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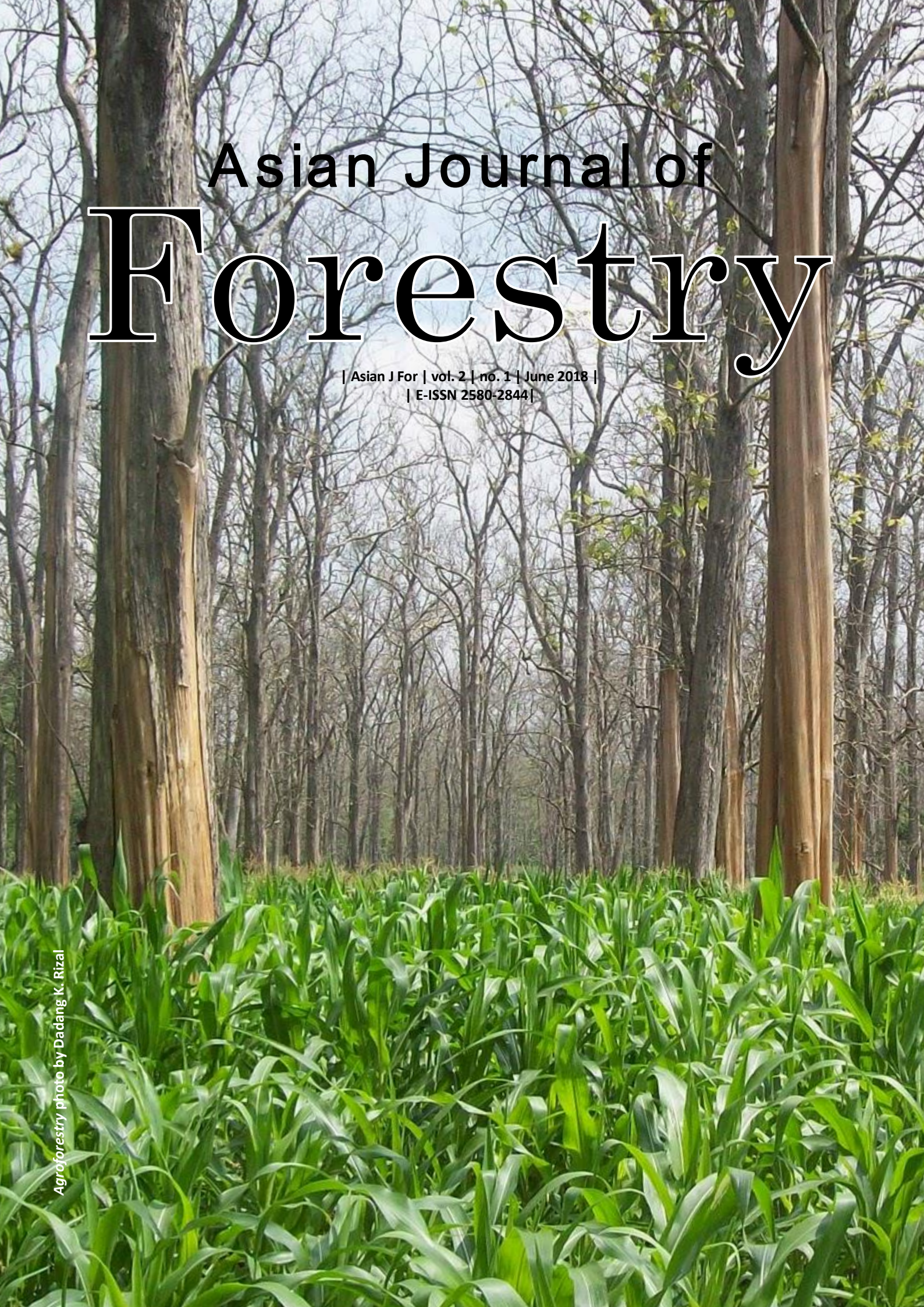

Agroforestry photo by Dadang K. Rizal

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Abstract should not be more than 200 words. **Keywords** is about five words, covering scientific and local name (if any), research theme, and special methods which used; and sorted from A to Z. All important **abbreviations** must be defined at their first mention. **Running title** is about five words. **Introduction** is about 400-600 words, covering the background and aims of the research. **Materials and Methods** should emphasize on the procedures and data analysis. **Results and Discussion** should be written as a series of connecting sentences, however, for manuscript with long discussion should be divided into subtitles. Thorough discussion represents the causal effect mainly explains for why and how the results of the research were taken place, and do not only re-express the mentioned results in the form of sentences. **Concluding** sentence should be given at the end of the discussion. **Acknowledgments** are expressed in a brief; all sources of institutional, private and corporate financial support for the work must be fully acknowledged, and any potential conflicts of interest are noted.

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Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta***.** 50th Annual Symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.) Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. www.molecularsystemsbiology.com. DOI:10.1038/msb.2008.24

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Plant diversity in betel leaf agroforestry of South Meghalaya, Northeast India

H. TYNSONG[1,♥], B.K. TIWARI[2], M. DKHAR[3]
[1]Ministry of Environment, Forest and Climate Change, North Eastern Regional Office. Shillong 793021, Meghalaya, India.
♥email: herotynsong@yahoo.com
[2]Department of Environmental Studies, North-Eastern Hill University, Shillong 793022, Meghalaya, India
[3]Union Christian College Umiam, Ri Bhoi, Shillong 793122. Meghalaya, India

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Abstract. *Tynsong H, Tiwari BK, Dkhar M. 2018. Plant diversity in betel leaf agroforestry of South Meghalaya, Northeast India. Asian J For 2: 1-11.* Large areas of lowland tropical forests in South Meghalaya, India have been converted into betel leaf agroforestry systems by the tribal people living in the area. Traditional betel leaf agroforestry still maintains high biodiversity and structurally complex shade canopies compared to other agricultural lands despite changes natural forests. Yet, it is not clear the state of biodiversity in betel leaf agroforestry compared to that in natural forests. This study aimed to assess plant diversity of betel leaf agroforestry in South Meghalaya, India and to compare it with a nearby natural forest. A total of 160 plant species were recorded in natural forests out of which 75 were trees, 40 shrubs, and 45 herbs, while in betel leaf agroforestry, a total of 159 plant species, 94 trees, 17 shrubs and 48 herbs were recorded. A total of 34 tree species, 13 shrub species, and 14 herb species were found in the two land uses. All the plant species were native species. The results of the study suggest that the conversion of natural forest into betel leaf agroforestry in South Meghalaya has no significant impact on tree and herb diversity. However, the basal area and density were affected to some extent. The land-use change had also affected the density and diversity of shrubs. The study concludes that betel leaf agroforestry in South Meghalaya developed by the indigenous War Khasi tribe through experiential learning over several generations has emerged as a fairly sustainable agroforestry system causing minimal impact on plant diversity.

Keywords: Betel leaf agroforest, cash crop, natural forest, South Meghalaya

INTRODUCTION

Biodiversity cannot be conserved effectively if conservation strategies are restricted to protected natural ecosystems alone (Moguel and Toledo 1999). Ryan (1992) reported that there are only about 7000 protected areas in the world, covering approximately 650 million ha and representing less than 5% of the earth's land surface. While the rest of terrestrial environments are affected by human activities, including agriculture and other developmental works.

Forest ecosystems can range from intact natural forests to slightly-disturbed forests to monoculture industrial plantations. The area under natural and semi-natural forests is decreasing by 13 million ha annually (FAO 2006). Contrary to this, the average annual rate of forest plantation establishment is 5 million ha (FAO 2014). Among such variety form of forestry landscapes, there is the traditional land-use system that combines agriculture and forestry (often so-called agroforestry) under indigenous management which provides relatively high and sustainable economic benefits with a seemingly diversified, productive system.

Most research in ecology and biodiversity has been focused on undisturbed ecosystems, while human-impacted and managed ecosystems have not received equal attention. Man-made landscapes that are part of the indigenous agricultural practices, such as agroforestry, home gardens, polycultures, also contribute a great deal in biodiversity conservation (Toledo 1990; Tynsong and Tiwari 2011). Toledo et al. (1994) and Tiwari et al. (2017) reported that there is increasing evidence that landscapes under indigenous and local knowledge-based management systems maintain and even improve biodiversity. There are indications that the area under agroforestry systems will continue to increase, making it important to assess its potentials to fulfill biological conservation as well as its economic purpose. The question is whether agroforestry systems can harbor biodiversity which is similar to that in natural forests or not.

Northeastern India is a part of the Indo-Burma biodiversity hotspot, harboring about 50% of plant biodiversity of India (ca. 8000 species), of which 31.58% (ca. 2526 species) is endemic (DE, MEDHI 2014). The region is rich in orchids, ferns, oaks (*Quercus* spp.), bamboos, rhododendrons (*Rhododendron* spp.), magnolias (*Magnolia* spp.), etc. According to Conservation International (2011), Indo-Burma is the most threatened hotspot with 5 % original habitat is remaining. Threats to species, sites, and landscapes are immediate and severe (Baltzer et al. 2001; Nooren and Claridge 2001; IUCN 2011). The combination of economic development and an increasing human population is exerting enormous pressure on the region's natural resources, and overexploitation has eradicated species from many areas.

Meghalaya is a region in northeastern India with high biodiversity importance. This area harbors 3128 species of angiosperms which include 1237 endemic species and 53 threatened plant species (Khan et al. 1997). The biodiversity of natural forests of Meghalaya has been

studied by Tiwari et al. (1998), Upadhaya (2002), Jamir and Pandey (2003) and Tripathi et al. (2006). However, biodiversity of agroforestry has not received due attention.

Betel leaf (*Piper betle* L.) is an important cash crop in India and Bangladesh with huge demand in the Middle East, Britain, Pakistan, and some African counties (Haider et al. 2013). This huge market demand has acted as a driver for conversion of a large chunk of natural forests into betel leaf-based agroforestry systems in India, Bangladesh, Sri Lanka, Malaysia, Philippines, and East Africa (Arambewela et al. 2005; Nath and Inoue 2009a). Betel leaf is traditionally consumed with slices of areca nut and a thin coating of lime by people of South and Southeast Asia, the Gulf States, and the Pacific islands (Nath and Inoue 2009b). Betel leaf has a trade worth of INR 7000 million in India alone (Balasubrahmanyam et al. 1994), where about 15-20 million people consume betel leaves on a regular basis (Jana 1996). Jeng et al. (2002) reported that worldwide, over 2 billion people consume betel leaf.

Although it changes natural forests, traditional betel leaf agroforestry still maintains high biodiversity and structurally complex shade canopies compared to other agricultural lands. The traditional betel leaf agroforestry is more likely to conserve a significant portion of the original forest biodiversity since the establishment of this land use is never clear-felled trees in the natural forests (Tynsong 2009). With a sustained increase of world betel leaf consumption and growing human population in many of the betel leaf consuming regions, pressures to intensify betel leaf production are likely to increase, which will result in more conversions of natural forests to betel leaf-based agroforestry.

In Meghalaya, the farming of plant betel leaf (*Piper betle* L.**)** is done without cutting of naturally growing trees or burning the field. Betel leaf grows along with trees, shrubs, and herbs on the same piece of land. During the first year after planting the betel leaf, the farmers prune the canopy of all trees except a few important timber trees, fruit trees, and non-coppicing tree species. The cutting of tree branches is done, so the newly planted betel leaf grown at the base of the trees may receive enough sunlight and nutrients from the decaying leaves and branches of the lopped trees. After three to four months, most trees start sprouting again and after one year the whole plantation looks like a natural forest again. Important timber trees include *Michelia cathcartii, Toona ciliata, Cedrela toona,* and *Schima wallichii,* fruit trees include *Artocarpus heterophyllus, Gynocardia odorata,* and *Baccuarea sapida* and non-coppicing trees include *Macaranga peltata, Macaranga hypoleuca, Lithocarpus elegans,* and *Ligustrum robustum.* The process involved in the cultivation of betel leaf and management of betel leaf agroforestry in Meghalaya is similar to that reported for the Khasia tribes of Bangladesh as described by Saha and Azam (2004) and Haider et al. (2013).

Other agroforestry systems viz., coffee, cocoa, and swidden cultivation have received considerable scientific and public attention for their ability to maintain biodiversity (Perfecto et al. 1996; Moguel and Toledo 1999; Schroth and Harvey 2007), yet the same situation has not happened on betel leaf agroforestry systems. Only a handful of researchers from Bangladesh and Sri Lanka have reported biodiversity of betel leaf agroforestry systems (Alam and Mohiuddin 1995; Nath et al. 2003; Arambewela et al. 2005; Nath and Inoue 2009b). In South Meghalaya, more than twenty thousand farmers are currently engaged in the cultivation of betel leaf on approximately fifteen thousand hectares of land. There is a noticeable gap in our understanding of the biodiversity of betel leaf agroforestry created and maintained by the local tribal communities of South Meghalaya. The objective of this study was to inventory the plant diversity of betel leaf agroforestry and to compare it with a nearby natural forest of the area.

MATERIALS AND METHODS

Study area

The plant diversity survey was conducted in one natural forest (hereafter NF) and one betel leaf agroforestry (hereafter BLA) in South Meghalaya, India. The NF was located in Siatbakon Village (latitude $25^0$16' N, longitude $91^0$56' E, altitude 1003 m asl) and the BLA was located in Nongkwai Village (latitude $25^0$20' N, longitude $91^0$39.54' E, altitude 600 m asl) (Figure 1). Cherrapunjee-Mawsynram Plateau, one of the wettest places in the world is located in this region. The mean annual maximum and minimum temperatures are 23ºC and 13ºC, respectively. The mean annual rainfall is 11565 mm. The slope of the area is predominantly towards the south and the angle of the slope varies between 10º and 40º. The area has a large number of rivers and rivulets, which drain into the plains of Bangladesh. At the present time, narrow and deep river valleys separate one hill range from the other.

The population density in the studied area is sparse. Horticulture, forestry, and fisheries are the principal occupations of the people. Agriculture is limited to some small valleys where mainly tuber crops are grown. Areca nut, orange, betel leaf, jackfruit, bay leaf, honey and broom grass are the important products of the region. The area is inhabited by *War Khasi* people, a tribal community having a long tradition of forest conservation. The local people collect, process, and market a large variety of non-timber forest products (NTFPs) and medicinal and aromatic plants (MAPs) such as *Cinnamomum tamala*, *Piper peepuloides*, *Phrynium capitatum*, bamboo, honey, mushrooms, nuts, wild tubers, edible worms, insects and leafy vegetables from the forests (Tynsong et al. 2012).

Data collection

An extensive survey was carried out during the months of January 2006 to October 2008. The data were collected once in every season of the year for a period of two years. The composition and structure of NF and BLA were determined within 100 m^2 plots (10 m × 10 m) for trees (dbh ≥5 cm); 25 m^2 plots (5 m × 5 m) for shrubs and 1 m^2 plot (1 m × 1 m) for herbs. The total sampled area for each study site was 1 ha for the tree, 0.05 ha for shrub and 0.01 ha for herbs. Tree species with > 10 cm diameter at breast height (dbh) were individually counted, measured and numbered. The density and frequency of occurrence of the species per plot were also estimated.

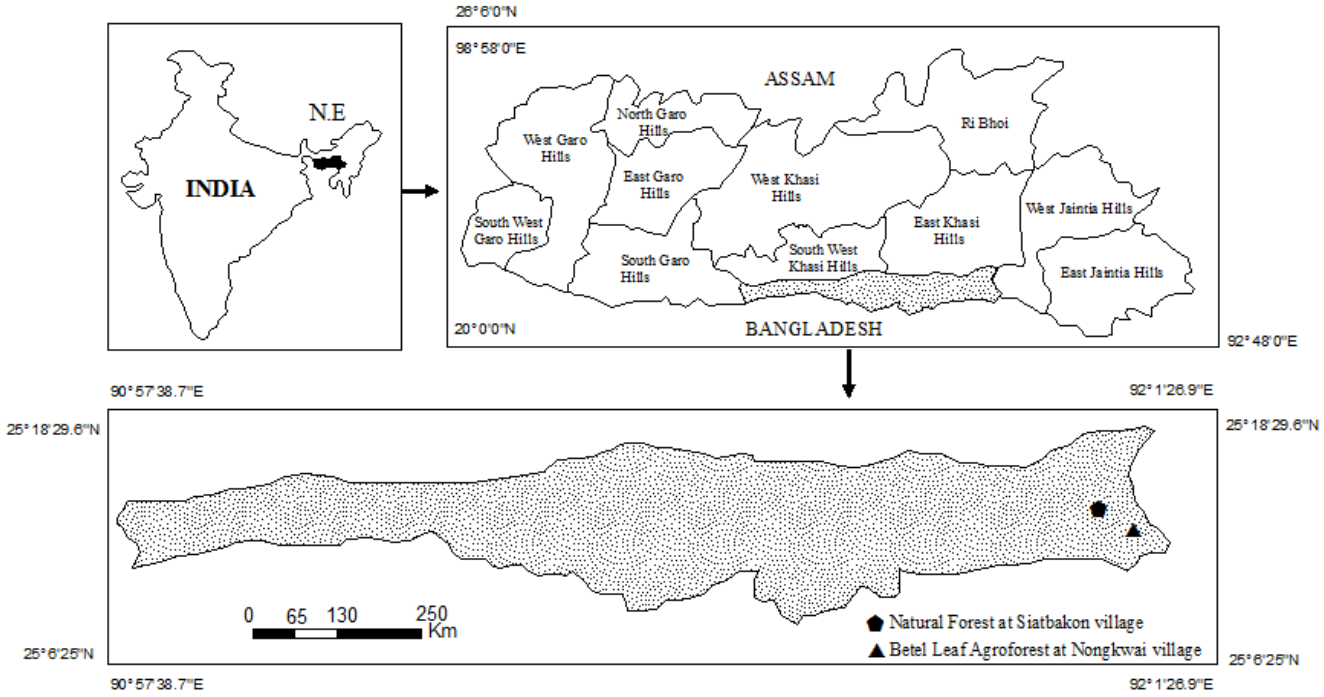

Figure 1. The study sites in natural forest (hereafter NF) and betel leaf agroforestry (hereafter BLA) in South Meghalaya, India

Data analysis

Plant specimens collected from the two forest types were identified with the help of Flora of Assam (Kanjilal et al.1934-1940) and Flora of Jowai (Balakhrishnan 1981-1983). The identifications were confirmed by consulting the herbaria at Botanical Survey of India, Northeastern Circle, Shillong, India. The nomenclatures of the species are as per the regional flora. Analysis of variance (ANOVA) and correlation coefficient values (r) was calculated using Statistica Version 6 (Serial no: BX1117619309D60).

Basal area: The basal area of each overstory tree was calculated using equation (1). The basal area values were then extrapolated to per hectare basis.

$$BA = \frac{\pi D^2}{4} \quad (1)$$

Where:
BA : basal area (m^2ha^{-1});
D : diameter at breast height (cm); and
Π : pi (3.142).

Frequency, density, and abundance: The frequency, density, and abundance of the species were determined following the methods of Misra (1968) and Muller-Dombois and Ellenberg (1974). The frequencies of occurrence were obtained to ascertain species abundance and species evenness. The following biodiversity indices were computed.

Simpson index of dominance (D): Simpson index of dominance (D) (Simpson 1949) was obtained using equation (2):

$$D = \sum (ni/N)^2 \quad (2)$$

Where:
ni : number of individuals of ith species.
N : total number of individuals of all the species.

Species relative density (RD): This refers to the number of individuals of a given species divided by the total number of individuals of all species. This was obtained using equation (3):

$$RD = \frac{n_i \times 100}{N} \quad (3)$$

Where:
RD : relative density;
n_i : number of individuals of species;
N : total number of individuals in the entire population

Importance value index (IVI): For tree species, it was obtained by summing RD and RDo, and then dividing it by 2 as given by Equation (4).

$$\textbf{IVI} = \frac{RD + RDo}{2} \quad (4)$$

For shrub and herb: **IVI** = relative frequency + relative density.

The Shannon-Wiener diversity index (H^-). Shannon-Wiener diversity index (Shannon and Wiener 1963) was obtained by using Equation (5):

Shannon-Wiener diversity index:

$$H^{-} = -\sum (ni/N) \log_e (ni/N) \quad (5)$$

Where:
ni : IVI of each species and
N : total IVI

Species evenness (E): Pielou's evenness index (Pielou 1975) was obtained by using equation 6.

Pielou's evenness index (E):

$$E = \sum (ni/N) \log_e (ni/N) / \log_e S \quad (6)$$

Where:
ni : IVI of each species,
N : total IVI and
S : Number of species

RESULTS AND DISCUSSION

Plant diversity

In the natural forest site (NF), a total of 160 plant species were recorded of which 75 were trees, 40 shrubs, and 45 herbs, while in the betel leaf agroforestry site (BLA) a total of 159 plant species, 94 trees, 17 shrubs and 48 herbs were recorded. A total of 34 tree species, 13 shrub species, and 14 herb species were present in both forest types. All the plant species encountered were native species (Table S1). For shrub components, species richness, number of families and number of genera were significantly higher in NF as compared to BLA. However, for tree and herb components, the number of families, number of genera and Shannon Diversity Index were slightly higher in BLA as compared to NF (Table 1). The similarity between NF and BLA for trees, shrubs and herbs species was 43.34%, 37.93%, and 38.32 % respectively. The one-way analysis of variance (ANOVA) showed significant variation ($P \leq 0.001$) of tree, shrub, and herb between NF and BLA. The correlation coefficient values (r) showed a positive significant correlation between tree ($r = 0.87$, $P \leq 0.001$), shrub ($r = 1.00$, $P \leq 0.001$) and herb ($r = 1.00$, $P \leq 0.001$).

Three dominant families of trees in NF were Lauraceae (11 species), Fagaceae (8 species) and Euphorbiaceae (8 species), while dominant families of trees in BLA were Euphorbiaceae (12 species) Lauraceae (12 species) and Moraceae (8 species). For shrubs, three dominant families in NF were Rubiaceae (9 species), Moraceae (4 species) and Poaceae (3 species), while in BLA they were Rubiaceae (6 species), Arecaceae (2 species) and Urticaceae (2 species). For herbs, three dominant families in NF included Zingiberaceae (5 species), Rubiaceae (4 species) and Melastromaceae (4 species), while dominant families in BLA included Asteraceae (5 species), Rubiaceae (4 species) and Poaceae (3 species). We encountered 24 families of trees, 9 families of shrubs and 9 families of herbs present in both forest types.

Tree species with high IVI in each forest type were: *Lithocarpus fenestatus*, *Lithocarpus elegans*, and *Sarcosperma griffithii (*NF); *Duabanga grandiflora*, *Sarcosperma griffithii*, and *Ficus glomerata (*BLA). The ten most important tree species in the two forest types are given in Table 2. A list of endemics (E) and rare (R) species found in both the forest types is given in Table 3.

Main uses of plants in betel leaf agroforestry

All 94 tree species recorded in BLA were maintained by local people as supporting trees for betel leaf to grow. However, it was observed that most preferred tree species well supporting the growth of betel leaf include *Artocarpus heterophyllus, Duabanga grandiflora, Ficus glomerata, Saraca indica and Sarcosperma griffithii.* It was also observed that in BLA, local people preserved the plant species for various purposes. Based on the usage patterns, the plant species present in the BLA were grouped into ten broad categories of usage, namely as, (i) timber divided into: high-value timber (HT) and low-value timber (LT), (ii) fuelwood divided into: high-value fuelwood (HFW) and low-value fuelwood (LFW), (iii) edible stuff (E), (iv) medicinal stuff (M), (v) tool-making stuff (T), (vi) ornamental stuff (O), (vii) craft (C), (viii) packing leaf (PC), (ix) latex producing plant (L) and (x) nonspecific use (NSU). Edible plants included: fruit, vegetable, and seed. In BLA as a whole, the usages were 21 as timber trees (HT = 10 and LT = 11), 51 as fuelwood (HFW = 20 and LFW = 31), 15 as edible stuff, 17 as medicinal stuff, 40 with nonspecific uses and as making tools stuff, ornamental stuff, craft, spice stuff, packing leaf and latex producing plant for the rests with a total of 15 plant species (Table 4).

Table 1. Diversity and community characteristics of plant species in natural forest (NF) and betel leaf agroforestry (BLA) in South Meghalaya, India

Parameter	NF			BLA		
	Tree	Shrub	Herb	Tree	Shrub	Herb
Sampling size (ha)	1	0.4	0.02	1	0.4	0.02
Number of families	33	21	27	41	13	31
Number of genera	61	33	37	78	21	42
Species richness	75	40	45	94	17	48
Density (ha^{-1})	1972	19280	347563	1788	7660	423688
Basal area (m^2ha^{-1})	52.26	-	-	50.06	-	-
Species evenness index (E)	0.83	0.90	0.93	0.90	0.45	0.95
Shannon diversity index (H^{-})	3.87	3.35	3.55	4.10	2.70	3.68

Table 2. Ten most important tree species in natural forest and betel leaf agroforestry

Tree species	Freq. (%)	DBH (cm)	Basal Area (m^2ha^{-1})	IVI
NF				
Lithocarpus fenestatus (Roxb.) Rehder	41	1.58	4.96	40.66
Lithocarpus elegans (Blume.) Soep.	48	1.37	4.29	30.8
Sarcosperma griffithii Benth.	51	0.94	2.94	16.58
Machilus bombycina King.	38	0.80	2.52	12.35
Schima walichii Choisy.	22	0.68	2.12	11.66
Quercus lanceofolia Roxb.	29	0.65	2.04	10.51
Castanopsis hystrix A.DC.	34	0.58	1.84	10.48
Helicia erratica Hk.f.	40	0.58	1.82	8.76
Castenea sativa Miller.	19	0.51	1.61	8.5
Quercus spicata Smith.	16	0.49	1.55	6.02
BLA				
Duabanga grandiflora (Roxb. Ex DC.) Walp.	33	50.94	11.82	32.78
Sarcosperma griffithii Benth.	58	79.59	5.97	16.36
Ficus glomerata Roxb.	28	18.78	3.66	10.99
Ficus benjamina L. var. *comosa* Kurtz.	8	68.98	2.24	10.51
Phoebe cooperiana U.N.Kanjilal ex A.Das.Nov.sp.	30	17.37	2.18	8.7
Wrightia tomentosa Roem & Sch.	33	31.84	2.07	8.19
Artocarpus lakoocha Roxb.	40	17.51	1.69	7.01
Toona ciliata Roem.	33	28.65	1.68	6.86
Caryota urens L.	28	22.29	1.17	6.85
Adenanthera pavonina L.	40	21.51	1.16	6.39

Table 3. Endemic and rare plant species present in the natural forest and betel leaf agroforestry

Plant species	Family	Status	Forest stands
Tree			
Acer oblongum Wall.	Aceraceae	R	NF
Citrus latipes (Swingle.)Tanaka.	Rutaceae	E, R	NF
Cyathea gigantea (Wall ex Hook.) Holttm.	Cyatheaceae	R	NF
Daphniphyllum himalayense Muell.	Euphorbiaceae	E	NF
Drimycarpus racemosus Hk.f	Anacardiaceae	E	BLA
Erythroxylon kunthianum Wall.	Malpighiaceae	E	NF
Euonymus lawsonii Clarke & Prain.	Celastraceae	E	BLA
Ilex embelioides Hk .f.	Aquifoliaceae	E, R	BLA
Sarcosperma griffithii Benth.	Sapotaceae	R	NF & BLA
Shrub			
Ardisia griffithii C.B.Clarke.	Myrsinaceae	E	NF
Ixora subsissillis Wall.	Rubiaceae	E	NF & BLA
Mahonia pycnophylla Fedde.	Berberidaceae	E	NF
Rubus khasianus Cordat.	Rosaceae	E	NF
Herb			
Eriocaulon cristatum Mast.	Eriocaulaceae	E	NF
Impatiens tripetala DC.	Balsaminaceae	E	BLA
Osbekia capitata Benth.	Melastromaceae	E	NF
Sonerilla khasiana Dyer.	Melastromaceae	E, R	NF

Note: NF- natural forest, BLA-betel leaf agroforestry, E- Endemic, R- Rare

Discussion

Tree species diversity and richness (H' = 4.10; 94 species) in BLA was higher than that in NF (H' = 3.87; 75 species). Also, herb species diversity and richness in BLA (H' = 3.68; 48 species) were higher than NF (H' = 3.55; 45 species). However, shrub species diversity and richness (H' = 2.70; 17 species) in BLA was slightly lower than that in NF (H' = 3.35; 40 species) (Table 1). A comparison between the tree species richness in BLA of South Meghalaya recorded in this study with other agroforestry systems shows that tree diversity of BLA is significantly higher (94 tree species) than cocoa agroforestry in southern Cameroon (21 tree species), betel leaf agroforestry in Bangladesh (61 tree species) (Nath et al. 2003) and betel

nut agroforestry in South Meghalaya (83 tree species) (Tynsong and Tiwari 2010) but it is lower than that in the coffee farms in Veracruz, Mexico (Lopez-Gomez et al. 2008). Species richness for herb in BLA in South Meghalaya recorded in this study (48 species) is similar to cocoa agroforestry in south Cameron (48 herb species) (Sonwa et al. 2007) and slightly higher than the betel nut agroforestry in South Meghalaya (41 herb species) (Tynsong and Tiwari 2010). Tree species in BLA are more diverse as compared to traditional agroforestry in Dellomenna District, Southeastern Ethiopia (H' = 2.53 to 2.73) (Molla and Kewessa 2015), home garden in Thailand (H' = 0.9 to 2.7) (Gajaseni and Gajaseni 1999) and traditional agroforestry in Kerala in India (H' = 1.12 to 3) (Kumar et al. 1994).

