0971-4693/94 Euro 10.00
*Allelopathy Journal* 39 (2): 231-242 (2016) *International Allelopathy Foundation* 2016
Table: 2, Figs : 4

# Allelochemicals in *Quercus variabilis* forest leachates

Dengzhi Wang and Lishui Nie[1]*
Department of Soil Science and Plant Nutrition, College of Forestry,
Beijing Forestry University. Beijing 100083, China
E. Mail: nielishui@bjfu.edu.cn

**(Received in revised form: October 26, 2016)**

## ABSTRACT

Allelochemicals of leachates collected from the litter layers of a *Quercus variabilis* stand, were identified by gas chromatography-mass spectrometry (GC-MS). In natural leachate of forest floor litter, aliphatic acids, phenolic acids, alcohol ether and esters were identified Bioassay of leachates of was also done to determine the allelopathic potential on seed germination and root length of *Pinus tabuliformis*. Prolonged exposure to Leachates inhibited seed germination and root length development of *P. tabuliformis*, while seeds germination and root length was stimulated when seeds were soaked in leachate and then germinated in water.

**Keywords**: Allelochemicals, bioassay, forest, leachate, litter, *Quercus variabilis*, *Pinus tabuliformis*, root length, seed germination, seedling growth.

## INTRODUCTION

*Quercus* tree (family Fagaceae) has 450 species worldwide (23). In China, there are 13 deciduous species, (9 indigenous including *Quecus variabilis*). In China, *Q. variabilis* often forms a part of a mixed forest with Pine. *Pinus tabulaeformis* Carr. While in Durango state of Mexico, the forests are mixture of pine and oak trees with *Q. variabilis* (1**).** In the mixed forests of *Quercus* and *Pine* in the west mountains of Beijing, the stand biomass, stand volume and storage capacity of nutrients are greater than in pure forests (10).

Plant litter affects plant-plant interactions. It directly affects plant performance by physically changing the environment (10,11,16,26) and also indirectly influences the environment through the release of nutrients (26,27) and phytotoxic substances (3,14) during the litter decomposition and leaching. Release of dissolved soluble organic matter (DOM) from the forest floor through leaching is the main source of DOM in forest soils (19,30). Hydrological conditions also play a role in both production and mobilization of DOM in the forest floor. The amount of moisture ins(Dear editor, delete letter s) soil and soil microbiological acivity also increases the formation of water soluble organic materials (4). Water fluxes are key drivers of DOM transport either from the forest floor (13) or from the whole soil profile (5,25).

*Correspondence author, [1] College of forestry, Beijing Forestry University, Beijing 100083,China

DOM may be divided into hydrophobic and hydrophilic fractions (8). The hydrophobic fraction is acidic and consists of fulvic acid with long-chained aliphatic acid, phenols and aromatic carboxylic acids, while the hydrophilic acids are a mixture of simple fatty acids, hydroxy acids and complex polyelectrolytic organic acids (hydroxyl and carboxyl functional groups). Hydrophilic neutral compounds include the aliphatic amides, alcohols, aldehydes, esters and ketons with < 5-carbon atoms, polyfunctional alcohols and carbohydrates (8,29).

The hydrophobic and hydrophilic materials have been characterised in the *Quercus* litter by various extraction methods and include phenolic compound (6,7), *n*-alkanoic acids, sterols and triacylglycerols (21) in *Quercus robur* and phenolics in *Quercus faginea litter* (33). The present work was aimed at identifying the kinds and relative contents of water soluble organic materials from the *Q. variabilis* forest in natural rainfall conditions and to determine the its effect on germination of *P. tabuliformis* seeds.

## MATERIALS AND METHODS

**I. Study site**: The study site (Latitude 39°54′N, Longitude 116°28′E) was in Yanerling, Jiufeng National Forest Park, 30 km west of Beijing. A major feature of the park is Miaofeng Mountain, a part of the West Mountain range that connects the northern part of Taihang Mountain and the eastern part of Yanshan Mountain. The peak of Miaofeng Mountain is 1153 m above sea level. The upper part of the mountain consists of granite, while the lower part is sandstone and limestone. The site has a typical warm-temperate, continental monsoon climate with distinct seasons. It is warm, dry and windy in spring, warm and humid in summer, sunny and humid in the fall and cold and dry in winter. The annual mean temperature is 11.7°C with maximum of 39.7°C and minimum of -19.6°C. Annual rainfall is about 480 mm with peak rainfall between July and September .

