

Changes in terpenes of three kinds of pine needles during litter decomposition

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This study was designed to evaluate changes in the terpene composition of 3 types of pines (*Pinus densiflora*, *Pinus thunbergii* and *Pinus rigida*), while decomposing their leaf litter. Needle litters were placed at two different organic layer depths, one on the surface and the other beneath the litter layer. Changes in the terpene composition of this litter were detected using a gas chromatograph-mass spectrometer. Among the monoterpenes acquired from the fresh needles of *P. densiflora* and *P. rigida*, α -pinene (12.05% and 19.87%, respectively) was the major one, followed by β -pinene (2.90% and 14.07%). However, from the needles of *P. thunbergii*, β -pinene (20.77%) was the major one, followed by α -pinene (10.79%). Among the sesquiterpenes detected in *P. densiflora*, trans-caryophyllene (3.12%) was the highest composition compound, whereas germacrene-D (6.09%) for *P. thunbergii* and 1,6-cyclodecadiene (7.41%) and endo-1-bourbonanol (7.41%) for *P. rigida* were the highest content compounds. However, the total amounts of terpenes decreased sharply by 40-85.4% in all three types of pine needle after 90-120 days of the experiment. The concentration of each terpene differed during decomposition, and the majority of compounds disappeared from beneath the litter layer. It was determined that three types of reducing patterns of each compound appeared on the rate of loss of concentration during decomposition; one pattern decreasing sharply during the initial period, another pattern steadily or slowly decreasing, and a newly detected pattern at low concentration occurring during decomposition.

Key words: decomposition, GC-MS, litter bag, monoterpene, sesquiterpene

INTRODUCTION

The decomposition of several chemical compounds in pine needle litter is a relatively long-term process (Coûteaux et al. 1998, McTiernan et al. 2003). The complete decomposition of needle litter takes a number of years. Overall, litter mass loss rates in pine forests vary widely, depending on the climate and litter quality (Coûteaux et al. 1995, Berg et al. 1996), and initial concentration of chemical compounds (Kainulainen and Holopainen 2002, Kainulainen et al. 2003, Koide et al. 2005). Coniferous species primarily generate sizable quantities of terpenes (Guenther et al. 1995, Kelkar et al. 2006, Kup-

kinskiene et al. 2008), which is a complex mixture of terpenes consisting of monoterpenes, sesquiterpenes, and diterpenes. Although highly volatile, monoterpenes can initially be present at relatively high concentrations in recently fallen litter, as has also been shown in previous studies (Wilt et al. 1993). Additionally, the composition of monoterpenes changes rapidly during the decomposition of needle litter (Kainulainen and Holopainen 2002, Ludley et al. 2008), and some compounds are also detected in soils (Wheatley et al. 1996, Asensio et al. 2008). The compounds remaining in soil play important

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ecological roles in the interactions between living soil microorganisms (Vokou et al. 2002, Hwang et al. 2004, Kim et al. 2006). Many authors (Koide et al. 1998, Kleinheinz et al. 1999, Owen et al. 2007) demonstrated that terpenes from forest -soil extracts and enriched cultures could be used by certain soil microorganisms as a source for growth. Therefore, a considerable change in terpene composition in soil is always accompanied by initial terpene concentrations in needle litter and microbial consumption. However, little is currently known regarding the quantitative and qualitative distribution of terpenes in different soil systems (Wilt et al. 1988, Smolander et al. 2006). Recently, monoterpene concentrations have been reported to be highest in surface litter (Asensio et al. 2008, Ludley et al. 2008). The most abundant monoterpenes were identified as α -pinene, β -pinene, and 3-carene in *Pinus sylvestris*, *Picea abies*, and *Picea sitchensis* (Ludley et al. 2008); α -pinene predominated in the roots and β -caryophyllene predominated in the litter layer from *Pinus halepensis* trees (Asensio et al. 2008). A comparative study of terpenes concentrations in different soil horizons and changes in terpenes of different species during litter decomposition was previously conducted (Ludley et al. 2009) on the basis of monoterpene contents and distribution in the litters and roots of three conifer species (*P. abies*, *P. sitchensis*, and *P. sylvestris*) via the litter bag method. However, no comparative studies of terpene content have thus far been conducted in the field, because such studies should be conducted with the same coniferous species at the same geographical location on the same series of soils.

Pinus densiflora, *Pinus thunbergii*, and *Pinus rigida* are common species in Korea. In particular, *P. densiflora* and *P. thunbergii* are native to Northeast Asia (Korea, China, and Japan); *P. rigida* was introduced from North America to Korea in 1906 and is now distributed widely. Owing to the differing rates of decomposition of the various compounds, the chemical quality of the litter changes over time and this continually influences the ongoing decomposition process. Additionally, the volatile compounds of essential oils extracted from the needles of *P. densiflora* have also been evaluated (Jung et al. 2003, Yu et al. 2004, Kim and Shin 2005, Lee et al. 2005, Lim et al. 2008). The principal monoterpene compounds from *P. densiflora* were α -pinene, myrcene, β -phellandrene, d-limonene, and α -terpinene, as previously reported by Lim et al. (2008). However, little is currently known regarding the essential oil concentration and composition of different pine needles during the decomposition process in Korea. In this study, we focused on investigating mono-

terpenes and sesquiterpenes, which are components of the essential oil. The principal objective of this study was to evaluate changes occurring in the essential oil composition of three pine species during different stages of decomposition. The study was conducted for one year in consideration of Korea's characteristic climate: wet and hot summer, warm spring and autumn, and relatively dry and cold winter.

MATERIALS AND METHODS

Site description

This study was conducted in a natural forest in Mt. JeongByeong (35°08'59" N-35°66'29" N, 128°45'13" E-128°36'19" E, elevation 567 m above mean sea level) in Gyeongnam Province, South Korea. The characteristics of its climate were mild and continental, and the annual average, maximum month, and minimum month temperatures of this region were 15.09°C, 26.7°C (July) and 6.7°C (January), respectively. Additionally, the annual mean precipitation in 2005 was recorded as 1,827 mm. Each of five 20- to 40-year-old trees of the species *P. densiflora*, *P. thunbergii*, and *P. rigida*, with diameters at breast height of 15-30 cm and heights of 3-10 m, were selected. Fresh (green) and senescent (recently dead and fallen) needles were obtained from each tree. Approximately 30 g quantities of fresh needles were enclosed in litter bags (20 cm × 20 cm) made of polypropylene shade cloth with a mesh size of 0.5 mm (Fig. 1). The litter bags were placed in the same plots in which the leaves were collected, and positioned at each of the surface litter layers and at a depth of 20 cm beneath the litter layer. The bags were attached to the soil with metal pins to prevent movement. The decomposition study encompassed one year, from April 2004 through April 2005. The bags were sampled 10 times at monthly intervals. On each sampling occasion, some of the needles from each of 9 bags were collected and placed in plastic bags, then carried to the laboratory for analysis of mono- and sesquiterpenes.