Tree basal area in BLA (50.05 m^2ha^{-1}) was marginally less than NF (52.26) (Table 1). However, in comparison with other agroforestry systems, the BLA has higher tree basal area. For example, in cocoa agroforestry and mixed food crops agroforestry in Southeastern Ghana, the basal area was recorded at 8.4 m^2ha^{-1} and 8.2 m^2ha^{-1} respectively (Asase and Tetteh 2010). It is also higher than in cocoa plantations in Indonesia (11.9 to 20.5 m^2/ha) (Merijn et al. 2007) and in cocoa plantations in the southern province of Cameroon (29.7 to 42.6 m^2/ha) (Van Gemerden 2004). Our results suggest that a better stock of forest tree species was maintained in the betel leaf agroforestry than that in the natural forest of the area. We also observed that tree species such as *Trema polytoria*, *Macaranga denticulata*, *Macaranga peltata*, *Adenanthera pavonina*, *Ficus roxburghii* and *Wrightia tomentosa* were found only in BLA.

Furthermore, some light-demanding second-storey tree species such as *Trema polytoria*, *Macaranga denticulata*, and *Macaranga peltata* grew luxuriantly in BLA and were absent in NF due to the higher density of trees resulting in the lower sunlight. Higher herb species diversity in BLA may be attributed to the fact that it was dominated by light-demanding plants, specifically those belonging to Asteraceae, Rubiaceae, and Poaceae. Thus in BLA, the species composition of trees and herbs seem to be directly related to the availability of light.

Table 4. Number of plant species and their main uses in BLA in South Meghalaya, India

Main uses		No of plant species
Timber	High value	10
	Low value	11
Fuelwood	High value	20
	Low value	31
Edible		15
Medicinal		17
Tool		6
Ornamental		5
Craft		2
Packing Leaf		1
Latex Producing Plant		1
No Specific Use		40
Total		159

A similar finding was reported in traditional cocoa forest gardens by Bisseleua et al. (2008). The lower number of shrub species in BLA could be explained by the traditional management practices of BLA by local people, such as by the weeding out of shrubs growing close to betel leaf plants twice a year by farmers, so the betel leaf have sufficient space and nutrients to grow. Even though in BLA all tree species were maintained as supporting trees, we observed that high percentage of highly economical useful plant species were retained by purpose. The preference for multipurpose tree species is understandable in the context that the owners of the agroforestry depend on such plants for timber, food, medicine, and fuelwood (Tiwari et al. 2004). Motiur et al. (2006) also found that agroforestry in Bangladesh supply important forest products like fruit, fuelwood, timber and bamboo to meet household demands. Besides supporting trees, tree species were maintained mainly for timber, fuelwood, and edible stuff purposes. *Artocarpus heterophyllus*, *Cedrela toona*, *Duabanga grandiflora*, and *Schima wallichii* were preferred timber trees, while *Macaranga denticulata*, *Macaranga hypoleuca*, *Macaranga peltata*, *Quercus dealbata*, and *Quercus lanceofolia* were most preferred as fuelwood trees. A total of 17 medicinal plant species were recorded in BLAs. BLAs are also the habitat for 14 endemic and 6 rare plants. Thus, these manmade ecosystems contribute to biodiversity conservation, while at the same time they also provide goods and services to the local inhabitants.

In conclusion, the betel leaf agroforestry harbors plant diversity comparable to the natural forests and provides habitat for endemic and rare plant and animal species. The land-use change has a negligible impact on tree and herb diversity. However, it has a significant impact on density and diversity of shrub species. Betel leaf agroforestry in South Meghalaya is best-suited land use practice with minimal impact on plant diversity and forest community structure. We conclude that for a more robust study and conclusions regarding the impact of betel leaf agroforestry on plant diversity, further research needs to be carried out across the region.

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Table S1: Plant species, their families, density (individual ha^{-1}) recorded in Natural Forest (NF) and Betel Leaf Agroforest (BLA) of South Meghalaya, Indonesia

Tree species	Family	Frequency		Density		IVI	
		NF	BLA	NF	BLA	NF	BLA
Acer oblongum Wall.	Aceraceae	3.00	-	4	-	0.48	-
Actinodaphne abovata (Nees.) Blume.	Lauraceae	4.00	10.00	7	16	0.77	2.93
Actinodaphne angustifolia Nees.	Lauraceae	3.00	-	7	-	0.61	-
Adenanthera pavonina L.	Mimosaceae	-	40.00	-	44	-	6.39
Aesculus assamica Griff.	Hippocastanaceae	-	7.50	-	6	-	1.02
Aglaia perviridis Hiern.	Meliaceae	-	37.50	-	36	-	5.29
Alstonia scolaris Brown.	Apocynaceae	-	10.00	-	8	-	1.32
Amoora rohituka W&A.	Meliaceae	-	12.50	-	10	-	1.71
Antidesma khasianum Hk.f.	Euphorbiaceae	-	15.00	-	12	-	2.10
Aporosa dioica (Roxb.) Muell. Arg.	Euphorbiaceae	11.00	7.50	18	6	2.24	1.03
Ardisia floribunda Wall.	Myrsinaceae	16.00	-	42	-	4.48	-
Artocarpus heterophyllus Ham.	Moraceae	-	12.50	-	20	-	2.45
Artocarpus lakoocha Roxb.	Moraceae	-	40.00	-	36	-	7.01
Baccuarea sapida (Roxb.) Muell.	Euphorbiaceae	24.00	20.00	46	18	5.52	3.34
Bambusa tulda Roxb.	Poaceae	12.00	-	58	-	5.82	-
Bambusa vulgaris Schrad.	Poaceae	-	2.50	-	80	-	5.06
Beilschmiedia brandisii Hk f.	Lauraceae	-	7.50	-	6	-	1.78
Bischofia javanica Blume.	Euphorbiaceae	-	15.00	-	12	-	2.60
Bombax malabaricum DC.	Malvaceae	-	2.50	-	4	-	0.82
Brassaiopsis glomerulata (Blume.) Regel.	Araliaceae	-	7.50	-	14	-	1.90
Bridelia monta Willd.	Euphorbiaceae	-	20.00	-	16	-	2.67
Calicarpa vestica Wall. ex Cl.	Verbenaceae	-	12.50	-	10	-	1.66
Canthium glabrum Blume.	Rubiaceae	6.00	-	11	-	1.17	-
Caryota urens L.	Arecaceae	-	27.50	-	26	-	6.85
Casearia kurzii C.B.Clarke.	Pittosporaceae	-	10.00	-	8	-	1.50
Castanopsis hystrix A.DC.	Fagaceae	34.00	-	59	-	10.48	-
Castenea sativa Miller.	Fagaceae	19.00	-	48	-	8.50	-
Cedrela toona Roxb.	Meliaceae	-	7.50	-	6	-	1.28
Chrysophyllum rozburghii G.Don.	Sapotaceae	5.00	-	7	-	0.96	-
Cinnamomum bejolghota Buch.-Ham.	Lauraceae	-	20.00	-	20	-	3.27
Cinnamomum camphora F.Nees.	Lauraceae	9.00	-	14	-	1.71	-
Cinnamomum tamala Fr. Nees.	Lauraceae	21.00	10.00	36	12	4.86	2.24
Citrus latipes (Swingle.)Tanaka.	Rutaceae	6.00	-	6	-	1.05	-
Citrus macroptera Lour.	Rutaceae	-	2.50	-	2	-	0.36
Cryptocarya amygdalina Nees.	Lauraceae	12.00	15.00	20	12	2.76	2.16
Cryptocarya andersoni King.	Lauraceae	-	25.00	-	20	-	3.46
Cryptocarya floribunda Nees.	Lauraceae	-	5.00	-	6	-	1.30
Cyathea gigantea (Wall ex Hook.) Holttm.	Cyatheaceae	2.00	-	5	-	0.37	-
Daphniphyllum himalayense Muell.	Euphorbiaceae	5.00	-	18	-	1.72	-
Derris robusta Benth.	Fabaceae	-	7.50	-	10	-	1.39
Diospyros kaki L.	Sapotaceae	8.00	35.00	11	28	1.49	4.43
Diospyros pilosula Wall.	Ebenaceae	-	10.00	-	8	-	1.26
Diospyros sp.	Ebenaceae	-	10.00	-	8	-	1.43
Drimycarpus racemosus (Roxb.) Hk.f.	Anacardaceae	-	10.00	-	10	-	1.71
Duabanga grandiflora (Roxb. Ex DC.) Walp.	Sonneratiaceae	6.00	32.50	15	58	2.32	32.78
Dysoxylum hamiltoni Hiern.	Meliaceae	-	20.00	-	30	-	4.14
Ehretia acuminata Br.	Boraginaceae	7.00	10.00	10	8	1.32	1.47
Elaeocarpus lancifolius Roxb.	Elaeocarpaceae	-	7.50	-	6	-	1.13
*Elaeocarpus sikkimensis*Mast.	Elaeocarpaceae	-	17.50	-	32	-	4.59
Elaeocarpus lancifolius Roxb.	Elaeocarpaceae	11.00	-	22	-	2.58	-
Engelhardtia spicata Blume.	Jaglandaceae	5.00	7.50	7	14	0.94	1.80
Erythroxylon kunthianum Wall.	Malpighiaceae	7.00	-	10	-	1.34	-
Erythroxylum kunthianum Wall.	Malpighiaceae	7.00	-	25	-	2.39	-
Euonymus lawsonii Clarke & Prain.	Celastraceae	-	10.00	-	8	-	1.37
Eurya acuminata DC.	Theaceae	8.00	5.00	14	26	1.64	1.98
Ficus benjavina L. var.*comosa* Kurtz.	Moraceae	-	7.50	-	12	-	10.51
Ficus faveolata Wall.	Moraceae	4.00	10.00	13	12	1.21	1.83
Ficus gibbosa Blume.	Moraceae	-	12.50	-	10	-	1.60
Ficus glomerata Roxb.	Moraceae	-	27.50	-	92	-	10.99
Ficus hirta Vahl.	Moraceae	5.00	-	5	-	0.82	-
Ficus roxburghii Wall.	Moraceae	-	20.00	-	46	-	5.72
Ficus sp.	Moraceae	13.00		27		3.15	
Garcinia gummi-gutta (L.) Roxb.	Clusiaceae	-	5.00	-	4	-	0.72
Garcinea spicata Hk.f..	Guttiferae	5.00	-	13	-	1.38	-
Garcinia lanceolarium Dalz.	Guttiferae	7.00	-	12	-	1.73	-
Garcinia paniculata (G.Don) Roxb.	Guttiferae	13.00	-	17	-	2.42	-

Garuga pinnata Roxb.	Burseraceae	4.00	-	4	-	0.65	-
Glochidion khasicum Hk.f.	Euphorbiaceae	6.00	12.50	13	10	1.37	1.66
Glochidion thomsoni Hk.f.	Euphorbiaceae	-	10.00	-	10	-	1.39
Gynocardia odorata R.Br.	Flacourtiaceae	-	22.50	-	22	-	3.43
Helicia erratica Hk.f.	Proteaceae	40.00	2.50	52	2	8.76	0.35
Hydnocarpus kurzii Warb.	Flacourtaceae	-	7.50	-	6	-	1.16
Ilex embelioides Hk .f.	Aquifoliaceae	-	7.50	-	6	-	1.33
Itea macrophylla Wall.	Saxiferaceae	7.00	-	21	-	2.42	-
Knema andamanica Hk.f.	Myristicaceae	2.00	-	2	-	0.33	-
Knema linifolia (Roxb.) Warb.	Myristicaceae	8.00	27.50	9	32	1.40	4.79
Ligustrum robustum (Roxb.)Blume.	Oleaceae	8.00	7.50	8	6	1.30	1.17
Lithocarpus elegans (Blume.) Soep.	Fagaceae	48.00	10.00	133	8	30.80	1.38
Lithocarpus fenestatus (Roxb.) Rehder	Fagaceae	41.00	-	169	-	40.66	-
Litsea citrata Blume.	Lauraceae	8.00	-	11	-	1.53	-
Litsea elongata Wall.	Lauraceae	-	7.50	-	10	-	1.40
Litsea laeta Benth. Hk.f.	Lauraceae	-	7.50	-	6	-	1.06
Litsea leiantha (Kurz) Hk.f.	Lauraceae	3.00	-	5	-	0.60	-
***Litsea** semecarpifolia* (Wall. ex Nees) Hook.f.	Lauraceae	4.00	7.50	4	6	0.70	1.04
Macaranga denticulata Muell. Arg.	Euphorbiaceae	-	2.50	-	2	-	0.34
Macaranga peltata (Roxb.) Muell.Arg.	Euphorbiaceae	-	10.00	-	20	-	2.07
Machilus bombycina King.	Lauraceae	38.00	22.50	79	46	12.35	5.46
Macropanax undulatus (Wall.ex D.Don) Seem.	Araliaceae	-	22.50	-	34	-	4.82
Magaranga indica Wight.	Euphorbiaceae	9.00	-	13	-	1.74	-
Magnolia pterocarpa Roxb.	Magnoliaceae	2.00		4		0.49	-
Magnolia sp.	Magnoliaceae	14.00	5.00	42	4	4.61	0.83
Mallotus ferrugineus Roxb.	Euphorbiaceae	-	20.00	-	20	-	2.90
Mesua ferrea L.	Clusiaceae	-	2.50	-	2	-	0.50
Michelia cathcartii Hk.f.&Th.	Magnoliaceae	-	25.00	-	20	-	3.58
Morus laevigata Wall.	Moraceae	-	25.00	-	22	-	3.87
Musa paradisiaca L.	Musaceae	-	12.50	-	12	-	2.30
Myrica esculenta Buch-Ham.ex D.Don.	Myricaceae	9.00	-	11	-	1.61	-
Myrica nagi Thunb.	Myricaceae	4.00	-	7	-	0.81	-
Oroxyrum indicum Vent.	Bignoniaceae	14.00	10.00	25	8	3.82	1.45
Ostodes paniculata Blume.	Euphorbiaceae	2.00	12.50	4	12	0.33	2.14
Pandanus odoratissimus L.	Pandanaceae	5.00	-	14	-	1.59	-
Phoebe cooperiana U.N.Kanjilal ex A.Das.Nov.sp.	Lauraceae	17.00	30.00	24	70	3.61	8.70
Phoebe lanceolata Nees.	Lauraceae	9.00	10.00	17	12	2.52	1.82
Pinus khasya Royle.	Pinaceae	1.00	-	4	-	0.16	-
Pittosporum glabratum Lindl.	Pittosporaceae	-	12.50	-	12	-	1.74
Pterospermum acerifolium Willd.	Sterculiaceae	-	2.50	-	2	-	0.85
Quercus dealbata Hk.f.&Th.	Fagaceae	8.00	-	24	-	3.06	-
Quercus dilatata Lindle.	Fagaceae	12.00	-	46	-	5.50	-
Quercus lanceaelia Roxb.	Fagaceae	-	12.50	-	10	-	1.54
Quercus lanceifolia Roxb.	Fagaceae	29.00	-	67	-	10.51	-
Quercus spicata Smith.	Fagaceae	16.00	5.00	53	6	6.02	0.98
Rhododendron arboreum Sm.	Ericaceae	1.00	-	5	-	0.21	-
Sapium baccatum Roxb.	Euphorbiaceae	6.00	7.50	12	6	2.27	3.80
Sapium insigne Benth.	Euphorbiaceae	7.00	25.00	9	30	1.61	5.13
Saprosma ternatum Hk.f.	Rubiaceae	-	12.50	-	14	-	1.98
Saraca indica L.	Caesalpiniaceae	-	12.50	-	10	-	1.71
Sarcosperma griffithii Benth.	Sapotaceae	51.00	57.50	106	132	16.58	16.36
Schima walichii Choisy.	Theaceae	22.00	10.00	64	8	11.66	1.66
Sterculia roxburghii Wall.	Sterculiaceae	3.00	12.50	6	10	0.54	1.81
Streospermum chelonoides (L.f.) DC.	Bignoniaceae	4.00	22.50	6	38	0.65	4.41
Styrax serrulatum Roxb.	Styraceae	14.00	10.00	25	10	3.06	1.44
Symplocos ramosissima Wall.	Symplocaceae	7.00	-	12	-	1.54	-
Tetrameles nudiflora R.Br.	Tetramelaceae	-	2.50	-	2	-	1.44
Toona ciliata Roem.	Ochnaceae	12.00	32.50	19	26	2.46	6.86
Travesia palmata (Roxb.) Vis.	Araliaceae	27.00	-	37	-	6.02	-
Trema polytoria Planch.	Ulmaceae	-	17.50	-	18	-	2.74
Unidentified1	Unidentified	-	15.00	-	16	-	3.01
Unidentified2	Unidentified	16.00	-	32	-	4.20	-
Unidentified3	Sapotaceae	-	15.00		16		2.18
Unidentified4	Unidentified	6.00	-	21	-	1.20	-
Unidentified5	Guttiferae	9.00	-	17	-	2.24	-
Unidentified6	Unidentified	2.00	-	6	-	0.52	-
Villebrunea intigrifolia Gaud.	Urticaceae	-	10.00	-	16	-	1.93
Wenderhardia tinctoria DC.	Rubiaceae	18.00	15.00	37	16	4.84	2.30
Wendlandia paniculata DC.	Rubiaceae	-	7.50	-	16	-	1.62
Wrightia tomentosa Roem & Sch.	Apocynaceae	-	32.50	-	76	-	8.19
Syzygium tetragonum (L.) Skeels.	Myrtaceae	42.00	2.50	86	2	12.50	0.36

Total		-	-	**1972**	**1788**	**300**	**300**
Shrub component							
Adenosaeme sp.	Rubiaceae	20.00	-	260	-	3.70	-
Ardisia griffithii C.B.Clarke.	Myrsinaceae	10.00	-	80	-	1.59	-
Ardisia sp.	Myrsinaceae	10.00	-	140	-	1.90	-
Boehmeria malabarica Wedd.	Urticaceae	10.00	10.00	180	1005	2.11	25.10
Calamus arborescence Griff.	Arecaceae	-	8.00	-	1480	-	28.01
Camellia caduca C.B. Clarke.	Theaceae	40.00	-	340	-	6.47	-
Cephalostachyium sp.	Poaceae	10.00	-	400	-	3.25	-
Cephalostachyum pallidum Munro.	Poaceae	15.00	-	280	-	3.22	-
Chloranthus glaber Thunb.	Chloranthaceae	35.00	-	1000	-	9.30	-
Clerodendron serratum Spreng.	Verbenaceae	55.00	3.00	2020	100	16.95	4.23
Coffea benghalensis Roxb.	Rubiaceae	20.00	-	360	-	4.22	-
Coffea sp.	Rubiaceae	25.00	-	480	-	5.43	-
Cyathea albosetacea Copel.	Cyatheaceae	35.00	-	440	-	6.40	-
Daphne involucrata Wall.	Thymelaeaceae	20.00	-	240	-	3.60	-
Daphne involucrata Wall.	Thymeleaceae	-	4.00	-	120	-	6.16
Dracaena fragrans (L.) Ker-Gawl.	Agavaceae	-	3.00	-	112	-	4.39
Eupatorium odoratum Spreng.	Asteraceae	-	3.00	-	280	-	6.27
Eurya japonica Thunb.	Theaceae	45.00	-	1400	-	12.56	-
Ficus clavata Wall.	Moraceae	5.00	-	40	-	0.80	-
Ficus hirta Vahl.	Moraceae	5.00	-	40	-	0.80	-
Ficus pyriformis Hook. &Arn.	Moraceae	5.00	-	20	-	0.69	-
Ficus sarmentosa Wall.	Moraceae	5.00	3.00	80	120	1.00	4.29
Flemingia macrophylla (Willd.) Prain.	Fabaceae	20.00	-	160	-	3.18	-
Goniothalamus sesquipidalis Hk.f.&Th.	Annonaceae	10.00	2.00	120	76	1.80	3.16
Hedychium coccineum Buch.-Ham.ex Sm.	Zingiberaceae	5.00	-	40	-	0.80	-
Hedychium thyrsiforme Buch.-Ham.ex Sm.	Zingiberaceae	20.00	6.00	340	401	4.12	12.38
Ixora subsissillis Wall.	Rubiaceae	20.00	7.00	-	340	-	12.09
Justicia sp.	Acanthaceae	15.00	-	560	-	5.26	-
Laportea crenulata (Roxb) Gaud.	Urticaceae	15.00	7.00	180	260	2.70	11.05
Luculia pinceana Hook.	Rubiaceae	5.00	3.00	20	123	0.69	5.01
Mahonia pycnophylla Fedde.	Berberidaceae	5.00	-	60	-	0.90	-
Medinilla rubicunda Blume.	Melastromaceae	5.00	-	100	-	1.11	-
Morinda angustifolia Roxb.	Rubiaceae	45.00	9.00	440	400	7.58	15.01
Mussaendra roxburghii Hk.f.	Rubiaceae	5.00	9.00	80	339	1.00	14.11
Myrioneuron nutans Kurtz.	Rubiaceae	20.00	5.00	640	224	5.67	8.03
Ophiorhiza hispida Hook.f.	Rubiaceae	10.00	-	160	-	2.01	-
Phrynium capitatum Willd.	Marantaceae	20.00	10.00	1020	860	7.64	8.01
Phrynium sp.	Marantaceae	35.00	-	980	-	9.20	-
Phyllanthus debilis Ham.	Euphorbiaceae	10.00	-	120	-	1.80	-
Pinanga cracilia (Roxb.) Blume.	Arecaceae	35.00	-	1200	-	10.34	-
Psychotria erratica Hk.f.	Rubiaceae	60.00	-	2140	-	18.16	-
Rubus khasianus Cordat.	Rosaceae	5.00	-	20	-	0.69	-
Sylvianthus bracteotus Hk.f.	Rubiaceae	-	2.00	-	120	-	3.08
Thysanolaena maxima (Rozb.)O.Ktze.	Poaceae	35.00	4.00	1680	600	12.83	19.07
Unidentified	Unidentified	20.00	-	760	-	6.29	-
Wallichia densiflora Mart.	Arecaceae	55.00	2.00	540	700	9.27	11.31
Zanthoxylum armatum DC.	Rutaceae	20.00	-	120	-	2.98	-
Total		**-**	**-**	**19280**	**7660**	**200**	**200**
Herb component							
Achyranthes japonica (Miq.) Nakai.	Amaranthaceae	29.37	14.38	15250	7938	9.80	4.01
Ageratum conzynoides L.	Asteraceae	-	10.63	-	11313	-	4.25
Ananas bracteatus (Lindl.) Schult. & Schult. f.	Bromeliaceae	-	6.88	-	2563	-	1.63
Ananas comosus (L.) Merr.	Bromeliaceae	-	12.50	-	6626	-	3.42
Andropogon glomeratus Walt.	Poaceae	-	13.75	-	7375	-	3.78
Anotis wightiana Hk.f.	Rubiaceae	-	3.75	-	1500	-	0.91
*Anthyrium drepanopterum (*Kuntze.) A.Brown.	Anthyriaceae	8.75	16.25	6188	15250	3.82	6.01
Begonia josephii A.DC.	Begoniaceae	16.88	20.63	7375	10625	5.23	5.57
Begonia palmata D.Don.	Begoniaceae	6.88	-	4650	-	2.83	-
Begonia picta Sm.	Begoniaceae	-	12.50	-	6313	-	3.35
Bidens pilosa (Blume.) Sherff.	Asteraceae	-	16.25	-	17250	-	6.49
Bolbitis appendiculata J.Sm.	Lomariosidaceae	12.50	15.63	10290	8876	5.85	4.42
Borreria articularis (L.f.) F.N. Will.	Rubiaceae	10.63	-	6885	-	3.93	-
Borreria pilosa K.Schum	Rubiaceae	11.25	24.38	7635	20625	3.98	8.49
Calanthe masuca (D.Don) Lindl.	Orchidaceae	18.75	-	8280	-	5.83	-
Carex vesiculosa Booth.	Cyperaceae	-	48.75	-	53000	-	19.75
Centella asiatica (L.) Urban.	Apiaceae	-	10.63	-	16563	-	5.49
Commelina beghalensis L.	Commelinaceae	6.88	20.00	6998	11563	3.76	5.70