The area was planted with *Q.variabilis* in 1950s -1960s and the trees are of uniform age in about 200 ha on southern slope. The mean *Quercus* tree height is 10.5 m and the mean breast diameter is about 13.4 cm. The tree density is 1335 trees/ha. Shrub undergrowth includes *Zizyphus jujube var. inermis*, and *Rhamnus parvifolia*. Herbaceous plants include *Arthraxon hispidus* and *Eriophorum vaginatun*. The litter layer (undecomposed and semi-decomposed material) was about 4 cm thick.

The Chemicals **(**Trimethylsilyl, ethyl acetate and Acetonitrile) were purchased from Sigma Chemical (St. Louis, MO).

**II. Sampling:** Three sample plots (20 m x 20 m) were selected on the parallel slope in each plot, three 1 m × 1 m areas were randomly selected as sample area, the litter composition (undecomposed and decomposed)**,** thickness and uniformity were considered in selecting the sampling area.

Litter leachates were collected from June to October in 2009 by zero tension lysimeter from three 1 $m^2$ plastic sheets covered with a polyethylene net (0.5 mm mesh size). The lysimeters were installed through horizontal tunnels excavated in the mineral soil from downslope positions. Plastic sheets were horizontally pushed into litter layer without damage to its natural structure. Each lysimeter was drained into 25 L polyethylene buckets housed in a wooden box that preserved surrounding soil (33). The rainfall, leachates were collected for 12 h and were transported to the laboratory, Beijing Forestry University, where these were filtered through a 0.45-μm membrane filter and mixed to get composite samples and stored in plastic bottles at 4℃ in refrigerators.

**III. Leachate preparation for Chemical Analysis:** Extraction of water soluble organic chemicals from the forest floor litter leachate was done as under: The filtered composite sample (50 ml) was put into a 50 ml volumetric flask and 1ml ethyl acetate was added. The mixture was shaken for 3 min and left to stand until it separated into two phases. The upper layer was collected and extracted with ethyl acetate three times as before, 100 µl extracts were mixed together, then evaporated to dryness using a vacuum rotary evaporator at room temperature (20℃),and finally re-dissolved in 10 µl of 1% trimethylsilyl (TMS) and were analysed using an Agilent-6890 GC-MS.

**IV. Leachate preparation in the laboratory for bioassay** In the laboratory, the litter was not ground to powder and was kept in natural state. Two Kg *Q. variabilis* litterfall was collected (up to 5 cm depth）randomly from the experimental field and spread on fine plastic mesh over a collecting tray (30 cm x 40 cm) and leached using tap water in ratio of 1: 3 (w/v) for 24 h at room temperature (20±1$^{o}$C). The leachate was collected and filtered through filter paper.

**V. Gas chromatography/ mass spectrometry:** Samples were analyzed using an Agilent GC6890 (coupled to Technologies 5973 Mass Selective Detector) equipped with a capillary column (30m*0.25mm*0.25µm). Sample volumes (µl /) were injected in a splitless mode for 1min with an ALS automatic injector. Helium (99.995%) was used as the carrier gas at a constant flow rate of 0.8ml $min^{-1}$. Oven temperature was initially set at 50℃ (5min isothermal), and then increased to 200℃ at 20℃$min^{-1}$ (10min isothermal), and continued to 290℃, where it remained for 15 min. Injector temperature was held 280℃. The mass spectrometer parameters were: ionisation energy set at 70 eV with the quadropole mass analyser scanning the range m/z 29–540 with a cycle time of 0.6 $s^{-1}$. Agilent Chemstation analysis software was Xcalibur1.2, NIST98 standard mass spectrum library.

**Bioassay of leachates:** *P. tabuliformis* seeds were obtained from the Agricultural Research Centre, Beijing. Non-viable and underdeveloped floating seeds were discarded by suspending the seeds in water. The seeds were surface–sterilized with 0.5% $KMnO_4$ solution (or 10% sodium hypochlorite solution) for 30 min, then rinsed five times with distilled water. The selected 270 sterilized seeds were devided into 3 lots (90 seeds each) for use in Treatments T1, T2 and T3. The *P.tabuliformis* seeds germination needs pre-soaking, that is why seeds were soaked in leachates or distilled water for 30 h. In the laboratory bioassays there were 3 treatments: T1 (seeds soaked in leachate for 30 h and later irrigated with leachate), T2 (seeds soaked in leachate for 30 h and irrigated with distilled water) and T3 (seeds soaked in distilled water for 30 h and irrigated with distilled water).