Analysis of terpenes

Foreign materials attached to the outsides of the bags were removed carefully; decaying needles were prepared for analysis by gently removing the soil. Three grams of needles were then collected from each litter bag, placed in a mortar with pure sand and 1% tetradecane as an internal standard, then extracted with 30-50 mL of pen-

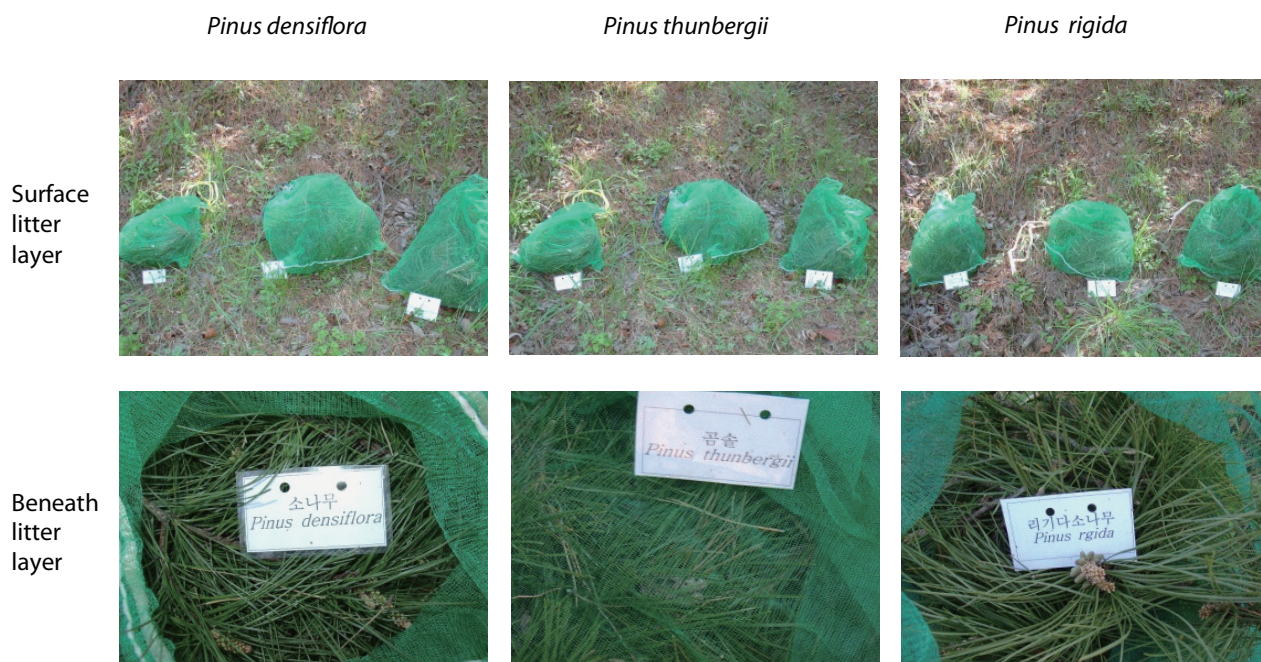


Fig. 1. Three litter bags with fresh needles placed on one subplot of forest floor at two soil depths (surface litter layer and 20 cm beneath litter layer).

tane. After extraction, extracts from the fresh and decaying needle samples were concentrated under nitrogen gas to 10 mL and 1.0-5.0 mL, respectively. One μL aliquot of each sample was injected into a gas chromatography-mass spectrophotometer (HP 5890II-5972 MS; Hewlett Packard, Wilmington, DE, USA) with an FID detector. A HP-5 capillary column (i.d. 0.25 mm, 30 m long) was used for the FID. The GC/FID oven program was initially set to 37°C for five minutes, increased to 180°C at a rate of 5°C/min, and then by 20°C/min up to 320°C.

Terpene calibrations were conducted via comparisons with the spectral data of the internal spectral library of the instrument (Wiley Library, ver. 7.0) and retention times, based on references. The concentration of peaks at selected retention times were estimated from peak areas using an internal standard curve for tetradecane. The differences in terpene concentration during the decomposition periods were analyzed for each species via analyses of variance (ANOVA).

RESULTS AND DISCUSSION

Terpenes in green and senescent needles from different pine species

The percentages were determined via the area normal-

ization method, and the obtained results were submitted for statistical analysis. We discussed the components with retention times of less than 30 minutes, since the peaks of components with retention times higher than 30-35 minutes were largely unstable. The components with concentrations in excess of 0.05% were expressed as “very low” or “trace”, although many components were detected in all three species.

Essential oil compositions in the fresh and senescent needles from 3 pine species are shown in Table 1. Seven monoterpenes and 10 sesquiterpenes were identified as components with concentrations higher or near 1% in fresh needles from three pine species. Among the monoterpenes, α -pinene (10.79-19.87%), β -pinene (2.90-22.86%), β -myrcene (1.01-6.95%), β -phellandrene (1.88-9.67%) are the major ones, and occur commonly in all three pine species. Among the monoterpenes obtained from *P. densiflora* fresh leaves and senescent needles, α -pinene (12.05% and 11.38%, respectively) is the principal one, followed by β -pinene (2.90% and 4.00%, respectively). However, in the fresh and senescent needles of *P. thunbergii*, β -pinene (20.77% and 16.12%, respectively) was detected as the primary monoterpene, followed by α -pinene (10.79% and 11.42%, respectively). The component found at the highest levels in the fresh needles from *P. rigida* was α -pinene (19.87%), followed by β -pinene (14.07%), but in the senescent needles β -pinene was the

Table 1. Composition of the total essential oil from fresh and senescent needle extracts of *P. densiflora* (P. d), *P. thunbergii* (P. t), *P. rigida* (P. r). Only compounds found at level > 0.05% are included (N = 3)

