, 2nd Edn. Geneva: Cornell University.

Mehmood U, Inam-ul-Haq, Saeed M, Altaf A, Azam F. 2018. A brief review on plant growth promoting rhizobacteria (PGPR): a key role in plant growth promotion. Plant Protection 2(2):77-82.

Moura, D.S., Bergey, D.R., Ryan, C.A., 2001. Characterization and localization of a woundinducible type I serine-carboxypeptidase from leaves of tomato plants (Lycopersicon esculentum Mill.). Planta 212, 222-230.

Nautiyal, C.S., Bhadauria, S., Kumar, P., Lal, H., Mondal, R., Verma, D., 2000. Stress induced phosphate solubilization in bacteria isolated from alkaline soils. FEMS Microbiology Letters 182, 291-296

Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., et al. (2001). Forecasting agriculturally driven global environmental change. Science 292, 281–284. doi: 10.1126/science.1057544.

Rodríguez, H., Fraga, R., 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology advances 17, 319-339.

Trivedi P, Delgado0Baqruerizo M, Anderson IC, Singh BK. 2016. Response of soil properties and microbial communities to agriculture: implications for primary productivity and soil health indicators. Frontiers in Plant Science 7: 1-13.

Vitousek, P. M., Naylor, R., Crews, T., David, M. B., Drinkwater, L. E., Holland, E., et al. (2009). Nutrient imbalances in agricultural development. Science 324:1519. doi: 10.1126/science.1170261

Yadav, K.S., Dadarwal, K.R., 1997. Phosphate slubilization and mobilization through soil microorganisms, Biotechnological approaches in soil microorganisms for sustainable crop production, pp. 293-308.

Submitted: 01.04.2020 Accepted: 16.06.2020

DOI: 10.3195/ejejfs.712992

Eurasian Journal of Forest Science

2020 8(2): 129-139

http://dergipark.gov.tr/ejejfs

An evaluation of ten estimators for fitting two-parameter weibull function to Nigerian forest stands

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Abstract
The quality fit produce by distribution function such as the Weibull depends to an extent the type of estimator used to derive its parameters. Inappropriate choice of estimator could affect management decision. Though several estimators have been developed for the Weibull function, their application to forestry have been relatively few. Therefore, this study evaluated ten estimators of the Weibull parameters using tree diameter data from five production forest plantations in Nigeria. The estimators were generalized least type I and type II, L-moment, moments, maximum likelihood, percentiles, rank correlation, least squares, U-statistics and weighted least squares. The quality of fits of the Weibull function were evaluated with Kolmogorov-Smirnov, Anderson-Darling, Cramer-von Mises, Akaike information criterion and Bayesian information criterion. Relative rank sum from the evaluation statistics of the methods was analysed using One-way analysis of variance. The results showed that weighted least square had the smallest statistics and relative rank, but not significantly different from L-moment, moments and maximum likelihood ($p > 0.05$). The performances of least squares, generalized least type I and type II, percentiles and U-statistics were relatively poor. Thus, either the weighted least squares, moments-based or MLE could be used for the Weibull function in the diameter distribution of forest stands in Nigeria.
Keywords: Moment-based methods; weighted least squares, maximum likelihood; Eucalyptus camaldulensis, Gmelina arborea; Tectona grandis

Introduction

The roles of diameter distribution in forest management and planning cannot be overemphasized. It provides information on product specification, value and volume production of forest stand (Gorgoso et al. 2012). It is also a useful tool for planning silvicultural treatments, determining age distribution and stand stability (Carretero and Álvarez 2013). Yield estimates are often derived from diameter distribution; and a common practice is to apply statistical function such as the Weibull to characterise the number of trees per ha into diameter classes. Class volume is then obtained by substituting the diameter class midpoint and mean height mean derived from height-diameter model into appropriate volume function (Burkhart and Tomé, 2012).

The accuracy and precision in characterising tree diameter using statistical function depends on the estimator used to derive the distribution (Zhang et al. 2003, Ogana and Gorgoso-Varela 2015). The Weibull function is the commonly used statistical function to characterise diameter distribution because of its relative flexibility, simplicity, ease of computing relative frequency of trees in diameter class and

has different parameter estimators (Rubin et al. 2006, Carretero and Álvarez 2013). Several estimators including generalized least type I and type II, L-moment, logarithmic moment, maximum likelihood estimator, method of moments, percentiles, rank correlation, least squares, U-statistics and weighted least squares, etc. have been developed for the Weibull function (Sadani et al. 2019). However, not all the methods have been evaluated in forestry, especially in Nigeria.

In Nigeria, most of the production forest plantations are predominantly of *Eucalyptus camaldulensis* Dehn, *Gmelina arborea* Roxb and *Tectona grandis* Linn. Large investments in the plantations have been made in the country to meet the demand of wood and wood products (Ogana, 2019). The diameter distributions of these stands have been described using the Weibull function fitted with maximum likelihood and percentiles estimators (e.g., Ajayi 2013, Ekpa et al. 2014, Saka 2014, Ogana et al. 2020, Ogana and Ekpa 2020). Other studies that have applied the moments, least squares, percentiles, maximum likelihood methods for the Weibull function outside Nigeria include Poudel and Cao (2013), Gorgoso-Varela and Rojo-Alboreca (2014), Sun et al. (2019), etc. Inappropriate choice of estimator to derive the diameter distribution could affect the quality of fit and overall management decision. Therefore, the aim of this study was to evaluate different estimators of the Weibull distribution to characterise tree diameters in five production forest plantations in Nigeria.

Methodology

Data

The data for this study were collected from five different production forest plantations of *Gmelina arborea*, *Tectona grandis* and *Eucalyptus camaldulensis* in Nigeria. Two plantations of *G. arborea* and *T. grandis* are situated in Omo Forest Reserve (FR), Ogun State. The reserve lies between Latitude 6°35′ – 7°05′ N and Longitude 4°10′ – 4°19′ E. The second *G. arborea* and *T. grandis* plantations are in Oluwa FR and Gambari FR, respectively. Oluwa FR is in Ondo State of Nigeria and lies between Latitude 6°55′ – 7°20′ N and Longitude 3°45′ – 4°32′ E (Onyekwelu 2001). While Gambari FR is in Oyo State of Nigeria and lies between Latitude 7°21′ – 7°55′N and Longitude 3°53′ – 3°9′E (Adedeji et al. 2015). The *E. camaldulensis* plantation is in Afaka FR situated between Latitude 10.58° – 10.68°N and Longitude 7.35° – 7.37°E of Kaduna State, Nigeria. Diameter at breast height (1.3m above the ground, dbh in cm) data of 1,052, 1,079, 1,370, 1,916 and 3,988 trees from *G. arborea* in Oluwa FR, *G. arborea* in Omo FR, *T. grandis* in Gambari FR, *T. grandis* in Omo FR and *E. camaldulensis*, respectively were available for this study. The descriptive statistics of the data are presented in Table 1.

Table 1: Descriptive statistics of the data set

Species	Mean	Max	Min	SD	N trees
G. arborea in Oluwa FR	23.0	54.5	3.0	10.4	1052
G. arborea in Omo FR	19.5	49.6	4.6	8.9	1079
T. grands in Gambari FR	19.6	39.2	5.8	6.2	1370
T. grands in Omo FR	17.9	37.9	6.0	5.3	1916
E. camaldulensis	10.5	47.4	2.0	6.3	3988
All species	15.8	54.5	2.0	8.4	9405

Two-Parameter Weibull Function

The probability density function (pdf) and cumulative distribution function (cdf) of the commonly used two-parameter Weibull function (Weibull 1951) are expressed as:

$$f(x) = \frac{c}{b}\left(\frac{x}{b}\right)^{c-1} exp\left(-\left(\frac{x}{b}\right)^{c}\right) \quad \text{Eq. [1]}$$

$$F(x) = 1 - exp\left(-\left(\frac{x}{b}\right)^{c}\right) \quad \text{Eq. [2]}$$

Where: $f(x)$ = pdf; $F(x)$ = cdf; c = shape parameter ($c > 0$); b = scale parameter ($b > 0$).

Estimation methods

Ten estimation methods of the Weibull function were evaluated in this study. These include: generalized least type I (GLS1) and type II (GLS2), L-moment, maximum likelihood estimator (MLE), method of moments, percentiles, rank correlation (Rank), least squares (LS), U-statistics (U-stat) and weighted least squares (WLS). Some these estimators are presented in Appendix. Detailed information including the derivations on the various estimators of the Weibull distribution can be found in Teimouri et al. (2013) and Sadani et al. (2019). Each method was used to fit the Weibull distribution to the diameter data from the five forest plantations and for all species combined. The 'ForestFit' package (Teimouri, 2020) implemented in R (R Core Team, 2017) was used for the analysis.

Evaluation statistics

Five evaluation statistics were used to assess the ten estimation methods of the Weibull distribution. For each estimation method, the Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), Anderson-Darling (AD), Cramer-von Mises (CvM) and Kolmogorov-Smirnov (KS) statistics were computed. The smaller the statistics are, the better the method.