The bioassay was done in Petri plates (20 cms dia) lined with two layers of Whatman filter paper. After soaking the seeds in leachate/distilled water for 30 h, the seeds were placed on top of the filter paper in the Petri plates. To each Petri plate, 15 ml leachate/ distilled water (as per treatments) were added on the first day and every two days thereafter. The germinated seeds were counted daily for 10 days and thereafter the root length was measured. The germination (%), germination potential and germination index were calculated as under.

(i). Germination (%) = $n_1/N * 100$

Where, $n_1$: Number of germinated seeds, N: Total number of seeds per replication.

(ii). Germination potential (%) = $n_2/N * 100$

Where, $n_2$: Number of germinated seeds when number of germinated seeds the day reach maximum, N: Total number of seeds per replication. Germination potential is indices of speed of germination

(iii). Germination index (GI) indicates the ratio of the daily germination rate to the maximum germination value. It was calculated as per AOAC (2) as under:

Germination Index (GI) =$N_1 / 1 + N_2 / 2 + N_3 / 3 + \ldots\ldots + N_n / n$

Where, $N_1$, $N_2$, $N_3$ and $N_n$ is the number of germinated seeds on day 1,2,3 …… n.

**VI. Statistical Analysis:** The effects of leachates of *Q.variabilis* litterfall on *P. tabuliformis* seed germination and root length were subjected to one-way ANOVA. Mean difference were tested separately with least significant difference (LSD) at $p < 0.05$. All significance analyses were done using SPSS version 17.0.

## RESULTS AND DISCUSSION

### Chemical analyses of litter leachate of *Q variabilis*

Total ion current chromatography of water soluble organic component in forest floor litter leachate was done with Agilent Chemstation (Fig. 1). Qualitative identification of the organic chemicals was confirmed by NIST98 standard mass spectrogram, data detecting system and relative content determined with peak area normalization method (Table 1).

Chemical components (aliphatic and phenolic acids, alcohols, esters, and amides) were determined by GC/MS, in the ethyl acetate extracts of floor litter leachate (Table 1). The litter contained phenolic acids (benzonic acid, 2/3/p-methylbenzoic acid, p-hydroxy benzoic acid, vanillic acid) and non-phenolic acids (octanoic acid, succinic acid, non anoic acid, glutaric acid, decanoic acid, dodecanoic acid, tetradecanoic acid, hexadecanoic acid, linolelaidic acid) (Table 1). The compounds detected in highest concentration were acids, which were more than half of the total. Aliphatic acids were 10-times more than phenolic acids. However, alcohols, esters, and amides were relatively less.

Earlier studies have reported the allelopathic potential of leachate from litterfall of *Quercus* (6,7,21,34). In this study, we identified the water soluble organic components in *Quercus variabilis* litter leachate (Table 2). Considerable differences in water soluble organic components were found in different or same *Quercus* litter, which may be attributed to interspecific difference, litter age and extraction methods used. Further, in woodland experiments *in situ*, besides climatic factors such as temperature (9,15), the intensity and amount of precipitation (9,31,32) influences the litter decay rate, besides topographical factors, such as slope, may produce different environmental conditions that can reduce or accelerate the litter decomposition (24) and alter the microbial population (17).