| | Compounds | Retention time | Fresh needle amount (%) | | | Senescent needle amount (%) | | |
|----|----------------------------|----------------|-------------------------|-------------|-------------|-----------------------------|--------------|-------------|
| | | | P. d | P. t | P. r | P. d | P. t | P. r |
| 1 | Tricyclene | 9.41 | 0.58 | - | - | 0.33 | 0.21 | 0.09 |
| 2 | α -pinene | 9.89 | 12.05 | 10.79 | 19.87 | 11.38 | 11.42 | 16.32 |
| 3 | Camphene | 10.34 | 1.75 | 0.87 | - | 1.30 | 1.12 | - |
| 4 | β -pinene | 11.32 | 2.90 | 20.77 | 14.07 | 4.00 | 16.12 | 22.86 |
| 5 | β -myrcene | 11.78 | 2.84 | 1.01 | 4.38 | 6.95 | 2.51 | 1.34 |
| 6 | Benzene | 12.82 | - | - | - | 0.02 | 0.04 | - |
| 7 | Sabinene | 13.03 | - | - | 1.55 | - | - | 0.03 |
| 8 | β -phellandrene | 13.17 | 2.90 | 7.26 | 1.88 | 9.67 | 4.90 | 2.81 |
| 9 | α -terpinene | 13.57 | 1.59 | 1.63 | - | 0.37 | 0.80 | - |
| 10 | 1,3,6-octatriene | 13.86 | - | - | - | - | 0.07 | - |
| 11 | γ -terpinene | 14.15 | - | - | - | 0.08 | 0.46 | - |
| 12 | 1-octanol | 14.64 | 0.05 | - | - | - | - | - |
| 13 | Camphor | 16.80 | - | - | - | - | 0.27 | - |
| 14 | Bicyclo heptan-3-one | 17.55 | 0.05 | - | 0.05 | 0.20 | 0.26 | 0.69 |
| 15 | Borneol | 17.64 | 0.05 | - | - | 0.14 | 0.05 | - |
| 16 | 3-cyclohexen-1-ol | 18.00 | - | - | - | - | 0.23 | - |
| 17 | 1,8-methadien-4-ol | 18.34 | - | - | - | 0.01 | - | - |
| 18 | Benzenemethanol | 18.43 | - | - | - | 0.55 | - | - |
| 19 | 2-methoxy-4-methylene | 19.77 | 0.10 | 0.12 | - | 0.10 | 0.12 | - |
| 20 | 1-decanol | 20.94 | 0.05 | - | 0.92 | - | 0.06 | - |
| 21 | Bicyclo heptan-2-ol | 21.35 | 1.23 | 1.80 | 2.82 | 0.55 | 0.30 | 0.57 |
| 22 | Bicycloelemene | 22.48 | 0.34 | 0.15 | 0.06 | 0.16 | - | 0.60 |
| 23 | α -cubebene | 23.10 | 0.57 | 0.08 | - | 0.14 | 0.16 | - |
| 24 | Vlangene | 23.35 | - | - | - | 0.04 | 0.07 | - |
| 25 | α -copaene | 23.85 | 0.10 | - | 0.09 | 0.27 | 0.26 | 0.24 |
| 26 | β -bourbonene | 24.11 | 0.06 | - | 4.02 | 0.17 | 0.12 | - |
| 27 | β -elemene | 24.30 | 0.51 | 0.25 | 0.92 | 0.82 | - | 0.76 |
| 28 | 1,4-methanoazulene | 24.70 | 0.14 | 0.70 | 3.42 | 1.44 | 0.44 | - |
| 29 | Trans-caryophyllene | 25.11 | 3.12 | 4.70 | 0.88 | 7.92 | 12.65 | 1.77 |
| 30 | δ -cadinene | 25.32 | - | 0.61 | - | 0.25 | 2.23 | 3.28 |
| 31 | Aromadendrene | 25.48 | 0.16 | - | 0.05 | 0.22 | - | 0.46 |
| 32 | 1,6-cyclodecadiene | 25.59 | 0.08 | 1.88 | 7.41 | - | 11.27 | 2.89 |
| 33 | α -humulene | 25.73 | 0.70 | 1.29 | - | 1.72 | - | 0.26 |
| 34 | β -cubebene | 25.98 | 0.05 | - | 1.08 | - | 0.55 | 0.11 |
| 35 | α -amorphene | 26.23 | 0.25 | 1.41 | 0.36 | 0.19 | 1.49 | 2.32 |
| 36 | Germacrene-D | 26.57 | 1.72 | 6.09 | 0.10 | 9.19 | 10.09 | 0.26 |
| 37 | β -selinene | 26.85 | 0.28 | - | 0.05 | 0.52 | 2.53 | 0.62 |
| 38 | Bicyclogermacrene | 27.11 | 1.09 | - | - | 1.83 | - | 3.89 |
| 39 | Cadina-1,4-diene | 27.40 | - | - | - | - | 0.15 | - |
| 40 | Naphthalene | 27.53 | - | - | 0.05 | 1.65 | 1.61 | 1.26 |
| 41 | γ -cadinene | 27.54 | 0.25 | 0.83 | 0.06 | 0.14 | 0.14 | 0.62 |

Table 1 (continued)

| | Compounds | Retention time | Fresh needle amount (%) | | | Senescent needle amount (%) | | |
|---|---------------------------------|----------------|-------------------------|-------------|-------------|-----------------------------|-------------|-------------|
| | | | P. d | P. t | P. r | P. d | P. t | P. r |
| 42 | α -cadinene | 28.12 | 0.16 | - | - | 0.60 | 0.16 | 1.57 |
| 43 | 1,6,10-dodecatrien-3-ol | 28.72 | - | - | - | 0.18 | - | 0.42 |
| 44 | Endo-1-bourbonanol | 29.14 | 2.11 | 0.38 | 7.41 | 2.07 | 0.11 | 2.89 |
| 45 | Spathulenol | 29.20 | - | - | - | 0.74 | - | 1.74 |
| 46 | Caryophyllene oxide | 29.35 | 0.16 | - | - | 1.50 | 2.57 | 0.60 |
| 47 | Salvial-4-en-1-one | 29.36 | - | - | - | - | 0.17 | - |
| 48 | Tau-muurolol | 29.57 | - | - | - | - | - | 1.53 |
| 49 | Copaene | 30.80 | 0.08 | - | - | 0.12 | - | - |
| 50 | Tau-cadinol | 30.94 | - | - | - | 0.60 | - | - |
| 51 | α -cadinol | 31.01 | 0.30 | 0.06 | - | 0.94 | - | - |
| 52 | Tridecanedial | 31.29 | 0.08 | - | - | 0.04 | - | - |
| 53 | 1-naphthalenepropanol | 32.66 | - | 2.82 | - | - | - | - |
| 54 | 4,2,5-secoobscurinervan-4-ol | 33.70 | - | 3.29 | - | - | - | - |
| 55 | Farnesyl acetate | 33.86 | - | - | - | - | 0.50 | - |
| 56 | 2-pentadecanone | 33.79 | - | - | - | 0.18 | 0.36 | 0.34 |
| 57 | 7-hexadecene | 34.35 | - | - | - | - | - | 1.00 |
| 58 | Dibuthyl phthalate | 34.39 | - | - | - | - | - | 0.07 |
| 59 | 7,11,15-trimethyl-3-methylen | 34.75 | 0.18 | - | - | - | - | - |
| 60 | 1,3,6,10-cyclotetradecatetraene | 35.00 | 0.41 | - | - | - | - | - |
| 61 | Cembrene | 35.12 | 0.24 | - | - | 0.36 | - | - |
| 62 | Cyclohexadecane | 35.45 | - | - | - | - | - | 0.19 |
| 58 | Thunbergol | 35.99 | 4.74 | - | 0.10 | 8.65 | - | - |
| 59 | Widdrene | 36.21 | - | 0.98 | - | - | - | - |
| 59 | Phytol | 36.22 | - | - | - | 0.13 | 0.44 | 0.58 |
| 60 | Labdadien | 36.38 | - | - | 0.10 | - | - | - |
| 61 | Geranyl linalool isomer | 36.66 | - | - | - | 0.21 | 0.27 | - |
| 62 | Neophytadiene | 36.84 | - | - | - | - | 0.27 | - |
| 63 | Labda-8,13-diene-ol | 36.94 | - | - | - | - | - | 0.76 |
| 64 | Bicyclo nonane | 37.10 | - | - | - | - | 0.11 | - |
| 65 | 1-phenanthrenecarboxylic acid | 38.10 | - | - | - | 0.73 | 2.01 | 1.72 |
| 66 | Dehydroabietic acid | 38.78 | - | - | - | - | 1.11 | - |
| 67 | 1-eicosanol | 38.16 | - | - | - | - | 0.81 | - |
| 68 | Eicosane | 38.46 | - | - | - | - | - | 0.13 |
| 69 | 1-docosene | 38.47 | - | - | - | - | - | 0.19 |
| 70 | Methyl abietate | 38.76 | - | - | - | - | 0.82 | - |
| 71 | Octadecane | 39.10 | - | - | - | - | - | 0.28 |
| Total compound No. of fresh needle | | | 42 | 24 | 26 | | | |
| Total compound No. of senescent needle | | | | | | 47 | 46 | 39 |

Bold values have been used to show the main representative compounds with contents higher than 1%.