Akaike Information Criterion (AIC):

$$AIC = 2\left(\sum_{i=1}^{n}\left[ln(b) - ln(c) + (1-c)ln\left(\frac{x_i}{b}\right) + \left(\frac{x_i}{b}\right)^{c}\right]\right) + 2p \quad Eq.[3]$$

Bayesian Information Criterion (BIC):

$$BIC = 2\left(\sum_{i=1}^{n}\left[ln(b) - ln(c) + (1-c)ln\left(\frac{x_i}{b}\right) + \left(\frac{x_i}{b}\right)^{c}\right]\right) + pln(n) \quad Eq.[4]$$

Kolmogorov-Smirnov (KS) *statistics*:

$$KS = max\left\{max_{1\le i\le n_i}\left[F_n(x_i) - F_0(x_j)\right], max_{1\le i\le n_i}\left[F_o(x_j) - F_n(x_{i-1})\right]\right\} \quad \text{Eq. [5]}$$

Anderson-Darling (AD) statistic:

$$AD = -n_i - \sum_{j=1}^{n_i}(2j-1)\left[ln\left(F_o(x_j)\right) + ln\left(1 - F_n(x_{i-1})\right)\right]/n_i \quad Eq.[6]$$

Cramer-von Mises (W^2) statistic:

$$W^2 = \sum_{i=1}^{n}\left\{\hat{F}(x_i) - \frac{(i-0.5)}{n}\right\}^2 + \frac{1}{12n} \quad Eq.[7]$$

Where $F(x_i)$ is the observed cumulative frequency distribution for x_i (i ranged from 1 to n); $F_0(x_i)$ is the theoretical cumulative frequency distribution; b and c are the scale and shape parameters of the Weibull distribution; p is the number of parameter; ln is the natural logarithm

Ranking of Methods

Relative rank introduced by Poudel and Cao (2013) was used in this study. It is given by:

$$R_i = 1 + \frac{(m-1)(S_i - S_{min})}{S_{max} - S_{min}} \quad \text{Eq. [8]}$$

where R_i = relative rank of method i (i = 1, 2, …, m); m = number of methods evaluated (10 estimation methods), S_i = evaluation statistics value of method i; S_{max} and S_{min} = maximum and minimum values of S_i, respectively. The relative rank is real number between 1 (best) and 10 (worst). For each estimation method, the relative ranks were summed across the five evaluation statistics, analysed and plotted. One-way analysis of variance (ANOVA) was then used to analysed the relative rank sum of the ten methods at 5% level of significant. Methods that were significantly different were separated with Duncan multiple range test (DMRT).

Results

The estimates of the parameters of the Weibull distribution from the ten fitting methods are presented in Table 2. The estimated Weibull shape and scale parameters from the ten methods ranged from 2.1418 to 2.4344 and 25.8840 to 26.50, respectively in the *G. arborea* stand in Oluwa FR. For *G. arborea* stand in Omo FR, the shape and scale parameters, respectively, ranged from 1.9808 to 2.5347 and 21.8721 to 22.3649. The values of the parameters ranged from 3.4436 to 3.7408 and 21.6373 to 21.8576, and 3.5456 to 4.0992 and 19.40 to 20.0427, respectively in the *T. grandis* stands in Gambari FR and Omo FR, respectively. In the case of the *E. camaldulensis* stand, the shape and scale parameters, respectively, ranged from 1.6306 to 2.2042 and 11.4237 to 12.0086. For the pooled data i.e., all species, the estimates of the shape and scale parameters ranged from 1.8245 to 2.1318 and 17.6678 to 17.8696, respectively. There was lack of fit for the Weibull distribution fitted with weighted least squares (WLS) to the pooled data.

The evaluation statistics of the ten methods for fitting the Weibull distribution by species and all species showed that the WLS and L-moment had in most cases smallest values (Table 3). However, L-moment, moment and Rank correlation (Rank) had the best evaluation statistics for all species combined. Larger AD, CvM and KS were observed in the least squares (LS), generalized least squares both type 1 (GLS1) and type 2 (GLS2) for most of the stands.

The plot of the relative rank sum (mean ± standard errors) for the ten estimation methods are presented in Figure 1. A boundary line was used to demarcate the methods with relative rank sum < 20 from those > 20. The L-moments, MLE, moment and WLS methods were within the lower region of 0 – 20. The method of WLS had the smallest value. Though Rank correlation had average value that was < 20, its upper limit was slightly beyond the boundary line. The GLS1, GLS2, LS, Percentiles and U-statistics (U-stat) methods were those above the boundary line. Further result from the analysis of variance (ANOVA) showed a significant difference in the relative rank sum of the ten methods. Methods within the same region were not significantly different; whereas methods between regions were significant (Figure 1). That is, no significant difference between L-moments, MLE, moment, Rank and WLS methods; but they differed significantly from those in the upper region, i.e., GLS1, GLS2, LS, Percentiles and U-stat methods.

The graph of the observed and fitted Weibull function with three best methods in the individual stand and all species combined are presented in Figure 2a to f. In all cases, the fitted Weibull function approximated the observed diameter distribution of the stands. Only in diameter class of 10 cm in the *E. camaldulensis* stand that the Weibull function underestimated the relative frequencies of trees.

Table 2: Estimated parameters from the fitting methods in the different forest stands

Methods	***G. arborea* in Oluwa FR**		***G. arborea* in Omo FR**		***T. grandis* in Gambari FR**		***T. grandis* in Omo FR**		***E. camaldulensis***		**All species**	
	Shape	**Scale**	**Shape**	**Scale**	**Shape**	**Scale**	**Shape**	**Scale**	**Shape**	**Scale**	**Shape**	**Scale**
Greg1	2.4344	25.9581	2.5347	21.9922	3.6167	21.8199	3.9603	19.9357	2.1847	12.0081	2.1318	17.8682
Greg2	2.4005	25.9667	2.5009	21.9986	3.5805	21.8227	3.9341	19.9368	2.1777	12.0086	2.1269	17.8696
L-moment	2.3308	25.9835	2.3064	21.9751	3.5413	21.7553	3.7872	19.8221	1.8560	11.8403	1.9562	17.7859
MLE	2.3957	26.0239	2.3729	22.0366	3.4436	21.7791	3.5456	19.8623	1.8145	11.9133	1.9813	17.8520
Moment	2.3640	25.9781	2.3320	21.9719	3.5128	21.7647	3.7346	19.8375	1.7267	11.7973	1.9472	17.7839
Percentiles	2.1418	26.5000	1.9808	22.3649	3.7408	21.6373	3.8542	19.4000	1.6306	11.4592	1.8245	17.7000
Rank	2.3294	25.8967	2.3051	21.9260	3.5398	21.8576	3.7860	20.0427	1.8557	11.9879	1.9561	17.7998
LS	2.4318	25.8840	2.4907	21.8721	3.6816	21.6825	4.0992	19.6996	2.1836	11.7236	2.1278	17.6678
U-stat	2.3792	26.0340	2.4373	21.9945	3.6348	21.7324	4.0082	19.7663	2.2042	11.6967	2.0771	17.7867
WLS	2.2765	26.0916	2.2185	21.9719	3.5336	21.6450	3.6885	19.6116	1.8468	11.4237		

Table 3: Evaluation statistics of the different methods in the forest stands

	***G. arborea* in Oluwa FR**					***G. arborea* in Omo FR**				
Methods	**AIC**	**BIC**	**AD**	**CvM**	**KS**	**AIC**	**BIC**	**AD**	**CvM**	**KS**
GLS1	7839	7849	2.6348	0.4243	0.0405	7689	7699	9.1294	1.3084	0.0738
GLS2	7838	7848	2.1462	0.3403	0.0382	7686	7696	8.0777	1.1563	0.0703
L-moment	7839	7849	1.5073	0.2088	0.0336	7681	7691	4.3173	0.5020	0.0480
MLE	7838	7848	2.0551	0.3267	0.0362	7680	7690	5.1622	0.6833	0.0570
Moment	7838	7848	1.7483	0.2642	0.0357	7680	7690	4.5788	0.5622	0.0509
Percentiles	7864	7874	2.7141	0.1839	0.0319	7741	7751	7.9843	0.5831	0.0642
Rank	7839	7849	1.5849	0.2217	0.0361	7681	7691	4.3238	0.5008	0.0468
LS	7839	7849	2.6767	0.4258	0.0426	7686	7696	7.8353	1.0976	0.0665
U-stat	7838	7848	1.8668	0.2908	0.0349	7681	7691	6.4073	0.8962	0.0633
WLS	7843	7853	1.3275	0.1386	0.0272	7688	7698	3.9870	0.3621	0.0485
	***T. grandis* in Gambari FR**					***T. grandis* in Omo FR**				
GLS1	8882	8892	2.2988	0.3040	0.0333	11943	11954	12.6178	2.1889	0.0697
GLS2	8879	8890	2.1600	0.2933	0.0320	11936	11947	12.0843	2.0950	0.0683
L-moment	8878	8888	1.7886	0.2166	0.0288	11912	11923	8.1486	1.1846	0.0554
MLE	8875	8886	2.0496	0.2794	0.0348	11892	11903	8.6319	1.1061	0.0498
Moment	8877	8887	1.8190	0.2307	0.0307	11904	11915	7.9756	1.1456	0.0546
Percentiles	8898	8909	2.7256	0.2082	0.0275	11955	11966	8.6557	0.6535	0.0388
Rank	8877	8888	2.2677	0.3333	0.0339	11908	11919	12.0068	2.1737	0.0698
LS	8890	8900	2.2199	0.1939	0.0279	12001	12012	12.8293	1.7148	0.0627
U-stat	8884	8895	2.0201	0.2111	0.0290	11965	11976	11.0646	1.6048	0.0621
WLS	8879	8889	1.6047	0.1567	0.0284	11907	11918	6.5598	0.6024	0.0383
	E. camaldulensis					**All species**				
GLS1	25147	25160	113.1166	20.0076	0.1395	65458	65472	46.9262	5.6606	0.0575
GLS2	25134	25147	111.5718	19.7491	0.1387	65451	65465	45.7733	5.5162	0.0569
L-moment	24818	24830	64.8736	9.6506	0.0923	65362	65376	22.0706	1.9715	0.0341
MLE	24812	24824	66.4468	9.8241	0.0909	65359	65373	23.8078	2.3434	0.0380
Moment	24830	24843	67.1428	8.8588	0.1009	65364	65378	21.8793	1.9121	0.0348
Percentiles	24895	24907	77.3515	9.3111	0.1221	65466	65480	30.3126	2.5521	0.0459
Rank	24815	24828	69.3386	10.9219	0.0991	65362	65376	22.1679	1.9914	0.0340
LS	25202	25214	100.8920	16.2469	0.1257	65473	65487	44.1083	4.8872	0.0533
U-stat	25252	25265	104.6804	16.6574	0.1267	65403	65418	34.3245	3.8464	0.0492
WLS	24845	24857	61.0646	7.5250	0.0918	nf	nf	nf	nf	nf