Costus speciosus (koenig) Smith.	Zingiberaceae	8.13	-	4063	-	2.38	-
*Crassocephalum crepioides (*Benth.)Moore.	Asteraceae	-	21.88	-	12000	-	6.08
Crotalaria ferruginea Garh.	Fabaceae	6.88	-	5125	-	2.45	-
Curculigo orchioides Gaertn.	Hypoxidaceae	15.63	31.88	10875	29750	6.71	11.76
Cyperus compressus L.	Cyperaceae	-	5.63	-	1875	-	1.28
Cyperus flavidus Retz.	Cyperaceae	35.00	-	34625	-	16.41	-
Cyperus odoratus L.	Cyperaceae	16.88	-	7813	-	5.35	-
Drymaria cordata (L.) Roem. & Schult.	Caryophyllaceae	-	6.88	-	3875	-	1.94
Dryopteris sp.	Dryopteridaceae	20.63	-	19750	-	9.48	-
Elatostema sesquifolium Hassk.	Urticaceae	6.25	-	5063	-	2.32	-
Elatostemma sessile Forst.	Urticaceae	15.00	15.63	7000	7625	4.78	4.12
Eleusine indica Gaertn.	Eragrosteae	-	15.00	-	4500	-	3.29
*Eragrostis gangetica (*Roxb.) Steud.	Poaceae	11.88	-	7563	-	4.36	-
Eriocaulon cristatum Mast.	Eriocaulaceae	8.75	-	6875	-	3.59	-
Ficus pumila L.	Moraceae	11.88	-	7563	-	4.36	-
Galingsoga parviflora Cay.	Asteraceae	-	8.13	-	12125	-	4.07
Gentiana tenella Fries.	Gentianaceae	18.13	-	11625	-	6.68	-
Globba clarkii Baker.	Zingiberaceae	9.38	-	4563	-	3.04	-
Globba racemosa Sm.	Zingiberaceae	15.00	-		10813	-	4.78
Globba sp.	Zingiberaceae	10.00	-	5688	-	3.48	-
Goodyera repens (Ker-Gawl.) Hook.	Orchidaceae	6.88	-	1750	-	1.77	-
Hemiphragma heterophyllum Wall.	Scrophulariaceae	-	13.13	-	8813	-	4.03
Hydrocotyle javanica Thunb.	Apiaceae	7.50	13.75	2813	6125	2.29	3.49
Impatiens balsamina L.	Balsaminaceae	-	12.50	-	4000	-	2.80
Impatiens benthamii V.Steenis.	Balsaminaceae	2.50	25.00	770	12375	0.78	6.63
Impatiens tripetala DC.	Balsaminaceae	9.38	27.50	3388	11188	2.64	6.73
Imperata cylendrica (L.)P.Beauv.	Poaceae	-	7.50	-	1938	-	1.57
Ipomea purpurea (L.) Roth.	Convolvulaceae	-	9.38	-	4688	-	2.50
Justicia sp.	Acanthaceae	-	5.63	-	1500	-	1.19
Kaulina pteropus (Blume) Nayar.	Polypodiaceae	-	6.25	-	1938	-	1.39
Knoxia corymbosa Willd.	Rubiaceae	16.25	-	9750	-	5.80	-
Miconia prasina (SW.) DC.	Melastromaceae	9.38	-	3375	-	2.70	-
Onoclea sensibilis L.	Onocleaceae	-	10.63	-	4875	-	2.73
Ophiorrhiza hispida Hk.f.	Rubiaceae	12.50	-	5938	-	4.01	-
Ophiorrhiza hispida Hk.f..	Rubiaceae	-	14.38	-	8063	-	4.04
Oplismenus compositus P.Beauv.	Paniceae	10.00	12.50	3188	4250	2.76	2.86
Osbekia capitata Benth.	Melastromaceae	5.00	-	5438	-	2.33	-
Oxalis corniculata L.	Oxalidaceae	8.13	-	7500	-	2.50	-
Paspalum dilatatum Pior.	Poaceae	13.13	17.50	13063	5250	6.17	3.84
Peperomia heyneana Miq.	Piperaceae	-	6.25	-	2500	-	1.52
Peperomia reflexa A.Dietr.	Piperaceae	14.38	-	6000	-	4.37	-
Phryma leptostachya L.	Verbenaceae	-	6.88	-	4375	-	2.05
Polygonum hydropiper L.	Polygonaceae	-	10.00	-	4125	-	2.46
Polygonum thunbergii Sieb. & Zucc.	Polygonaceae	-	18.13	-	7374	-	4.43
Polystichum aculeatum (L.)Roth.	Aspidiaceae	-	20.00	-	8500	-	4.98
Pronephrium nudatam (Roxb)Holttum.	Thelypteridaceae	-	3.75	-	2813	-	1.22
Pteris quadriaurita Retz.	Pteridaceae	-	3.13	-	4500	-	1.53
Richardia brasiliensis Gomes.	Rubiaceae	-	6.88	-	3875	-	1.94
Selaginella chrysocaulos Hk. et. Grev.) Spring.	Selaginellaceae	11.25	-	8663	-	4.91	-
Sida cordifolia L.	Malvaceae	-	6.88	-	5751	-	2.38
Smithea ciliata Royle.	Fabaceae	18.13	-	16475	-	8.97	-
Sonerila khasiana Clarke.	Melastromaceae	21.25	-	9895	-	6.99	-
Sonerilla khasiana Dyer.	Melastromaceae	8.13	-	5090	-	2.94	-
Spilanthes paniculata DC.	Asteraceae	5.63	14.38	6188	5564	1.95	3.45
Srobilanthus sp.	Acanthaceae	15.63	-	10813	-	5.99	-
Tacca laevis Roxb.	Taccaceae	-	9.38	-	4115	-	2.37
Torenia diffusa D.Don.	Scrophulariaceae	-	14.38	-	10188	-	4.54
Trillium erectus L.	Liliaceae	5.63	-	3974	-	1.85	-
Urena labata L.	Malvaceae	-	10.63	-	5062	-	2.77
Viola palmaris Ging.	Violaceae	3.13	-	560	-	0.78	-
Viola sikkimensis W.Becker	Violaceae	6.25	-	3650	-	1.82	-
Zingiber rubens Roxb.	Zingiberaceae	12.50	-	6750	-	4.24	-
Zingiber sp.	Zingiberaceae	4.38	-	790	-	1.02	-
Total		-	-	**347563**	**423688**	**200**	**200**

Note: '-' : indicates absent

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Nutrient distribution on soil and aboveground biomass of *Macaranga gigantea* five years after planting

DWI SUSANTO[1,♥], RATNA KUSUMA[1], RUDIANTO AMIRTA[2]
[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University. Jl. Barong Tongkok No. 4, Gunung Kelua, Samarinda Ulu, Samarinda-75123, East Kalimantan, Indonesia. Tel./fax.: +62-541-749140, 749152, 749153, ♥email: susantodwiki@yahoo.com
[2]Faculty of Forestry, Mulawarman University. Jl. Ki Hajar Dewantara, Kampus Gunung Kelua, Samarinda 75123, East Kalimantan, Indonesia

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Abstract. *Susanto D, Kusuma R, Amirta R. 2018. Nutrient distribution on soil and aboveground biomass of* Macaranga gigantea *five years after planting. Asian J For 2: 12-19.* Despite the high potentials of *Macaranga gigantea* to be developed as biofuel plantation, there is limited understanding of the growth of this species especially when it is planted as uniform stand and treated with silvicultural application. This research aimed to investigate the growth and accumulation of aboveground biomass of *M. gigantea* in varying fertilization treatments five years after planting, and to evaluate the distribution of nutrients in soil and aboveground biomass. Five treatments at randomized design were established with each treatment having three blocks, and each block contained 20 plants, making 300 plants in total. The five treatments of *M. gigantea* were the application of NPK fertilizer at five measures, i.e.: P0: 0 g, P1: 40 g, P2: 80 g, P3: 120 g and P4: 160 g. Aboveground biomass and nutrient contents of N, P, K, Ca and Mg accumulated the plants were measured across the treatments, while soil sampling was conducted to analyze the nutrients accumulated in the soil. The research findings revealed that treatment P4 had the best growth performance with accumulation of N, P, K, Ca and Mg in wood, bark, branches, and leaves twice as higher than those in treatment P0. The most distributed nutrients in the soil were magnesium, nitrogen, calcium, and phosphorus. Whereas the most accumulated nutrient in plant was potassium. The relative portion of K stored in the soil is quite small (44.18%) while K accumulated in plant was 55.82%. These findings imply that if *M. gigantea* plantation is harvested at five years rotation, it needs to give attention to the availability of potassium nutrients for the next planting cycles.

Keywords: Biomass, *Macaranga gigantea*, distribution nutrient, soil

INTRODUCTION

Macaranga gigantea is a pioneer and fast-growing tree species widely distributed in lowland of tropical rainforest, usually growing within the gaps after shifting cultivation (Susanto et al. 2016b), forest fires (Silk 2008) and timber harvestings (Susanto et al. 2017a). The plant reproduces by forming flower buds initiated in the dry season and the fruits ripened in the rainy season (Bentos et al. 2008).

Because of the rapid growth and high accumulation of biomass, *M. gigantea* has the potentials to be developed as raw source for bioethanol by establishing large-scale plantation forestry (Amirta et al. 2016). So far, only limited number of tree species have been developed as biofuel in the tropical region with several of them are recognized as non-native species. The hindrance of such development is mainly due to the limited understanding of the early growth of *M. gigantea* especially when it is planted as uniform stand compared to when it grows naturally. The lack of knowledge is particularly eminent from the seedling development stage into juvenile age.

The seeds of *M. gigantea* are classified as orthodox seeds and have low water content. The seeds that fell under the tree are germinating in approximately 24 days (Susanto et al. 2016a). Susanto et al. (2016a) reported that wet extraction without fruit drying treatment is able to increase seeds germination to 65%) with germination time first seeds (GTFS) 7.67 ± 1.15 days, germination time last seeds (GTLS) 17.33 ± 4.04, and mean germination times (MGT) 11.97 ± 1.93 days. However, previous studies demonstrated that additional seed treatments do not necessarily increase germination rate. Using dry seed extraction treatment, the seeds produce low seed germination rate of 2-10% (Suita and Nurhasiby 2009), while soaking the seeds into 0.2% potassium nitrate solution for 20 minutes before germinating on sand medium is only able to increase seed germination rates up to 20% (Mindawati et al. 2010). During the germination stage, *M. gigantea* seedlings showed the highest relative growth rate when planted on mushroom spawn waste media, followed by compost, topsoil, and sand media (Susanto et al. 2016a).

The experimental planting of *M. gigantea* seedlings in the field also showed mixed results. Lawrence (2001) reported that the seedlings of *M. gigantea* grew rapidly over the first of 18 weeks when planted in polybags enough supplied with a combination of nitrogen and phosphorus fertilizers. While, Nussbaum et al. (1995) found that nutritional deficiency is an important factor that inhibits early growth *M. gigantea* at the age 6 months after planting in degraded land of unused log piles in Malaysia.

Susanto et al. (2016b) reported that in secondary forests after shifting cultivation, *M. gigantea* plants accumulated phosphorus and potassium nutrient mostly in the leaves. On the other hand, in secondary forests after selective logging, most accumulated nutrients in *M. gigantea* were potassium, calcium, and magnesium. It suggested that bases nutrients,

potassium, calcium, and magnesium are by *M. gigantea* and extremely important to its growth (Susanto et al. 2017a). In monoculture system, *M. gigantea* was fertilized with NPK at the age of 1 year, the most nutrient element accumulated were potassium, followed by phosphorus and then nitrogen (Susanto et al. 2017b).

There is a lack information about aboveground biomass of *M. gigantea* treated with silvicultural application in relation to nutrient content stored in the soil. This research aimed to investigate the growth and accumulation of aboveground biomass of *M. gigantea* in varying fertilization treatments five years after planting, and to evaluate the distribution of nutrients in soil and aboveground biomass. This information is very important as reference for the development of *M. gigantea* plantation.

MATERIALS AND METHODS

Experimental site description

This research was conducted at the research forest of Faculty of Forestry, Mulawarman University, Samarinda, East Kalimantan, Indonesia (000.44.71,11" South and 1170.21; 67,50" East). The experimental plot had an extent of 0.7 ha, consisting planting trial of *M. gigantea* at the age of 5 years old. The trial was set up with five randomized treatments plots, each treatment had three blocks, and each block contained 20 plants, making 20 x 5 x 3 = 300 plants in total. The five treatments of *M. gigantea* were the application of NPK fertilizer at five measures, i.e.: P0: 0 g, P1: 40 g, P2: 80 g, P3: 120 g and P4: 160 g. Fertilization was conducted two times, i.e., directly after planting and 6 months after planting. Weed cleaning was done once for 4 months until the plant age was 2 years old (Susanto et al. 2017b).

Procedures

Plant measurement and soil analysis

The samples of the five-year-old *M. gigantea* were observed through direct measurement. The stem heights, diameter, height increment and diameter increment were the parameters in this research. For soil analysis, soil samples were taken at a depth of 0-30 cm and 30-60 cm at each plot. Soil analysis was conducted after the samples were oven-dried with a temperature of 150°C until the weight was constant. Composite sample was wind-dried and its total Nitrogen (Kjeldahl), available phosphorus (Bray), available potassium, calcium, and magnesium were analyzed (Susanto et al. 2017b).

Measuring Macaranga gigantea *biomass*

Biomass measurements were limited only to above-ground biomass of the average tree after stratification method. The determination of strata boundaries for sample selection was performed using cumulative method. The trees sampled from one plot were selected after all the available trees were grouped into three stages based on their estimated size according to the formula D^2H (diameter squared multiplied by height).

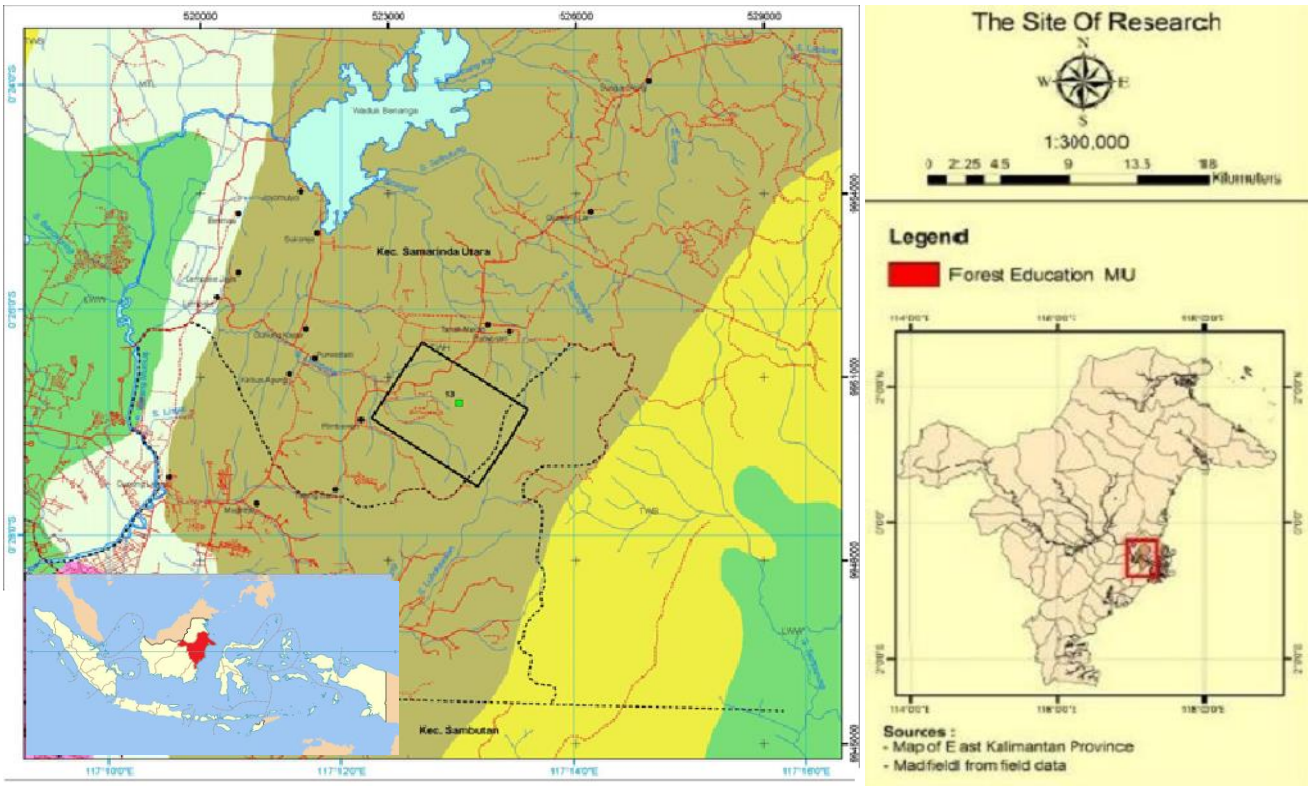

Figure 1. Map of the experimental site in the research forest of Faculty of Forestry, Mulawarman University, Samarinda, East Kalimantan, Indonesia

The entire stand biomass was calculated by multiplying the dry weight of the components of the sampled tree by the number of trees in each stage, and then it was converted into a biomass per hectare. The wet weight of every component including wood, barks, branch, and leaves was measured according to Ruhiyat (1996) methods. Samples of the wood, barks, branch, and leaves were weighed in wet and dry condition. The plant samples were taken to the laboratory and their nutrient contents (N, P, K, Ca, Mg) were analyzed.

Measuring nutrients in plant

The total N concentration was measured using Kjeldahl method (extraction, distillation, titration). To measure element of P and K, the plant components were extracted using High-Pressure Digestion method at the temperature of 180°C for 10 hours with HNO3 as a reductant. Phosphorus was measured by calorimetric technique using nitrate-molybdate-vanadate acid as a coloring agent, and was measured using spectrophotometer at the wavelength of 470 nm. Potassium, calcium, and magnesium were measured using Atomic Absorption Spectrophotometer at the wavelength of 766.5 nm, 489.5 nm, and 245.2 nm. To calculate macronutrient elements (N, P, K, Ca, Mg) that were accumulated in the three components in the stand, the dry weight of the tree component was multiplied by its nutrient concentration (Susanto et al. 2017b).

Data analysis

Nutrient distribution in soil and in the *M. gigantea* plants at the five-year-old was observed. The content of macronutrients (N, P, K, Ca, Mg) in the plant parts were calculated by multiplying the dry weight of the part with nutrient concentration. Based on the nutrients stored in the soil and the nutrient accumulated in the *M. gigantea* stands, it can be obtained information about the number of nutrients stored in the soil (kg.ha-1) and its relative portion (%) accumulated in the plant.

RESULTS AND DISCUSSION

Plant growth

At the age of 5 years, the best growth of *M. gigantea* is shown by the treatment P4 with mean stem diameter of 12.88 ± 4.2 cm, stem height of 10.25 ± 2,9 m, increment diameter 2.58 $cm.y^{-1}$ and hight increment 2.05 $m.y^{-1}$ (Figure 2).

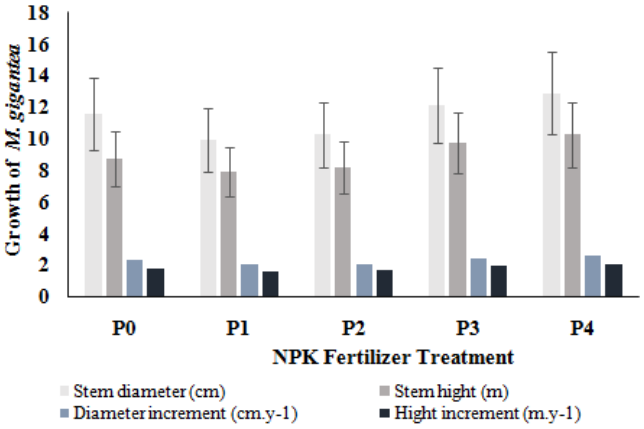

Figure 2. The growth parameters of *Macaranga gigantea* across five different treatments at the age of five years old.

Figure 3. The stand of *Macaranga gigantea* plants at planting plots at the age of five years

Plant biomass

The result on aboveground biomass estimation of *M. gigantea* showed that the highest biomass was produced by treatment P4 with 64.51 ton.ha^{-1}, comprising 27.58 tons stored in main stem, 5.078 tons in bark, 22.938 tons in branch (wood and bark), and 8.914 tons in leaves. Woody biomass had the largest biomass component with 50.52 ton.ha^{-1}, consisting of 27.58 tons stored in the stem and 22.938 tons in the branches. The lowest biomass was produced by treatment P0 with 25.039 ton.ha^{-1}. Based on this result, it can be concluded that fertilization of 160 g per plant (P4) increases biomass production by 250 percent.

Nutrient content in plant components

Potassium was the most accumulated nutrient in the five-year-old stands, followed by nitrogen, calcium, phosphorus, and magnesium. The amount of nutrients absorbed by *M. gigantea* stands were potassium, ranged from 239.88 to 676.20 kg.ha^{-1} with an average of 413.50 kg.ha^{-1}; calcium, ranged from 66.51 to 114.56 kg.ha^{-1} with an average of 232.62 kg.ha^{-1}; nitrogen, ranged from 155.91 to 344.00 kg.ha^{-1} with an average of 232.62 kg.ha^{-1}; and magnesium, ranged from 36.09 to 77.64 kg.ha^{-1} with an average of 51.93 kg.ha^{-1}. The highest concentration of nitrogen, phosphorus, potassium, calcium and magnesium occurred at treatment P4 with fertilization of NPK at 160 g per tree (Table.1).

Soil nutrient content

Soil nutrient concentration at treatment plot varied in value. There is an increasing tendency of dosage of NPK fertilizer to decrease soil nutrient concentration at each treatment plot. In terms of N, the highest concentration at the depth of 0-60 cm was at the P2 plot with 13405.4 kg.ha^{-1}, followed by the P1 plot with 12744.5 kg.ha^{-1}, P3 plot with 12210.7 kg.ha^{-1}, plot P0 with 12136. 5 kg.ha^{-1} and the lowest was at plot P4 with 10888.7 kg.ha^{-1}. In terms of P, the highest concentration at the depth of 0-60 cm was at the plot P0 with 441.3 kg.ha^{-1}, followed by plot P1 with 435.4 kg.ha^{-1}, plot P2 with 386.5 kg. ha^{-1}, plot P4 with 377.4 kg.ha^{-1} and the lowest was at plot P3 with 291.8 kg.ha^{-1}. The highest K nutrient occurred at plot P0 with 1034.2 kg.ha^{-1}, followed by plot P2 with 603.7 kg.ha^{-1}, plot P1 with 573.9 kg.ha^{-1}, plot P4 with 535.2 kg.ha^{-1} and the lowest was at plot P3 with 448.8 kg.ha^{-1}. The highest Ca occurred at plot P0 with 26953 kg.ha^{-1}, followed by plot P1 with 26232 kg.ha^{-1}, plot P3 with 25085 kg.ha^{-1}, plot P2 with 23630 kg. ha^{-1} and the lowest was at plot P4 with 21963 kg.ha^{-1}. While the concentration of Mg was the highest at plot P0 with 51292 kg.ha^{-1}, followed by plot P1 with 48111 kg.ha^{-1}, plot P2 with 45603 kg.ha^{-1}, plot P3 with 39800 kg.ha^{-1} and the lowest was at plot P4 with 38520 kg.ha^{-1}.

The nutrient content in *M. gigantea* is positively correlated with biomass production at 5 years of age. The higher the nutrient content of biomass the higher the biomass production (Figure 5). On the other hand, the relationship between soil nutrient content and biomass production shows negative correlation, i.e., the higher the biomass production, the lower nutrient content in the soil (Figure 6). The same is also shown in the relationship between soil nutrient and nutrient content in plant (Figure 7).

Nutrients stored in the soil and nutrients accumulated in aboveground biomass

The nutrient content stored in the soil and nutrients accumulated in *M. gigantea* is shown in Figure 8.

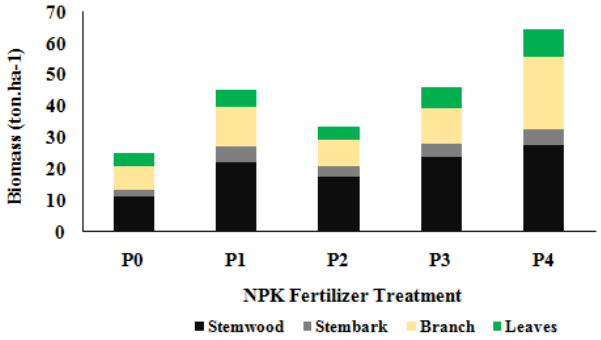

Figure 4. The distribution of aboveground biomass stored in plant components in *Macaranga gigantea* (five-year-old) across five different treatments.

Table 1. Nutrient content stored in plant components in *Macaranga gigantea* (five-year-old) across five different treatments

Nutri-ents	Fertilizer appli-cation	Nutrient content of plant components (kg.ha^{-1})				
		Stem wood	Stem bark	Branch	Leaf	Total
N	P0	39.48	13.16	33.81	69.48	155.92
	P1	63.59	24.18	44.05	91.74	223.56
	P2	48.75	21.40	35.50	70.69	176.34
	P3	65.28	23.92	53.96	120.13	263.29
	P4	79.16	24.74	89.54	150.57	344.01
Mean		**59.25**	**21.48**	**51.37**	**100.52**	**232.63**
P	P0	6.08	1.30	7.07	6.46	20.91
	P1	4.94	1.34	3.35	8.17	17.79
	P2	2.16	1.35	5.79	6.95	16.23
	P3	3.47	1.99	8.52	8.53	22.52
	P4	11.72	3.14	14.79	13.39	43.04
Mean		**5.67**	**1.82**	**7.90**	**8.70**	**24.10**
K	P0	69.86	28.80	66.17	75.06	239.89
	P1	165.96	32.47	104.23	97.07	399.72
	P2	57.04	40.48	104.65	86.63	288.79
	P3	144.57	49.58	145.97	122.81	462.93
	P4	198.95	66.95	240.08	170.23	676.20
Mean		**127.28**	**43.66**	**132.22**	**110.36**	**413.51**
Ca	P0	27.08	13.12	14.20	12.11	66.51
	P1	11.78	28.02	20.97	15.06	75.83
	P2	22.36	16.85	11.27	13.07	63.55
	P3	14.91	24.21	20.94	16.59	76.65
	P4	30.47	17.87	40.16	26.07	114.57
Mean		**21.32**	**20.01**	**21.51**	**16.58**	**79.42**
Mg	P0	14.46	4.50	8.318	8.82	36.10
	P1	18.99	11.47	12.66	12.57	55.69
	P2	16.44	6.81	10.79	10.52	44.55
	P3	13.97	6.82	11.18	13.72	45.69
	P4	25.17	8.49	25.80	18.19	77.64
Mean		**17.81**	**7.62**	**13.75**	**12.76**	**51.93**

Note: P0 = control without fertilizer, P1 = NPK fertilizer 40 g, P2 = NPK fertilizer 80 g, P3 = NPK fertilizer 120 g and P4 = NPK fertilizer 160 g.