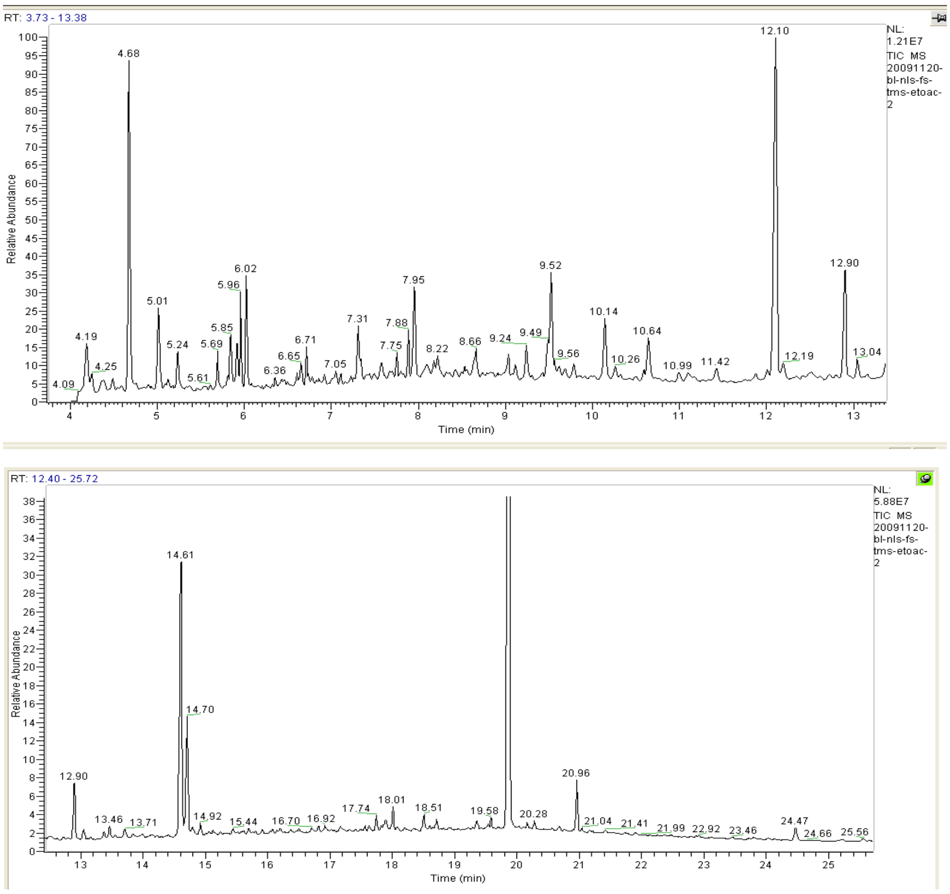


Figure 1. Total ion current chromatogram of O horizon leachate under *Q variabilis*

The leaching of fresh and decomposing litter is the main sources of organic acids in forest soils. Phenolic acids (benzoic acid, p-hydroxy benzoic acid, vanillic acid) have been reported in litter leachates of different *Quercus* spp. (6,7)**.** In O horizon leachate, other phenolic acids were not detected. Kuiters & Sarink (7) found different phenolic acids in litter collected in October and January months. González *et al*. (6) and Kuiters & Sarink (7) have examined the phenolic acids as allelochemicals and not as aliphatic acids. Aliphatic acids (oxalic, malonic, fumaric, succinic, maleic, malic, citric, C16: 0, C18: 0, C18: 1, C18: 2, C18: 3 and C20: 0 acids) have been reported in forest litters (Larch, Manchurian ash, Korean pine and White birch) in Northeast China (28). Succinic acid was also detected in *S. pulcherrima* litter (22).

Table1. Chemical compounds in leachate of O horizon under *Q variabilis*

| No. | RT min. | Compounds | Molecular formula | Relative content（%） |
|---|---|---|---|---|
| 1 | 4.68 | Glycerine | $C_3H_8O_3$ | 9.09 |
| 2 | 5.01 | Diethylene glycol | $C_4H_{10}O_3$ | 2.08 |
| 3 | 5.24 | Octanoic acid | $C_8H_{16}O_2$ | 0.87 |
| 4 | 5.69 | Carbamide | $CO(NH_2)_2$ | 0.77 |
| 5 | 5.85 | Benzoic acid | $C_7H_6O_2$ | 1.05 |
| 6 | 5.96 | Succinic acid | $C_4H_6O_4$ | 1.46 |
| 7 | 6.02 | Nonanoic acid | $C_9H_{17}O_2$ | 2.17 |
| 8 | 6.60 | Glutaric acid | $C_5H_8O_4$ | 0.17 |
| 9 | 6.65 | 2/3/p-Methylbenzoic acid | $C_8H_8O_2$ | 0.39 |
| 10 | 6.71 | Decanoic acid | $C_{10}H_{20}O_2$ | 0.53 |
| 11 | 6.78 | 2-phenoxy ethanol | $C_8H_{10}O_2$ | 0.15 |
| 12 | 7.95 | Dodecanoic acid | $C_{12}H_{24}O_2$ | 2.21 |
| 13 | 8.22 | P-hydroxy-benzoic acid | $C_7H_6O_3$ | 0.24 |
| 14 | 9.24 | Monocaprylin | $C_{11}H_{22}O_4$ | 0.77 |
| 15 | 9.52 | Tetradecanoic acid | $C_{14}H_{28}O_2$ | 1.73 |
| 16 | 9.78 | Vanillic acid | $C_8H_8O_4$ | 0.40 |
| 17 | 12.10 | Hexadecanoic acid | $C_{16}H_{32}O_2$ | 12.33 |
| 18 | 12.90 | Heptadecanol | $C_{17}H_{36}O$ | 3.51 |
| 19 | 14.92 | Linolelaidic acid | $C_{18}H_{32}O_2$ | 0.65 |
| 20 | 18.01 | Glyceryl monostearate | $C_{21}H_{42}O_4$ | 1.10 |