most abundant component (22.86%). The results reported by several researchers (Yu et al. 2004, Lee et al. 2005, Bao et al. 2008), were consistent with our results for *P. densiflora*. They reported that the content of α -pinene was relatively higher than β -pinene in *P. densiflora* leaves. On the other hand, different results were reported by Kang and Kim (1997), Tani et al. (2002) and Kim and Shin (2005) for *P. densiflora* leaves. Kang and Kim (1997) reported that the concentrations of β -pinene were higher than the concentrations of α -pinene in all three species, and Tani et al. (2002) suggested that the most abundant monoterpenes from *P. densiflora* in Japan were β -phellandrene (37.4%), although the content of α -pinene (28.7%) was higher than that of β -pinene (19.8%). The primary monoterpene components of *P. densiflora* fresh needles detected by diethyl ether at pH 3.6 were α -ocimene (29.3%), sabinene (10.9%), β -myrcene (9.6%) and β -pinene (4.3%) (Kim and Shin 2005). Ludley et al. (2009) also insisted that there were typically two dominant monoterpenes (α - and β -pinene) in the green needles from conifer species (*P. abies*, *P. sitchensis*, and *P. sylvestris*). As many researchers (Janson 1993, Lee et al. 2005, Randrianalijaona et al. 2005) have reported, our results also suggested that the observed differences between the primary components and the concentrations of essential oil from the needles of *Pinus* species were based on variations in geographical conditions, sampling time, and extraction methods. As can be seen in Table 1, fresh needle monoterpenes from *P. densiflora* and *P. thunbergii* as compared to *P. rigida* were characterized by high contents in terpenic compounds, including camphene (1.75%, 0.87% vs. 0%, respectively) and α -terpinene (1.59%, 1.63% vs. 0%, respectively). On the other hand, tricyclene (0.58%), 1-octanol, borneol and 2-methoxy-4-methylene were detected only in *P. densiflora* and sabinene (1.55%) was detected only in *P. rigida*. Among the principal terpenes in fresh needles, β -myrcene, β -phellandrene, and α -terpinene, bicycloheptan-2-ol, α -copaene, α -cadinene, and trans-caryophyllene did not differ significantly ($P > 0.05\%$) between the three pine species. Camphene, bicycloheptan-2-ol, and naphthalene in senescent needles did not differ significantly ($P > 0.05\%$) among all three pine species. Additionally, the contents of most terpenes differed significantly (at 0.05%) among the three pine species. The difference in terpene composition between species might be based on systematic and genetic differences (Scrivanti et al. 2009), owing to the fact that *P. thunbergii* and *P. densiflora* are native species, but *P. rigida* is an induced species. Therefore, a systematic study will be necessary to further investigate terpene composition distances and/or corre-

lations among species.

Bicycloheptan-2-ol (0.30-2.82%), trans-caryophyllene (0.88-12.65%), germacrene-D (0.10-9.19%), 1,6-cyclodecadiene (0.08-11.27%) and endo-1-bourbonanol (0.11-7.41%) were the principal sesquiterpenes detected commonly in the fresh and the senescent needles of all 3 species. Among the fresh needle sesquiterpenes detected in *P. densiflora*, trans-caryophyllene (3.12%) was the most abundant compound, along with germacrene-D (6.09%) in *P. thunbergii* and 1,6-cyclodecadiene (7.41%) and endo-1-bourbonanol (7.41%) in *P. rigida*. Germacrene-D (9.19%) was the highest content compound in the senescent needles from *P. densiflora*, trans-caryophyllene (12.65%) in the senescent needles from *P. thunbergii*, and bicyclogermacrene (3.89%) was the highest content compound in *P. rigida*.

There have been many studies regarding monoterpene contents in coniferous trees (Wilt et al. 1993, Asensio et al. 2008), but few studies of sesquiterpene contents and profiles for coniferous plants have been conducted thus far. A previous study performed by Kim and Shin (2005) showed that β -caryophyllene (8.0%), β -cadinene (7.3%), α -terpinolene (4.9%) and 2-hexanal (4.3%) were the principal sesquiterpene compounds of *P. densiflora* needles in Korea, but this was not consistent with our results. However, no studies have been done thus far comparing sesquiterpene contents and profiles from needles of *P. thunbergii* and *P. rigida* in Korea. Three other studies on the sesquiterpene contents of *Pinus* species are worth considering in this regard (Barnola and Cedeño 2000, Dob et al. 2005, Asensio et al. 2008). The primary sesquiterpene compounds of needles from *Pinus caribaea* and *P. halepensis* were identified as β -caryophellene and α -humulene, although no comparative studies were conducted. Kupcinskiene et al. (2008) reported that (*E*)-caryophellene and δ -cadinene were the highest-level sesquiterpenes from *P. sylvestris*.

As is shown in Table 1, the total components detected from senescent leaves of three pine species were increased as compared to the components acquired from fresh needles. A total of 47 components were detected for *P. densiflora*, 46 for *P. thunbergii*, and 39 for *P. rigida*, which were obtained via the addition of new compounds to the data of the fresh needle components. These results showed that the biological degradation of monoterpenes and sesquiterpenes was induced by microorganisms such as fungi and bacteria (Harder and Probian 1995, Cleveland and Yavitt 1998, Osono et al. 2006). Harder and Probian (1995) previously performed an investigation into the anaerobic degradation of natural monoterpenes

with *Pseudomonas citronellois*. They determined that the consumption of monoterpenes, including linalool, menthol, menth-1-ene, α -phellandrene, limonene, 2-carene, α -pinene, and fenchone, depended on the presence of living microorganisms and nitrates. They also reported that geraniol and geranial were generated from linalool via biotransformation. However, it is difficult to evaluate its correlation with individual compounds in terms of microbial activity.

The content ratios of δ -cadinene, bicyclogermacrene, naphthalene, and caryophyllene oxide in senescent needle extracts were higher than those of fresh needle extracts in all three species. In particular, trans-caryophyllene and germacrene-D compositions of senescent needles of all three types of pines were elevated as compared to those detected in the fresh needles. The proportional content of 1,6-cyclodecadiene detected in senescent needles from *P. thunbergii* was approximately six times higher and the germacrene-D content from *P. densiflora* was approximately five times higher than that of the fresh needles. Bicyclogermacrene (3.89%) and δ -cadinene (3.28%) from *P. rigida* were detected at markedly higher levels in the senescent needles. The increased levels of sesquiterpene compounds in the senescent needles indicate that those compounds become more abundant during the decomposition process. Additionally, although the relevant data are not shown in this study, the ratio of total sesquiterpenes was increased above that of total monoterpenes during decomposition in all three species. Li et al. (2008) asserted that sesquiterpenes might originate from decaying needle litter or fungal hyphae. Our results also revealed that elevated sesquiterpene levels and/or new sesquiterpenes might be produced

and consumed by microbial activity in soils (Schade and Custer 2004, Sidorenko and Buzoleva 2008), and may have occurred as the result of litter-fall inputs from the aboveground needle parts, as was suggested previously by Asensio et al. (2008).