nf = no fit

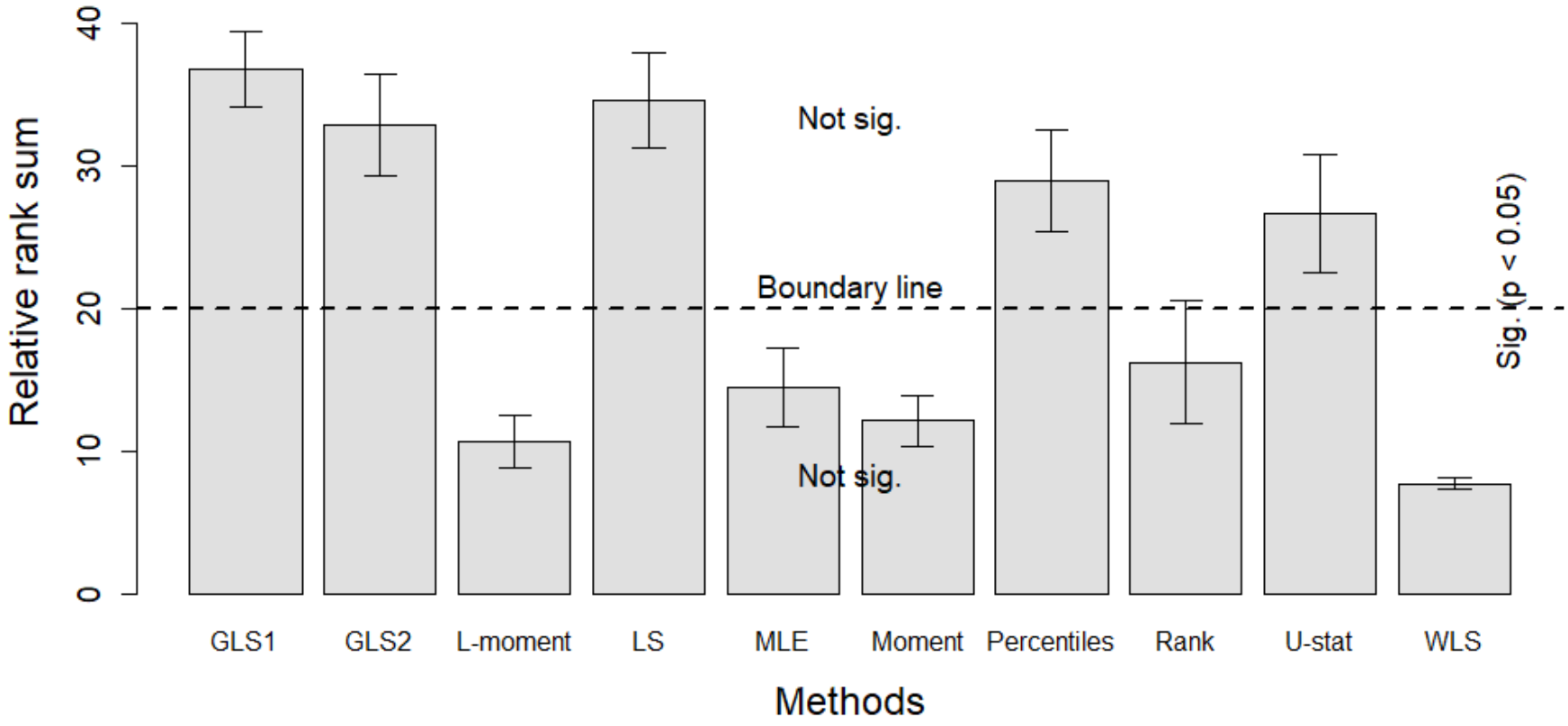

Figure 1: Bar graph of relative rank sum of ten Weibull parameter estimation methods. Methods in the same region are not significant, while between regions are significant.

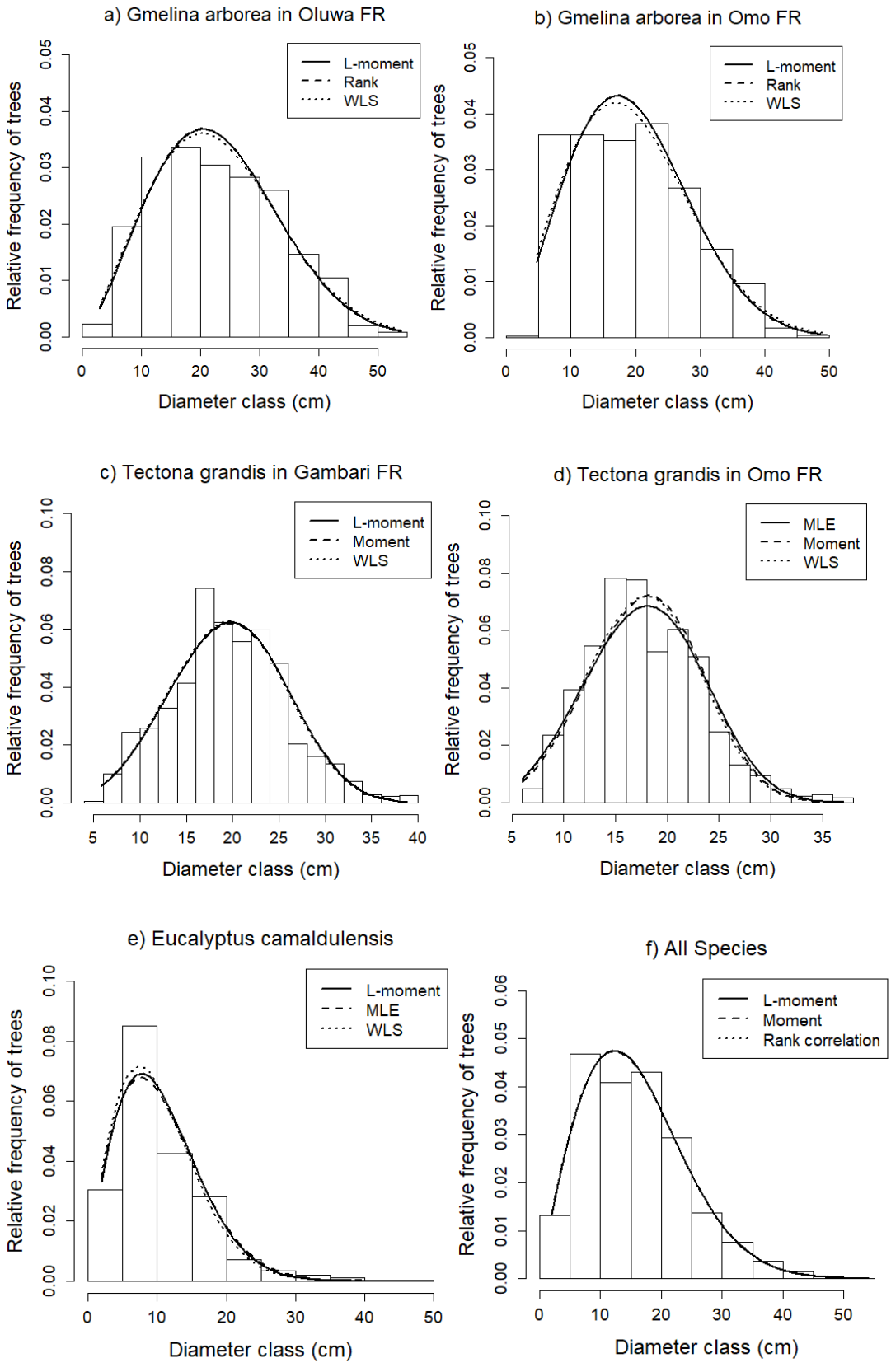

Figure 2: Observed and fitted Weibull function with three best methods across the stands.