RT: Retention time in min. Relative content: Ratio of peak area of chemical component to total peak area.

**Seed germination**

Soaking seeds in leachate of *Q. variabilis* significantly reduced the germination (%) of *P. tabuliformis seeds*. There was 29% and 32% reduction in T1 as compared to T2 (leachate-water) and T3 (water-water control), while seed germination in T2 and T3 was similar (Fig. 2). Seed germination in T1 (leachate-leachate) was 22% and 24% lower than in T2 and T3, respectively. It indicates that the leachate inhibited seed germination, while the germination of seeds soaked first in leachate and then soaked in distilled water was stimulated.

Table 2. Chemical components of litter in different *Quercus* species using various extraction methods

| Litter | Extraction method | Compounds | Reference |
|---|---|---|---|
| *Querous variabilis* | Rainfall through litter *in situ* | benzoic acid, p-hydroxy benzoic acid, vanillic acid, 2/3/p-methylbenzoic acid, glycerine, diethylene glycol, octanoic acid, carbamide, succinic acid, nonanoic acid, glutaric acid, decanoic acid, dodecanoic acid, 2-phenox ethoxy, dodecanoic acid, monocaprylin, tetradecanoic acid, hexadecanoic acid, heptadecanol, linolelaidic acid**,** glyceryl monostearate | 6 |
| *Qurercus robur* | Powdered litter, methanol-water | 3,4-dihydroxybenzoic, vanillic, ellagic acids, 4-hydroxybenzaldehyde, quercetin and kaempferol | 6 |
| *Qurercus robur* | Shaked litter, distilled water | benzoic acid, p-hydroxy benzoic acid, vanillic acid, gallic acid, fi-resorcylic acid, gentisic acid, p-anisic acid ,syringic acid fendic acid, caffeic acid, p-coumaric acid , o-coumaric acid, catechol; p-hydroxybenzaldehyde; syringaldehyde; vanillin | 7 |
| *Qurercus robur* | Crushed litter, dichloromethane (DCM): acetone | n-tetracosanol (C22-30), n-alkanols (C14–C30), n-aldehydes (C24-C32), Sitosterol， wax esters （C38 to C46), triacylglycerol peaks (C52 and C54), n-alkane (C18 to C33), glucose and other C6-monosaccharides, glutamic acid | 20 |

Germination potential and germination index (GI) are more reliable indicators of vigour than germination (%). Germination potential of seeds treated with leachate was lowest (28%) and highest with distilled water (40%). Germination potential in seeds in T2 (leachate and water) was slightly higher than T1 (leachate-leachate) (Fig. 2).

Germination index (GI) of seeds of *P. tabuliformis* in T2 (leachate and water) was 20, almost similar to seeds in T3 (water-water, control). The germination index in T1 (leachate-leachate) was 40% and 42% lower than in T1 and T2, respectively (Fig. 3).

Germination potential of seeds was 43% lower in leachate and 25% lower when soaked in leachate and distilled water than in distilled water. While the GI of seeds was lowest in leachate but the GI of seeds soaked in leachate and then in distilled water was identical to control. Germination potential and GI values further confirm the inhibition of seed germination of *P tabuliformis* by *Q. variabilis* litter leachates.