Terpenes loss from needles during decomposition

Kim et al. (2006) previously conducted a study of monoterpene concentrations in litterbags over a one-year period. According to that report, total monoterpene concentrations in all three species evidenced a rapid decrease for the first 90 days, and some monoterpenes remained at a low concentration throughout the entirety of the experimental period. The quantities of main essential oil loss from the remaining litter at 30-day intervals are provided in Fig. 2a and 2b. The concentration of essential oils in the litters of all three species as well as the monoterpene concentrations evidenced a rapid reduction for the initial 90 days after litter decomposition. We noted some slight differences in the soil horizons and the species. The total essential oil concentration in the litter of *P. densiflora* and *P. thunbergii*, respectively, decreased to 44.7% (390.7 $\mu\text{g/g f.w.}$) and 43.1% (377.4 $\mu\text{g/g f.w.}$) of the initial litter concentration determined in the first sampling. The total essential oil concentration in litter of *P. densiflora* decreased to 4.82% (24.6 $\mu\text{g/g f.w.}$) by day 120 and that of *P. thunbergii* was reduced to 4.92% (29.0 $\mu\text{g/g f.w.}$) by day 150. Concentration of the total essential oil in the needle litter of *P. rigida* evidenced a sharper reduction. At the first sampling (after day 30), the essential oil concentration of needle litter declined to 35.5% (301.8

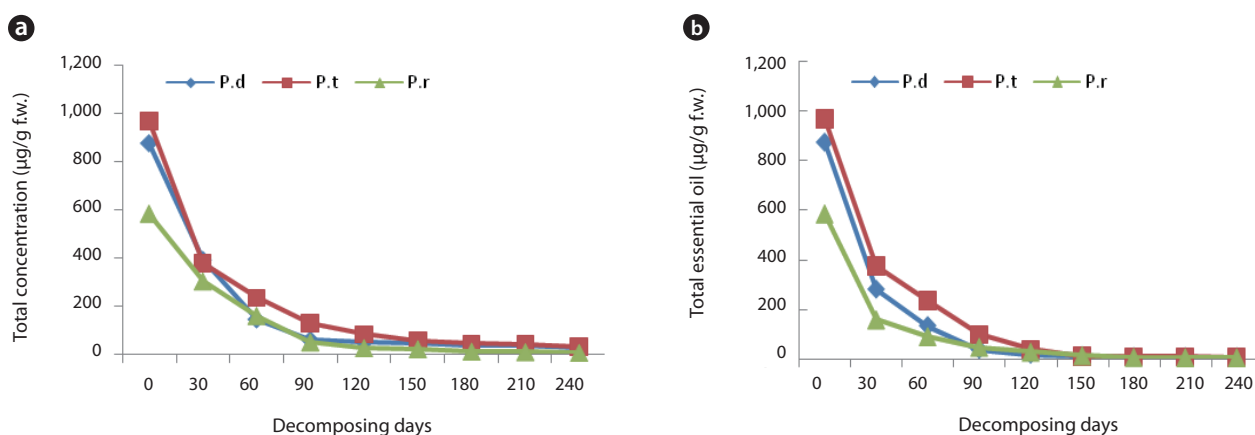


Fig. 2. The concentration of total essential oils ($\mu\text{g/g f.w.}$) in needle litter between litter layers (a) and 20 cm beneath litter layer (b) of *Pinus densiflora* (P.d), *Pinus thunbergii* (P.t), and *Pinus rigida* (P.r) over 240 days via the litterbag method. Each data was produced using all three replicates at each time point.

Table 2. Concentration of main terpenes ($\mu\text{g/g}$ f.w.) in litter (L) and ground layers (G) of *Pinus densiflora* (P. d), *Pinus thunbergii* (P. t), and *Pinus rigida* (P. r) during decomposition ($N = 3$)

| Compounds | Reten- tion time | Species | Decomposing days | | | | | | | | |
|-----------------------|---------------------|---------|------------------|-------|-------|-------|------|------|------|------|------|
| | | | 0 | 30 | 60 | 90 | 120 | 150 | 180 | 210 | 240 |
| α -pinene | 9.89 | P. d L | 132.55 | 32.97 | 9.59 | 4.57 | 3.30 | 1.51 | 1.98 | 2.03 | 1.24 |
| | | G | 132.5 | 36.68 | 10.42 | 2.48 | 0.42 | 0.25 | 0.01 | 0.05 | 0.07 |
| | | P. t L | 100.67 | 32.67 | 20.78 | 7.38 | 6.11 | 4.80 | 4.06 | 2.59 | 1.07 |
| | | G | 100.67 | 30.50 | 20.49 | 7.41 | 2.24 | 0.06 | - | - | - |
| | | P. r L | 113.46 | 19.39 | 11.95 | 2.54 | 0.21 | - | - | 0.02 | 0.01 |
| | | G | 113.46 | 18.26 | 2.65 | 0.82 | - | - | - | - | - |
| Camphene | 10.34 | P. d L | 19.72 | 5.96 | 3.19 | 0.71 | 0.70 | 0.51 | 0.47 | 0.19 | 0.18 |
| | | G | 19.72 | 2.87 | 1.96 | 0.50 | 0.11 | - | - | - | - |
| | | P. t L | 8.40 | 4.08 | 2.11 | 0.69 | 1.16 | 0.88 | 0.48 | 0.12 | 0.11 |
| | | G | 8.40 | 4.04 | 2.56 | 1.13 | 0.53 | - | - | - | - |
| | | P. r L | - | - | - | - | - | - | - | - | - |
| | | G | - | - | - | - | - | - | - | - | - |
| β -pinene | 11.32 | P. d L | 32.14 | 10.01 | 2.86 | 0.77 | 0.55 | 0.24 | 0.28 | 0.33 | 0.26 |
| | | G | 32.14 | 6.56 | 2.07 | 0.56 | 0.18 | - | - | 0.02 | 0.03 |
| | | P. t L | 191.65 | 51.42 | 24.99 | 15.35 | 6.75 | 5.33 | 3.05 | 1.08 | 0.89 |
| | | G | 191.65 | 23.94 | 34.61 | 10.23 | 1.90 | 0.33 | - | - | - |
| | | P. r L | 78.60 | 23.79 | 1.91 | 2.26 | - | - | 0.02 | 0.02 | - |
| | | G | 78.60 | 29.0 | 3.74 | 1.04 | - | - | - | - | - |
| β -myrcene | 11.78 | P. d L | 31.91 | 7.36 | 2.79 | 0.34 | 0.22 | - | - | - | 0.05 |
| | | G | 31.91 | 10.56 | 2.46 | 0.60 | 0.08 | - | - | - | - |
| | | P. t L | 8.96 | 27.05 | 2.12 | 0.69 | 0.23 | - | - | 0.04 | - |
| | | G | 8.96 | 26.0 | 2.74 | 0.95 | 0.19 | - | - | - | - |
| | | P. r L | 24.30 | 19.18 | 0.33 | - | - | - | - | - | - |
| | | G | 24.30 | 11.64 | 0.38 | - | - | - | - | - | - |
| β -phellandrene | 13.17 | P. d L | 41.79 | 24.36 | 5.45 | 0.99 | 0.55 | - | - | 0.28 | - |
| | | G | 41.79 | 0.32 | 3.70 | 0.89 | 0.14 | - | - | - | 0.03 |
| | | P. t L | 67.09 | 31.69 | 11.73 | 5.46 | 2.33 | - | 1.18 | 0.95 | - |
| | | G | 67.09 | 28.55 | 13.08 | 3.74 | 1.19 | - | - | - | - |
| | | P. r L | 12.02 | 0.21 | 2.87 | 0.51 | - | - | - | - | - |
| | | G | - | - | - | - | - | - | - | - | - |
| α -terpinene | 13.57 | P. d L | 22.92 | 0.05 | 1.93 | 0.31 | - | - | - | 0.43 | - |
| | | G | 22.92 | 1.19 | 2.30 | 0.48 | - | - | - | - | - |
| | | P. t L | 14.65 | 4.28 | 2.92 | 0.66 | 0.63 | 0.57 | 0.33 | 0.08 | - |
| | | G | 14.65 | 4.28 | 4.13 | 1.02 | 0.10 | - | - | - | - |
| | | P. r L | - | - | - | - | - | - | - | - | - |
| | | G | - | - | - | - | - | - | - | - | - |
| Bicycle heptan-2-ol | 21.35 | P. d L | 15.44 | 5.03 | 2.39 | 1.27 | 0.30 | 0.15 | 0.34 | 0.07 | 0.03 |
| | | G | 15.44 | 4.89 | 1.42 | 0.84 | - | - | - | - | - |
| | | P. t L | 15.25 | 5.27 | 1.58 | 0.41 | 0.77 | 0.85 | 0.39 | 0.67 | 0.11 |
| | | G | 15.25 | 1.03 | - | 0.49 | 0.06 | - | - | - | - |
| | | P. r L | 15.04 | 2.80 | - | - | - | - | - | - | - |
| | | G | 15.04 | 1.18 | 1.42 | 0.44 | 0.08 | 0.09 | 0.06 | 0.01 | 0.02 |
| α -cubebene | 23.10 | P. d L | 6.00 | 5.44 | 0.11 | 0.33 | 0.08 | 0.03 | - | 0.10 | 0.09 |
| | | G | 6.00 | 0.31 | 0.13 | 0.10 | 0.10 | 0.10 | - | 0.02 | 0.01 |
| | | P. t L | - | 0.39 | - | 0.36 | 0.25 | 0.31 | 0.26 | 0.10 | 0.09 |
| | | G | - | 0.55 | 0.23 | 0.21 | 0.14 | - | - | - | - |
| | | P. r L | - | - | - | - | - | - | - | - | - |
| | | G | - | - | - | - | - | - | - | - | - |