Discussion

Ten methods have been evaluated for fitting the two-parameter Weibull function to five forest stands in Nigeria. There was little variation in the estimates of the shape and scale parameters from the ten methods. However, the estimate of the shape parameter from Percentiles tend to have lower value for relatively skewed stands e.g., *G. arborea* in Omo FR, *E. camaldulensis* and for all species combine. Much of the variabilities in relative rank sum of the ten methods came from these skewed stands. Whereas only little variations exist in the methods for stands with gaussian shape (symmetric) such as the *T. grandis* stands in Gambari FR and Omo FR. This shows that some of the estimators (e.g., GLS1, GLS2, LS, Percentiles and U-stat) of Weibull parameters are most appropriate for stands with gaussian structure (symmetric). Other methods such as L-moment, MLE, Moment, Rank and WLS estimators can be used to fit Weibull function to both symmetric and asymmetric stands.

Heavily skewed or asymmetric structure for plantations could be due to thinning, poor forest management, illegal exploitation and other forms of disturbances. The *E. camaldulensis* and *G. arborea* stands have been previously reported to have suffered from severe disturbance – both anthropogenic and wind damage (Ogana et al. 2017, 2018). Though silvicultural practices such as selection cutting can be used to convert even-aged stand to uneven-aged (reverse J-shaped), it is not a common practice in Nigeria.

Among the suitable methods identified for fitting the Weibull function, only the MLE and moments have been frequently used to model diameter distribution of forest stands especially in Nigeria (e.g. Ajayi 2013, Ige et al. 2013, Ogana et al. 2015, Ogana and Gorgoso-Varela 2015). In Spain, Carretero and Álvarez (2013) found MLE and moment to be less efficient compared to LS for fitting two-parameter Weibull to Cork oak stands. Gorgoso et al. (2007) reported similar result for beech stand in northwest Spain. Recently, Gorgoso-Varela et al. (2020) observed smallest KS value for MLE in three species - *E. globulus* Labill, *Pinus radiata* D. Don. (temperate forest) and *G. arborea* (tropical forest). Though numerically the L-moment and WLS methods had the best results in all the stands, their relative ranks are not significantly different from MLE and moments. The application of L-moment and WLS to fit the Weibull function has been limited in forestry.

One important factor to consider in the selection of an estimator is relative simplicity (i.e., ease of estimation) without compromising the quality of fits. The estimation procedures of the five suitable methods (estimators below the boundary line) vary in complexity. The moment-based estimators are handier compared to MLE and WLS. In consequence, when complex estimators do not outperform a simpler alternative, the simpler method should be selected (Gorgoso-Varela et al. 2019, 2020).

Conclusion

This study has evaluated the performance of ten estimators for fitting the Weibull function to some production stands in Nigeria. The quality of fit produced by the Weibull function varies with the different estimators. While some estimators such as the least squared, generalized least squared type 1 and 2, percentiles and the U-statistics are more appropriate for stands with gaussian structure; other estimators - the moment-based, MLE, rank and weighted least square can be used to fit Weibull function to stands with either symmetric or asymmetric structure.

References

Ajayi, S. (2013). Diameter Distribution for *Gmelina arborea* (Roxb) plantation in Ukpon River Forest Reserve, Cross River state, Nigeria. *AFREV STECH (An International Journal of Science and Technology*). 2(1): 64-82, ISSN 2225-8612 (print); ISSN 2227-5444 (online)- (www.afrrevjo.net/stech; www.arronet.info.)

Adedeji, O.H., Tope-Ajayi, O.O. and Abegunde, O.L. (2015). Assessing and predicting changes in the status of Gambari Forest Reserve, Nigeria using remote sensing and GIS techniques. *Journal of Geographic Information System* 7: 301-318

Burkhart, H.E. and Tomé, M. (2012). *Modeling Forest Trees and Stands* (2nd Ed). Springer Dordrecht Heidelberg New York, 271p.

Carretero, A.C. and Álvarez E.T. (2013). Modelling diameter distributions of *Quercus suber* L. stands in "Los Alcornocales" Natural Park (Cádiz-Málaga, Spain) by using the two parameter Weibull functions. *Forest Systems* 22(1): 15-24.

Ekpa, N.E, Akindele, S.O. and Udofia, S.I. (2014). Gmelina arborea Roxb. Graded stands with the Weibull distribution Function in Oluwa Forest Reserve, Nigeria. *International Journal of Agroforestry and Silviculture* 1(9)110-113pp.

Gorgoso-Varela, J.J., Ogana, F.N. and Ige, P.O. (2020). A comparison between derivative and numerical optimization methods used for diameter distribution estimation. *Scandinavian Journal of Forest Research* 35(3-4): 156-164.

Gorgoso-Varela, J.J., Ogana, F.N. and Alonso Ponce, R. (2019). Evaluation of direct and indirect methods for modelling the joint distribution of tree diameter and height data with the bivariate Johnson's SBB function of forest stands. *Forest Systems* 28(1) e004.

Gorgoso-Varela, J.J. and Rojo-Alboreca, A. (2014). Short communication: A comparison of estimation methods for fitting Weibull and Johnson's S_B functions to pedunculate oak (*Quercus robur*) and birch (*Betula pubescens*) stands in northwest Spain. *Forest Systems* 23(3): 500-505

Gorgoso, J.J., Rojo, A., Camara-Obregon, A. and Dieguez-Aranda, U. (2012). A comparison of estimation methods for fitting Weibull, Johnson's S_B and beta functions to *Pinus pinaster, Pinus radiata* and *Pinus sylvestris* stands in northwest Spain. *Forest Systems* 21(3): 446-459

Gorgoso, J.J., Alvárez-González, J.G., Rojo, A. and Grandas-arias, J.A. (2007). Modelling diameter distributions of *Betula alba* L. stands in northwest Spain with the two parameter Weibull function. *Investigación Agrarian: Sistemas y Recursos Forestales* 16(2): 113-123

Ige, P.O, Akinyemi, G.O. and Abi, E.A. (2013). Diameter Distribution Models for Tropical Natural Forest trees in Onigambari Forest Reserve. *Journal of Natural Sciences Research*, 3(12): 14 – 22.

Ogana, F.N. and Ekpa, N.E. (2020). Modelling the non-spatial structure of Gmelina arborea Roxb stands in Oluwa Forest Reserve, Nigeria. *Forestist* (In press)

Ogana, F.N., Chukwu, O. and Ajayi, S. (2020). Tree size distribution modelling: moving from complexity to finite mixture. *Journal of Forest and Environmental Science* 36(1): 7-16

Ogana, F.N. (2019). Bivariate modelling of diameter and height distributions of Eucalyptus plantations in Afaka forest reserves, Nigeria. [Postgraduate Thesis] University of Ibadan, Ibadan, Nigeria.

Ogana, F.N., Osho, J.S.A. and Gorgoso-Varela, J.J. (2018). Application of extreme value distribution for assigning optimum fractions to distributions with boundary parameters: an eucalyptus plantations case study. *Siberian Journal of Forest Sciences* 4: 39-48.

Ogana, F.N., Itam, E.S. and Osho, J.S.A. (2017). Modelling diameter distributions of *Gmelina arborea* plantation in Omo Forest Reserve, Nigeria with Johnson's S_B. *Journal of Sustainable Forestry*, 36(2): 123-133.

Ogana, F.N. and Gorgoso-Varela, J.J. (2015). Comparison of estimation methods for fitting Weibull distribution to the natural stand of Oluwa Forest Reserve, Ondo State, Nigeria. *Journal of Research in Forestry, Wildlife and Environment*, 7(2): 81-90.

Ogana, F.N., Osho, J.S.A. and Gorgoso-Varela, J.J. (2015). Comparison of beta, gamma and Weibull distributions for characterising tree diameter in Oluwa Forest Reserve, Ondo State, Nigeria. *Journal of Natural Sciences Research* 5(4): 28-36.

Onyekwelu, J.C. (2001). Growth Characteristic and management scenarios for plantation grown *Gmelina arborea* and *Nauclea diderichii* in south-western Nigeria. Ph.D. thesis; Hieronymus Publishers, Munich; ISBN 3-89791-235-X

Poudel, K.P. and Cao, Q.V. (2013). Evaluation of methods to predict Weibull parameters for characterising diameter distributions. *Forest Science* 59(2): 243-252.

R Core Team. (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. (Visited on date: 30 /06/ 2017).

Rubin, B.D., Manion, P.D. and Faber-langendoen, D. (2006). Diameter distributions and structural sustainability in forests. *Forest Ecology and Management* 222(1-3): 427-438

Sadani, S., Abdollahnezhad, K., Teimouri, M. and Ranjbar, V. (2019). A new estimator for Weibull distribution parameters: comprehensive comparative study for Weibull distribution. *Statistics Computation* arXiv:1902.05658

Saka, M.G. (2014). Growth and yield models for sustainable management of plantation-grown Eucalyptus species in Afaka Forest Reserve, Kaduna State, Nigeria. [Postgraduate Thesis] University of Ibadan, Ibadan, Nigeria.