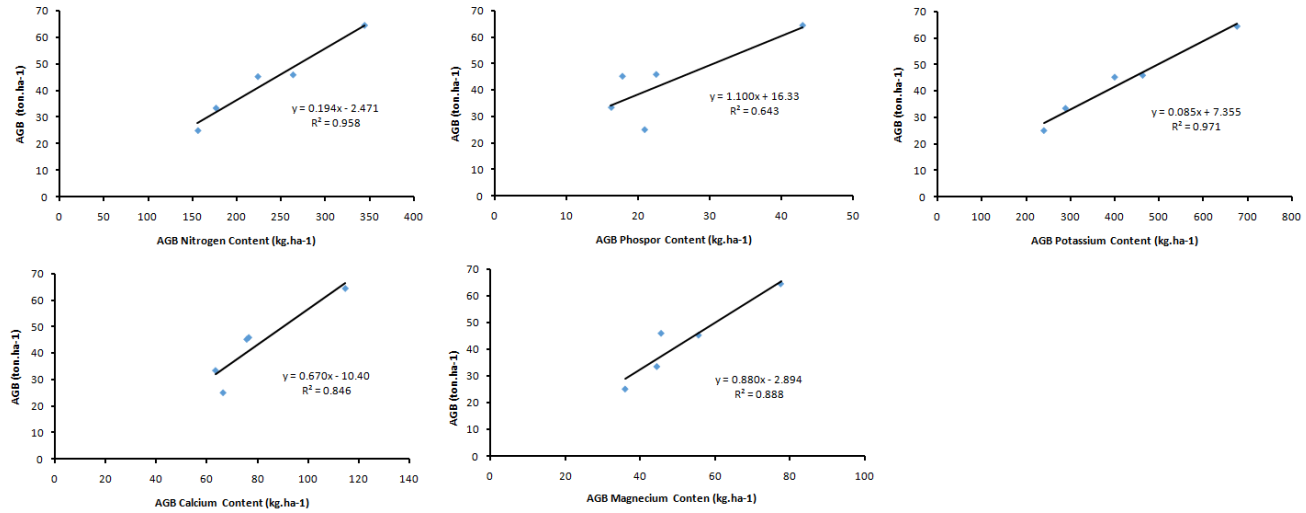

Figure 5. Corelation between aboveground biomassa and nutrient content stored in the plant of *Macaranga gigantea* at five years old

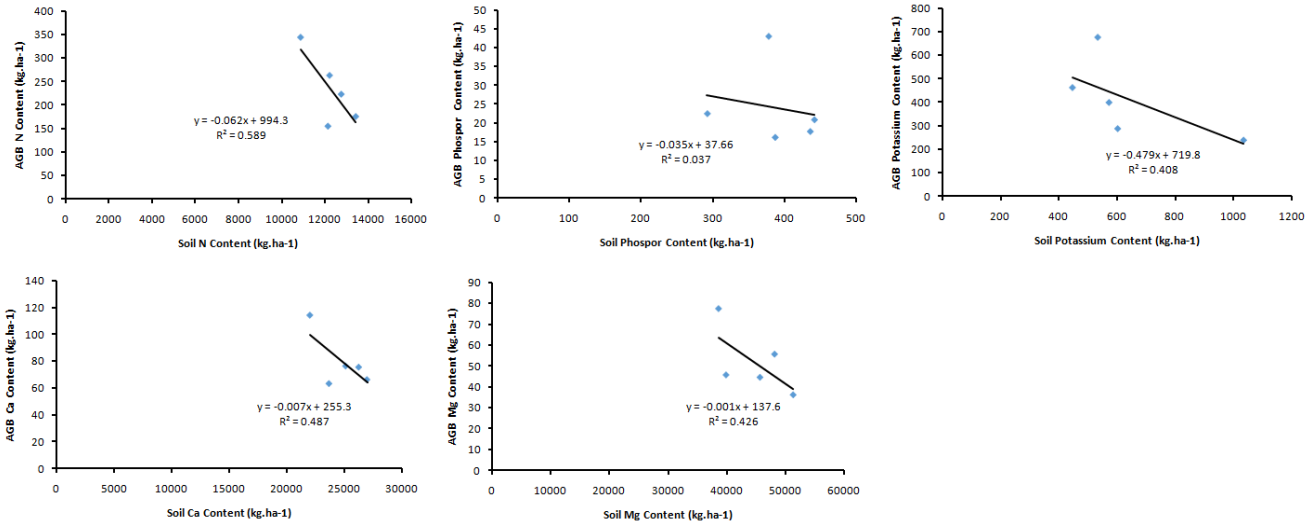

Figure 6. Corelation between soil nutrient and plant nutrient content of *Macaranga gigantea* at five years old

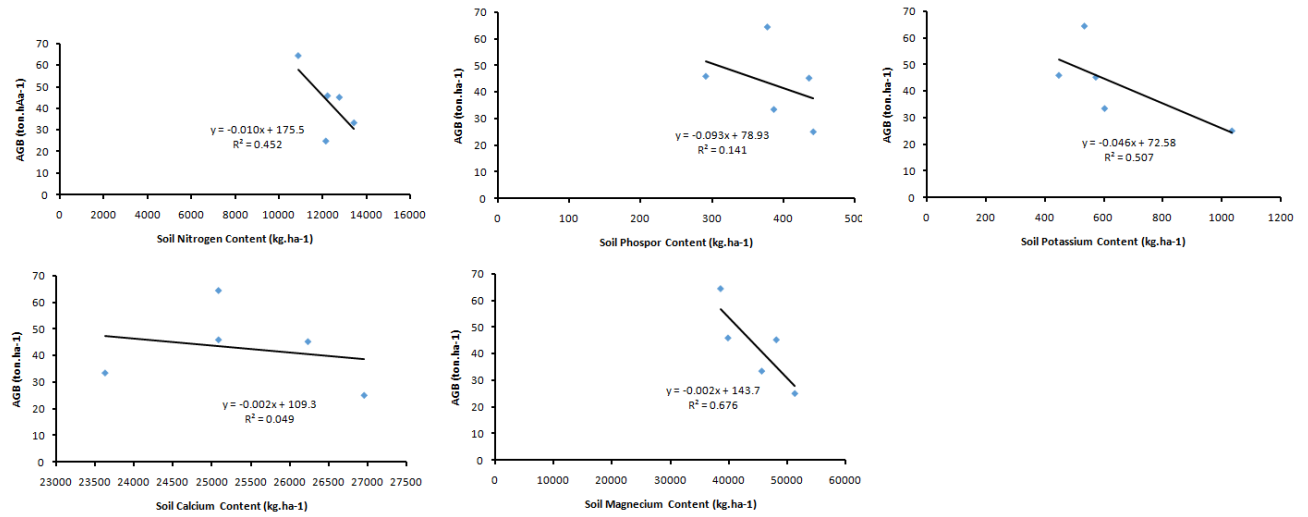

Figure 7. Corelation between soil nutrient content and aboveground biomass of *Macaranga gigantea* at five years old

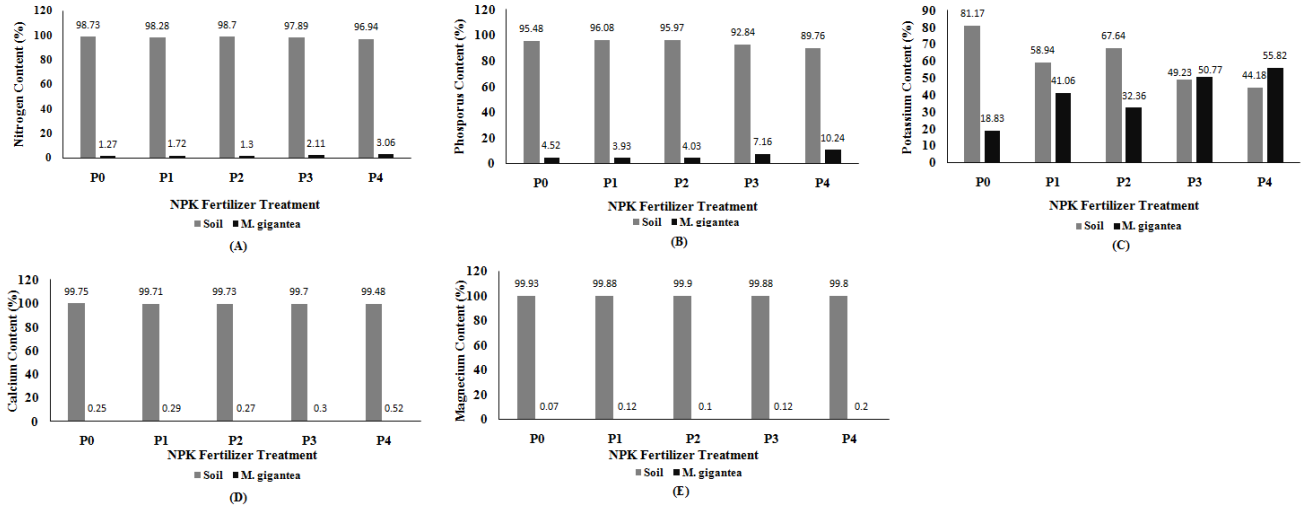

Figure 8. The proportion of nutrients stored in the soil and nutrient accumulated in *Macaranga gigantea* plants at five years old.

Table 2. Soil nutrient contents in *Macaranga gigantea* plant at the age of five years

Fertilizer application	Soil depth	Soil nutrient content (kg.ha^{-1})				
		N	P	K	Ca	Mg
	30-60	5385.7	290.7	705.9	10159	24100
	0-60	12136.5	441.3	1034.2	26953	51292
P1	0-30	6561.9	191.8	280.8	8710	24068
	30-60	6182.6	243.7	293.1	17522	24043
	0-60	12744.5	435.4	573.9	26232	48111
P2	0-30	7667.8	140.9	373.1	15770	22234
	30-60	5737.6	245.6	230.6	7860	23369
	0-60	13405.4	386.5	603.7	23630	45603
P3	0-30	6867.9	96.9	239.4	11872	18774
	30-60	5342.8	195.0	209.5	13213	21026
	0-60	12210.7	291.8	448.8	25085	39800
P4	0-30	6045.9	153.8	281.5	12169	21444
	30-60	4842.8	223.6	253.7	9794	17076
	0-60	10888.7	377.4	535.2	21963	38520

Note: P0 = control without fertilizer, P1 = NPK fertilizer 40 g, P2 = NPK 80 g fertilizer, P3 = NPK fertilizer 120 g and P4 = NPK 160 g fertilizer.

The relative portion of N, P, Ca, and Mg nutrients stored in the soil was still very large, ranging from 89.76 to 99.93%, whereas that accumulating in *M. gigantea* stands was very small at 0.20% - 10.24%. It means that the absorbed nutrients of N, P, Ca, and Mg by *M. gigantea* were relatively small. On the other hand, the relative portion of accumulated K nutrients in the soil was quite small, ranging from 44.18 to 81.17%, while that in *M. gigantea* stands was large enough, ranging between 18.83 and 55.82%. It means that K was highly absorbed from the soil and was accumulated in *M. gigantea* plant tissue, followed by phosphorus, nitrogen, calcium, and magnesium (Figure 8).

Discussion

At the age of five years, the best growth of *M. gigantea* was found in P4 plot which was treated with NPK fertilizer at 160 g dosage (Figure 1). The same result was also found in aboveground biomass accumulation (Figure 3). In contrast to Susanto et al. (2017b) which found that at the age of one year the best growth and production of above-ground biomass were found in the application of NPK fertilizer with the dosage of 120 g. Nusbaum et al. (2005) reported that the application of fertilizer in degraded soil is able to increase dry weight, basal diameter, and height for all plant species at the age of 6 months. The increase in height and basal diameter in fertilized *M.gigantea* seedlings are four times higher than those in unfertilized plants.

The result also showed that the accumulation of nutrients N, P, K, Ca, and Mg in *M. gigantea* was the highest at the P4 treatment (NPK 160 g fertilizer). The highest N accumulation was found in the leaves, followed by branches of wood and bark. The highest P nutrient was stored in the branch followed by leaves, wood, and bark. On the other hand, K, Ca, and Mg nutrients were more accumulated in branches, wood, leaves, and bark. The highest absorbed nutrient by M. gigantea from the soil was K with 676.20 kg.ha-1, followed by N with 344.01 kg.ha-1, Ca with 114.57, Mg with 77.64 kg.ha-1 and P with 43.04 kg.ha-1.

The content of N, P, K, Ca and Mg in soil (0-60 cm) showed that plot P4 had the lowest nutrient content compared to Plots P0, P1, P2, and P3. Elements of K and P had a lower content than N, Ca and Mg nutrients. It means that *M. gigantea* plant more easily absorbs nutrients in the soil for its growth. This plant is a fast-growing pioneer species that requires high levels of light within forest gaps of secondary (Davies et al. 1998; Romell et al. 2008). Fast-growing tree species are generally considered to accumulate soil nutrients faster than slow-growing ones when multiple rotations are implemented (Cossalter and Pye-Smith 2003). Inagaki and Tange (2014) also reported that fast-growing Eucalyptus trees can accumulate more aboveground biomass than N_2-fixing and other non-N_2-fixing broadleaved trees while storing less aboveground N. Furthermore, some Acacia and Eucalyptus trees can produce more aboveground biomass than other non-N_2-

fixing broadleaved trees while using less Phosphorus. Montagnini (1998) reported that four years after planting, decrease in soil nutrients was apparent in pure plots of some of the fastest-growing species. On the other hand, some fast-growing tree species have strategies that allow them to grow on degraded soils. Montagnini (2000) reported that decreases in soil P, K and Ca were apparent in pure plots of the fastest-growing species with the largest accumulation of nutrients in above-ground biomass, such as *J. copaia* and *V. guatemalensis*, five years after planting.

In this study, the highest accumulation of nutrients was also found at P4 plot. Nitrogen was accumulated at 344.01 kg.ha-1, 121% higher than non-fertilized control plants which accumulated only 155.9 kg.ha-1. The accumulation of phosphorus in the biomass of *M. gigantea* at P4 plot was 43.04 kg.ha-1, twice higher than the phosphorus content in control plot with 20.9 kg.ha-1. Potassium accumulation was 676.20 kg.ha-1, three times higher than that at control plot (239.89 kg.ha-1). While for the calcium and magnesium nutrients, the accumulation of each nutrient in plant biomass was 114.57 kg.ha-1 and 77.64 kg.ha-1 at plot P4, twice higher compared to those at P0 control with 66.51 kg.ha-1 and 36.1 kg.ha-1. The addition of NPK fertilizer higher than 160 g significantly increased N, P, K, Ca and Mg content of *M. gigantea* plant biomass. This nutrient has important role in photosynthesis. The finding of this study is in accordance with Cossalter and Pye-Smith (2003), which states that fast-growing tree species are generally considered to accumulate soil nutrients faster than slow-growing ones.

Potassium is the most accumulated nutrient in *M. gigantea* biomass with 676.20 kg.ha^{-1}, consisting of 64% in stem and branch, and 36% in leaf and bark. On the other hand, the relative portion of K nutrients that were accumulated in *M. gigantea* stands was quite large, ranging between 18.83 and 55.82% (Figure 7) compared to other nutrients. This result shows that nutrient K is the most absorbed nutrient by *M. gigantea*. Hertemink (2001) reported that fast-growing species *Piper aduncum* also accumulated large amounts of biomass and nutrients, particularly K.

When *M. gigantea* plant is harvested at the age of five years with stems and branches taken out of the system, the greatest nutrient that will be reduced from the soil is potassium, followed by phosphorus, nitrogen, calcium, and magnesium. In the first cycle of harvesting *M. gigantea*, the soil will lose nutrients by 438 kg.ha-1 (64%), assuming that the leaves and barks were left on the site. Meckensen, (1999) and Meckensen et al. (2001) reported that nutrient content of fast-growing *Eucalyptus deglupta* in industrial plant forests in East Kalimantan (100 m^3 log and barks) was N with 44. 4 kg.ha-1, P with 2.3 kg.ha-1 and K with 125 kg.ha-1. Uri et al. (2003) found that the amount of nutrients accumulated by 1-year-old grey alder in producing one tonne of biomass at an N:P:K ratio of 100:9:43. The uptake of nutrient K by *M. gigantea* is more abundant than *Eucalyptus deglupta*. Montagnini (2000) reported that in monoculture plantations, *Vochysia guatemalensis* had the greatest accumulation of K and Ca. In *V. guatemalensis* plantation, stem harvest would remove less than 30% of N for 50% of total above-ground tree Ca, K, Mg and P. Branches and foliage summed together contribute only 25 to 35% of total above-ground tree biomass, but they generally represent about 50% of above-ground tree nutrients. Mackensen and Foster (2000) reported that cost of fertilization is different across species. For example, fertilization costs for *Eucalyptus deglupta* are generally higher than *Acacia mangium*. Alriksson and Eriksson (1998) suggested that the choice of tree species harvest and stem-wood harvest is when they reach similar rates of stem-wood biomass production. On the other hand, Montagnini (2000) reported that continued sampling will be needed to assess the long term effects of plantation treatments on soil chemistry, especially near the end of the rotation

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Woody biomass and elements uptake in phytoremediation of compost leachate

TOOBA ABEDI[1,♥], NAZI AVANI[2,♥♥]
[1]Environmental Research Institute, Academic Center for Education, Culture and Research. Rasht, I.R. Iran. ♥email: tooba.abedi@gilan.ac.ir, abeditooba@gmail.com
[2]Teaching fellow, School of Distance Education, Universiti Sains Malaysia, Penang, Malaysia. ♥♥email: avani.nazi@yahoo.com

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Abstract. *Abedi T, Avani N. 2018. Woody biomass and elements uptake in phytoremediation of compost leachate. Asian J For 2: 20-24.* The utilization of wastewater for irrigation to develop biomass plantations is a win-win solution to reduce planting costs while mitigating the impacts of waste pollution. This study examined the performance of growth, biomass accumulation and nutrient absorption in *Alnus glutinosa* and *Taxodium distichum* treated with leachate water. Randomized research design with three watering treatments, namely tap water as control (C), pure leachate (P) and a mixture 1:1 of tap water and leachate, were applied to one-year-old seedlings of *Alnus glutinosa* and *Taxodium distichum* planted in pots. The parameters of diameter and height growth, aboveground and root biomass, and elements uptake were observed for eight months. The results showed that leachate had a positive influence on the growth of *A. glutinosa* and *T. distichum* in terms of an increase in diameter and height, and aboveground biomass accumulation although there was no significant difference with the other two treatments. This indicated that the irrigations by pure leachate and mixture liquid stimulated growth in the same way as irrigation by water. The results of elements absorption showed no statistical difference except the absorption of Ca concentration in root which was higher than the absorption of other elements and showed significant difference in 1: 1 treatment. The results of this study suggest that pure compost leachate can be used to water the studied species although further studies that escalate the laboratory scale into field-scale are required.

Keywords: Treatment, growth, compost leachate, seedling

INTRODUCTION

The production of renewable energy sources has been increasingly promoted in Iran including in the form of biomass. In order to achieve this, large-scale planting activities are needed which require sufficient inputs, such as artificial fertilizers, for the optimal growth of biomass crops being developed.

In the development of biomass plantations, fertilizers represent an important production cost. As such, replacement of artificial fertilizers with alternative ones becomes the most favorable option for stakeholders in reducing the cost. There is a win-win solution on the use of fertilizers at minimum cost while reducing the problems of wastes, i.e., using landfill leachate or wastewater from compost production, sludge, etc. (Justin et al. 2010; Holm and Heinsoo 2013). The substitution of artificial fertilizers with waste sources could be a promising option with regard to the reduction of planting costs and simultaneously the reduction of spending on the treatment of wastes.

Previous studies reported positive effects of leachate irrigation on tree growth, showing its fertilizing potential. Zalesny and Bauer (2007b) found that Salix clones S287 and S566 exhibited responses favoring leachate irrigation over water. Justin et al. (2010) detected that the use of landfill leachate treatments resulted in a considerably increased aboveground biomass compared to the control tap water treatment. Further, Abedi et al. (2014, 2015) investigated *Populus deltoides*, *P. euramericana*, and *Salix alba* in phytoremediation, suggesting a potential of pollution removal of wastewater while establishing vegetation.

This paper aimed to investigate the response of *Alnus glutinosa* and *Taxodium distichum* to different concentrations of compost leachate with respect to growth and biomass accumulation. The choice of the two species was because of their high biomass production capacity and their status as endemic species of Hyrcanian forests of Iran. *Taxodium distichum* becomes one of the most well-known conifer trees due to its economic and ecological usefulness. This species also has high tolerance to flooding, waterlogging and salinity (El-Dayem 2003). *Alnus glutinosa* has tolerance to prolonged submergence of its roots in water for up to 30 cm deep. This plant can also grow in much drier sites, though, in such conditions, it will usually not live for a long period and will die soon. *Alnus* can grow well in heavy clay soils, and is in toleration to lime and very infertile sites. It tolerates a wide range of soils but prefers an above 6 pH. It is very tolerant to maritime exposure and can grow very rapidly in early stage (http://www.pfaf.org/user/plant.aspx).

MATERIALS AND METHODS

Study area and period

This research was conducted in Safrabaste Poplar Research Station. It is located in eastern part of Gilan province in north of Iran (37º 19´N, 49º 57´E). The experiment was done in growing season in 2013.

One-year-old seedlings of *Alnus* and *Taxodium* were used as experiment materials and they were collected from the nursery of the Safrabaste Poplar Research Station. At the beginning of the planting season (i.e., in the middle of March 2013), the seedlings were planted in experiment pots filled with loamy-sandy soil obtained from the surrounding area with a depth of 40 cm. The initial substrate used in the experiment was analyzed in the soil laboratory. In accordance with the standard procedure described by the Soil Science Society of America, the main physical and chemical characteristics of the soil were determined (Page et al. 1982). Table 1 shows a list of substrate analysis, physical characteristics, and analytical methods applied in this study.

Compost leachate was gathered from a leachate reservoir belonging to Compost Plant of Municipal Waste Management of Rasht, North of Iran (37º 10´N, 49º34´E). The analysis for its chemical content was performed in the Laboratory of Guilan Department of Environment (Rasht, North of Iran) using approved Standard Methods for the Examination of Water and Wastewater (Eaton et al. 2005).

Table 2 shows the composition of leachate for the experiment. It had dark brown colour with smelly odour. A 20-liter plastic tank was used to store the leachate while it was mixed with tap water to create a mixture with specified degree of dilution. Before filling the tank, chemical analysis of leachate was performed.

In the beginning, tap water was used to water the plants for eight weeks. When the experiment started in mid-May 2013, three watering treatments were applied to the plant, namely: (C) tap water (control), (P) pure leachate, and (1: 1) a mixture of one unit of leachate with one unit of tap water (by volume). The experiment layout was a completely random design consisting of ten replicates for each treatment. The experiment lasted until early December. The pots with the plants inside were placed randomly on an experiment field under a transparent roof to avoid rainfall but still be exposed to sunlight. The plants were irrigated with a mixture of water for as much as the absorption capacity of substrate against water (0.5 l per pot) in the first week of the experiment. Pure leachate was given without dilution. Tap water for C treatment and for the preparation of the water mixture is from the public drinking water supply.

The growth in diameter and height of the trees was monitored bi-monthly. The diameter was measured at the breast-high, and the height was recorded from the soil surface to the apical bud at the terminal shoot (Zalesnyet al. 2007a). For eight months of the growth phase, the average growth rate of diameter and height of the trees for each treatment was calculated bi-monthly.

Table 1. Soil chemical and physical properties of the substrate used in the experiment

Component	Unit	Amount
pH		8.31
EC	mS cm^{-1}	0.128
C_{org}	%	0.08
N_{tot}	%	0.01
P	mg kg^{-1}	0.69
K	mg kg^{-1}	57.60
Ca	mg kg^{-1}	400
Mg	mg kg^{-1}	24
Soil texture		Loamy sand
sand	%	86
silt	%	5
clay	%	9

Table 2. The composition of pure compost leachate

Parameter	Unit	Amount
pH		5.22
EC	mS cm^{-1}	1.26
N_{tot}	mgL^{-1}	21.384
NO_2	mgL^{-1}	0.08
NO_3	mgL^{-1}	21.3
SO_4	mgL^{-1}	7101
PO_4-P	mgL^{-1}	22.11
Na	mgL^{-1}	310
K	mgL^{-1}	250
Ca	mgL^{-1}	152
Mg	mgL^{-1}	1103
Pb	mgL^{-1}	0.27
Ni	mgL^{-1}	0.342
Cd	mgL^{-1}	0.0047
Cr	mgL^{-1}	Trace
COD	mgL^{-1}	260500
BOD	mgL^{-1}	130000
TSS	mgL^{-1}	3060.6
Turbidity	mgL^{-1}	12500

After eight months, all plants were cut and were separated into two parts, namely: aboveground (stems, branches, and leaves) and root system. The parts of the root system were separated carefully and washed thoroughly with distilled water. Next, the roots and the stems were dried in an oven at 60º C for 48 hours. The biomass and absorbed elements of aboveground and of root were calculated carefully. Data were calculated using SPSS 16.0 statistical package. The statistical dissimilarity among treatments is ascertained by the analysis of variance. The outcomes were regarded significant at $p < 0.05$. The tree growth rate was displayed on graphs of the diameter and of height against time. The test of Tukey was applied to get the dissimilarity between level means.

RESULTS AND DISCUSSION

High quantity of chemical contents was given to plants in the pots. The contents of N, P and K were much greater in the leachate than in the soil, but Ca content was lower

compared to that in soil-filled in the pots. The higher ion concentration in leachate also reflected higher electrical conductivity (1.26 mS cm^{-1}) compared to that in soil (0.128 mS cm^{-1}). Metal contents were low in the leachate. After eight months, pure leachate treatment (P) showed the highest diameter growth of *A. glutinosa* and *T. distichum* with an average of 1.356 and 1.128 cm respectively (Figure 1). In terms of height, a higher rate of growth was found in the treatment of pure leachate (P) and a mixture of leachate and water (1:1) (Figure 2). Nonetheless, all three treatments have no significant differences in terms of the growth in diameter and height for both species (Figures 3 and 4).

The greatest accumulation of aboveground biomass was obtained in the treatment of 1:1, but it was not significantly different from other treatments ($p<0.05$) (Figure 5). For root biomass, the greatest accumulation was in the treatment of 1:1, which was not significantly different from the treatment of C, but gave a significant difference from P treatment ($p<0.05$) (Figure 6).

The outcome of elements absorption shows no statistical differences among treatments and across species. Lower K absorption was found on the control (C) treatment while the most absorption of Ca happened above ground (Figure 7). The absorption of Ca concentration on root was higher than the absorption of other elements and show significant difference in 1:1 treatment (Figure 8).

Discussion

The positive effects of leachate irrigation on tree growth and its fertilizing potential for plants have been reported by many studies. Justin et al. (2010) found that the utilization of landfill leachate treatment increased the amount of surface biomass significantly compared with the control tap water treatment, but tree growth and biomass accumulation in wastewater compost treatment decreased compared to the treatment of tap water and compost leachate. The results of Abedi et al. (2014, 2015) showed a positive effect of compost leachate on tree species. Depend on the constituents of the leachate and soil, as well as the nutrient demands of the genotypes tested, the concentrations and amounts of leachate can be determined (Zalesny and Bauer 2007b).

In all treatments, no statistically significant differences in the aboveground biomass were found (Figure 5). High concentration in the pure leachate (P) treatment turned out to be toxic, meaning that the water mixture in P treatment already had too high concentration of salts and other elements (Table 2).

The compost leachate was a by-product of composting of organic matter, having a low pH (5.22) which is a sign of unfinished degradation processes of raw organic matter, where due to the inadequate oxygen levels. The comparison of the plant growth in the several treatments showed apparently normal and healthy-looking trees.

The nitrogen content was also elevated in compost leachate. There is acknowledged in common agricultural practice that supplementary nitrogen is utilized to manage distinctive toxicity issues and boost vegetation growth (Ayers and Westcot 1994). Kadlec and Wallace (2009) notified that more elevated concentrations of sulfate (402 mg SO_4/L) in compost leachate could bring negative effect on plant absorption in water-saturated root part and should also be highlighted. In this study, the sulfate concentration was 7101 mg SO_4/L. Still, the plant continued to accrue.