Table 2 (continued)

| Compounds | Reten- tion time | Species | Decomposing days | | | | | | | | | |
|---------------------|---------------------|---------|------------------|-------|-------|-------|------|------|------|------|------|--|
| | | | 0 | 30 | 60 | 90 | 120 | 150 | 180 | 210 | 240 | |
| α -copaene | 23.85 | P d L | 1.12 | 0.64 | 0.15 | 0.08 | 0.10 | 0.12 | 0.09 | 0.13 | 0.05 | |
| | | G | 1.12 | 0.23 | 0.09 | 0.08 | 0.08 | 0.11 | 0.04 | 0.02 | 0.01 | |
| | | P t L | - | 0.51 | 0.25 | 0.37 | 0.22 | 0.38 | 0.27 | 0.13 | 0.11 | |
| | | G | - | 0.35 | 0.28 | 0.13 | 0.09 | - | - | - | - | |
| | | P r L | - | - | 0.34 | - | - | - | - | - | - | |
| | | G | - | 0.24 | - | - | - | - | - | - | - | |
| β -elemene | 24.30 | P d L | 5.28 | 2.45 | 0.66 | 0.13 | 0.17 | - | 0.12 | 0.08 | 0.04 | |
| | | G | 5.28 | 1.51 | 0.52 | 0.12 | 0.02 | - | - | - | - | |
| | | P t L | 1.78 | 0.90 | 0.65 | - | 0.31 | - | - | 0.08 | - | |
| | | G | 1.78 | 0.90 | 1.06 | 0.24 | - | - | - | - | - | |
| | | P r L | 6.22 | 1.65 | 0.37 | - | - | - | - | 0.01 | - | |
| | | G | 6.22 | 1.79 | 0.69 | 0.39 | - | - | - | - | - | |
| Trans-caryophyllene | 25.11 | P d L | 32.95 | 13.65 | 4.86 | 0.95 | 1.52 | 1.68 | 1.23 | 0.38 | 0.28 | |
| | | G | 32.95 | 17.81 | 3.87 | 1.05 | 0.36 | 0.33 | 0.18 | 0.03 | 0.02 | |
| | | P t L | 41.01 | 43.36 | 12.34 | 6.85 | 5.92 | 5.2 | 3.74 | 1.65 | 1.48 | |
| | | G | 41.01 | 38.11 | 16.22 | 6.04 | 2.36 | 0.68 | 0.31 | 0.19 | - | |
| | | P r L | 4.43 | 2.92 | 7.33 | 0.39 | 0.10 | - | - | 0.02 | - | |
| | | G | 4.43 | 4.49 | 0.95 | 0.31 | 0.13 | 0.06 | 0.04 | 0.01 | - | |
| α -humulene | 25.73 | P d L | 7.42 | 4.39 | 1.13 | 0.22 | 0.30 | 0.23 | 0.26 | 0.11 | 0.03 | |
| | | G | 7.42 | 3.74 | 0.99 | 0.24 | 0.07 | - | - | - | - | |
| | | P t L | 12.47 | - | 2.73 | 1.73 | 1.25 | 1.12 | - | - | - | |
| | | G | 12.47 | - | 3.86 | 1.15 | 0.28 | 0.23 | - | - | - | |
| | | P r L | 0.50 | 0.66 | - | - | - | - | - | - | - | |
| | | G | 0.50 | 1.49 | 0.24 | - | - | - | - | - | - | |
| α -amorphene | 26.23 | P d L | 2.60 | 5.21 | 1.24 | 0.22 | 0.22 | 0.53 | 0.41 | 0.20 | 0.07 | |
| | | G | 2.60 | 0.64 | 0.84 | 0.15 | - | - | 0.02 | 0.02 | 0.03 | |
| | | P t L | 15.5 | 5.39 | 2.28 | 0.65 | 0.52 | 4.33 | 2.13 | 0.84 | 0.94 | |
| | | G | 15.5 | 5.16 | 2.81 | 1.0 | 0.36 | 0.41 | - | 0.08 | - | |
| | | P r L | 4.03 | 1.79 | 2.11 | 0.40 | 0.23 | - | - | 0.02 | - | |
| | | G | 4.03 | 3.11 | 1.03 | 0.28 | - | 0.06 | 0.09 | 0.11 | - | |
| Germacrene-D | 25.73 | P d L | 17.81 | 19.91 | 2.10 | 0.04 | 0.47 | 0.13 | 0.22 | 0.04 | 0.04 | |
| | | G | 17.81 | 6.71 | 2.12 | 0.56 | 0.16 | 0.12 | 0.17 | 0.02 | 0.03 | |
| | | P t L | 56.91 | 54.97 | 1.32 | 10.23 | 4.16 | 1.85 | 1.55 | 0.04 | 0.42 | |
| | | G | 56.91 | 54.43 | 13.84 | 3.68 | 0.60 | - | - | - | - | |
| | | P r L | 4.87 | 0.42 | 0.92 | - | - | - | - | 0.02 | - | |
| | | G | 4.87 | 1.33 | 0.22 | 0.08 | 0.03 | - | - | - | - | |
| Bicyclogermacrene | 27.11 | P d L | 6.84 | 5.50 | - | - | - | - | - | - | - | |
| | | G | 6.84 | 2.65 | 1.00 | 0.39 | - | - | - | - | - | |
| | | P t L | - | - | - | - | - | - | - | - | - | |
| | | G | - | - | - | - | - | - | - | - | - | |
| | | P r L | - | 4.90 | - | - | - | - | - | - | - | |
| | | G | - | 20.68 | 4.78 | 2.21 | 0.69 | 0.12 | 0.29 | 0.09 | 0.06 | |

$\mu\text{g/g}$ f.w.) of the initial content, and to 5.59% (5.3 $\mu\text{g/g}$ f.w.) by day 60. However, the initial concentrations in the freshly fallen litter of *P. rigida* were far lower than those of the other two species (583 $\mu\text{g/g}$ f.w. compared to 875 $\mu\text{g/g}$ f.w. and 968 $\mu\text{g/g}$ f.w. in *P. densiflora* and *P. thunbergii*, respectively) (Fig. 2a). These results are consistent with a study conducted by Ludley et al. (2009), in which it was reported that the total monoterpene concentrations from *P. abies*, *P. sitchensis*, and *P. sylvestris* declined rapidly to approximately 4% of the initial concentration in 6 months.