**Seedling growth -Root length:**

Root length was significantly affected by leachate, compared to either T2 (leachate-water) or T3 (water-water, control), which showed that leachate in high concentration inhibited the root development (Fig. 4). While low concentration stimulated the root length. It was shortest in T1 (leachate-leachate) and longest in T2 (leachate-water). Root length in T2 (leachate-water) and T3 (water-water, control) was 2-3 times longer than in T1 (leachate-leachate). There were significant differences between the T1 (leachate-leachate) and T2 (leachate-water), but no differences between T2 and T3, T1 and T3 ($P< 0.05$).

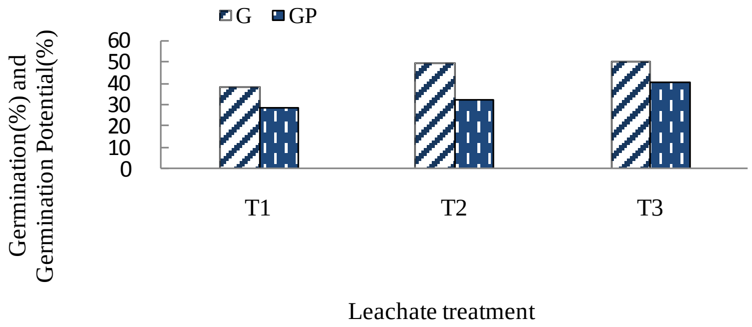


Figure 2. Effects of leachate concentrtions from *Q. variabilis* litterfall on seed germination (%) and germination potential (GP) of *P. tabuliformis*

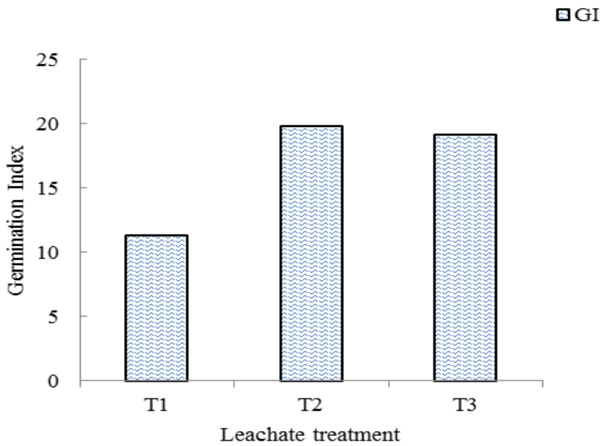


Figure 3. Effects of leachate of *Q variabilis* litterfall on seed germination index (GI) of *P. tabuliformis*

This study of water soluble organic components in forest floor under *Q. variabilis* provides new information of what occurs under natural conditions. A variety of water soluble components are present in the organic matter of forest floor in varying amounts. The litter leachates act as carriers of these water soluble organic components into the soil. The analysis of leachates under natural conditions (*in situ*) using the non-aggressive extraction methods, showed the presence of a variety of components in the litter leachate and their effect on of Pine seed germination.

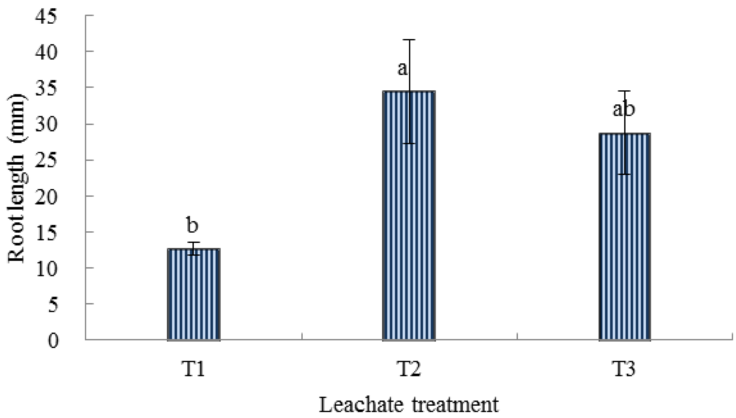


Figure 4. Effects of leachate of *Q variabilis* litterfall on root length (RT) of *P. tabuliformis.* bars with different letters are significantly different at the 0.05 level.