Sun, S., Cao, Q.V. and Cao, T. (2019). Characterizing diameter distributions for uneven-aged pine-oak mixed forests in the Qinling Mountains of China. *Forests* 10: 596, doi:10.3390/f10070596

Teimouri, M. (2020). ForestFit: Statistical modelling for plant size distributions. R package version 0.5.7. https://CRAN.R-project.org/package=ForestFit. Accessed 15 March 2020.

Teimouri, M., Hoseini, S.M. and Nadarajah, S. (2013). Comparison of estimation methods for the Weibull distribution. *Statistics*, 47:93-109.

Weibull, W. (1951). A statistical distribution function of wide applicability. *Journal of Appliced Mechanics*, 18: 293-297.

Zhang, L., Kevin, C.P. and Chuanmin, L. (2003). A comparison of estimation methods for fitting Weibull and Johnson's S_B distributions to mixed spruce-fir stands in north-eastern North America. *Canadian Journal of Forest Research.* 33: 1340-1347.

Submitted: 01.04.2020 Accepted: 14.06.2020

DOI: 10.3195/ejejfs.696768

Eurasian Journal of Forest Science

2020 8(2): 140-151

http://dergipark.gov.tr/ejejfs

DNA barcoding, phytochemical screening and antimicrobial activity of *Rhododendron arboreum*, a high altitudinal medicinal plant from Nepal

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Abstract

DNA barcoding has been proposed as a powerful taxonomic tool for species identification genetically. The Consortium for the Barcode of Life (CBOL) Plant Working Group has recommended the combination of rbcL & matK as the core plant barcode. Rhododendron of Nepal shows genetic diversity due to which 31 different species were reported till date, found in Nepal based on morphological character only. For the genetic identification first time, we collected different samples from the central and western parts of Nepal and also based on its phytochemical too. Cold extraction was performed using methanol as solvents. The crude extracts were tested for the presence of phytochemicals such as alkaloids, Saponin, glycosides, tannins, flavonoids, and Coumarins. The extract was also used for antimicrobial assay into five different pathogenic micro-organisms. MIC was done for calculating the minimum effective concentration of the extract. Then TLC was done to separate the compound. The methanol extract was analyzed for core secondary metabolites by HPLC-QTOF-MS and found more than 20 important medicinal compounds. DPPH assay was also done with crude extracts for identifying antioxidant potency of the extracts. Among samples compared, Gulmi and Palpa showed the best result for antioxidant assay with minimum IC50. Similarly, phenolic content was also checked and samples from Palpa showed high phenolic content. Finally with the rbcL and MatK gene loci was amplified and sequenced and based on alignment with NCBI-BLAST databased it show the 99.98% similarly for the *R.arboreum*. The final contig was submitted to NCBI and get the accession number LC456605.1 and were submitted to the BOLD database for the final authentication and succeed for the first time to publish DNA barcode of Nepalese plants.

Keywords: DNA Barcode, rbcL +matK, Antimicrobial, Antioxidant, and DPPH.

Introduction

Rhododendron is classified as the earliest of the flowering plants. It has been in existence for 800 million years ago (Vikas Kumar, 2019) .The first rhododendron was observed in the temperate zone of northern hemisphere and the fossil of oldest rhododendron was found in the southern China, in a place where three Chinese province of Yunnan, Sichuan and Tibet meet (Wang et al., 2014) the area is situated around 1000 kilometers of Nepal). The first rhododendron was brought into Britain in 1656 from the European Alps, where the plant was classified and named as Rhododendron hirsutum. (Liu et al., 2012) Correspondingly, after Linnaeus introduced the classification of plants under Species Plantarum, genus Rhododendron was included for the first time. However,

at that time, only five species of Rhododendron were known. Coming to this time, 1024 species of rhododendron has been found (Meijón et al., 2010)

Nepal can be classified into several climatic conditions because of varying range of altitude i.e. from 67m in the lowly plain to 8848 m on the top of Mount Everest. It is known that temperature decreases by 6^0C for every 1000 m rise in temperature, Nepal foster the growth of several vegetation that include various species (Liu et al., 2012) of Rhododendron is also termed as "Laliguras" in Nepal and is the national flower of Nepal.

Nowadays, this taxonomic system is generally accepted by *Rhododendron* specialists. (COX PA, 1997) However, the *Rhododendron* genus still has some problems at various systematic classification levels and there is no simple or universal manner to discriminate the various species within the genus. Rhododendron arboreum is a rapidly evolutionary genus within the angiosperms in recent years with many closely related species and there are many artificial and natural hybrids.(Kress et al. 2005)

The DNA barcoding, based on a short DNA sequence to identify species (Hebert et al. 2003) has been proposed as a rapid, accurate, and convenient taxonomic tool.(Hebert et al. 2003) The Consortium for the Barcode of Life (CBOL) Plant Working Group recommended the *rbcL + matK* combination as a barcode sequence in the plant kingdom, and they also suggested that ITS (ITS2) and psbA-trnH were good candidates for plant DNA barcoding, because of their fast evolution rates. (Kress et al., 2005) One of the problems for plant DNA barcoding was that the previous studies were mainly carried out on a large scale and rarely on a specific genus, with many closely related species, so some studies suggested that species identification using standard DNA sequences should be carried out within a narrow taxon (such as the genus) (Li, Tong, & Xing, 2016). One of the challenges for any DNA barcode is its utility in discriminating closely related species.(Newmaster et al. 2008; Wong et al. 2013). One of the challenges for any DNA barcode was its ability in discriminating closely related species (i.e., sister-species).(Liu et al., 2012; Newmaster et al., 2008).

The stem and roots of Rhododendron arboreum have medicinal importance. They have been used to cure illness. Even though, there are some cases but there is substantial evidence to support.

Rhododendron arboreum is also a source of traditional medicine in the Himalayas. One method of preparation is making a powder from the flower which is mixed with the starch of the boiling rice and given to the patient suffered from dysentery. Another remedy is to make a paste from the leaves and apply it to the forehead in the treatment of headaches. It is also used to treat skin diseases. An extract of the bark is used in the treatment of coughs, diarrhea and dysentery. In Ayurvedic medicine rhododendron plants are used to treat jaundice, diabetes, piles, enlargement of the spleen, liver disorder and worms. According to common folklore in Nepal a sip of the juice of the Lali Guras (Garg, 2017) flower dissolves fish bones stuck in the throat. Some Rhododendron species in the high mountains are used to make herbal teas. According to herbalist Amchi Lhakpa the tea "clears your stomach and improves digestion. It is helpful with lung and stress disorders involving general weakness of the body. It helps reduce fever and swelling of the abdomen due to the indigestion of food, restores the natural balance after changes in climate or water, and relieves numbness of the extremities, swelling and itching of the throat, or a feeling of thirst".(Scott, 2010) Rhododendron arboreum experience natural and anthropogenic pressures and the later factors are realized as the main cause of their population decline (Mainra et al. 2010; Pokhrel, 1999). The color of the flower ranges from blood red, or pink to white. Rhododendron bears large compact clusters of about 20 flowers. The corolla is tubular, bell-shaped, 4-5 cm long and wide, 5 lobed. The grained wood of Rhododendron arboreum is utilized in making handles of 'Khukhri', packsaddles, gift-boxes, gunstocks and posts (Paul et al. 2005). Flowers and leaves are used as decorations around the houses and temples by adjusting into ropes made of munja grass (Shrivastav, 2012).

Phytochemical processing of raw plant materials is essentially required to optimize the concentration of known constituents and also to maintain their activities (Andriani et al., 2019) Extraction is an important step in the itinerary of phytochemical processing for the discovery of bioactive constituents from plant materials. Selection of a suitable extraction technique is also important for the standardization of herbal products as it is utilized in the removal of desirable soluble constituents, leaving out those not required with the aid of the solvents.

Medicinal plants are currently in considerable significance view due to their special attributes as a large source of therapeutic (Deepak et al. 2019) phytochemicals that may lead to the development of novel drugs. Most of the phytochemicals from plant sources such as phenolics and flavonoids have been reported to have positive impact on health and cancer prevention(Venugopal et al. 2012) as also found in Rhododendron arboreum too. Plants are able to produce a large number of diverse bioactive compounds (Sharma et al. 2016). High concentrations of phytochemicals,(Deepak and Lamichhane 2019) which may protect against free radical damage, accumulate in fruits and vegetables(Kathirvel and Sujatha 2016) .

Phytochemical consumption is associated with a decrease in risk of several types of chronic diseases due to in part to their antioxidant and free radical scavenging effects (Zhang et al., 2015). Recent research has also highlighted their potential role in improved endothelial function and increased vascular blood flow (Shrivastav 2012).

The use of plant extracts, as well as other alternative forms of medical treatments, was of great popularity in the late 1990s. The reason for this is due to increase in antimicrobial resistance, and the need of treatments for new emerging pathogens. Antimicrobial susceptibility testing is carried out to screen whether the extracts are potential antimicrobial agents and measure the antimicrobial activity of the substance. An antimicrobial susceptibility test is a determination of the least amount of an antimicrobial chemotherapeutic agent that will inhibit the growth of a microorganism in vitro using a tube dilution method, agar cup or disc diffusion method. The test may function as an aid in the selection of chemotherapeutic agent by the physician (Prajapati et al., 2018).