In the litter under layers, the total essential oil concentration in the *P. densiflora*, *P. thunbergii*, and *P. rigida* samples, respectively, decreased to 32.1% (280.6 $\mu\text{g/g}$ f.w.), 43.0% (376.4 $\mu\text{g/g}$ f.w.) and 17.9% (156.4 $\mu\text{g/g}$ f.w.) of the initial litter concentrations determined in the first sampling. The total essential oil concentration in the remaining litter of *P. densiflora* and *P. rigida* was also greatly reduced; the rate of loss of essential oils was higher than that of the litter layer (Fig. 2b). On the other hand, concentrations in the needle litter of *P. thunbergii* evidenced a similar rate of loss to the litter layers. Concentrations of *P. densiflora*, *P. thunbergii*, and *P. rigida* in litter layers declined to 2.8%, 3.3% and 0.6% of original litter, respectively, at the end of the sampling period. However, those of all three species in the under layers of the litter were 0%.

All these results demonstrated clear changes in the essential oil contents in needle litter from *Pinus* species during the decomposition process. Terpene concentration in the litter is susceptible to seasonal changes due to variations in environmental conditions and decay status throughout the year (Kainulainen and Holopainen 2002). Therefore, terpenes concentrations in deeper soil horizons might depend on seasonal patterns of litter-fall inputs from the aboveground parts of the tree to the soil surface. In this study, our results show that the highest rate of loss in concentration from all three species occurred by day 90 (3 months later), which was in about July, since the litter bag experiment began in April. July is a very hot and humid summer season in Korea. It was difficult, in this study, to delineate factors that might have influenced the changes in terpene concentration from *Pinus* species, but seasonal factors may be one of the more important factors, as previously asserted by Asensio et al. (2008). The rate of monoterpenes loss is thought to be largely related to the initial monoterpene concentration (Ludley et al. 2009). Despite the fact that the initial concentrations in *P. densiflora* and *P. thunbergii* litter were higher than that measured in *P. rigida* litter,

the loss rate of essential oil from *P. rigida* was higher than any of the other samples evaluated. As mentioned above, this might translate to a genetic difference; *P. rigida* is an induced species, but the others are native.

Each terpene had a small proportion of its original content remaining in the surface litter layer and the litter under layer at the end of the sampling period (Table 2). It was determined that the three types of reducing patterns of each compound depend on the rate of loss of concentration during the decomposition process. One of them is a sharp reduction in the initial period (like α -pinene, β -pinene, β -myrcene, β -phellene, bicycle heptan-2-ol, trans-caryophellene and germacrene-D), another one involves a steady or slow decline (camphene, α -cubebene, α -copaene, β -elemene, α -humulene and α -amorphene), and the final one is a newly detected pattern at low concentrations during decomposition (α -cubebene for *P. thunbergii*, α -copaene for *P. thunbergii* and *P. rigida*, and δ -cadinene and bicyclogermacrene for *P. rigida*). In particular, the remaining terpene contents in the litter layer from *P. rigida* disappeared completely at the final sampling days as compared to *P. densiflora* and *P. thunbergii* (with the exception of α -pinene). The α -pinene concentrations in the litter layers from *P. densiflora* and *P. thunbergii* were 1.24 and 1.07 $\mu\text{g/g}$ f.w., respectively, at the final sampling. This may be attributable to the sizeable quantities of remaining monoterpenes; the trans-caryophellene contents from *P. thunbergii* (1.48 $\mu\text{g/g}$ f.w.) identified it as the most abundant sesquiterpene remaining in the litter until the end of the experiment. Other studies have considered the loss of terpenes from litter of conifer species over time in the field (Kainulainen and Holopainen 2002, Ludley et al. 2009), although no similar studies have been published for *P. densiflora*, *P. thunbergii*, and *P. rigida*. Moreover, little information is currently available regarding the concentrations of each terpene in different soil horizons and decomposition periods. Both of the aforementioned studies reported a rapid initial loss of monoterpenes, which is consistent with the results of the present study. The loss rate of each compound might be related to its initial concentration, as mentioned above. However, we detected significant differences in the loss rates of concentration between the litter layer and the litter under layer (Table 2), although the initial concentrations were the same. Therefore, other factors influencing the rates of terpene loss might have been present during decomposition in this study. These factors include temperature, the difference in the volatility of the dominant compound in the litter, and the availability of compounds as a carbon source for micro-

organisms in the soil (e.g. α -pinene, the most abundant compound in the litter of *P. densiflora* and *P. rigida* is more volatile than β -pinene, which is the most abundant compound in the litter of *P. thunbergii*). The loss data of each compound (Table 2) demonstrated that the *P. densiflora* and *P. thunbergii* needles in litter decomposed more slowly than those of *P. rigida*, which may have contributed to the slightly higher amounts of monoterpene than sesquiterpene (except for endo-1-bourbonanol) remaining in the *P. rigida* litter at the final sampling. The variations in the concentrations of each terpene obtained in the litter bag experiment showed that the majority of terpene contents were lost from the litter of all three species in the litter under layers. This is consistent with the research conducted by Asensio et al. (2008); it suggested that higher terpene concentrations for all compounds from *P. halepensis* trees were observed in the top mineral horizon, as compared to the low mineral horizon.

In conclusion, the concentration of each essential oil in all three species was clearly shown to be affected by the depth of the layer and the decomposition stage of the needles. There is, therefore, transparently a need for further investigations into the sources of terpenes, particularly on the needle-degrading microorganisms and their physical and chemical factors in soil systems.