The leachates in high or low concentration affect seeds germination and root length of *P. tabuliformis* by either inhibition or stimulation, respectively. However, seasonal dynamics of water soluble organic components in the litter leachates of the forest floor under natural rainfall, leaching or other environmental conditions (such as water stress) need to be further studied.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge the National Science Foundation Committee (NSFC) Project approval number 30872010. The authors wish to thank Forestry Farm, Beijing Forestry University for fieldwork. We also thank Prof. Konnie Andrews for helpful comments and remarks on earlier versions of the manuscript.

## REFERENCES

1. Corral, S. and Navar Chaidez, J.J., (2005). Analisis del crecimiento e incremento de cinco pinaceas delos bosques de Durango. *Madera y Bosques*, **11**: 29-47.
2. Dezfuli, P., Sharif-Zadeh., F. and Janmohammadi, M. (2008). Influence of priming techniques on seed germination behaviour of maize inbred lines (*Zea mays* L.). *Journal of Agricultural Science and Technology* **3**: 22-25.
3. Djurdjević, L., Mitrović, M., Gajić, G., Jarić, S., Kostić, O., Oberan, L. and Pavlović, P. (2011). An allelopathic investigation of the dominance of the introduced invasive *Conyza canadensis* L. *Flora* **206**: 921-927.
4. Falkengren, G.U. and Tyler, G. (1993). The importance of soil acidity moisture exchangeable cation pools and organic matter solubility to the cationic composition of beech forest (*Fagus sylvatica* L) soil solution. *Zhurnal Pflanze. Bodenkunde* **156**: 365-370. (German).
5. Goller, R., Wilcke, W., Fleischbein, K., Valarezo, C. and Zech, W. (2006). Dissolved nitrogen, phosphorus, and sulfur forms in the ecosystem fluxes of a montane forest in Ecuador. *Biogeochemistry* **77**: 57-89.

6. González, L., Souto, X.C. and Reigosa, M.J. (1995). Allelopathic effects of *Acacia melanoxylon* R.Br. phyllodes during their decomposition. *Forest Ecology and Management* **77**: 53-63.
7. Kuiters, A.T. and Sarink, H.M. (1986). Leaching of phenolic compounds from leaf and neeedle litter of several deciduous and coniferous trees. *Soil Biology and Biochemistry* **18**: 475-480.
8. Leenheer, J.A. and Huffman, E.W.D. (1979). Analytical Method for Dissolved-Organic Carbon Fractionation. Water-Resources Investigations. Pp. 79-84. U.S. Geological Survey, Denver Clorado, USA.
9. Liski, J., Nissinen, A., Erhard, M. and Taskinen, O. (2003). Climatic effects on litter decomposition from arctic tundra to tropical rainforest. *Glob. Change Biol* **9**: 575-584.
10. Liu, C.J. (1987). *Studies on the Biomass and Nutrient Cycling of the Mixed Plantation of Pinus tabulaeformis* Carr. and *Quercus variabilis* in Xishan Region, Beijing. Master's thesis. Beijing Forestry University, Beijing Pp. 1-10. (Chinese).
11. Mahmoud, A., Singh, S.D. and Muralikrishna, K.S. (2016). Allelopathy in jatropha plantation: Effects on seed germination, growth and yield of wheat in north-west India. *Agriculture Ecosystems and Environment* **231**: 240-245.
12. Martell, A.E., Motekaitis, R.J. and Smith, R.M. (1988). Structure-stability relationships of metal complexes and metal speciation in environmental aqueous solutions. *Environ. Toxicol. Chem* **7**: 417-434.
13. Michalzik, B., Kalbitz, K., Park, J.H., Solinger, S. and Matzner, E. (2001). Fluxes and concentrations of dissolved organic carbon and nitrogen- a synthesis for temperate forests. *Biogeochemistry* **52**: 173-205.
14. Molina, A., Reigosa, M.J. and Carballeira, A. (1991). Release of allelochemical agents from litter, throughfall and topsoil in plantation of *Eucalyptus globulus* labill in Spain. *Journal of Chemical Ecology* **17**: 147-160
15. Moore, A.M., (1986). Temperature and moisture dependence of decomposition rates of hardwood and coniferous leaf litter. *Soil Biology and Biochemistry* **18**: 427-435.
16. Nunes, L., Lopes, D., Rego, F.C. and Gower, S.T. (2013). Aboveground biomass and net primary production of pine, oak and mixed pine-oak forests on the Vila Real district, Portugal. *Forest Ecology and Management* **305**: 38-47
17. Pandey, R.R., Sharma, G., Tripathi, S.K. and Singh, A.K. (2007). Litterfall, litter decomposition and nutrient dynamics in a subtropical natural oak forest and managed plantation in northeastern India. *Forest Ecology and Management* **240**: 96-104.
18. Pohlman, A.A. and McColl, J.G. (1988). Soluble organics from forest litter and their role in metal dissolution. *Soil Science Society of America Journal* **52**: 265-271.
19. Qualls R.G. and Haines B.L. (1991). Geochemistry of dissolved organic nutrients in water percolating through a forest ecosystem. *Soil Science Society of America Journal* **55**: 1112-1123.
20. Qualls, R.G. and Haines, B.L. (1992). Biodegradability of dissolved organic matter in forest throughfall, soil solution and stream water. *Soil Science Society of America Journal* **56**: 578-586.
21. Rawlins, A.J., Bull, I.D., Poirier, N., Ineson, P. and Evershed, R.P. (2006). The biochemical transformation of oak (*Quercus robur*) leaf litter consumed by the pill millipede (*Glomeris marginata*). *Soil Biology and Biochemistry*, **38**: 1063-1076.
22. Ruprecht, E., Józsa, J., Ölvedi, T.B. and Simon, J. (2010). Differential effects of several''litter''types on the germination of dry grassland species. *Journal of Vegetation Science* **21**: 1069-1081.
23. Sánchez-Burgos, J.A., Ramrez-Maresb, M.V., Larrosac, M.M., Gallegos-Infantea, J.A., Gonzalez-Laredoa, R.F., Medina-Torres, L. and Rocha-Guzman, N.E. (2013). Antioxidant, antimicrobial, antitopoisomerase and gastroprotective effect of herbal infusions from four Quercus species. *Industrial Crops and Products* **42**: 57-62.
24. Sariyildiz, T. and Küçük, M. (2009). Influence of slope position, stand type and rhododendron *(Rhododendron ponticum*) on litter decomposition rates of Oriental beech (*Fagus orientalis Lipsky*.) and spruce [*Picea orientalis* (L.) Link]. *European Journal of Forest Research* **128**: 351-360.
25. Savric, I. (2001). *Einflussfaktoren auf die Bindung und Mobilität Organischer und Anorganischer, Stoffe in Kontaminierten Rieselfeldböden*. Ph.D. thesis. Berlin University of Technology, Germany. (German).
26. Schmidt, M., Veldkamp, E. and Corre, M.D. (2015). Tree species diversity effects on productivity, soil nutrients availability and nutrient response efficiency in a temperate deciduous forest. *Forest Ecology and Management* **338**: 114-123.