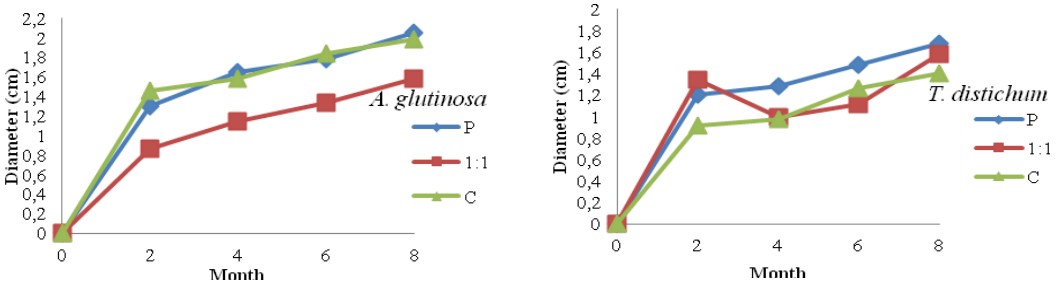

Figure 1. Mean growth in diameter (cm) of *Alnus glutinosa* and *Taxodium distichum* with three watering treatments

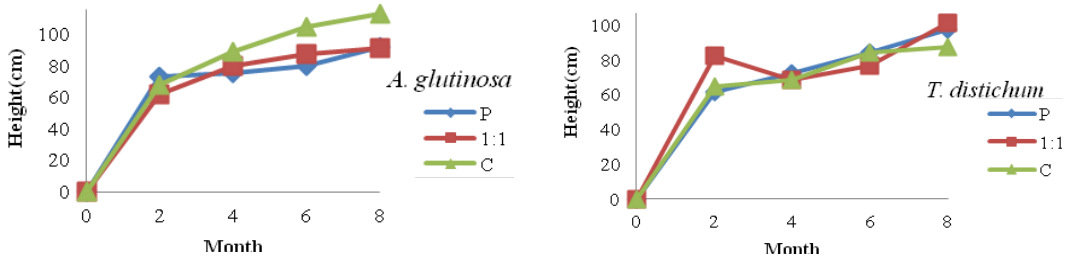

Figure 2. Mean growth in height (cm) of *Alnus glutinosa* and *Taxodium distichum* with three watering treatments

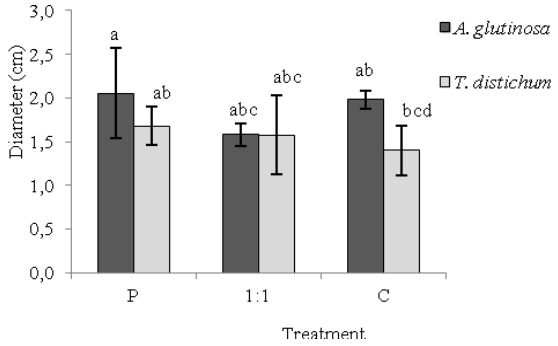

Figure 3. Comparison of mean of diameter in all treatments. Note: bars with similar letters indicate no significant differences at the level of confidence of 95%

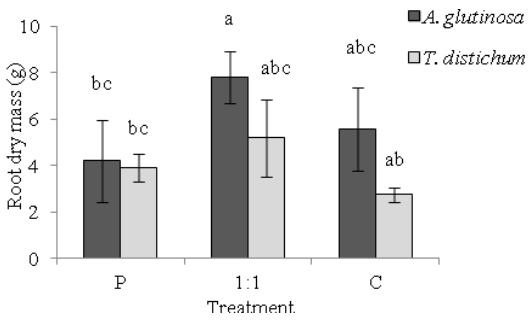

Figure 6. Comparison of mean of root biomass components. Note: bars with similar letters indicate no significant differences at the level of confidence of 95%

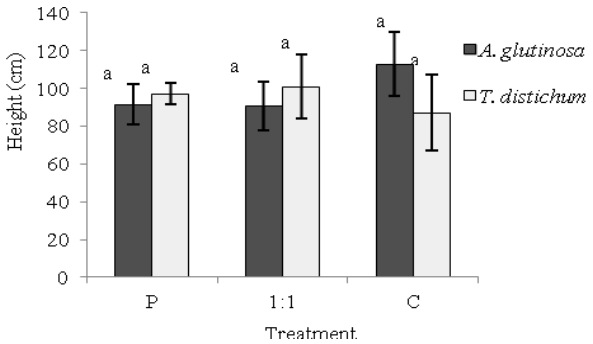

Figure 4. Comparison of mean of height in all treatments. Note: bars with similar letters indicate no significant differences at the level of confidence of 95%

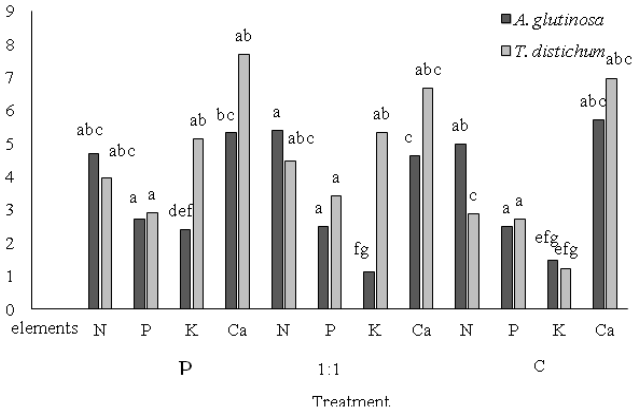

Figure 7. Comparison of aboveground elements uptake. Note: bars with similar letters indicate no significant differences at the level of confidence of 95%

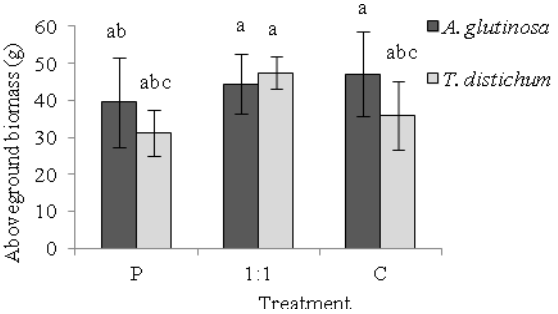

Figure 5. Comparison of mean of aboveground biomass components. Note: bars with similar letters indicate no significant differences at the level of confidence of 95%

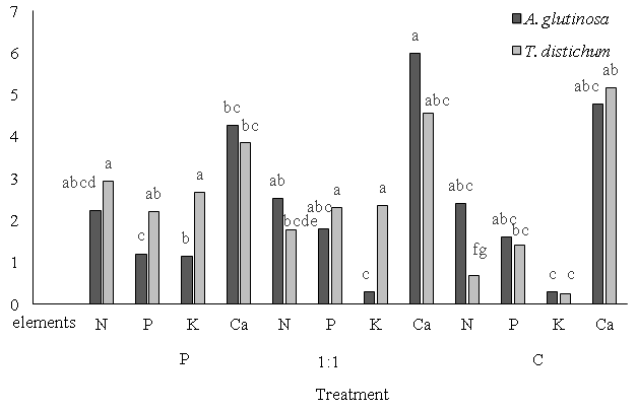

Figure 8. Comparison of root elements uptake. Note: bars with similar letters indicate no significant differences at the level of confidence of 95%

Fung et al. (1998) and Abedi et al. (2014) stated that elevated rates of salt (1.0% NaCl) quickly lessened the absorption of *Populus* and owned an instantaneous result on predawn leaf water potency, photosynthesis and stomatal resistance. It has been notified that the *Populus* was sensitive to salt, but *A. glutinosa* which was hardwood tree species and *T. distichum* which was softwood tree species have no negative reactions to elevated concentrations of salts.

Zalesny and Bauer (2007b) used fast-accretion *Populus* and *Salix* clones and their genomic groups to be treated with waste leachate for one growing season. In such experiment, *Populus* resulted greatest in diameter and dry mass. The highest level of phytoremediation showed no significant relation with the highest biomass yield (Greger and Landberg 1999; Klang-Westin and Eriksson 2003; Zalesny and Bauer 2007b), and the concentration of iron and nutrients is piled up in the bark which is higher than the wood tissue (Pulford and Dickinson 2005; Dimitriou et al. 2006; Adler et al. 2008). This should be examined in the future elemental analysis of the plant material.

The results of this study suggest that pure compost leachate can be used to water the studied species. However, upscaling the experiment to the field would enable leaching of the excess water from the root zone, and the washing-out of salts by precipitation to the lower soil layers, thus better survival with the same amounts of pure compost leachate as used in the pot experiment. The development of aboveground biomass is important from the leachate consumption and phytoremediation point of view (Justin et al. 2010).

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The effects of fires on plant and wildlife species diversity, and soil physical and chemical properties at Aberdare Ranges, Kenya

WANGARI FAITH NJERI[1], J. M. GITHAIGA[1,♥], AGGREY K. MWALA[2]
[1]School of Biological Sciences, University of Nairobi, Nairobi, Kenya. ♥email: jmgithaiga@uonbi.ac.ke, mainagithaiga@gmail.com
[2]Department of Land Resource Management and Agricultural Technology, University of Nairobi. Nairobi, Kenya

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Abstract. *Njeri WF, Githaiga JM, Mwala AK. 2018. The effects of fires on plant and wildlife species diversity and soil physical and chemical properties at Aberdare Ranges, Kenya. Asian J For 2: 25-38.* Forest fires have devastating impacts on the biodiversity and biophysical elements of forest ecosystems. Yet, little is known of the impacts of forest fires in a lesser-known region such as Kenya. This study was aimed to determine the effects of fires on species diversity (plants, animals, birds), and soil physical and chemical properties at the Aberdare Ranges forest, Kenya. Data were collected on five sites that experienced fires in 2002, 2009, 2012, 2013, and 2014 from both burnt and unburnt areas. Point Centered Quarter and quadrant methods were used for woody vegetation sampling and herbaceous vegetation sampling, respectively. Foot count was done for animal census and point count for birds. The data showed that the herbaceous vegetation in burnt sites had significantly higher species diversity than the unburnt sites in the areas that experienced fire before 2014. The fire had triggered the regeneration of the herbaceous plants. The burnt sites had a significantly higher percentage cover. The fire had an immediate adverse effect on the population of animals as demonstrated on the site consumed in 2014. No animal species were found on the site seven days after the fire when data was collected. The animal diversity was proportional to the vegetation density caused by the vegetation regeneration due to fires. All the burnt sites had fewer birds than the unburnt sites. Effects of fires were prominent in the upper layer of the soil for all the soil properties under study. Burning caused an increase in pH, potassium, organic carbon and cation exchange capacity. The study demonstrated that fires lead to an immediate adverse effect on vegetation, wildlife and soil chemical properties. Postfire management is necessary on sites that have recently experienced fires to rehabilitate them. Authorities responsible for the management of forests must ensure that people are kept out of those sites to allow vegetation to recover without interference. Reforestation can also be done on the burnt sites to increase plants and habitat for the wildlife.

Keywords: Animal diversity, birds, fire, plant diversity, soil properties

INTRODUCTION

Forests are indispensable sources of products and they deliver a variety of ecosystem services including provision of food, timber, fuel, genetic materials among others, regulating services (e.g., protection of watershed and carbon storage), cultural values and supporting services (Koziowski 2002). Forest ecosystem services have been shown to be of high economic value since they provide raw materials for food, fuel, and shelter (Constanza et al. 1997). In forest valuation studies, non-tangible service components such as carbon storage or hydrological protection frequently fetch higher values than forest products.

Old-growth forests are a valuable component of biodiversity, and one way to assess the effectiveness of forest management is by evaluating the proportion of old-growth forests currently present concerning that was historically present (Lesica 1996). However, forests throughout the world, and especially old-growth forests in the tropics, are threatened by natural-induced disturbances such as windthrow, droughts, floods, disease and outbreaks, and human-induced disturbances such as fires, logging, charcoal burning and forest clearance for farming (Koziowski 2002).

One phenomenon that threatens forests worldwide is forest fires. Forest fire can be defined as the widespread uncontrolled fire in forest ecosystems, caused by accidental, natural or intentional causes (Chuvieco 2009). Forest fires are a severe hazard and can cause considerable damage to ecological, economic, cultural, and human resources (Long et al. 2001).

Knowledge on the impacts of forest fire has attained increased importance to land managers because fire as a disturbance process is a part of ecosystem management. Fire can initiate changes that affect the composition, structure, and pattern of vegetation on the landscape. A disturbance is necessary to maintain a diversity of living things and processes (Botkin 1990; Morgan et al. 1994). Anthropogenic activities create fire-prone ecosystems in the tropics through the alteration of vegetation cover by logging, burning, and development. The new ecosystems differ substantially in carbon budget, nutrient cycling, fuel and habitat characteristics (Swetnam et al. 1999).

More than 220,000 events of fires occur in a year around the world with burning extent of more than 6 million ha of forests (Gonzalez et al. 2005). Many studies have been conducted on the assessment and management of forest fires. Some countries like Russia, the United States of America and Canada have led the way in forest fire research (Sturtevant et al. 2009) by developing forest fire management systems and forest fire risk forecast systems temporally and spatially. Kenya is also adopting a fire

management system through Kenya Forest Service by recording all the fire incidences and stocking every station with fire equipment although the progress has been slow.

As one of Kenya's five main water towers, the forests of the Aberdares play a critical role in supporting the country's economy. They are the primary source of water for Nairobi. Furthermore, 55% of Kenya's electricity is generated by water flowing from Aberdares and Mt. Kenya. The Aberdares are the main catchments for Sasumua and Ndakaini dams, which mainly act as the water source for Nairobi, a city of more than three million people that accounts for 60% of Kenya GDP. The energy, water and some raw materials used to drive some economic activities in the city and its environment are derived from the Aberdare ecosystem. Proper management of this ecosystem is crucial for the national economy (Ark 2011). Nevertheless, fires may interfere with the ability of Aberdares to continue providing its services hence affecting the national economy.

This study was carried out in the western Aberdares at Geta forest zone. The specific objectives were (i) to determine the effect of fire on plant species diversity at different time periods; (ii) to assess the impact of fire on a population of herbivores and birds at different time periods; (iii) and to determine the effects of fire on soil physical and chemical properties at a different period.

In doing so, five sites that had been burnt at different periods were identified, and data were collected in both burnt sites and unburnt sites. The data were collected during the wet season and dry season. Point Centered Quarter method was used for the woody vegetation, whereas the quadrant method was employed for the herbaceous plant. Herbivores were counted using foot count, and the birds were counted using point count. Soil samples were collected from all the sites and taken to a soil laboratory for physical and chemical analysis. The soil properties analyzed include pH, nitrogen, organic matter, potassium, phosphorous, and Cation Exchange Capacity.

MATERIALS AND METHODS

Study area

Geographic location

The study was carried out in the Aberdare Range, part of Kenya which is close to the equator (Figures 1 and 2). It is the third highest mountain in Kenya, with two central peaks: Oldonyo Lesatima (also known as Sattima) and Kinangop, which are at altitudes of 4,001 and 3,906 meters above sea level respectively. The Range presents a deeply dissected topography sloping gradually to the east. In contrast, the western side drops along impressive fault escarpments towards the Rift Valley **(**www.britannica.com retrieved 2014-06-17). The Aberdare mountain range lies between Latitude 00° 00”-01°00” South and Longitude 36° 30”-36°55" East running in an NNW-SSE direction. Altitude varies from 1850 min at the lower parts to about 4000 m at the highest point.

In the context of this study, data collection was done at Geta forest zone which is at the western side of the Aberdares and the most significant part of the Aberdares hills. Data was also collected from Kipipiri hill which lies next to the Aberdare ranges.

Climate of Aberdare Ranges

Altitude largely determines the environment of the Aberdare Ranges. The rainfall distribution is greatly influenced by the movement of inter-tropical convergence zones of air masses of the southern and northern hemispheres. It is characterized by two rainy seasons: long rains from April to May, and short rains from October to November. Rainfall reaches a maximum of around 2,600 mm annually on the south-eastern slopes but varies with altitude and exposure to the dominant wind from the Indian Ocean (Butynski 1999). On the western part, rainfall reduces sharply from about 1,400 mm at the forest border to less than 700 mm in the valley of the Malewa River.

The northern end of the range has 3-4 dry months per year with the seasonal distribution showing three rainfall peaks: March-May (long rains), July-August, and November. Elsewhere, the rainfall distribution is bimodal with peaks in April-May and October-November and only 1-2 dry months each year. Temperature decreases with increase in altitude and rainfall also decline with elevation.

Vegetation in Aberdare Ranges

Vegetation zones in the Aberdare Ranges include the bamboo zone, the closed-canopy forest belt, and the sub-alpine and alpine vegetation. The forest belt covers a significant part of the range. Most of the forest is gazetted as forest reserves. The key trees and shrubs species at the Aberdares are shown in Table 1.

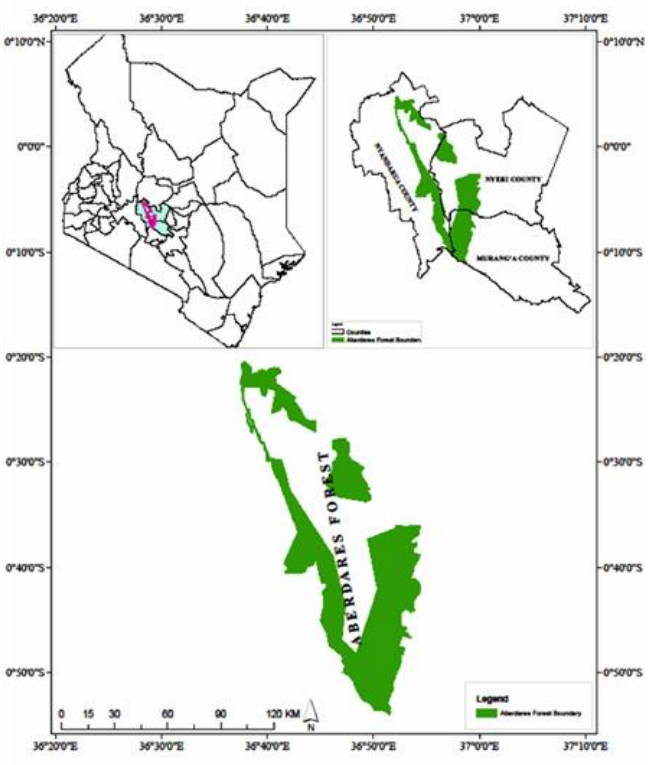

Figure 1. The location of Aberdares in Kenya

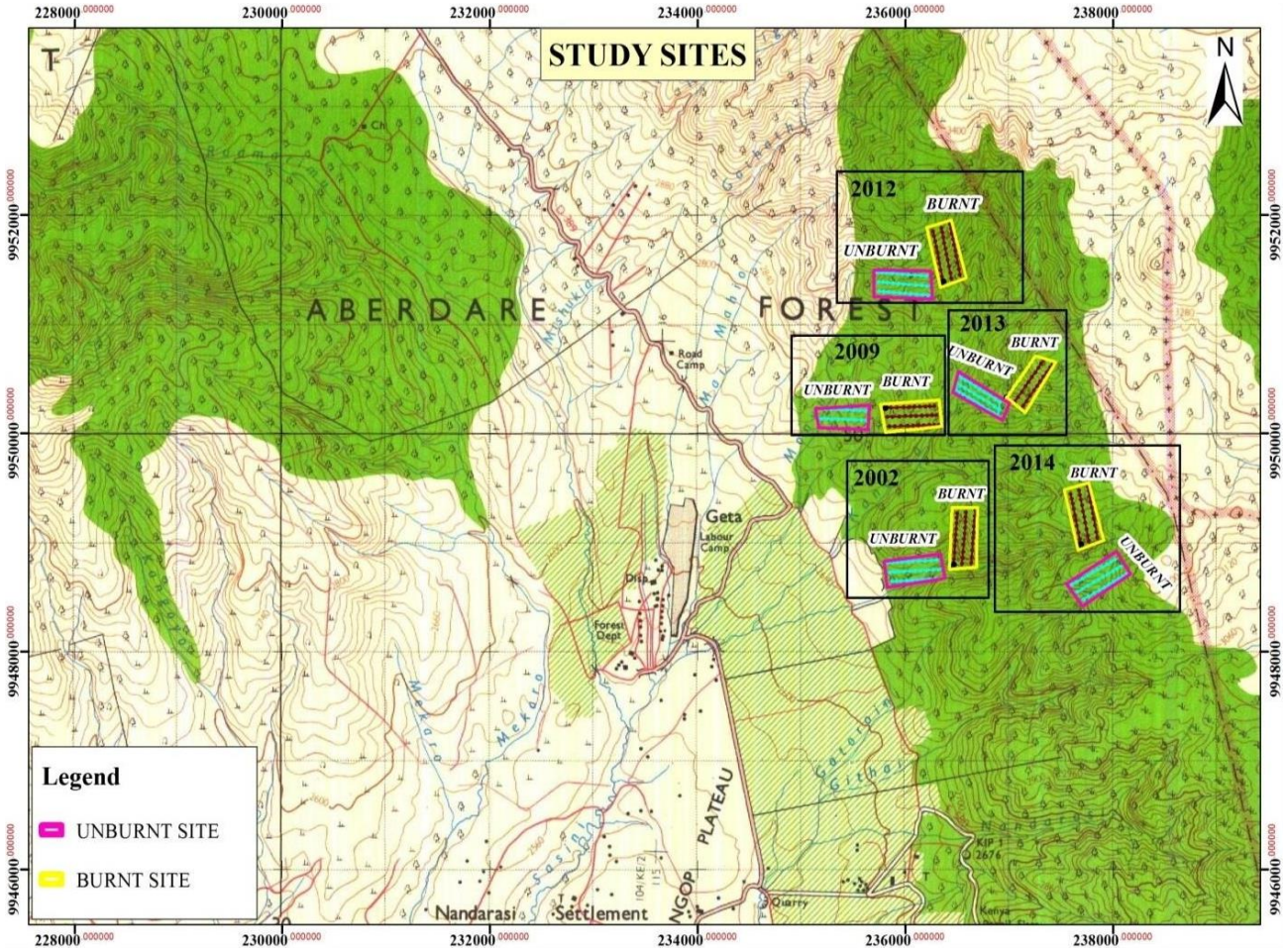

Figure 2. Map of the study sites in Western Aberdares, Kenya

Table 1. The key vegetation species at the Aberdare ranges, Kenya (Ng'ang'a 1990)

Vegetation zone	Altitude in m asl and location	Key trees and shrub species
Montane forest zone	1900-2500 / East	*Cassipourea malosana, Ekebergia capensis, Teclea nobilis,*
Moist forest	2100-2500 / South-East	*Calodendrum capense, Podocarpus latifolius, Nuxia congesta Ocotea usambarensis, Macaranga kilimandscharica, Neoboutonia macrocalyx, Tabernaemontana stapfiana, Prunus Africana*
Dry forest	1800-2400 / South-West	*Juniperus procera, Calodendrum capense, Teclea simplicifolia Juniperus*
	2400-3300 / West	*procera, Olea europaea (africana), Podocarpus*
	2300-3200 / North, North-East	*falcatus, Nuxia congesta*
Bamboo zone	2400-3000 / East, South-East	*Arundinaria alpina with scattered trees, including Podocarpus latifolius*
	2700-3300 / West	*and Nuxia congesta*
Hagenia-Hypericum zone	2950-3500 (discontinuous)	*Hagenia abyssinica, Hypericum revolutum, Rapanae melanophloeos*
Ericaceous zone	2900-3560 (discontinuous)	*Erica excelsa, Erica trimera, Erica arborea, Cliffortia nitidula, Helichrysum nandense, Stroebe kilimandscharica*

Some parts of the upper forest zone fall within the Aberdare National Park. A high diversity of forest types characterizes the forest belt of the Aberdare Range due to the wide altitudinal range and the climatic differences between the slopes. The Aberdare Range is heavily-forested, much of them have been protected within the Aberdare National Park since its creation in 1950 (Ng'ang'a and Kamande 1990).

Vegetation zones and species distribution are characterized according to the different climatic zones and altitudes, mostly through variation in vegetation structure, cover, and composition. A total of 778 species, sub-species and varieties of vascular plants belonging to 421 genera and 128 families, have been documented in the Aberdare.

Animals in Aberdare Ranges

The Aberdare Range forests host some threatened fauna species. The Jackson mongoose (*Bdeugale jacksoni*), endemic to Kenya's montane forests and the rarely seen golden cat (*Felix aurata*) are two threatened mammals. Other large threatened mammals of international conservation interest that occur in Aberdare forests are bongo (*Tragelaphus euryceros*), giant forest hog (*Hylochoerus meinertzhageni*), black rhino (*Diceros bicornis*), elephant (*Loxodonta africana*), leopard (*Panthera pardus*) and African hunting dog (*Lycaon pictus*). Also, the forest harbors bushbuck (*Tragelaphus scriptus*), mountain reedbuck (*Redunca fulvorufula*), waterbuck (*Kobus ellipsiprymnus*), suni (Neotragus moschatus), cape buffalo (*Syncerus cafferNeotragus moschatus*), side-striped jackel (*Canis adustus*), eland (*Taurotragus oryx*), and varieties of duikers and bushbabies. The forests are rich in primates, and the common ones include the black-and-white colobus monkey (*Colobus guereza*), vervet monkey (*Cercopithecus aethiops*), sykes monkey (*Cercopithecus mitis*), and baboons (*Papio anubis neumanni*) (Waithaka 1994).

The Aberdare Range is internationally recognized as an Important Bird Area (IBA). The Range is a home for 52 of Kenya's 67 Afrotropical highland species and six of the eight restricted-range species in the Kenyan montane endemic bird areas. Over 270 species of birds have been documented in the Aberdares including the following globally threatened and restricted-range species: Sharpe's Longclaw, Aberdare Cisticola, Abbott's Starling, and Jackson's Widowbird. Regionally threatened species found in the Abedares include Cape Eagle Owl, African Crowned Eagle, and African Green Ibis. Jackson's Francolin, Hartlaub's Turaco and Bar-tailed Trogon are characteristic and spectacular birds of the Aberdare Range.

Soils in Aberdare Ranges

The grounds are polygenetic and occur in profoundly undulating topography, are subjected to intense leaching and have a low base saturation. The grounds are derived from massive lava flows, thick beds of volcanic tuffs and ash showers of geological formation. Soils are deeply weathered, highly porous, stone-free, free draining and support deep rooting associations of forest vegetation.

Soils on the upper eastern slopes of the Aberdare Ranges is highly fertile, being of basaltic origin. They are well-drained, usually very deep, dark reddish-brown, friable clays with a humid topsoil layer. On the western boundary of the Ranges, soils are also of medium to high inherent fertility, but are more variable and interspersed with poorer draining soils and lower productivity. The grounds of the moorlands are umbric andosols, derived from volcanic glass. The area is characterized by a high content of organic matter and is very porous.

The soils of the Northern Aberdare are rich in clay content (82.7%) and consist almost exclusively of kaolinite. Red kaolinite soils are found on slopes, and dark grey, swelling montmorillonite (black cotton) grounds are located in areas of impeded drainage.

The soils of the southern area are characterized by dark surface horizons and are rich in organic matter. Their bulk density is low and includes Leptosols which are distinguished by a continuous coherent hard rock at very shallow depth, strong brown loams, eutrophic brown soils on volcanic ash and Gleysols which show hydromorphic properties within 50 cm of the surface and are found in valley bottoms.

Methods

The data were collected between September 2013 and March 2014. Both the wet season (September 2013 to December 2013) and dry season (January 2014 to March 2014) were covered. Five locations in the forest that had been burnt in 2002, 2009, 2012, 2013 and 2014 were selected. These sites were chosen since they were close to each other and had similar topography and vegetation. In every location, data were collected from both the burnt area and unburnt area that acted as a control. The unburnt regions were separated from burnt areas by buffers of at least 100m to avoid fire effects.

Woody vegetation sampling

Point Centered Quarter (PCQ) sampling technique was utilized for the woody vegetation sampling. Three line transects (500 m long) were randomly set at each site, and sampling points along the transect lines were randomly set. Each sampling point was divided into four quarters using a perpendicular line placed at right angles to the line transect. Individual woody plants species closest to the point in each quarter were identified, and the points to individual plant distances were measured using a tape measure (Kevin 2007). The data that was recorded included: the nearest tree species to the sampling point to individual plant distance measured, the height of tree estimated measured using clinometer, diameter at breast height (DBH) measured by Vernier calipers or a tape measure, and canopy cover measured by tape measure.

Herbaceous plants sampling

Quadrat sampling method was used (Cox 1990). Sampling was done on areas affected by fire in 2002, 2009, 2012, 2013 and 2014. Three line transects of 500 m long were randomly set in both burnt and unburnt areas at each site. Sampling points along the transect lines were randomly placed. Herbaceous layer species were sampled at each location using a quadrat measuring 0.25m^2. The percentage cover of the species was determined through estimation in all the quadrats, while the heights of the plants were measured using a tape measure.

Animal census

Direct foot count was done on all study sites to estimate the number of animals. The calculation was done once every week where the species name and the number of individuals were recorded. An indirect animal count was also be done by use of animal dung as described by Barnes, (1996). The indirect technique gave an index of abundance rather than a measure of animal density (Sutherland 1996). The length of fine dung may vary between habitat and between time periods, weather, dung decomposition rate, fiber content and the number of dung beetles and termites. Very fresh dung was marked, and during the second visit,

the fresh dung scored in the previous visit was compared to the dung in the site and ignored any dung that looked more decayed. The indirect animal count gave a good indication of the study site use. The following equation was used to convert the number of dungs into the number of animals.

$$\text{Number of animals} = \frac{\text{Number of dung}}{\text{Number of days between visits x defecation rate}}$$

The species diversity was calculated using Shannon Weiner diversity index.

Bird count

Bird count was done once every week in the morning hours between 9.00 AM to 12.00 Noon. Point count was done along two transects in each study site. In every place, the bird count was done on the same day in both burnt and unburnt areas. The sampling points were chosen systematically on the transect after every 100m. In every sampling point, the birds were observed for 10 minutes by use of naked eyes and 8x40 binoculars. The bird species found and the heard calling was recorded. The distance between the observer and the birds and the activity of the bird were also recorded. Species diversity was calculated using Shannon Weiner diversity index.