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LITERATURE CITED

- Asensio D, Owen SM, Llusà J, Peñuelas J. 2008. The distribution of volatile isoprenoids in the soil horizons around *Pinus halepensis* trees. *Soil Biol Biochem* 40: 2937-2947.
- Bao H, Kondo A, Kaga A, Tada M, Sakaguti K, Inoue Y, Shimoda Y, Narumi D, Machimura T. 2008. Biogenic volatile organic compound emission potential of forests and paddy fields in the Kinki region of Japan. *Environ Res* 106: 156-169.
- Barnola LF, Cedeño A. 2000. Inter-population differences in the essential oils of *Pinus caribaea* needles. *Biochem Syst Ecol* 28: 923-931.
- Berg B, Ekbohm G, Johansson M, McClaugherty C, Rutigliano F, DeSanto AV. 1996. Maximum decomposition limits of forest litter types: a synthesis. *Can J Bot* 74: 659-672.
- Cleveland CC, Yavitt JB. 1998. Microbial consumption of atmospheric isoprene in temperate forest soil. *Appl Environ Microbiol* 64: 172-177.
- Coûteaux M, McTiernan KB, Berg B, Szuberla D, Dardenne P, Bottner P. 1998. Chemical composition and carbon mineralization potential of Scots Pine needles at different stages of decomposition. *Soil Biol Biochem* 30: 583-595.
- Coûteaux MM, Bottner P, Berg B. 1995. Litter decomposition, climate and litter quality. *Trends Ecol Evol* 10: 63-66.
- Dob T, Berramdane T, Chelgoum C. 2005. Chemical composition of essential oil of *Pinus halepensis* growing in Algeria. *Comptes Rendus Chimie* 8: 1939-1945.
- Guenther A, Hewitt CN, Erickson D, Fall R, Geron C, Graedel T, Harley P, Klinger L, Lerdau M, McKay WA, Pierce T, Scholes B, Steinbrecher R, Tallamraju R, Taylor J, Zimmerman P. 1995. A global model of natural volatile organic compound emissions. *J Geophys Res* 100: 8873-8892.
- Harder J, Probian C. 1995. Microbial degradation of monoterpenes in the absence of molecular oxygen. *Appl Environ Microbiol* 61: 3804-3808.
- Hwang JY, Kim JH, Yun KW. 2004. The effect of selected monoterpenoids on the cellular slime mold, *Dictyostelium discoideum* NC4. *J Chem Ecol* 30: 1153-1163.
- Janson RW. 1993. Monoterpene emissions from Scots pine and Norwegian spruce. *J Geophys Res* 98: 2839-2850.
- Jung MJ, Chung HY, Choi JH, Choi JS. 2003. Antioxidant principles from the needles of red pine, *Pinus densiflora*. *Phytother Res* 17: 1064-1068.
- Kainulainen P, Holopainen JK. 2002. Concentrations of secondary compounds in Scots pine needles at different stages of decomposition. *Soil Biol Biochem* 34: 37-42.
- Kainulainen P, Holopainen T, Holopainen JK. 2003. Decomposition of secondary compounds from needle litter of Scots pine grown under elevated CO₂ and O₃. *Global Change Biol* 9: 295-304.
- Kang HN, Kim JH. 1997. The monoterpenoids in *Pinus thunbergii*, *Pinus rigida* and *Pinus densiflora*. *Korean J Ecol* 20: 323-328. (in Korean with English abstract)
- Kelkar VM, Geils BW, Becker DR, Overby ST, Neary DG. 2006. How to recover more value from small pine trees: essential oils and resins. *Biomass Bioenergy* 30: 316-320.
- Kim JH, Hwang JY, Jo GG, Kang HN. 2006. Monoterpenoids concentration during decomposition and their effect on *Polysphondylium violaceum*. *J Ecol Field Biol* 29: 337-342.
- Kim YS, Shin DH. 2005. Volatile components and antibacterial effects of pine needle (*Pinus densiflora* S. and Z.) extracts. *Food Microbiol* 22: 37-45.
- Kleinheinz GT, Bagley ST, St John WP, Rughani JR, McGinnis GD. 1999. Characterization of alpha-pinene degrading

- microorganisms and application to a bench-scale bio-filtration system for VOC degradation. *Arch Environ Contam Toxicol* 37: 151-157.
- Koide K, Osono T, Takeda H. 2005. Fungal succession and decomposition of *Camellia japonica* leaf litter. *Ecol Res* 20: 599-609.
- Koide RT, Suomi L, Stevens CM, McCormick L. 1998. Interactions between needles of *Pinus resinosa* and ectomycorrhizal fungi. *New Phytol* 140: 539-547.
- Kupcinskiene E, Stikliene A, Judzentiene A. 2008. The essential oil qualitative and quantitative composition in the needles of *Pinus sylvestris* L. growing along industrial transects. *Environ Pollut* 155: 481-491.
- Lee JG, Lee CG, Kwag JJ, Buglass AJ, Lee GH. 2005. Determination of optimum conditions in pine needles by double-shot pyrolysis-gas chromatography-mass spectrometry. *J Chromatogr A* 1089: 227-234.
- Li GH, Duan M, Yu ZF, Li L, Dong JY, Wang XB, Guo JW, Huang R, Wang M, Zhang KQ. 2008. Stereumin A-E, sesquiterpenoids from the fungus *Stereum* sp. CCTCC AF 207024. *Phytochemistry* 69: 1439-1445.
- Lim JH, Kim JC, Kim KJ, Son YS, Sunwoo Y, Han JS. 2008. Seasonal variations of monoterpene emissions from *Pinus densiflora* in East Asia. *Chemosphere* 73: 470-478.
- Ludley KE, Jickells SM, Chamberlain PM, Whitaker J, Robinson CH. 2009. Distribution of monoterpenes between organic resources in upper soil horizons under monocultures of *Picea abies*, *Picea sitchensis* and *Pinus sylvestris*. *Soil Biol Biochem* 41: 1050-1059.
- Ludley KE, Robinson CH, Jickells S, Chamberlain PM, Whitaker J. 2008. Differential response of ectomycorrhizal and saprotrophic fungal mycelium from coniferous forest soils to selected monoterpenes. *Soil Biol Biochem* 40: 669-678.
- McTiernan KB, Couteaux MM, Berg B, Berg MP, de Anta RC, Gallardo A, Kratz W, Piussi P, Remacle J, De Santo AV. 2003. Changes in chemical composition of *Pinus sylvestris* needle litter during decomposition along a European coniferous forest climatic transect. *Soil Biol Biochem* 35: 801-812.
- Osono T, Hirose D, Fujimaki R. 2006. Fungal colonization as affected by litter depth and decomposition stage of needle litter. *Soil Biol Biochem* 38: 2743-2752.
- Owen SM, Clark S, Pompe M, Semple KT. 2007. Biogenic volatile organic compounds as potential carbon sources for microbial communities in soil from the rhizosphere of *Populus tremula*. *FEMS Microbiol Lett* 268: 34-39.
- Randrianalijaona JA, Ramanoelina PAR, Rasoarahona JRE, Gaydou EM. 2005. Seasonal and chemotype influences on the chemical composition of *Lantana camara* L.: essential oils from Madagascar. *Anal Chim Acta* 545: 46-52.
- Schade GW, Custer TG. 2004. OVOC emissions from agricultural soil in northern Germany during the 2003 European heat wave. *Atmos Environ* 38: 6105-6114.
- Scrivanti LR, Anton AM, Zygodlo JA. 2009. Essential oil composition of *Bothriochloa Kuntze* (Poaceae) from South America and their chemotaxonomy. *Biochem Syst Ecol* 37: 206-213.
- Sidorenko ML, Buzoleva LS. 2008. Character of interactions of saprophytic soil microflora via gaseous metabolites. *Microbiology* 77: 235-239.
- Smolander A, Ketola RA, Kotiaho T, Kanerva S, Suominen K, Kitunen V. 2006. Volatile monoterpenes in soil atmosphere under birch and conifers: effects on soil N transformations. *Soil Biol Biochem* 38: 3436-3442.
- Tani A, Nozoe S, Aoki M, Hewitt CN. 2002. Monoterpene fluxes measured above a Japanese red pine forest at Oshiba plateau, Japan. *Atmos Environ* 36: 3391-3402.
- Vokou D, Chalkos D, Karamanlidou G, Yiangou M. 2002. Activation of soil respiration and shift of the microbial population balance in soil as a response to *Lanvandula stoechas* essential oil. *J Chem Ecol* 28: 755-768.
- Wheatley RE, Millar SE, Griffiths DW. 1996. The production of volatile organic compounds during nitrogen transformations in soils. *Plant Soil* 181: 163-167.
- Wilt FM, Miller GC, Everett RL. 1988. Monoterpene concentrations in litter and soil of single-leaf pinyon woodlands of the western great basin. *Great Basin Nat* 48: 228-231.
- Wilt FM, Miller GC, Everett RL, Hackett M. 1993. Monoterpene concentrations in fresh, senescent and decaying foliage of single-leaf pinyon (*Pinus monophylla* Torr. & Frem.: Pinaceae) from the western great Basin. *J Chem Ecol* 19: 185-194.
- Yu EJ, Kim TH, Kim KH, Lee HJ. 2004. Aroma-active compounds of *Pinus densiflora* (red pine) needles. *Flavour Fragr J* 19: 532-537.