27. Slazak, A., Freese, D., Matos, E.S. and Hüttl, R.F. (2010). Soil organic phosphorus fraction in pine-oak forest stands in Northeastern Germany. *Geoderma* **158**: 156-162
28. Song, J.F. and Cui, X.Y. **(**2003). Analysis of organic acids in selected forest litters of Northeast China. Journal of Forestry Research **14**: 285-289.
29. Thurman, E.M. (1985). *Organic Geochemistry of Natural Waters*. Martinus-Nijhoff/Dr W. Junk Publishers, Dordrecht.
30. Traversa, A., D'orazio, V. and Senesi, N. (2008). Properties of dissolved organic matter in forest soils: Influence of different plant covering. *Forest Ecology and Management* **256**: 2018-2028.
31. Tukey, Jr. H.B. (1970). The leaching of substances from plants. *Annual Rev*iew of *Plant Physiology* **21**: 305-332.
32. Vanlauwe, B., Vanlangenhove, G., Merckx, R. and Vlassak, K. (1995). Impact of rainfall regime on the decomposition of leaf litter with contrasting quality under subhumid tropical conditions. *Biology and Fertility of Soils* **20**: 8-16.
33. William, S., Currie, John, D. Aber, William, H. and Dcdowell, E. (1996). Vertical transport of dissolved organic C and N under long-term N amendments in pine and hardwood forests. *Biogeochemistry* **35**: 471-505.
34. Zimmer, M., Oliveira, R., Rodrigues, E. and Graca, M.A.S. (2005). Degradation of leaf litter phenolics by aquatic and terrestrial isopods. *Journal of Chemical Ecology* **31**: 1933-1952.