Soil sampling and pH

At every site, the burnt and unburnt sites (three sampling points at random) were selected such that their terrain was as similar as possible. Area for sampling was cleared of vegetation. The soil was dug up to 45cm in depth. Samples were taken from 0-15cm, 15-30cm and 30-45cm at every sampling point for further analysis at the University of Nairobi soil laboratories. The grounds were air-dried, sieved through 2mm screen and analyzed for physical and chemical properties. For each sample, pH was determined using distilled water in the soil to water ratio of 1: 2.5 (Peech 1965).

Total nitrogen

Total Nitrogen content was determined by Kjeldahl digestion method (Fleige et al. 1971). For 1 g of each soil sample, 3.5 mL of phenolic-sulphuric acid (36N) was added and left to stay for 15 minutes. Then, 0.5 g of Sodium thiosulphate was added and the samples left to rest for another 15 minutes. 0.5g of potassium sulfate, 0.5g of selenium reaction mixture and 3.5 mL of concentrated sulphuric acid were thereafter added. The samples were then digested using an electric Kjeltric digestion block. The digested samples were distilled after addition of 40 mL of 10N NaOH solution. A 1% boric acid solution captured the released nitrogen in the form of NH_3 aqueous. The trapped NH_3 was titrated with the 0.01N solution of standard sulphuric acid. The results were expressed as total percentage nitrogen. The amount of nitrogen was calculated from the stoichiometric relationship that 1 mL of 0.01N sulphuric acid used in the titration is equivalent to 0.14 mg of nitrogen.

Organic carbon

The organic carbon was measured using the Walkley Black wet oxidation method (Black 1965). The soil samples were crushed and passed through a 0.6 mm sieve. Ten mL of dichromate solution was added into 1g of the soil sample. Twenty mL sulphuric acid was added into the solution and mixed gently and the mixture was allowed to stand for 30 minutes. It was then diluted to 200 mL with deionized water. Ten mL of Phosphoric acid, 0.2g ammonium fluoride, and ten drops of diphenylamine indicator were added.

The solution was titrated using a standard solution of ferrous sulfate. This procedure was repeated for all the soil samples, and the organic carbon content was calculated using the equation below:

$$\%C = \frac{\text{Me of } K_2Cr_2O_7 - \text{Me of } Fe_2SO4SO_4 * 0.39}{\text{weight of soil}} * 100$$

Where:
Me : Milliequivalents (normality * mLs of solution)
0.39 : Correction factor

Potassium

Potassium was extracted from air-dried soil samples by shaking the sample with 0.5M ammonium acetate acid solution for 30 minutes to effectively displace the potentially available potassium ions. The potassium content of the filtered extract was then determined using a Jenway PFP7 Flame Photometer.

Phosphorous

Phosphorus content was determined at an acidity of 0.20M H_2SO_4 by reacting with ammonium molybdate using ascorbic acid which acts as a reductant in the presence of antimony using spectrophotometer (Mehlich 1984). Air-dried soil measuring 2g was passed through < 2.0 mm into a 50 mL glass Erlenmeyer flask. 20.0 mL of Mehlich 3 extracting solution was added. The extraction flask was placed on a mechanical shaker for five minutes. The suspension was filtered immediately and the extract was collected in 40 mL plastic vials.

Cation exchange capacity

Following the ammonium extraction method, the soil samples were equilibrated with 1N ammonium acetate of an adjusted pH of 7.0, then washed using four 50 mL portions of ethanol, or until no NH_4^+ ions were seen in the supernatant liquid after centrifuging as tested by Nessler's reagent.

The soil samples were then distilled using the Kjeldahl distilling unit after addition of magnesium oxide. Distillate (200 mL) was collected over 2% boric acid-indicator solution. The distillate was then titrated to the endpoint with 0.1 N standard HCl solution. One mL of 0.1N HCl used in titration is equal to 1 milliequivalent per 100g of soil for an original soil sample site of 10g. The amount was expressed in milliequivalents per 100g of soil (Black 1965).

Data analysis

Species diversity of the vegetation and wildlife was estimated using Shannon Weiner diversity index:

$$H' = -\Sigma Pi \times LogPi$$

$$Pi = \frac{ni}{N}$$

Where:
ni is number of individuals of species *i*
N is total number of species

The data were analyzed using SPSS version 20 and Microsoft Excel spreadsheet statistical packages. For the woody species diversity, T-test was used to examine whether there was a difference between the burnt sites and unburnt sites. Univariate Analysis of Variance (ANOVA) was used to determine whether there were differences in the DBHs, heights, and canopies between the burnt and unburnt sites. ANOVA was also used to determine whether there were differences in DBHs, heights, and canopies among the five burnt sites.

For the herbaceous vegetation, T-test was applied to determine whether there was a difference in species diversity between the burnt sites and unburnt sites. Percentage cover of the herbaceous vegetation was first transformed utilizing arcsin transformation before it was analyzed. ANOVA was used to determine the differences in cover, number of individual plants and heights between the burnt sites and unburnt sites and among the five burnt sites. The possible sources of variances where there were more than two groups were determined using the Post Hoc method. The average of individuals was separated by the LSD method.

A t-test was also used to determine whether there was a difference in wildlife species diversity. For the soil properties, ANOVA was used to determine if there was any difference between the burnt and unburnt sites. ANOVA was also used to assess differences among the three depth levels. It was also used to determine differences in the soil properties among the five burnt sites.

RESULTS AND DISCUSSION

Woody species diversity

The result demonstrates that fires did not affect the species diversity of the woody plants as the calculated species diversity index did not show any significant difference between the species diversity in the burnt sites and the unburnt sites (Table 2). The mean species diversity of all burnt places was 2.045 ± 0.102, and that of all unburnt sites was 2.040 ± 0.102.

Community structure of woody plants

A total of 7 different woody plant species belonging to 7 different families were counted. The woody species found in all the sites are shown in Table 3.

DBH

The mean DBH of all the woody plant species at the burnt sites was less than that at the unburnt sites. The mean DBH of the burnt sites increased with the years after burning with the one of 2014 being the smallest at 0.34±0.1m and that of 2009 is the highest at 0.60±0.2m. Although time had caused recovery of the woody plant species in burnt sites, there was no burnt site whose DBH had recovered to be equal or more than that of the unburnt site. The DBH of all species is shown in Figure 3.

There was a significant difference in the DBH of all species between all burnt sites and all unburnt sites with the species in the unburnt locations having a higher mean DBH of 0.63 ± 0.026m and those in the burnt locations having a mean DBH of 0.49 ± 0.026m. There was also a significant difference in the DBH between all the five burnt sites with the maximum mean difference being between 2002 and 2014 and the minimum mean difference being between 2002 and 2009.

Height

The heights of the woody plant species were smaller in the burnt sites than those in the unburnt sites. The average height of the burnt sites increased with the years after burning with the one of 2014 being the shortest at 21±5.2m and that of 2002 is the tallest at 24±5.1m. There is no burnt site whose height had recovered to be equal or more than that of the unburnt site. Figure 4 listed all the heights of all woody species. There was no significant difference in the heights among all burnt sites and all unburnt sites and between all the burnt sites at different years.

Canopy

The mean canopies of all the burnt sites were smaller than the unburnt sites. The mean canopies of the burnt sites increased with the years after burning with the one of 2014 being the lowest at 1.5±0.4m and that of 2002 is the highest at 4.7±1.0m. There is no burnt site whose the canopy had recovered to be equal or more than that of the unburnt site. The values of the canopy of all species are shown in Figure 5.

Table 2. The species diversity (H') of the woody vegetation in burnt and unburnt sites

Year	Burnt (h')	Unburnt (h')
2002	1.786	1.778
2009	2.341	2.362
2012	1.894	1.848
2013	2.018	2.114
2014	2.185	2.098

Table 3. The woody plant species and family found in all the sites

Family	Species	Burnt sites	Unburnt sites
Bignoniaceae	*Markhamia lutea*	101	94
Canellaceae	*Warburgia ugandensis*	113	95
Cupressaceae	*Juniperus procera*	62	85
Lauraceae	*Ocotea usambarensis*	95	86
Oleaceae	*Olea africana*	105	97
Podocarpaceae	*Podocarpus falcatus*	91	100
Stilbaceae	*Nuxia congesta*	36	39

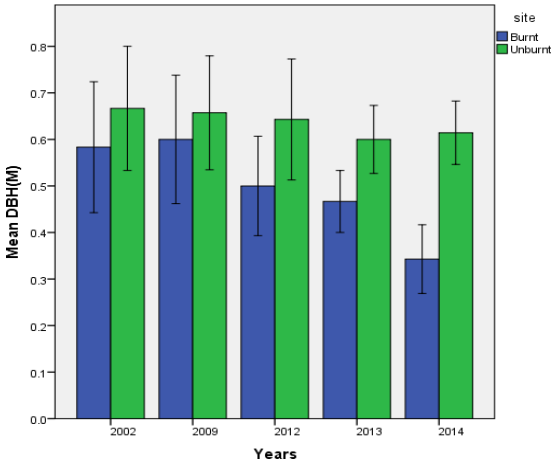

Figure 3. The mean DBH of the woody species in all the sites

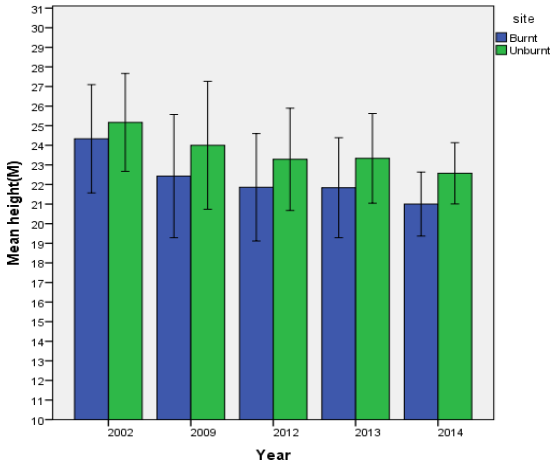

Figure 4. The average height of the trees in all the burnt and unburnt sites

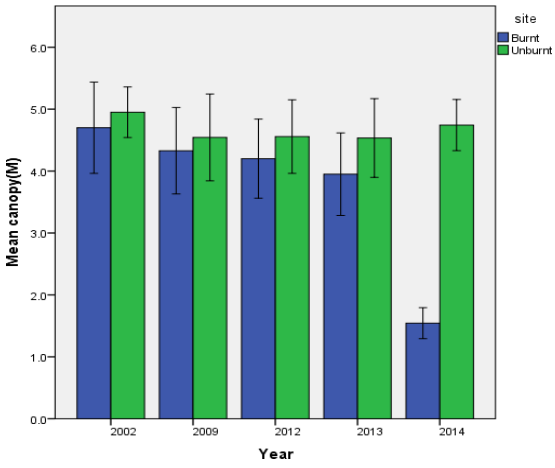

Figure 5. The mean canopy of trees in all the sites

There was a significant difference in the canopy between all burnt sites and unburnt sites ($F_{1, 64}$ =12.467; P<0.05). There was also a substantial difference in the canopy between all the five burnt sites ($F_{4, 28}$ =17.418; P<0.05) with the maximum mean difference being between 2002 and 2014 of 3.157 ± 0.44m and the minimum mean difference being at 2002 and 2009 of 0.371± 0.44m.

Herbaceous species diversity

Fire markedly reduced species diversity of the herbaceous plants immediately after it occurred. However, the species diversity quickly recovered within one year and got higher than the unburnt sites as plants regenerated after the fires. The place that had been burnt less than a year ago, i.e., in 2014, is the only one that had lesser species diversity than the unburnt site. Within a year, the herbaceous vegetation had already recovered and was more in the places that had been burnt. The species diversity of the herbaceous plant of all sites is shown in Table 4.

Community structure of herbaceous species

In all the burnt sites, a total of 39 species were counted while in all unburnt places, a total of 22 species were counted. Only seventeen species were found in the burnt sites. All the species found in the unburnt places were also found in the burnt locations. Table 5 is a list of the herbaceous plant species that were found in the burnt sites and not found in the unburnt locations.

Table 4. The species diversity (H') of the herbaceous vegetation in burnt and unburnt sites.

Year	**Burnt** (H')	**Unburnt (** H')
2002	2.441	1.682
2009	2.469	1.716
2012	2.369	1.837
2013	2.298	1.603
2014	0.950	1.737

Table 5. The herbaceous species and family found in the burnt locations and not found in the unburnt locations

Family	**Species**
Apiaceae	*Ferula communis*
Apocynaceae	*Gomphocarpus stenophyllus*
Asteraceae	*Cardius keniensis*
	Crepis aurea
	Dichrocephala chrysanthemifolia
	Gnaphalium purpureum
Fabacea	*Crotalaria paniculata*
	Rhynchosia minima
	Trifolium cryptopodium
	Trifolium tembense
	Adenocarous manii
Lamiaceae	*Leucas venulosa*
Poaceae	*Oplismenus compositus*
Ranunculaceae	*Delphinium macrocentron*
Rhamnaceae	*Rhamnus prinoides*
Rosaceae	*Alchemilla gracilipes*
Rubiaceae	*Pentas lanceolata*

Cover

The site burnt on 2104 was the only one that had a lower mean percentage cover compared to its unburnt places. All the other burnt places had more percentage cover than their unburnt sites as the herbaceous vegetation in those burnt sites had already regenerated and exceeded the unburnt sites. It took only one year for the herbaceous vegetation to restore its cover increase. The percentage cover of all the species found in every place is shown in Figure 6.

After Arcsin transformation of the percentage cover, there was a significant difference between the percentage cover of the burnt sites and the unburnt sites with the burnt sites having a higher mean cover 8.39±0.94% than the unburnt sites which had a mean cover of 5.47±0.84 %.

There was also a significant difference in the cover between all the burnt sites in the different years ($F_{4, 73}$=8.309; P< 0.05). The most considerable mean difference was between the year 2014 and 2002 which was 20.23 ± 4.09%, and the smallest mean difference was between the year 2009 and 2002 which was 1.42 ± 2.13%.

Number of individual plants

The location burnt in 2014 was the only one showing a lower number of herbaceous plants compared to the unburnt places (Figure 7). All the other burnt places had a higher number of herbaceous plants than the unburnt sites as fire led to regeneration in a year's time leading to a higher number of individuals in the burnt places.

There was a significant difference between the number of individuals in the burnt sites and the unburnt sites with the burnt sites having a higher mean number of 58.42±8.37 plants than the unburnt places which had a mean number of 35.83±7.53 plants.

There was also a significant difference in the number of individuals between all the burnt sites in the different years (F4, 73=3.101; P< 0.05). The maximum mean difference was between the year 2014 and 2002 which was 58.23 ± 20.97 plants and the minimum mean difference was between the year 2009 and 2002 which was 16.48 ± 18.34 plants.

Height

The site burnt in 2014 was solely the one that had a smaller mean height of herbaceous plants compared to the unburnt sites. All the other burnt sites had a bigger mean height of herbaceous plants than the unburnt sites. The height of every species is displayed in Figure 8.

There was a significant difference between the height of the herbaceous plants with the burnt sites having a higher mean height of 81.56±5.05cm than the unburnt sites which had an average number of 61.85±4.47cm.

There was also a significant difference in the height of the plants between all the burnt sites in the different years. The maximum mean difference was between the year 2014 and 2002 which was 41.34 ± 14.19cm, and the minimum mean difference was between the year 2009 and 2002 which was 10.74 ± 13.04 cm.

Animals

Nine different species from 5 families were counted in all the sites (Figure 9). There were no animals that were recorded in the place that was burnt in 2014. Only the sites that had been burnt in 2014 and 2013 had fewer animals than their corresponding unburnt locations. All the other burnt places had more animals compared to their similar unburnt sites. The site that was burnt in 2012 had the highest number of animals which was 132. Amongst the unburnt sites, 2014 had the highest number with 86.

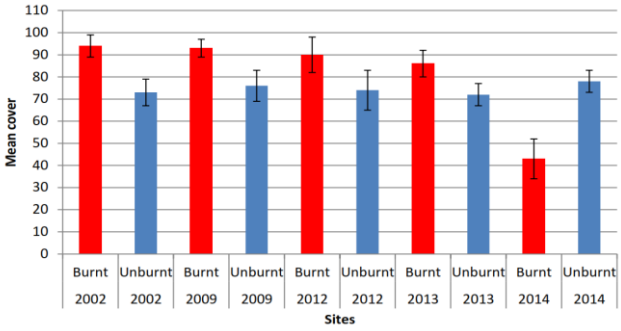

Figure 6. The mean percentage cover of the herbaceous vegetation in all sites

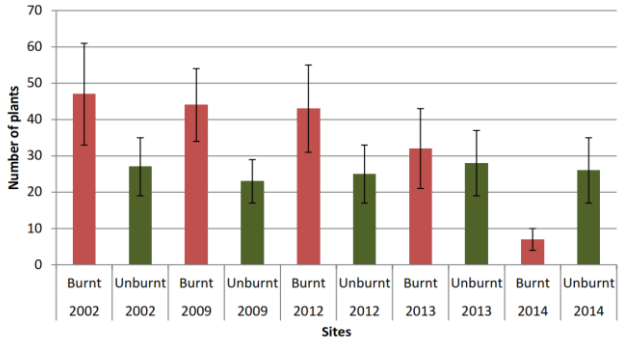

Figure 7. The mean number of plants found in all sites

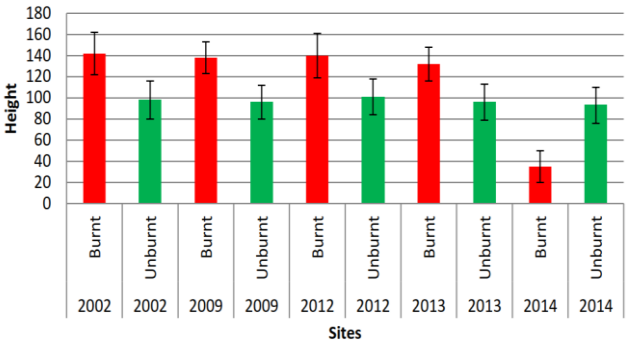

Figure 8. The mean height (cm) of the species found in all sites

Animals species diversity

Table 6 shows no animal was observed on the site that had recently been burnt (less than two weeks after the fire-2014). In the place that had been burnt one year ago, the species diversity of the animals was less than the unburnt site. In all the other areas that had been burnt more than one year ago, the species diversity of the animals was more than in their corresponding unburnt sites.

Birds

Thirty-four species belonging to 13 different families were counted in all sites. All the unburnt sites had a higher number of birds than the unburnt sites (Figure 10).

The species diversity of the birds is shown in Table 7. In all sites, the diversity was higher which had been burnt more than a year ago than in the unburnt sites. Only the sites that had been burnt less than a year ago had less species diversity than the unburnt sites because there was more vegetation in the burnt sites that provided habitat and food for the birds.

Soil properties

pH

The mean pH values of soils from both the burnt sites and unburnt sites at the three levels of depth are displayed in Figure 11. The soils were acidic as their pH ranged from 3.4 in 2014 unburnt site to 6.5 in the site which was burnt. At 0-15 cm depth, the mean pH of the burnt sites was higher in the sites burnt in 2014, 2013, 2012 and 2009. The locations that were burnt in 2002 were the only that had a lower pH compared to the unburnt site. The mean pH at 0-15 cm of the burnt and unburnt sites was 5.28±0.68 and 4.26±0.57 respectively. There was a significant difference in pH of the soil at the 0-15cm depth between burnt sites and unburnt sites (t 0.05 (1), 14=3.619; P<0.05).

At 15-30cm, the mean pH of all the burnt sites was higher than the unburnt sites. The mean pH of the burnt sites at this depth was 4.99±0.28, and that of the unburnt sites was 4.68±0.27. The pH at a depth of 15-30cm had a significant difference between the burnt sites and unburnt sites (t 0.05 (1), 14=3.961; P<0.05).

At 30-45cm, the mean pH of the burnt sites was higher than unburnt sites apart from the sites burnt in 2002 and 2009. The mean pH of the burnt sites was 4.85±0.27 while that of the unburnt sites was 4.82±0.32. The pH at a depth of 30-45cm did not have a significant difference between the burnt and the unburnt sites (t 0.05 (1), 14=0.349; P>0.05).

Table 6. The species diversity (H') of animals in burnt and unburnt sites

Year	Burnt (H')	Unburnt (H')
2002	1.6771	1.5924
2009	1.6315	1.5997
2012	1.9407	1.7144
2013	1.2613	1.7926
2014	0	1.8623

Table 7. The species diversity (H') of birds observed burnt and unburnt sites

Site	Burnt (H')	Unburnt (H')
2002	2.7352	2.6989
2009	2.8815	2.8291
2012	2.7871	2.6314
2013	2.1923	2.2248
2014	2.2036	2.6927

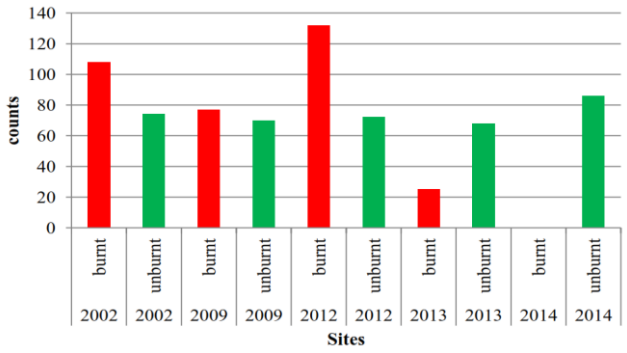

Figure 9. The total number of animals found in all the burnt and unburnt sites

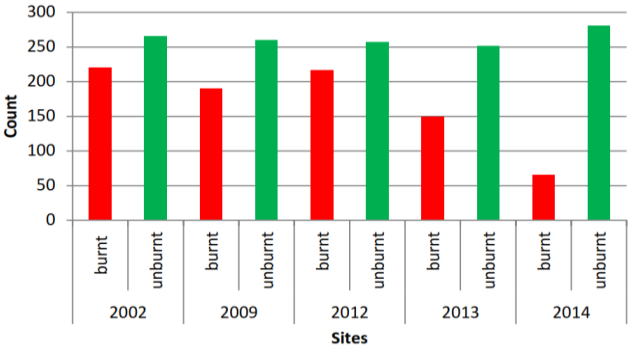

Figure 10. The total count of birds in all burnt and unburnt sites.

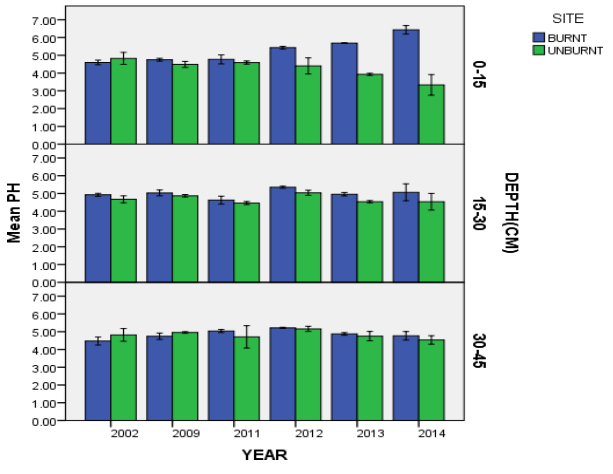

Figure 11. The pH of soils from both the burnt sites and unburnt sites at the three levels of depth

Nitrogen

Data for percent nitrogen is shown in Figure 12. The data indicate that at 0-15 cm deep, the amount of nitrogen in the soils from the burnt sites was lower than in soils from unburnt sites in the sites burnt in 2014, 2013 and 2012. However, on the sites that were burnt in 2009 and 2002, the soils had slightly higher nitrogen content compared to the unburnt site. The mean nitrogen at 0-15 cm of the burnt sites was 0.68±0.19%, and that of the unburnt sites was 1.10±0.66%. There was a significant difference in soil nitrogen at the 0-15cm depth between burnt sites and unburnt sites ($F_{1, 34}$=5.353; P<0.05).

At 15-30cm, the mean nitrogen of all the burnt sites was lower than the unburnt sites apart from the site burnt in 2014 where the mean nitrogen was higher in the burnt site than in the unburnt site. The mean nitrogen of the burnt sites at this depth was 0.58±0.21%, and that of the unburnt sites was 0.70±0.17%. At this depth, nitrogen amount was comparable between burnt and unburnt sites.

At 30-45cm, the mean nitrogen of the burnt sites was lower than unburnt sites apart from the sites burnt in 2014. The mean nitrogen of the burnt sites was 0.45±0.26% while that of the unburnt sites was 0.67±0.26%. The nitrogen at a depth of 30-45cm had a significant difference between the burnt and the unburnt sites ($F_{1, 34}$=29.55; P<0.05).

Univariate tests of nitrogen between depths showed that there was a significant difference between the three levels of depths ($F_{2, 51}$=7.819; P<0.05). After post hoc tests, the significant difference was between 0-15cm and 30-45cm and also between 0-15cm and 15-30cm. Between 15-30cm and 30-45cm, the mean difference was 0.14 and P>0.05 meaning there was no significant difference.

The nitrogen level between the sites burnt at different years was significantly different. The maximum mean difference was observed between 2002 and 2014, and the minimum mean difference was between 2002 and 2012.

Organic carbon

The mean organic carbon values in the soils from all the sites are shown in Figure 13. The data indicate that at 0-15 cm deep, the mean organic carbon of the burnt sites was higher in the sites burnt in 2014, 2013 and 2012 than in their unburnt sites. The sites that were burnt in both 2009 and 2002 had a lower mean organic carbon compared to the unburnt site. The mean organic carbon at 0-15 cm of the burnt sites was 7.05±0.93, and that of the unburnt sites was 6.07±1.59. At a depth of 0-15cm, there was a significant difference between the amount of organic carbon in the burnt sites and unburnt sites ($F_{1, 34}$=30.085; P<0.05).

At 15-30cm, the mean organic carbon of the sites burnt in 2014, 2013 and 2012 was higher than the unburnt sites. The sites burnt in 2009 and 2002 had a lower mean organic carbon than in the unburnt sites. The mean organic carbon of the burnt sites at this depth was 6.07±1.26%, and that of the unburnt sites was 5.60±1.52%. At this depth, the amount of organic carbon between the burnt sites and the unburnt sites was relatively the same.

At 30-45cm, the mean organic carbon of the sites burnt in 2014 and 2012 was higher than the unburnt sites. The sites burnt in 2009, 2002 and 2013 had a lower mean organic carbon than in the unburnt sites. The mean organic carbon of the burnt sites was 5.19±1.94% while that of the unburnt sites was 5.27±1.31%. The organic carbon at a depth of 30-45cm had no significant difference in the amount of organic carbon between the burnt sites and the unburnt sites ($F_{1, 34}$=0.141; P>0.05).

Univariate tests of organic carbon between depths showed that there was a significant difference between the three levels of depths ($F_{2, 51}$=6.374; P<0.05). After post hoc tests, the main difference was between 0-15cm and 30-45cm as well as between 0-15cm and 15-30cm. On the other hand, there was no significant difference between 15-30cm and 30-45cm depth.

There was a significant difference in the organic carbon between the sites burnt at different years. The maximum mean difference was between 2002 and 2014 which was 1.76±0.69%, and the least mean difference was between 2002 and 2013 which was 0.54±0.69%.