The effectiveness of antibiotics can be assessed by their ability to suppress bacterial growth, described by the MIC, or by their ability to kill bacteria, characterized by the minimal lethal concentration (MLC). MIC is usually derived by means of tests in solid media.

It involves the application of antibiotic solutions of different concentrations to cups, wells or paper discs, placed on the surface of or punched into agar plates seeded with the test bacterial strain. Antibiotic diffusion from these sources into the agarose medium leads to inhibition of bacterial growth in the vicinity of the source and to the formation of clear ’zones’ without bacterial lawn. The diameter of these zones increases with antibiotic concentration.(Contreras-Lynch et al., 2017).

Material and Methods:

Study Area : Sample were collected in the month of February 206-17 from Bagmati province and Province no 5 (Lumbini Zone i.e Palpa /Gulmi and Rolpa) at altitude of 1350m (Palpa) to 3400 (Rolpa) and 1200m (Kathmandu valley).

Sample Preparation: Sample were air dried after collection for a week in dark room and herbarium file was created and identified by Tirtha Maiya Shrestha and deposited to the Kathmandu University to get the voucher number.(KU_2017_Rho P5 and KU_2016_Rho P3).

Extract preparation: The shade dried whole plant sample was taken, crushed in Grinder to make it in powder form and mixed with methanol. The mixture was stirred for about half an hour. Then the mixture was kept for 48 hours at room temperature. After 48 hours, the solution was filtered and fresh methanol was added to crude extract. The filtrate extract was evaporated using shaker. The final extract was weighted to and yield of extract was calculated.

Phytochemical screening : Test for Sterols and Triterpenes (Leibermann- Burchard's Test).
Extract solution was concentrated to yield a residue. This residue was dissolved in 1ml of acetic anhydride followed by 1 ml of Chloroform. Appearance of brown ring between the junctions of the two liquids with upper layer green indicates the presence of sterols while the brown ring with upper layer violet indicates the presence of triterpenes.

Test for the Coumarins
Extract solution is concentrated to yield a residue. The residue was dissolved in hot water.
After cooling, the solution was divided into two test tubes. To one of the test tubes, 10% (v/v) Ammonium hydroxide (NH4OH) was added until the solution becomes basic. The other test tube was used as control. Observation of fluorescence indicates the presence of Coumarins.

Test for Tannins and Polyphenols (or Ferric Chloride Test)
1 ml of the extract solution was mixed with 1 ml of water. To this solution, 3 drops of 1% (w/v) Ferric Chloride (FeCl3) was added. Appearance of Blue-Black or Violet color indicates the presence of Tannins and/or Polyphenols.

Test for Reducing sugars (or Fehling's Test)
1 ml of the extract was mixed with 1 ml of water. To this solution, 1ml of Fehling's reagent (1:1 mixture of Fehling's reagent A and Fehling's reagent B) was added. The resulted mixture was warmed over a water bath for half an hour. Appearance of Brick-red precipitate indicates the presence of reducing compounds.

Test for Saponins
2 ml of the extract was shaken vigorously for 30 seconds in a test tube. Persistence of thick froth (about 1 cm) height even after 30 minutes indicates the presence of Saponins.

Test for Glycosides
4ml of the extract solution was dried till 2 ml. 1-2 ml of NH4OH was added and shaken. Appearance of Cherish Red color indicated the presence of Glycosides.

Test for Flavonoids
To 4 ml of the extract solution, 1.5 ml of 50% Methanol solution was added and warmed.
Metal Magnesium was added to the solution. Then 5-6 ml of conc. HCl was added. Appearance of the following was sought: Black color – indication of Flavonoids .Orange color – indication of Flavones .Violet color – indication of Flavanones.

Test for Alkaloids:
5 ml of extract was concentrated to yield a residue. Residue was dissolved in 3 ml of 2% (v/v) HCL.

Few drops of Mayer's reagent were added. Appearance of the dull white precipitate indicated the presence of basic alkaloids.

Microbial analyses

Bacillus subtilis, Pseudomonas, K. pneumonia, Streptococci, Candida albicans strains used for microbial analyses.

Sample Preparation

- 200 mg of each extracts was dissolved in 1000μl of DMSO separately i.e 200mg/ml
- This sample was used as crude to check for its antimicrobial activity

Preparation of microbial culture

- First respective strain of bacterial sample with ATCC (American Type Culture Collection) number was obtained. Nutrient Broth of desired volume were prepared in culture tubes and autoclaved at 121^0 c at 15lbs pressure. With the help of cotton swab, a bacterial sample from standard sample was scrapped and it was dipped in broth. Then all culture tubes were tagged correspondingly and incubated at 37^0 C for 12 hours

a) Disc Diffusion protocol

Filter paper disc of (6mm in diameter) are made .MH agar of desired volume was prepared and autoclaved at 121^0C and 15lbs pressure. Autoclaved media was then poured into sterilized petri plates under LAFH and allowed to set. The microbial sample from culture tube was swabbed in MH agar using sterilized L shaped spreader.15μl of each extracts of the preparation under test poured and dried in the paper disc with the help of a micropipette, It was then gently placed at the different sites of the Petri dish containing the inoculated medium .The plates were then incubated at a temperature of 37^0C. After 6 hours, the zones of inhibitions were measured with the help of a measuring ruler from the circumference of the disc

b) MIC (Minimum inhibitory Concentration)

After observation of crude extract, the sample showing antimicrobial activity was processed to its MIC.

- Various lowered concentration i.e. 100mg/ml, 50mg/ml, 20mg/ml, 10mg/ml, 5mg/ml from crude sample was prepared as per following calculation:

  For 100mg/ml
  Concentration of crude sample (s1) = 200mg/ml
  Desired concentration (s2) = 100mg/ml
  Final volume (v2) = 1ml
  Volume to take (v1) = x
  S1*v1=s2*v2
  V1= (s2*v2)/s1
  V1= 500μl
  For 50mg/ml
  Concentration of crude sample (s1) = 100mg/ml. Desired concentration (s2) = 50mg/ml
  Final volume (v2) = 1ml .Volume to take (v1) = x
  S1*v1=s2*v2
  V1= (s2*v2)/s1
  V1= 500μl
  For 20mg/ml
  Concentration of crude sample (s1) = 50mg/ml. Desired concentration (s2) = 20mg/ml

Final volume (v2) = 1ml. Volume to take (v1) = x
S1*v1=s2*v2
V1= (s2*v2)/s1
V1= 400μl

For 10mg/ml
Concentration of crude sample (s1) = 20mg/ml. Desired concentration (s2) = 10mg/ml
Final volume (v2) = 1ml.Volume to take (v1) = x
S1*v1=s2*v2
V1= (s2*v2)/s1
V1= 500μl

For 5mg/ml.
Concentration of crude sample (s1) = 10mg/ml. Desired concentration (s2) = 5mg/ml
Final volume (v2) = 1ml .Volume to take (v1) = x
S1*v1=s2*v2
V1= (s2*v2)/s1
V1= 500μl

- As the calculation respective volume was taken and final volume was made to be 1ml by adding DMSO. Then Disc diffusion method was carried.

c) Antioxidant analysis

a) Free radical scavenging activity (DPPH Assay)

The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm. When antioxidant reacts with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor and is reduced to the DPPH and as consequence the absorption is decreased from the DPPH. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Diphenyl picrylhydrazine; non radical) with the loss of violet color.

b) Preparation of DPPH solution

DPPH solution of 100Um was prepared by dissolving 3.94mg of DPPH in 100ml of methanol. It was protected from light by covering the bottle with aluminum foil.

c) Preparation of standard solution

100mg/ml stock solution of Ascorbic acid was prepared by dissolving 10mg Ascorbic acid in 1ml methanol. Test solution of 1,2,4,6 and 8 µg /ml of Ascorbic acid was prepared from stock by dilution.

d) Preparation of test sample

10mg of plant extract was dissolved in 1ml of methanol to prepare stock solution of 10mg/ml. Test solution of 1,2,4,6 and 8 µg /ml was prepared from the stock by dilution.
Experiments were done in triplicate.

e) Estimation of DPPH scavenging activity

Estimation was done in two ratios (1:1 and 1:3)

a. For 1:1 ratio

- 1ml methanol and 1 ml of DPPH solution were mixed as control.
- 2ml methanol was taken in Eppendorf tube as blank.
- 1ml ascorbic acid was mixed with 1 ml DPPH as standard.
- 1ml plant extract was mixed with 1ml DPPH as sample.

b. For 1:3 ratio

- 0.5ml of methanol and 1.5ml of DPPH solution were mixed as control.
- 2ml of methanol was taken in Eppendorf tube as blank.
- 0.5 ml ascorbic acid was mixed with 1.5ml DPPH as standard.
- All of these mixtures were immediately kept in dark to prevent from light.
- After 30minutes, absorbance was noted in 517nm.

f) Calculation of IC50

IC50 was calculated from % inhibition. Absorbance at 517nm was determined after 30 minutes using UV-Visible Spectrophotometer and IC50 was determined. The capability to scavenge the DPPH radical was calculated using following equation.