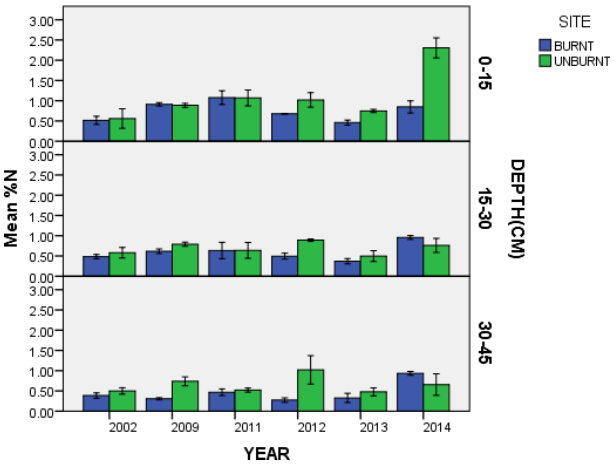

Figure 12. The nitrogen (%) of soils from all burnt and unburnt sites at the three depth levels

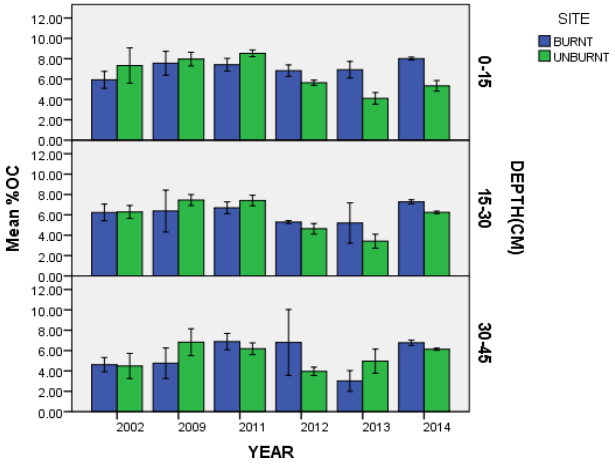

Figure 13. The organic carbon of soils from all burnt and unburnt sites at different depths

Potassium

The average values of the potassium in the soils from all the sites are shown in Figure 14. The data demonstrate that at 0-15 cm deep, the mean potassium was higher in all the burnt sites than in their unburnt sites apart from the site burnt in 2009. The mean potassium at 0-15 cm of the burnt sites was 2.68±1.78and that of the unburnt sites was 1.14±0.61.

At 15-30cm, the mean potassium of all the burnt sites was higher than the unburnt sites. The mean potassium of the burnt sites at this depth was 1.58±0.98 Cmol/kg, and that of the unburnt sites was 1.21±0.81 Cmol/kg. At 15-30cm, there was no significant difference in the amount of potassium between the burnt sites and the unburnt sites ($F_{1, 34}$=0.766; P>0.05).

At 30-45cm, the mean potassium of the sites burnt in 2014, 2012 and 2002 was higher than the unburnt sites. The sites burnt in 2013 and 2009 had lower mean potassium than that of unburnt sites. The mean potassium of the burnt sites was 1.31±0.97 Cmol/kg while that of the unburnt sites was 1.03±0.61 Cmol/kg. At 30-45cm depth, there was also no significant difference in the amount of potassium between the burnt sites and the unburnt sites ($F_{1, 34}$=1.659; P>0.05).

Univariate tests of potassium between depths showed that there was a significant difference between the three levels of depths ($F_{2, 51}$=5.951; P<0.05). After post hoc tests, the main difference was between 0-15cm and 30-45cm where the mean difference was 1.32 Cmol/kg and P<0.05. Between 0-15cm and 15-30cm, there was also a significant difference. However, between 15-30cm and 30-45cm, there was no significant difference.

There was a significant difference in the potassium between the sites burnt at different years. The maximum mean difference was between 2002 and 2014 which was 2.71±0.43 Cmol/kg, and the minimum means difference was between 2002 and 2009 which was 0.46±0.43 Cmol/kg.

Phosphorous

The mean phosphorous values in the soils from all the sites are shown in Figure 15. The data shows that at 0-15 cm depth, the mean phosphorous was lower in all the burnt sites than in their unburnt sites apart from the site burnt in 2009. The mean phosphorous at 0-15 cm of the burnt sites was 8.26±2.49 ppm and that of the unburnt sites was 13.32±3.83 ppm. The amount of phosphorous was significantly different between the burnt sites and unburnt sites at this depth.

At 15-30cm, the mean phosphorous was lower in all the burnt sites than in their unburnt sites apart from the site burnt in 2009. The mean phosphorous at 15-30 cm of the burnt sites was 7.59±3.86 and that of the unburnt sites was 10.11±2.89 ppm. There was no significant difference in the amount of phosphorous between the burnt sites and the unburnt sites ($F_{1, 34}$=3.55; P>0.05).

At 30-45cm, the mean phosphorous of the sites burnt in 2014, 2012 and 2002 was lower than the unburnt sites. The areas burnt in 2013 and 2009 had higher mean phosphorous than in the unburnt sites. The mean phosphorous of the burnt sites was 8.49±3.46ppm while that of the unburnt sites was 9.01±1.72 ppm.

Univariate tests of phosphorous between depths showed that there was no significant difference between the three levels of depths. There was a significant difference in the phosphorous between the sites burnt at different years. The maximum mean difference was between 2002 and 2014 which was 5.93±0.73 ppm, and the minimum mean difference was between 2002 and 2009 which was 0.14±0.73 ppm.

Cation Exchange Capacity (CEC)

The mean CEC values in the soils from all the sites are shown in Figure 16. The data shows that at 0-15 cm deep, the mean CEC was higher in all the burnt sites than in their unburnt sites apart from the site burnt in 2002.

At 15-30 cm, the mean CEC was higher in all the burnt sites than in their unburnt sites apart from the site burnt in 2002. The mean CEC at 15-30 cm of the burnt sites was 20.16±2.78 mol/kg, and that of the unburnt sites was 18.34±3.41 mol/kg. The CEC at a depth of 15-30cm did not have a significant difference between the burnt sites and unburnt sites ($F_{1, 34}$=1.523; P>0.05).

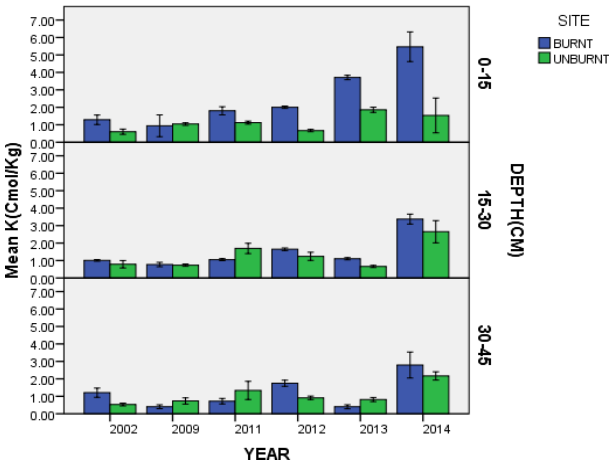

Figure 14. The potassium of soils from all the burnt and unburnt sites at different depths

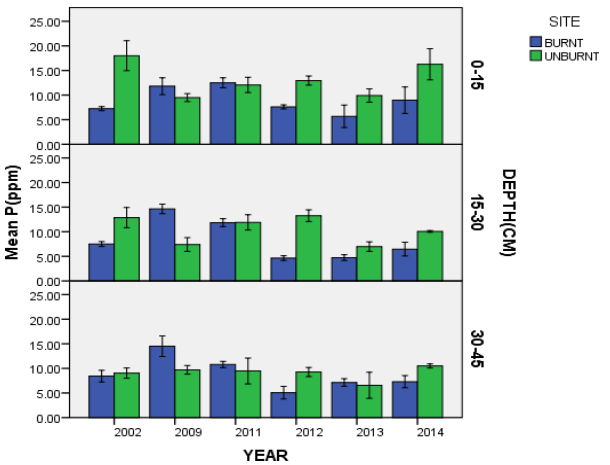

Figure 15. The phosphorous of soils observed in all the burnt and unburnt sites at different depths

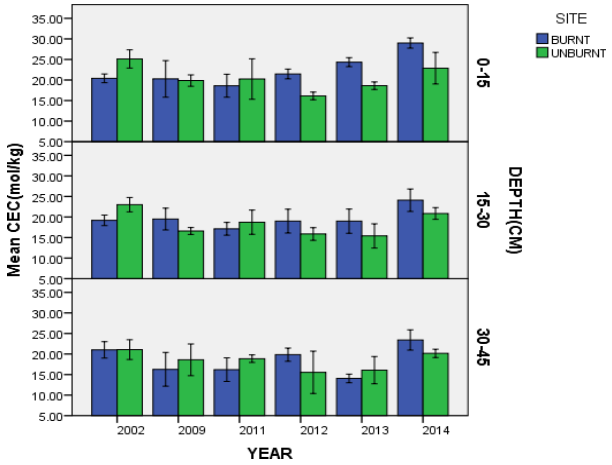

Figure 16. The CEC of soils from all the burnt and unburnt sites at different depths

At 30-45cm, the mean CEC of the sites burnt in 2014, 2012 and 2002 was higher than the unburnt sites. The sites burnt in 2013 and 2009 had lower mean CEC than in the unburnt sites. The mean CEC of the burnt sites was 18.91±3.90 mol/kg while that of the unburnt sites was 18.28±3.39 mol/kg. The CEC at a depth of 30-45cm did not have a significant difference between the burnt and the unburnt sites.

Univariate tests of CEC between the three levels of depths showed that there was a significant difference in the CEC between depths. After post hoc tests, the major difference was between 0-15cm and 30-45cm. There was also a significant difference between 0-15cm and 15-30cm, with the mean difference was 2.691 and P=0.027. There was a significant difference in the CEC between the sites burnt at different years. The maximum mean difference was between 2002 and 2014 which was 5.29±1.46 mol/kg, and the minimum mean difference was between 2002 and 2012 which was 0.09±1.46 mol/kg.

Discussions

The effect of fire on plant species diversity

Woody vegetation

The results indicated that fires did not contribute to the major adverse effect on the species diversity of the woody species. The reason may be because most fires experienced at Aberdare Ranges are usually ground fires that do not burn down the woody trees but the barks and sometimes small canopies of the woody trees are burnt. As a consequence, there was no difference in the species diversity of the woody species between the burnt sites and the unburnt sites. Tiny woody plants and seedlings are the only ones usually burnt down by the fires occurring at the Aberdares. The large trees found in Aberdares are resilient to ground fires and are not much affected by the fires. The results agreed with the studies that show that the effects of fire on woody vegetation depend on the intensity and frequency of the fire (Lehmann et al. 2014). Intensity could be low at Aberdares such that they do not have much effect on woody species diversity.

However, there was a significant difference in DBH between the woody species in the burnt sites and the unburnt sites with the unburnt sites having a higher DBH than that of the burnt sites. All the five burnt sites had a lower mean DBH than their corresponding unburnt sites, meaning that DBH takes long to increase. DBH of a tree is crucial in estimating the amount of timber volume in a tree and also in predicting the age of a tree because diameter increment is the only constant non-reversible feature of tree growth (White 1998). This indicates that the fires at Aberdares led to reduce in the timber volume of the trees and also could lead to a wrong prediction of the age of the trees at Aberdares.

The height and canopies of the trees were also smaller in all the burnt sites. There was no burnt site that the height and canopy had recovered. This means that the fires at Aberdares had a negative effect on both height and canopy. The negative impact of fire on DBH, height, and canopy of trees affects the carbon storage ability of tree because carbon is allocated preferentially to the new leaves and roots and then to storage and stem diameter growth (Waring and Pitman 1985). This means that the fires at Aberdares contribute to global warming, by releasing carbon dioxide to the atmosphere during the fires and by reducing carbon storage.

Herbaceous plants

The data showed that fire causes a decrease in the herbaceous plant species diversity, but one year later, the burnt sites had regenerated and the species diversity of the burnt areas was more diverse than the unburnt areas. The fire burnt almost all herbaceous plants, except *Micromera imbricata*, *Oplisnemus compositus*, and *Erica arborea*. Fire tends to favor species that can tolerate heat stress. Immediately after a fire, the percentage of bare ground is increased which made the land prone to erosion depending on its topography and weather.

In all the other sites except the one that was burnt in 2014, the species diversity of the herbaceous plants on the burnt sites was higher than on the unburnt sites; an indication that fire triggered the recovery of herbaceous plants. The statistical analysis showed that vegetation cover and height differ significantly between the burnt sites and the unburnt sites. In the sites that had been burnt recently, the height of the vegetation was shorter than the height of the vegetation in their corresponding unburnt sites. In the sites where vegetation had already recovered, the height of the vegetation was even more than the unburnt sites. Fires triggered the regrowth of the herbaceous vegetation, and one year later, the vegetation was taller in the burnt areas other than the site burnt in 2014.

Fires also had the same effect on the number of individuals as the cover and height. The sites that had already recovered had a higher number of individuals compared to the unburnt areas. Only the locations that were recently burnt had fewer numbers of individuals. This was an indication that fire has a positive long-term effect on the herbaceous vegetation. It triggers regeneration of pioneer species whose seeds could be lying dormant in the soil or on the soil surface.

Effects of fires on animals

Effects of fires on mammals

The burnt sites that had not yet regenerated possessed fewer animals when compared to the unburnt sites. No animal was found in the site that was burnt in 2014 as the data was collected several days after the fire. They had emigrated out of that area as a consequence of the fire since no vegetation could support the herbivores that support the rest of the food chain. No animals carcass were found at the site meaning that no animal succumbed to the fire.

The site that was burnt in 2013 also had lesser animals compared to the un-burnt site of the same year because the vegetation cover of that burnt site was less than the un-burnt site. Furthermore, animals prefer an area with higher vegetation cover where there are more resources.

Individuals were remaining in the burned forest dealt with a different set of problems. Fires brought an immediate negative impact on the wildlife, which in turn can affect the tourism of the country considering that wildlife is one of the major attractions to tourists. Fire leads to the emigration of the wildlife from the burnt areas to the unburnt areas which will lead to the completion of resources in the unburnt areas leading to the exclusion of the weaker wildlife. This ends up in a reduction of the wildlife population.

Effects of fires on birds

All the burnt sites had fewer birds than the unburnt sites. The number of birds in the other sites burnt in 2013, 2012, 2009 and 2002 had increased although they were still fewer than the unburnt sites. This could be as a result of the reduced canopy of the trees in all the burnt sites considering that vegetation structure and floristic composition influence the availability of food, the risk of predation and availability of nest sites.

Aberdares is an Important Bird Area where many visitors go for bird watching. Fires can lead to negative impacts on bird watching as a result of a decline in bird's diversity which can have disadvantages to the country's economy.

Effects of fire on soil properties

The fire affected mostly the upper layer at 0-15cm. Very few effects were observed only at 15-30cm. This is probably because most fires that occur at Aberdares are less severe and cannot exert effects beyond 15cm deep as the effects of fires depend on the fire severity which is determined by the intensity and the duration of the fire.

pH

After two years, there was not much difference in pH between burnt sites and unburnt sites. This indicated that fire resulted in an increase in pH and it took almost two years for the pH to return to its initial levels. The increase in pH could be a result of ash accretion. Certini (2005) suggests that the response depends on the amount of ash and buffering capacity of the soil. This rise in pH is because mineral substances are released as oxides or carbonates that usually have an alkaline reaction.

Nitrogen

The decrease in nitrogen in the recently burnt sites is a result of nitrogen volatilization caused by fire. Nitrogen has low-temperature thresholds and is easily volatilized. Significant losses of nitrogen during the fires could adversely affect long-term site productivity in Aberdare forest ecosystem, particularly if nitrogen replenishment mechanisms are not provided for during post-fire management. Nitrogen is considered the most limiting nutrient in wildland ecosystems, and as such it requires special consideration when managing fire, particularly in nitrogen-deficient ecosystems (Maars et al. 1983)

Organic carbon

The organic carbon was high in the burnt sires than in the unburnt sites, and this was more evident at 0-15cm where there was a significant difference. This is a result of the rapid decomposition of organic matter on the soil during burning to release organic carbon into the underlying soil (Certini 2005). The results were similar to that of a study by Bird et al. (2000), which was carried out on tropical savanna sites in Africa. Low-frequency burning increased soil carbon by about 10%

The effect of organic carbon by fire is determined by the severity of a fire. Fire severity influences the amount of organic matter that is lost. Groeschl and others (1990) revealed that areas burned by a low-severity fire, the forest floor Oi and Oe layers were completely combusted, but the Oa layer remained. High-severity burning also consumed the Oa layer. Of the 10.1 tons/acre (22.6 Mg/ha) of Carbon present in the forest floor in the unburned areas, no Carbon remained in the high-severity burned areas compared to 9.3 tons/ acre (20.8 Mg/ha) Carbon that was left on the burned areas at low severities.

Potassium

Increase in potassium in the burnt sites could be due to rapid decomposition of organic matter during burning due to elevated temperatures releasing nutrients to the soil. The organic matter acts as the primary reservoir for several nutrients and, thus, is the source of most potassium (Certini 2005).

Phosphorous

Phosphorous at 0-15cm was significantly lower in the burnt sites than in the unburnt sites. The differences were also observed in the sites that had been burnt less than two years ago but in the sites burnt more than two years ago did not have much difference between the burnt and unburnt sites. Just like nitrogen, phosphorous has low-temperature thresholds and is readily volatilized. This led to a decrease in the amount of phosphorous in the burnt sites.

Cation Exchange Capacity

As much as fire can make water-soluble cations available for plant uptake, light burns like the ones experienced at Aberdares do not affect the exchange system.

In conclusion, fires occur every year at Aberdares during the dry season mainly due to arsonists when they want to ferry poles from the forest, charcoal burning, accidents during honey harvesting as the harvesters use fire to drive bees away, and clearing of the forest to create farmlands. Fires brought an immediate negative effect on the herbaceous vegetation but later leads to a positive effect after the vegetation regenerated. The sites that were burnt in 2013, 2012, 2009 and 2002 had more herbaceous vegetation and a higher cover than their unburnt sites. The cover, height and the number of plants increase with time with the site burnt in 2002 having the highest and that burnt in 2014 having the lowest. Animals are also affected by fires. The decrease in vegetation led to a reduction in the wildlife population and diversity. Once vegetation regenerates, wildlife population and diversity tend to increase again. The sites burnt in 2013, 2012, 2009 and 2002 had more wildlife diversity than the unburnt sites since vegetation had regenerated. Loss of vegetation after a fire leads to the emigration of wildlife to the unburnt sites. All the sites burnt had fewer birds than the unburnt sites. This is as a result of the reduced canopy of the trees in the burnt forests. Immediately after a fire, the number of birds utilizing that area reduces drastically as a result of the reduced amount of food and destruction of their nests. Fires also affect the soil properties investigated: nitrogen, potassium, phosphorous, pH, organic carbon, and cation exchange capacity. Fire promotes an increase in pH, potassium, organic carbon and cation exchange capacity due to rapid decomposition of organic matter and leads to a reduction of nitrogen and phosphorous since these two elements are easily volatilized at a low-temperature threshold. Prescribed burning can be adopted by ecosystem managers where controlled fires can be used to burn an ecosystem to increase the herbaceous vegetation, despite the immediate negative effects of fires on species diversity and some soil properties.

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Ice Nucleation Active bacteria in Mount Lawu forest, Indonesia: 2. Identification and characterization of *ina* gene bacteria isolated from lichens

TEGUH NUR ARIFIN, ARI SUSILOWATI♥, SUTARNO
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57126, Central Java, Indonesia. Tel./fax.: +62-271-663375, ♥email: arisusilowati@staff.uns.ac.id

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Abstract. *Arifin TN, Susilowati A, Sutarno. 2018. Ice Nucleation Active bacteria in Mount Lawu forest, Indonesia: 2. Identification and characterization of* ina *gene bacteria isolated from lichens. Asian J For 2: 39-46.* Ice nucleation active (INA) bacteria can catalyze ice formation. Moreover, these bacteria cause frost injury in plants. This study aimed to determine the INA bacteria species of Mount Lawu forest, Java, Indonesia, based on the 16S rRNA gene and the characters of ice nucleation active gene coding of INA bacteria on lichens used *ina* gene primer. First, the isolates of INA bacteria were grown in NAG, and the DNA could be isolated. After that, genes coding for 16S rRNA and *ina* gene were amplified, and the amplification products were sequenced. Furthermore, the sequences were analyzed with BLAST program to know the similarity of bacteria species and *ina* gene characters. The result showed that the isolated INA bacteria had similarities with *Pantoea*, *Pseudomonas*, and *Rahnella*. The *ina* gene from N.2.1.B-13 isolate consists of 189 amino acids, dominated by a common amino acid found in other *ina* genes, like Alanine, Glycine, Tyrosine, Serine, Leucine, and Threonine. N terminal and C terminal are not found because *ina* gene from N.2.1.B-13 isolate is similar to the ice nucleation protein gene *Pseudomonas borealis* from bases 2947 bp to 3491 bp or 871 to 1060 amino acids.

Keywords: Amplification, INA bacteria, *ina* genes, sequencing

INTRODUCTION

The microbial community on leaf surfaces is diverse and includes many different species, such as bacteria, filamentous fungi, yeasts, algae, and a few protozoa and nematodes (Andrews and Harris 2000). Bacteria with the largest population are phyllosphere bacteria which differ greatly in size and number in plant parts of the same species (Lindow et al. 1978). One of the components of the leaf bacterial community that can be found is ice-nucleation-active (INA) bacteria (Lindow and Brandl 2003).

INA bacteria are a group capable of catalyzing ice formation at temperatures above -10°C. These bacteria can express ice nucleation proteins on the cell surface, lowering the water temperature and freezing it. If there is no ice nucleation, pure cold water (H_2O) can only be supercooled and will not spontaneously freeze until the temperature reaches -40°C. The INA bacteria can function in helping to accelerate the ice freezing process (Stephanie and Waturangi 2011).

INA bacteria have been shown to cause frost damage in some plants (Hirano and Upper 2000). Bacteria commonly found in plants consist of several species (such as *Pseudomonas syringae* and *Erwinia herbicola*), which can produce a protein that can induce ice core formation at temperatures above -2°C. The population on the leaf surface of this species of bacteria is limited and can form supercooling in some parts of the plant where INA bacteria are present by forming ice that can damage at temperatures of -2 to -4°C. Since plants lack the intrinsic elements of ice core formation at these temperatures, these bacteria play an important role in initiating frost damage formation (Lindow et al. 1978).

Various Gram-negative bacteria can trigger ice formation at temperatures of -2 to -12°C in nature (Lindow et al. 1982a,b; Hirano et al. 1985). Most INA bacteria are associated with plants (Lindow et al. 1978; Lindermann et al. 1982; Loper and Lindow 1994; Waturangi et al. 2008) or animals (Lee et al. 1995). However, other organisms, including several species of fungi (Pouleur et al. 1992) and lichens (Kieft 1988), have been reported for their ability to induce ice formation.

INA bacteria that have been found include *P. syringae*, *Pseudomonas viridiflava*, *Pseudomonas fluorescens, E. herbicola* (synonymous with *Pantoea herbicola*), and *Xanthomonas campestris* (Edwards et al. 1994). These five bacterial species can catalyze ice formation at a temperature of -1.5°C to -10°C. Even at temperatures above -5°C, these species can cause frost injury on the leaf surface. It is due to changes in the water between and inside the leaf cells to the ice at a temperature of -5°C (Gurian-Sherman and Lindow 1993).

Some INA bacteria are also considered important in condensation and ice core formation in clouds. Ice formation in tropospheric clouds is required to form snow and most precipitation. Studies on the biological formation of ice cores in snowfall have been carried out. Biological ice core-forming at warm temperatures is abundant in fresh

snow samples (Christner et al. 2008). According to Morris et al. (2004), INA bacteria also play a role in atmospheric processes that cause rain, given that these bacteria are easily spread in the atmosphere and have been found in clouds at an altitude of several kilometers. Furthermore, INA bacteria participate in the biological cycle of precipitation; they are transported to the cloud from the plant canopy.

Besides having an important role in bioprecipitation, INA bacteria can make artificial rain and snow by seeding clouds with INA bacteria as a substitute for salt sowing, which is now widely used. In addition, INA bacteria can be used in the food preservation industry by freezing, which is initiated by INA bacteria (Wahyudi 1995). It can be seen because INA bacteria can cause freezing at a temperature of -6°C in samples containing 10% sucrose. These results may indicate that with the application of INA bacteria, several types of food can be frozen through this process by modifying the slightly high temperature a few degrees below 0°C for periodic freezing, saving energy, and improving the quality efficiency of food products (Li et al. 1997).

Lichens are a symbiosis between fungi and algae that form a unified whole morphologically and physiologically. Lichenes can stick to rocks or other plants (Setyawan 2000). Samsali's research (2008) showed that along the hiking trail of Cemoro Sewu, Mount Lawu, Java, Indonesia, there were 12 epiphytic species, namely 4 from the Lichens division, 1 from the Bryophyta division, 5 from the Pteridophyta division, and 2 from the Spermatophyta division. A study by Kieft (1988) showed that some lichens in the southwest region of the United States could induce ice core formation. It indicates that the lichens may contain microorganisms capable of assisting the formation of ice cores, namely INA bacteria.

Most research on INA bacteria is carried out in subtropical areas, so research on INA bacteria from the tropics, especially in Indonesia, is very necessary (Stephanie and Waturangi 2011); considering the role of INA bacteria is quite a lot, one of which is to help the bioprecipitation process. The INA bacteria have been isolated from lichens on the climbing route of Cemoro Sewu, Mount Lawu by Fu'adah (2017), and isolates have been found in the Biology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Central Jawa, Indonesia. However, the isolates of INA bacteria have not been identified and characterized, so the information on these INA bacteria is still very little. Therefore, it is necessary to research the identification and characterization of INA bacteria from these lichens. This research data can be used to understand species diversity and the role of INA bacteria in nature.

The aims of this study were: (i) to determine the INA bacterial species found in lichens on the hiking trail of Cemoro Sewu, Mount Lawu based on the identification with the 16S rRNA gene; (ii) to know the character of the gene encoding the ice core-forming protein of INA bacteria in lichens on the hiking trail of Cemoro Sewu, Mount Lawu by using the *ina* gene primer.

MATERIALS AND METHODS

Materials

The materials used in this study include: bacterial isolates from research conducted by Fu'adah (2014) with isolate codes K.2.1.B-5, N.2.1.B-13, N.2.1.B-15, K .2.2.B-2, K.2.2.B-4, N.2.2.B-6, N.2.2.B-7, media

Procedure

Equipment and materials sterilization

Equipment and materials used for research must be sterilized first to prevent contamination. The sterilized types of equipment include test tubes, Erlenmeyer, measuring cups, microcentrifuge tubes, PCR tubes, and tips. In addition, sterilized materials were distilled water, Nutrient Agar Glycerol (NAG) media, and ddH_2O. Sterilization was carried out using an autoclave at a temperature of 121°C and a pressure of 1 atm for 15 minutes.

Preparation of media

NA medium was prepared by mixing 6 g of NA powder with 5 mL of glycerol in a 250 mL Erlenmeyer tube and adding distilled water to a limit of 200 mL. Erlenmeyer is placed on a hot plate until the solution boils and is clear. Erlenmeyer tube holes are covered with cotton and aluminum foil. Then, this tube was sterilized using an autoclave at 121°C for 15 minutes.

To make the slant media, the NA medium was left until the solution temperature was reduced. After being warm enough, 4 mL of media was poured into each test tube and autoclaved at 121°C for 15 minutes at a pressure of 1 atm. After sterilization, the tube is slanted at 45° until the media hardens.

Isolate rejuvenation

Seven isolates, namely K.2.1.B-5, N.2.1.B-13, N.2.1.B-15, K.2.2.B-2, K.2.2.B-4, N.2.2.B-6, and N.2.2.B-7 were rejuvenated to prolong the life of bacteria. The rejuvenation process was carried out by taking one ose of each isolate and scratching it on seven slant NAs. After that, the slant agar was incubated at 27°C for 3 days and then stored in a refrigerator for 4°C.

Bacterial DNA isolation

Before the DNA of the bacterial isolates was isolated, the bacteria were first cultured on NB media for 24 hours at a temperature of 27°C. After 24 hours, the bacterial culture is ready to proceed to the DNA isolation process.

The bacterial DNA isolation process is divided into several procedures based on the kit used, namely:

Sample preparation. A total of 1 mL of bacterial culture was put into a 1.5 mL microcentrifuge tube. The sample was centrifuged for 1 minute at 10,000 rpm, and the formed supernatant was discarded. Then, 200 µL of GT Buffer was added and homogenized with the pellet by shaking or using a micropipette. Then it was incubated at room temperature for 5 minutes and continued with the lysis process.

Cell lysis. 200 µL of GB Buffer was added to the sample and vortexed for 5 seconds. Then the samples were incubated at 60°C for 10 minutes. During incubation, shake the tube every 3 minutes. At the same time, the Elution Buffer (200 µL per sample) was heated to 60°C (for DNA elution).

DNA binding. 200 µL of absolute ethanol was added to the sample and vortexed. The GD Column is placed in a 2 ml Collection Tube. The mixed solution from the cell lysis process was then transferred to the GD Column and centrifuged at 10,000 rpm for 2 minutes. The 2 mL Collection Tube containing the liquid deposit was then placed back into the new 2 mL Collection Tube.

DNA cleansing. 400 µL of W1 Buffer was inserted into the GD Column. Then it was centrifuged at 10,000 rpm for 30 seconds, and the liquid residue formed was discarded. Next, 600 µL of Wash Buffer (which had been added by absolute ethanol) was added to the GD Column and centrifuged again at 10,000 rpm for 30 seconds, and the formed liquid deposit was discarded. Then the GD Column was placed back into the 2 mL collection tube and centrifuged at 10,000 rpm for 3 minutes under dry conditions.

Elution. The GD Column was transferred to a 1.5 mL microcentrifuge tube, then 100 L of Elution Buffer (which had been heated) was added to the center of the matrix in the GD Column and allowed to stand for 3 minutes. Then the GD Column was centrifuged at 10,000 rpm for 30 seconds to obtain pure DNA.

16S rRNA gene amplification

The 16S rRNA gene amplification was performed by mixing 9 µL of ddH_2O, 12.5 µL of 2X KAPA2G Fast ReadyMix (containing DNA Polymerase 0.5 U per reaction, PCR Buffer dNTP 0.2mM, and $MgCl_2$ 1.5 mM), 1.25 µL of forward primer 63f, 1.25 µL of reverse primer 1387r, and 1 µL of DNA template. The primers used in this amplification were forward primer 63f (5- CAG GCC TAA CAC ATG CAA GTC -3) and reverse primer 1387r (5- GGG CGG WGT GTA CAA GGC -3)

Amplification was carried out under PCR conditions, namely, initial denaturation at 95°C for 3 minutes, denaturation at 95°C for 15 seconds, annealing at 55°C for 15 seconds, extension at 72°C for 5 seconds, and final extension at 72°C for 5 minutes. Denaturation, annealing, and extension were carried out for 30 cycles.

ina gene amplification

Primers used to amplify this gene include *ina*Z F (5'GCA GAC TGC GGG TTA TGA GAG C 3'); *ina*Z R (5'CGC CGG TCA GTT TGC TTC TAT C 3'); *ina*A F (5'AGG CTT TGA GAA CGG ACT AAC G 3'); *ina*A R (5' TTT CTG TCG GCT GCG TAC TG 3'); *ina*W F (5'GCA GTA CGC AGA CGG CAC AG 3'); *ina*W R (5' TTT CGT AGC CAG CAG TTG ATG TG3'); *ina*X F (5'GCA AGG GCA GCG ATG TCA C 3'); and *ina*X R (5' TCT GCG TGC TGC CGT AAC C 3').

The *ina* gene amplification was carried out by mixing 9 µL of ddH_2O, 12.5 µL of 2X KAPA2G Fast ReadyMix (containing 0.5 U DNA Polymerase per reaction, 0.2 mM dNTP Buffer PCR, and 1.5 mM $MgCl_2$), 1.25 µL of forward primer, 1.25 µL of reverse primer, and 1 µL of DNA template.

PCR carried out amplification with the provisions of initial denaturation at 95°C for 3 minutes, denaturation at 95°C for 15 seconds, annealing for *ina*X at 55.2°C for 15 seconds, annealing for inaZ at 55°C for 15 seconds, annealing for inaW at 55°C for 15 seconds, annealing for inaA at 55°C for 15 seconds and extension at 72°C for 6 seconds, and final extension at 72°C for 5 minutes. Denaturation, annealing, and extension were carried out for 40 cycles.

Sequencing of 16S rRNA and ina *coding genes*

The PCR products of 16S rRNA and *ina* genes that were successfully amplified were sent to P.T. Genetika Science Indonesia, which will then be sequenced by 1st Base Singapore using the ABIprism™ 310 Automated DNA Sequencer (PE Applied Biosystem).

Data analysis

The results of the 16S rRNA and *ina* gene sequences were then compared with the database using the BLAST program on the National Center for Biotechnology Information website (http://www.ncbi.nlm.gov/BLAST/). The sequence similarity was then analyzed descriptively based on the existing database. Finally, the phylogenetic tree was created using MEGA 5.1 software.

RESULTS AND DISCUSSION

Isolate rejuvenation

This study used seven positive isolates of INA bacteria found in lichens on the climbing route of Cemoro Sewu, Mount Lawu, by Fu'adah (2014). The list of bacterial isolates used in this study and the classification of *ina* protein based on freezing temperature can be seen in Table 1.

Table 1 shows that all bacterial isolates came from lichens of the *Parmelia* sp. encountered at sampling station two. *Parmelia* sp. It lives as an epiphyte in pine trees (*Pinus* sp.). This station is located at an altitude of 2,532 m above sea level. Shrubs and large trees dominate it with a dense enough canopy, so sunlight is sufficiently blocked to reach plant vegetation. The temperature is around 20°C, and the light intensity is around 3676 Lux (Fu'adah 2014).

Table 1. List of bacterial isolates and their *ina* protein classification

Isolate code	Freezing temperature	INA protein class
K.2.1.B-5	-10°C	C
N.2.1.B-13	-5°C	B
N.2.1.B-15	-8°C	C
K.2.2.B-2	-5°C	B
K.2.2.B-4	-9°C	C
N.2.2.B-6	-10°C	C
N.2.2.B-7	-10°C	C

Note: K.2.1.B-5. K: Growth medium (K: King's B; N:NAG), 2: Sampling station number, 1: Repetition number, B: Lichen species name (A: Usnea; B: Parmelia), 5: Isolate number

The existing INA bacteria must be rejuvenated to be used in this study. Rejuvenation was carried out by regrowing INA bacteria on slant NAG media. NAG media is NA media added with 2.5% glycerol. Glycerol is a carbon source for bacterial growth and is commonly used to isolate ice-core bacteria (Lindow 1990). The rejuvenation result is a new bacterial colony with the same morphological characteristics as the previous bacterial colony.

Bacterial DNA isolation

DNA isolation is the first stage of various DNA analysis technologies. To extract DNA, laboratory steps are needed to break the cell wall, cell membrane and nuclear membrane, followed by the separation of DNA from other cell components (Fatchiyah et al. 2011).

In this study, DNA isolation was carried out on INA bacteria which were incubated for 3 days using Presto™ Mini gDNA Bacteria Kit (Geneaid Biotech Ltd.) and following the procedure recommended by the manufacturer. Presto™ Mini gDNA Bacteria Kit (Geneaid Biotech Ltd.) can optimally isolate the genomic DNA of Gram-negative and gram-positive bacteria.

The results of DNA isolation in Table 2 showed that the DNA of the seven INA bacteria was successfully isolated because the A260/280 ratio of isolated DNA ranges from 1.90 to 1.99. According to Sambrook et al. (1989), DNA isolates can be pure and meet the requirements to proceed to molecular analysis if the value of the A260/280 ratio ranges from 1.8 to 2.0.

16S rRNA gene amplification

Each INA bacterial DNA was amplified using PCR to obtain the 16S rRNA gene. The 16S rRNA gene is one of three types of ribosomal RNA present in prokaryotes. This gene is most often used for species determination. In addition, 16S rRNA can be used as a molecular marker because this molecule is ubiquitous (Pangastuti 2006). In this study, primers 63f (5- CAG GCC TAA CAC ATG CAA GTC -3) and 1387r (5- GGG CGG WGT GTA CAA GGC -3) were used to amplify the 16S rRNA gene. According to Marchesi et al. (1998), this primer was used because it can amplify the 16S rRNA gene better than other primers and consistently amplifies the 16S rRNA gene from various organisms.

The 16S rRNA gene amplification results by PCR were analyzed by agarose gel electrophoresis 0.8% (w/v) for 45 minutes at a voltage of 90 volts and a current of 400 mA. The results of the electrophoresis of the 16S rRNA gene amplification by PCR can be seen in Figure 1.

Table 2. Results of INA bacterial DNA isolation

Bacteria isolate	DNA concentration (µg/mL)	A260/A280 ratio
N.2.2.B-6	19.5	1.99
K.2.2.B-4	28.9	1.95
N.2.1.B-13	59.6	1.98
K.2.1.B-5	52.2	1.90
N.2.2.B-7	103.6	1.97
N.2.1.B-15	41.3	1.91
K.2.2.B-2	402.6	1.98

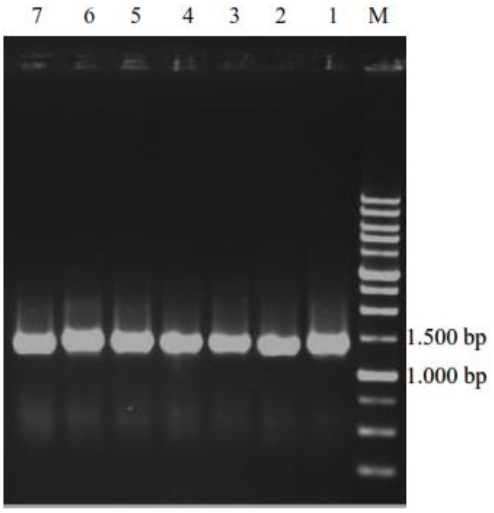

Figure 1. Electrophorogram of 16S rRNA gene using primers 63f and 1387r: M. *Marker* DNA 1 Kb, 1. N.2.2.B-6, 2. N.2.1.B-13, 3. K.2.2.B-2, 4. N.2.2.B-7, 5. K.2.1.B-5, 6. K.2.2.B-4, 7. N.2.1.B-15.

Figure 1 shows that bacterial DNA was successfully amplified. It is indicated by the presence of a bright and thick band. This success indicates that the primer attaches to a specific site on the DNA template with the optimum temperature used for primer annealing. The optimum temperature for the primer to anneal the DNA template can be known by looking at the information listed on the primer package.

The size of the PCR product can be determined by comparing the migration length of the DNA band with DNA markers of known size and concentration. For example, according to Marchesi et al. (1998), amplifying the 16S rRNA gene with primers 63f and 1387r was about 1,300 bp. Therefore, this study used a 1 Kb DNA marker. From Figure 1, it can be seen that in this study, all the obtained PCR products were about 1,300 bp in size with a concentration of about 92 ng/5 µL. Therefore, it can be known by comparing the DNA band with the DNA marker used.

***ina* gene amplification**

The *ina* gene possessed by INA bacteria has been identified according to its type. The known genes for each include inaA in Pantoea ananas (Abe et al. 1989), *ina*W in *P. fluorescens* (Warren et al. 1986), *ina*Z in *P.* syringae (Green and Warren 1985), and *ina*X on *X. campestris* (Zhao and Orser 1990). Therefore, different primers are needed for each isolate to detect the presence of this *ina* gene in it.

This study used four pairs of primers, namely inaZ F (5'GCA GAC TGC GGG TTA TGA GAG C 3'); inaZ R (5'CGC CGG TCA GTT TGC TTC TAT C 3'); inaA F (5'AGG CTT TGA GAA CGG ACT AAC G 3'); inaA R (5' TTT CTG TCG GCT GCG TAC TG 3'); inaW F (5'GCA GTA CGC AGA CGG CAC AG 3'); inaW R (5' TTT CGT AGC CAG CAG TTG ATG TG 3'); and *ina*X F (5'GCA AGG GCA GCG ATG TCA C 3'); and *ina*X R (5' TCT GCG TGC TGC CGT AAC C 3'). According to Nejad et al. (2006), these four pairs of primers can amplify four types of *ina* genes that are commonly found.

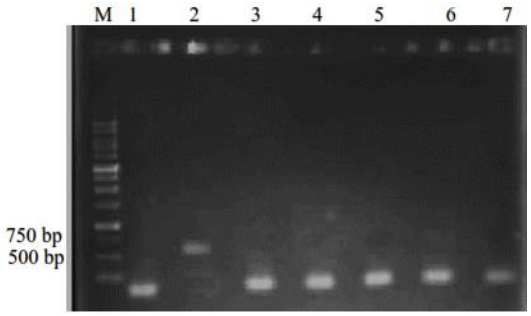

Figure 2. Electropherogram of the *ina*Z gene using primers *ina*Z f and *ina*Z r: M. DNA marker 1 Kb, 1. N.2.2.B-6, 2. N.2.1.B-13, 3.K.2.2.B-2, 4. N.2.2.B-7, 5. K.2.1.B-5, 6. K.2.2.B-4, 7. N.2.1.B-15

The amplification result of the *ina* gene showed that 1 of 7 bacteria whose genes were detected using four pairs of specific primers contained one of the *ina* genes. The bacteria had an isolate code of N.2.1.B-13. Figure 2 shows the results of agarose gel electrophoresis.

Based on the research conducted by Nejad et al. (2006), the four primary pairs of *ina* genes, namely *ina*Z, *ina*W, *ina*A, and *ina*X, can amplify the *ina* gene present in the sample. In this study, the researchers used PCR with program settings including pre-denaturation at 94°C for 5 minutes, post-extention at 72°C for 5 minutes, denaturation at 94°C for 45 seconds, annealing at 60°C for 45 seconds, and extension at 72°C for 1 minute. The PCR cycle lasted for 35 cycles. However, with this procedure, the amplification of the desired genes was not obtained in this study. Therefore, the researchers performed several PCR optimizations to produce the desired product.

The optimization of PCR was carried out according to the procedure given by Kapabiosystems as the master mix producer for PCR amplification. After PCR optimization, one pair of primers of the *ina* gene was obtained and amplified in the bacteria with the code N.2.1.B13. The amplified primer was the *ina*Z primer commonly used to amplify the *ina*Z gene (Nejad et al. 2006). The PCR conditions were carried out so that the *ina* gene was amplified, namely pre-denaturation at 95°C for 1 minute, post-extension at 72°C for 10 minutes, denaturation at 95°C for 15 seconds, annealing at 55°C for 15 seconds, and extension at 72°C for 6 seconds with a PCR cycle of 40 cycles. The comparison results of temperature used in PCR optimization to determine the best annealing temperature for amplifying the *ina* gene with *ina*Z f and *ina*Z r primers can be seen in Figure 3.

It can be seen from Figure 3 that the best temperature for annealing in the amplification of the *ina*Z gene is 55°C to 56°C. It is very different from the PCR conditions used in the study of Nejad et al. (2006) in amplifying the *ina* gene. Optimizing the use of temperature for annealing was also carried out on other *ina* gene primers but still did not produce the desired amplification product. The factor that allows this to happen is a primer that is not suitable. This mismatch can occur because no specific site on the DNA template matches the bases in the primer, so the annealing process during amplification does not occur, and PCR products are not formed (Zein et al. 2013).

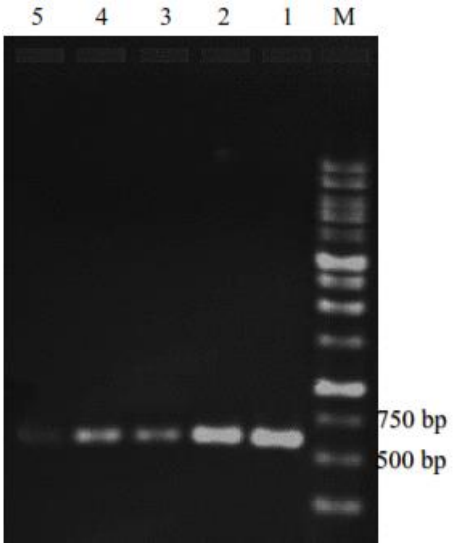

Figure 3. Electropherogram of *ina* gene in N21B-13 isolates using *ina*Z f and *ina*Z r primers with annealing temperature variation: M. *Marker* DNA 1 Kb, 1. 55°C, 2. 56°C, 3. 57°C, 4. 58°C, 5. 59°C.

Sequences analysis of 16S rRNA coding gene and *ina* gene

DNA sequencing is the process or technique of determining the sequence of nucleotide bases in a DNA molecule. These sequences are known as DNA sequences, which are the most basic information of a gene or genome because they contain the instructions needed for forming living organisms (Madigan et al. 1997).

In this study, the DNA sequencing process obtained from amplifying the 16S rRNA gene and the *ina* gene was carried out by PT Genetics Science Jakarta-1st Base Singapore. The sequencing cycle was performed on the ABIprism™ 310 Automated DNA Sequencer (PE Applied Biosystem).

The nucleotide base sequences of the 16S rRNA and *ina* genes were analyzed and compared with the database on GenBank using the BLAST program to determine the identity of the species being studied. According to Stephen et al. (1990), BLAST is a software algorithm for comparing primary information on biological sequences, such as amino acid sequences from different proteins or DNA sequences based on nucleotide bases. Following are the results of the analysis using BLAST. The results of the BLAST analysis can be seen in Table 3.

Table 3. Results of BLAST analysis based on 16S rRNA gene encoding of INA bacteria

Isolate code	Close relatives	Accession number	% Sim.
N.2.2.B-6	*Pantoea* sp. 57916	AF227860	99%
K.2.2.B-4	*Pantoea* sp. 57917	DQ094146	98%
N.2.1.B-13	*Pseudomonas* sp. BF81Fb	KC311267	96%
K.2.1.B-5	*Pseudomonas* sp. R1SpM3P2C2	KF147061	98%
N.2.2.B-7	*Pseudomonas* sp. R2SsM3P1C2	KF147058	95%
N.2.1.B-15	*Rahnella* sp. DmB 16	KF720908	95%
K.2.2.B-2	*Pseudomonas* sp. R2SsM3P1C13	KF147057	96%

Based on the database in GeneBank, four of the seven isolates had similarities with INA bacteria from several subtropical countries (Table 3). The four isolates were N21B-13 which was identified to have 96% similarity with *Pseudomonas* sp. BF81Fb, K21B-5 which was identified to have 98% similarity with *Pseudomonas* sp. R1SpM3P2C2, N22B-7 which was identified to have 95% similarity with *Pseudomonas* sp. R2SsM3P1C2 and K22B-2 which was identified to have 96% similarity with *Pseudomonas* sp. R2SsM3P1C13. *Pseudomonas* sp. BF81Fb was an *ina* bacterium isolated from the air at the moment of rain in the pine forest of Manitou, Colorado, USA, while *Pseudomonas* sp. R1SpM3P2C2, *Pseudomonas* sp. R2SsM3P1C2 and *Pseudomonas* sp. R2SsM3P1C13 were INA bacteria originating from the air of Lyon, France. The other three isolates have similarities with *Rahnella* sp. DmB 16, *Pantoea* sp. 57916 and *Pantoea* sp. 57917 were not INA bacteria. Although some *Pantoea* genera have *ina* capability, the emerging database showed they were not INA bacteria.

All the results of the BLAST analysis showed similarities to many INA bacteria originating from the subtropics (Table 4). It is because research on INA bacteria is mostly carried out in subtropical areas while the research in the tropics is still very little (Stephanie and Waturangi 2011), so the data in the NCBI database all come from research conducted in subtropical areas.

In general, the seven isolates belonged to 3 kinds of genera, namely *Pantoea*, *Pseudomonas*, and *Rahnella*. *Pantoea* is a genus of Gram-negative bacteria in the family Enterobacteriaceae. These bacteria are facultative anaerobes, rod-shaped, catalase-positive, and oxidase-negative. These bacteria can be found in soil, water, plants, and some animals (Paradis et al., 2005). In this genus, one species, Pantoea ananatis, is reported to cause browning of pineapple roots and is also classified as an *ina* bacterium with the *ina*A gene. In addition, *Pantoea* is the name of a new genus of *Erwinia* that is currently used so that bacteria that were previously in the genus *Erwinia*, such as *Erwinia ananas*, *E. herbicola*, and *E. uredovora*, are now in the genus *Pantoea* (Watanabe and Sato 1998).

Pseudomonas is one of the most well-known genera of INA bacteria. *Pseudomonas* is a Gram-negative bacterium, rod-shaped and oxidase negative. Soil, water, and air can be a habitat for these bacteria. Several species of this genus are plant pathogenic bacteria commonly found on leaf surfaces. In addition, these bacteria are thought to be able to be in the air around plants and contribute as a source of forming ice cores (Waturangi and Tjhen 2009). Several species of bacteria from this genus that have been reported to form ice cores include *P. syringae*, *P. fluorescens* (Maki et al. 1974), and *P. viridiflava* (Obata et al. 1989).

Rahnella is a genus of Gram-negative bacteria. This bacterium is rod-shaped, tolerant of low temperatures, and grows optimally at 30°C. This bacterium has been reported to cause decay in several plants (Oladoye et al. 2013), but no studies have reported that this bacterium can form ice nuclei.

Table 4. BLAST analysis results based on the ice nucleation gene of INA bacteria

Isolate code	Identified	Accession number	% Similarity
N.2.1.B-13	Complete sequence of ice core protein-coding gene in *Pseudomonas borealis*	EU573998.1	86%

DNA sequences using *ina*Z primers obtained from isolate N.2.1.B-13 had a similarity of 86% with the gene encoding the ice nucleation protein in *Pseudomonas borealis*. The *P. borealis*, used as a comparison in this study, is a bacterial isolate isolated from the Danu Daring Tundra Ecosystem Research Station, North West Territories, Canada. The gene encoding the ice nucleation protein in *P. borealis* isolates is similar to the *ina*Z gene in *P. syringae* (Wu et al. 2009).

The *ina* gene sequence using the *ina*Z primer showed the suitability of the base sequence with the *P. borealis ina* gene sequence in the database at GeneBank (access no. EU573998). The match starts from the base sequence 2947 to 3491 bp and is located in the middle region of the ice nucleation protein gene complete sequence of *P. borealis*.

The character of the *ina* gene obtained in this study has fewer amino acids compared to the *ina* gene in *P. borealis* in GeneBank (access no. EU573998). The *ina* gene fragment belonging to *P. borealis* encodes 1244 amino acids, each of which composes the N end of 163 amino acids, 41 amino acids make up the C end, and 1040 amino acids make up the core of the *ina* gene (Wu et al. 2009), while the *ina* gene present in isolate N.2.1.B-13 only amounted to 189 amino acids consisting of 16 amino acids. The *ina* gene from the N.2.1.B-13 isolate was not found to have an N tip and a C tip. It was because the amino acid sequencing of the *ina* gene from the N.2.1.B-13 isolate with the *P. borealis* gene showed compatibility at bases 2947 to 3491. bp or from the 871st amino acid to the 1060th amino acid. The translation product of the obtained *ina* gene structure is also dominated by amino acids commonly found in other *ina* genes, namely alanine, glycine, tyrosine, serine, threonine, and leucine.

The *ina*Z gene is one of the *ina* genes commonly found in INA bacteria. This gene is commonly found in the bacterium *P. syringae*. According to Green and Warren (1985), the product of translation of this gene structure is dominated by the octapeptide Ala-Gly-Tyr-Gly-Ser-Thr-Leu-Thr. The 24 bases that make up the octapeptide are GCCGGTTATGGCAGCACGCTGACC. The repeated presence of this octapeptide in the gene is thought to affect the catalytic function and the ability to form ice core formations in supercooled water. It has implications for the ability of INA bacteria to cause frostbite in plants.

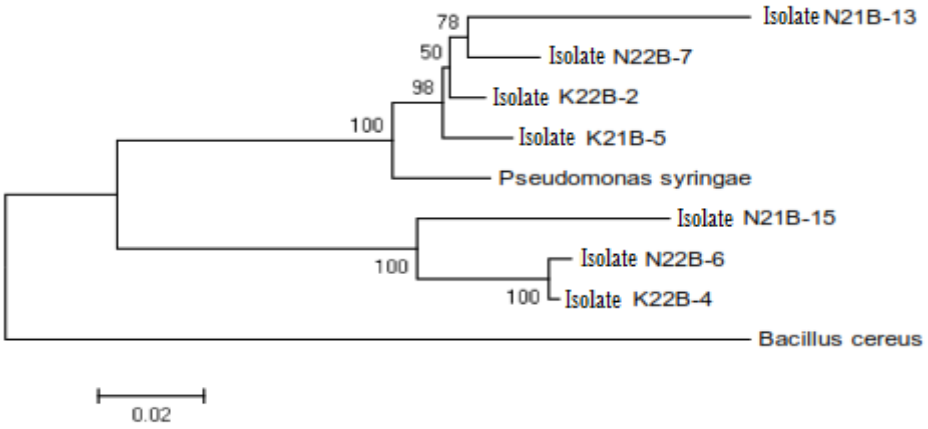

Figure 4. Phylogenetic tree of INA bacteria isolated from lichens on the climbing route of Cemoro Sewu, Mount Lawu, Jawa, Indonesia, with an outgroup of *Bacillus cereus* (Accession number AJ277908)

A phylogenetic tree was created based on the genetic distance between INA bacterial isolates using the MEGA 5.1 program. The purpose of making a phylogenetic tree is to determine the relationship of INA bacteria between existing isolates. The phylogenetic tree based on the 16S rRNA gene can be seen in Figure 4.

Based on the phylogenetic tree, *P. syringae*, known to form ice cores, are in the same group as bacterial isolates K21B-5, K22B-2, N22B-7, and N21B-13 which can form ice cores, while isolates N21B-15, N22B-6 and K22B-4 are in different group with *P. syringae*. Therefore, it indicates that K21B-5, K22B-2, N22B-7, and N21B-13 have a close relationship with INA bacteria of the *P. syringae* species.

The numbers in the branching of the phylogenetic tree in Figure 4 were bootstrap values. Bootstrap value is used to test how well the model data set is used in research. For example, the bootstrap value on branches of a phylogenetic tree is significant if the resampled data set repeatedly predicts the same branch for over 70% (Felsenstein 1988).

In conclusion, the INA bacterial species found in lichens on the climbing route of Cemoro Sewu, Mount Lawu, based on the results of identification with the 16S rRNA gene, respectively, were N.2.2.B-6 which had 99% similarity with *Pantoea* sp. 57916, N.2.1.B-13 which had 96% similarity with *Pseudomonas* sp. BF81Fb, K.2.2.B-2 which had 96% similarity with *Pseudomonas* sp. R2SsM3P1C13, N.2.2.B-7 which had 95% similarity with *Pseudomonas* sp. R2SsM3P1C2, K.2.1.B-5 which had 98% similarity with *Pseudomonas* sp. R1SpM3P2C2, K.2.2.B-4 which had 98% similarity with *Pantoea* sp. 57197, and N.2.1.B-15 which had 95% similarity with *Rahnella* sp. DmB 16. *Pseudomonas* sp. BF81Fb, *Pseudomonas* sp. R2SsM3P1C13, *Pseudomonas* sp. R2SsM3P1C2 and *Pseudomonas* sp. R1SpM3P2C2 were INA bacteria. The character of the protein gene coding for the ice core of INA bacteria in lichens on the climbing route of Cemoro Sewu, Mount Lawu, successfully amplified using *ina*Z primer from isolate N.2.1.B-13, was similar to the ice nucleation protein gene complete sequence from *P. borealis*. The *ina* gene from isolate N.2.1.B-13 had 189 amino acids consisting of 16 amino acids that were repeated, the N tip and the C tip were not found because the amino acid sequence of the *ina* gene isolate N.2.1.B-13 with the *ina* gene *P. borealis* showed compatibility at bases of 2947 to 3491 bp or from amino acids of 871 to amino acids to 1060, and was composed of amino acids commonly found in other *ina* genes, namely Alanine, Glycine, Tyrosine, Serine, Threonine, and Leucine.

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