%scavenging= $(A_o-A_1)/A_0*100$

Where, A_0= absorbance of DPPH solution

A_1= absorbance of DPPH along with different concentration of extract. IC50 was calculated from equation of line obtained by plotting a graph of concentration verses % scavenging of DPPH.

g) Determination of Total Phenol content.

Total phenol content estimation was done using Folin Ciocalteu's (folin) technique with little modification. Aliquots of 1ml and standard Gallic acid (20, 40, 60, 80 and 100) µg /ml were placed in test tube. Then 5 ml of Folin Ciocalteu's reagent and 4.5ml of distilled water was mixed. 4 ml of 7% sodium carbonate was added after 5 minutes. Then after shaking it was incubated at 40^0c in water bath for few minutes. Then absorbance was measured at 760nm using UV-Visible spectrophotometer. The experiments were done in triplicates and the blank was made using reagent blank with solvent. Standard Gallic acid was used for calibration curve plotting. Total phenol content was expressed as milligrams of Gallic acid equivalents per gram of fresh weight (mg $GAEg^{-1}FW$).

h) Determination of Total Flavonoid content

Total flavonoid content was measured by using Aluminum chloride colorimetric assay. 1 ml of standard rutin solution (100,200,400,600 and 800) µg /ml and 1 ml of aliquots was positioned into test tube. Then 200ul distilled water was added in each tube.150ul of $NaNO_2$ was added and incubated for 5 minutes at room temperature. Then 150ul of $AlCl_3$ was added. After 6 minutes 2ml of sodium hydroxide was added and final the volume of each test tube was made to 5 ml by adding distilled water. The solution was shake well and incubated at room temperature

for 15 minutes. Finally observance was measured at 510nm using UV-Visible spectrophotometer. Total flavonoid content was measured as mg rutin equivalent (mg RT/g).

- **DNA Isolation and Sequence analysis**

Genomic DNA extraction. was done with Zymo mini prep kit as per manual .(Quick-DNA™ Plant/Seed Miniprep Kit Catalog No. D6020). (kit).

For PCR and sequencing with reference to I bold Manual (Hollingsworth et al., 2009; Kress, 2017; Kress et al., 2005) . We followed the same criteria to get plant authentication by using rbcL and matK gene loci amplification. We used Mega X (Stecher, Tamura, & Kumar, 2020) for phylogenic analysis and online tool of I bold Database (Bold, 2013) for identification. NCBI databased is used for identification and Blankt for sequence submission. EMBL-ebi tools were used for identification of 6 ORF and amino acid recognition site identification.

Results and Discussion

Extractive values of the extract from Rhododendron

The extractive values and the percentage yield of the leaves of various sample of Rhododendron arboreum. The highest yield was 6.8% from the Palpa. The total phytochemical extract yield is shown in table 1.

Table 1: Extract Percentage yield

Plants	Weight of leaf sample (gm)	Weight of crude extract (gm)	% yield
Dhu1	41.69	0.86	2.06
Dhu2	40.29	1.24	3.07
Gul1	30.96	0.68	2.20
Gul2	20.15	0.81	4.02
Pal1	45.91	1.67	3.63
Pal2	31.52	2.15	6.8
Rol1	31.51	1.48	4.70
Rol2	31.47	1.06	3.37

Phytochemical Screening

We have carried out qualitative test for phytochemicals present in Rhododendron arboreum extract. Analysis was carried out for methanol extract. Saponin, Glycosides, Alkaloid, Steroids and Flavonoids were present except Coumarins. The presence or absence of seven different phytochemical are shown in table 2.

Table 2: Phytochemical screening of Rhododendron arboreum sample

Plants/ Properties	Coumarins	Saponin	Glycosides	Alkaloids	Steroids	Reducing Sugar	Flavonoids
Dhu1	--	++	++	++	++	++	++
Dhu2	--	++	++	++	++	++	++
Gul1	--	++	++	++	++	++	++
Gul2	--	++	++	++	++	++	++

Pal1	--	++	++	++	++	++	++
Pal2	--	++	++	++	++	++	++
Rol1	--	++	++	++	++	++	++
Rol2	--	++	++	++	++	++	++

Antibacterial Assay

From extract of each sample, 200 mg was taken and dissolved in 1ml DMSO to make concentration of 200mg/ml. After incubation zone of inhibition was observed and radius of zone of inhibition was measured in mm from center of disc. Finally it was found that the biggest zone of inhibition was found from sample of Gulmi1 *i.e.* 14 mm with respect to Bacillus subtilis.

The antioxidant activity of the extract was analyzed using the standard DPPH solution and compare with standard Ascorbic acid. The highest scavenging Activity was shown by Rhododendron arboreum species from Gulmi (gul1) sample and found to be 77.27%.

DNA isolation and PCR: Genomic DNA was extracted from the raw explant of the sample by Zymo plant DNA extraction kit and was found to be 80ng/uL, by spectrophotometric method and for amplification and sequencing it was send to Barcode Bioscience Pvt.Ltd laboratory, and final sequence was assemble and developed the contig of rbcL gene and introduced as DNA barcode after bioinformatics analysis using online and offline tool.

Contig Sequence of rbcL gene

The pcr product of rbcL gene was sequenced by Sanger sequencing and the final contig prepared with compilation of reverse and forward region which is as show below.

```
ATCAAGCTGGTGTTAAGATTACAAATTGAATTATTATACTCCTCAGTATGAAACCAAAGATACTGATATC
TTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTCCACCTGAAGAAGCAGGGGCCGCGGTAGCTGCGG
AATCTTCTACTGGTACATGGACAACAGTGTGGACTGATGGACTTACTAGCCTTGATCGTTACAAAGGGCG
ATGCTACCACATCGAGCCTGTTGCTGGAGACGAAAATCAATATATTGCTTATGTAGCTTATCCTTTAGAC
CTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGGTTCAAAGCCC
TGCGCGCTCTACGTCTGGAAGATCTACGAATCCCTGTAGCGTATGTTAAAACGTTCCAAGGACCGCCTCA
TGGTATCCAAGTTGAAAGAGATAAATTGAACAAGTACGGTCGTCCCCTGTTGGGATGTACTATTAAACCT
AAATTGGGGTTATCTGCTAAAAA
```

DNA Barcoding: Sequence analysis with NCBI BLAST OUTPUT

The above contig was submitted in NCBI for GI accession number and finally gets LC 456605.1 submitted to nBLAST mode in NCBI database for Rhododendron arboreum plant identification based on rbcL gene sequence alignment.

Phylogenetic Analysis: To find the close ancestor relationship among the available data source from NCBI , we compare rbcL gene of Rhododendron arboreum sequence with 10 closest similar sequence with Mega X software and found that the sequence similarity among them,which finally endorse for the development of rbcL gene DNA Barcode formation of Nepalese sample.

DNA Barcode of rbcL Gene: With due analysis of rbcL gene sequence with NCBI Blast and Mega X it is confirmed that the provide sequence belong to Rhododendron arboreum, since we develop the DNA barcode with of it.

The project has helped to reveal various biological activities of Rhododendron arboreum collected from different locations of the country. All the Samples showed presence of secondary metabolites (Table 2) such as Saponins, Glycosides, Alkaloids, Sterols, Reducing Sugars and flavonoids but not Coumarins which was further analyzed by different related tests. The antibiotic activity ranging from 2 mm to 14 mm in inhibition radii against *Bacillus subtilis*, 5 to 14 mm against *Pseudomonas*, 4 to 9 for *Klebsiella pneumonia*, and 4 to 10 mm for Streptococcus using 200 mg/ml solution of each sample. Sample 1 (Pal1) collected from Palpa region of Nepal showed the highest activity against all these bacteria. However, none of them showed any activity against *Candida albicans* showing the antibiotic tolerance that may be the key finding as it can be further implementation for new drug discovery. The same sample showed highest activity in DPPH bioassay very close to Ascorbic acid which can be stated from the regression analysis of DPPH for the IC50 value analysis of Ascorbic acid. All this might be due to the high content of Phenol in this sample. The extract yield of Palpa show the highest percentage among all the location of Nepal (Table 1). This detailed results show that Rhododendron arboreum has medicinal importance and the traditional healers use in specified community proves the facts. These results should be further validated scientifically by more rigorous scientific studies in days to come.

Also, this project highlights the necessity of assessing the right model for data evaluation and calculation which is justified by the results of MIC. As all the tests were performed using crude extract and so, the result cannot be validated. It is advised to test using isolated pure compound extracts for confirming the results presented here.

The promising result obtained from the research stimulates further research on the plant and its metabolites, and their safety concerns in human health. The great medicinal importance of this plants provokes the serious concerns for its preservation and conservation from the concerned authority. As evident from the above results and the PCR products of matK and rbcL gene) using specific primers of DNA demanded the barcoding of the genes in the given sample the genus of the sample is Rhododendron arboreum. The Phylogenic analysis with Mega X also support the close relationship among the Rhododendron species available data bases. This is first time ever reported in Nepal for any DNA barcoding of Medicinal plant, among the all genes rbcL and matK barcoding used for the analysis, all showed similarity to arboreum species. Yet more to elaborate among the genetic diversity of Rhododendron species of Nepal as we get success only in one species out of 31 as registered in flora of Nepal.

Acknowledgement

The research part of this project has been supported by UGC- Institutional Research Grant 2017.

Conflict of Interest: The authors declare no conflict of interest

References

Andriani, Y., Ramli, N. M., Syamsumir, D. F., Kassim, M. N. I., Jaafar, J., Aziz, N. A., . . . Mohamad, H. (2019). Phytochemical analysis, antioxidant, antibacterial and cytotoxicity properties of keys and cores part of Pandanus tectorius fruits. *Arabian Journal of Chemistry, 12*(8), 3555-3564. Retrieved from http://www.sciencedirect.com/science/article/pii/S1878535215003159. doi:https://doi.org/10.1016/j.arabjc.2015.11.003

Bold. (2013). <BOLD_Handbook_Oct2013.pdf>.

Contreras-Lynch, S., Smith, P., Olmos, P., Loy, M. E., Finnegan, W., & Miranda, C. D. (2017). A Novel and Validated Protocol for Performing MIC Tests to Determine the Susceptibility of Piscirickettsia salmonis Isolates to Florfenicol and Oxytetracycline. *Frontiers in Microbiology, 8*(1255). Retrieved from https://www.frontiersin.org/article/10.3389/fmicb.2017.01255. doi:10.3389/fmicb.2017.01255

COX PA, C. K. (1997). Encyclopedia of Rhododendron species.

Deepak, S., Lamichhane J. (2019). <qualitative-analysis-of-rhododendron-arboreum-leaves-extracts-using-hplcesiqtofms. *Biotechnology an Indian Journal*, *15*(6).
Deepak, S., Meena maiy, S., & Hitesh, B. (2019). The Effectiveness of Anti-Cancer Compounds Taxol Derived from Natural Products from Nepal. *Acta Scientific Pharmaceutical Sciences*, *3*(8), 116-123. doi:10.31080/asps.2019.03.0350
folin. <folin catechu reagent.pdf>.
Garg, N. (2017). Chapter 8 - Technology for the Production of Agricultural Wines. In M. R. Kosseva, V. K. Joshi, & P. S. Panesar (Eds.), *Science and Technology of Fruit Wine Production* (pp. 463-486). San Diego: Academic Press.
Hebert, P. D., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proc Biol Sci*, *270*(1512), 313-321. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12614582. doi:10.1098/rspb.2002.2218
Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings. Biological sciences*, *270*(1512), 313-321. Retrieved from https://pubmed.ncbi.nlm.nih.gov/12614582

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1691236/. doi:10.1098/rspb.2002.2218
Hollingsworth, P. M., Forrest, L. L., Spouge, J. L., Hajibabaei, M., Ratnasingham, S., van der Bank, M., . . . Little, D. P. (2009). A DNA barcode for land plants. *Proceedings of the National Academy of Sciences*, *106*(31), 12794-12797. Retrieved from https://www.pnas.org/content/pnas/106/31/12794.full.pdf. doi:10.1073/pnas.0905845106
Kathirvel, A., & Sujatha, V. (2016). Phytochemical studies, antioxidant activities and identification of active compounds using GC–MS of Dryopteris cochleata leaves. *Arabian Journal of Chemistry*, *9*, S1435-S1442. Retrieved from http://www.sciencedirect.com/science/article/pii/S1878535212000640. doi:https://doi.org/10.1016/j.arabjc.2012.03.018
kit, Z. p. d. <quick-dna_plant-seed_miniprep_kit.pdf>.
Kress, W. J. (2017). Plant DNA barcodes: Applications today and in the future. *Journal of Systematics and Evolution*, *55*(4), 291-307. doi:10.1111/jse.12254
Kress, W. J., Wurdack, K. J., Zimmer, E. A., Weigt, L. A., & Janzen, D. H. (2005). Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(23), 8369-8374. Retrieved from https://pubmed.ncbi.nlm.nih.gov/15928076

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1142120/. doi:10.1073/pnas.0503123102
Li, Y., Tong, Y., & Xing, F. (2016). DNA Barcoding Evaluation and Its Taxonomic Implications in the Recently Evolved Genus Oberonia Lindl. (Orchidaceae) in China. *Frontiers in Plant Science*, *7*(1791). Retrieved from https://www.frontiersin.org/article/10.3389/fpls.2016.01791. doi:10.3389/fpls.2016.01791
Liu, Y., Zhang, L., Liu, Z., Luo, K., Chen, S., & Chen, K. (2012). Species identification of Rhododendron (Ericaceae) using the chloroplast deoxyribonucleic acid PsbA-trnH genetic marker. *Pharmacognosy Magazine*, *8*(29), 29-36. Retrieved from http://www.phcog.com/article.asp?issn=0973-1296;year=2012;volume=8;issue=29;spage=29;epage=36;aulast=Liu. doi:10.4103/0973-1296.93311
Mainra, A., Badola, H., & Mohanty, B. (2010). *RHODODENDRONS: CONSERVATION AND SUSTAINABLE USE International Conference RHODODENDRONS: CONSERVATION AND SUSTAINABLE USE*.
Meijón, M., Cañal, M. J., Fernández, H., Rodríguez, A., Fernández, B., Rodríguez, R., & Feito, I. (2010). Hormonal Profile in Vegetative and Floral Buds of Azalea: Levels of Polyamines, Gibberellins, and Cytokinins. *Journal of Plant Growth Regulation*, *30*(1), 74-82. doi:10.1007/s00344-010-9169-5
Newmaster, S. G., Fazekas, A. J., Steeves, R. A. D., & Janovec, J. (2008). Testing candidate plant barcode regions in the Myristicaceae. *Molecular ecology resources*, *8*(3), 480-490. Retrieved from https://pubmed.ncbi.nlm.nih.gov/21585825. doi:10.1111/j.1471-8286.2007.02002.x
Paul, A., Khan, M., & Arunachalam, A. (2005). Biodiversity and conservation of rhododendrons in Arunachal Pradesh in the Indo-Burma biodiversity hotspot. *Current Science*, *89*.
pokhrel. (1999). <pokhrel 1999.pdf>.
Prajapati, K., Thapaliya, S., Dhakal, D., Acharya, S., Shrestha, S., Hora, R., & Sakha, H. (2018). Antimicrobial Activity of Ethanolic Extract of Medicinal Plants against Human Pathogenic Bacteria. *Tribhuvan University Journal of Microbiology*, *5*, 1-6. doi:10.3126/tujm.v5i0.22292
Scott. (2010). NEPAL'S MAGNIFICENT RHODODENDRON.
Sharma, D., Shrestha, T., Rapal, N., Poudel, S. C., Shrestha, S., Sharma, S., . . . Lamichane, J. (2016). Screening and bioactivity measurement of high altitude medicinal plants of Nepal. *Vegetos- An International Journal of Plant Research*, *29*(4). doi:10.5958/2229-4473.2016.00098.7
Shrivastav, P. (2012). <Pallavi shrivastav.pdf>. *Journal of Applied Pharmaceutical Science*.
Stecher, G., Tamura, K., & Kumar, S. (2020). Molecular Evolutionary Genetics Analysis (MEGA) for macOS. *Mol Biol Evol*. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/31904846. doi:10.1093/molbev/msz312

Venugopal, C., McFarlane, N. M., Nolte, S., Manoranjan, B., & Singh, S. K. (2012). Processing of primary brain tumor tissue for stem cell assays and flow sorting. *Journal of visualized experiments : JoVE*(67), 4111. Retrieved from https://pubmed.ncbi.nlm.nih.gov/23051935

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3490257/. doi:10.3791/4111

Vikas Kumar, S. S., Rasane Prasad, Yogesh Gat, Chesi Sangma, Heena Jakhu and Manjri Sharma. (2019). <s40066-019-0251-3.pdf>. *Agriculture & Food Security, (2019) 8:6*(8). doi:10.1186/s40066-019-0251-3

Wang, Y., Wang, J., Lai, L., Jiang, L., Zhuang, P., Zhang, L., . . . Baskin, C. C. (2014). Geographic variation in seed traits within and among forty-two species of Rhododendron (Ericaceae) on the Tibetan plateau: relationships with altitude, habitat, plant height, and phylogeny. *Ecol Evol*, *4*(10), 1913-1923. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/24963385. doi:10.1002/ece3.1067

Wong, K.-L., But, P., & Shaw, P.-C. (2013). Evaluation of seven DNA barcodes for differentiating closely related medicinal Gentiana species and their adulterants. *Chinese medicine*, *8*, 16. doi:10.1186/1749-8546-8-16

Zhang, Y.-J., Gan, R.-Y., Li, S., Zhou, Y., Li, A.-N., Xu, D.-P., & Li, H.-B. (2015). Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. *Molecules (Basel, Switzerland)*, *20*(12), 21138-21156. Retrieved from https://pubmed.ncbi.nlm.nih.gov/26633317

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6331972/. doi:10.3390/molecules201219753

Submitted: 01.03.2020 Accepted: 10.06.2020