

Needle Litter Decomposition of Native *Pinus sylvestris* L. and Alien *Pinus mugo* at Different Ages Affecting Enzyme Activities and Soil Properties on Dune Sands

DALIA JANUŠAUSKAITĖ^{1*}, VIRGILIJUS BALIUCKAS^{2,3} AND ZENONAS DABKEVIČIUS¹

¹ Department of Plant Pathology and Protection, Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Akademija, LT-58344, Kėdainiai distr. Lithuania,

² Department of Forest Tree Genetics and Breeding, Institute of Forestry, Lithuanian Research Centre for Agriculture and Forestry, Liepų str. 1 Girionys, LT-53101, Kaunas distr., Lithuania

³ Aleksandras Stulginskis University, Institute of Forest Biology and Silviculture, Studentų str. 11, LT-53361 Akademija, Kaunas distr., Lithuania

*E-mail: dali@lzi.lt, phone +370 437 37263; fax. +370 347 37096

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Abstract

Drifting dunes in the Curonian Spit of western Lithuania were covered by native *Pinus sylvestris* and alien *Pinus mugo* species. With planting of non-native species there is a need for studies which could evaluate the effect of alien and native plant species on soil ecosystem properties. We measured soil fungi amount, microbial enzyme activity, needle decomposition rate, respiration intensity, and soil and needle chemical composition from nearby pairs of native and alien pine species at different ages from several sites on the dunes (Lithuania). A total of 18 sampling sites of close-growing *P. sylvestris* and *P. mugo* were selected with stand ages ranging from 14 to 120 years, representing three age classes. Needles of *P. sylvestris* showed a faster decomposition rate than *P. mugo*. Needles of *P. mugo* had lower nitrogen content, higher C/N ratio and lignin content compared to *P. sylvestris*. The mass loss of needle litter was also positively correlated with C/N ratio and with fungi abundance in soil. Soil under *Pinus sylvestris* has higher C and N concentration than under *P. mugo*. The tendencies showed that there were more fungi in the soil under *P. sylvestris* than under *P. mugo*. Soil enzyme activity was lower in the *P. mugo* plots. The activities of invertase, dehydrogenase, alkaline phosphatase, protease, potential nitrification activity increased gradually with the development of forests after afforestation. These results suggest that due to the differences in litter properties and decomposition rates, changes may occur in microbial properties and nutrient cycling in the forest ecosystems.

Key words: litter decomposition, enzyme activity, Scots pine, mountain pine, litter quality.

Introduction

Coastal dunes are among the most valuable elements of natural environment and natural resources in Europe. The Curonian Spit is a 98 km long (area 180 km²) thin sand-dune spit situated between the Curonian Lagoon and the Baltic Sea. Dune systems are very sensitive and management both for the maintenance of landscape diversity and for the conservation of the heritage features of the Curonian Spit as a World Heritage site is important (Povilanskas et al. 2009). Curonian Spit is famous for the highest moving (drifting) sand dunes in Europe. National Park was established in 1991 to protect the unique ecosystems of the Curonian Spit and Curonian Lagoon. Several herbal

species are endemic to Baltic dunes. Forest occupies up to 70% of Curonian Spit land area. Scots pine stands prevail and some of them are over 200-year-old. To know how to behave in handling dunes is critical, seeing that theories exist resulting in a paradigm from afforestation to the removal of pine plantations.

The Curonian Spit is well known for its large-scale shifting sand dunes. The introduction of alien plant species on coastal dunes for sand stabilization was a common practice in the past and also in the present days. *Pinus mugo*, also known as mountain pine, is native to the mountains of central Europe and the Balkan Peninsula. In Lithuania, the first plantations of *P. mugo* were established on the coastal sand dunes about 200 years ago, and at present are considered

useful for protection of sandy dunes and human settlements. *P. mugo* does not spread extensively throughout the Curonian Spit, occurring only in locations where it was deliberately planted, however naturally regenerating seedlings of *P. mugo* appear near mature trees, demonstrating the adaptability of the mountain pine to coastal dune conditions (Aučina et al. 2011). Some investigations also demonstrated the facilities of the non-native pines to modify the dune habitats (Leege and Murphy 2001).

The dune soils (Arenosols) are poor in nutrients as well as carbonate and susceptible to acidification (Piotrowska 1988, Peyrat 2007). In the case of drifting dunes, no soil organic layer is formed and can be more difficult to afforestation of the dunes. Creation or improvement of soil fertility occurs mainly through addition of plant biomass, particularly as litter-fall, which is a major source of organic matter to soil (Xuluc-Tolosa et al. 2003). Accumulation of litterfall as well as the magnitude of benefits of organic matter onto the soil depends on the several factors i.e., plant species, climate, mainly temperature and humidity. Tree species influence ecosystem ecology in different ways, i.e. through differences in the quantity and quality of organic matter that they produce (Wardle et al. 2004, Madritch and Lindroth 2011), in particular nitrogen, lignin and polyphenol concentrations (Sariyildiz and Anderson 2003), differences in root activity, by below-ground cycling of carbon and nitrogen (Schweitzer et al. 2004), soil nutrient availability (Fioretto et al. 2005), the qualitative and quantitative composition of decomposer communities (Knoepp et al. 2000). Moreover, plants have species-specific feedback interactions with their soil decomposer organisms (Wardle et al. 2004) when the releases of mineral nutrients through litter-fall decomposition are processed by soil organisms, which are of crucial importance for the functioning of ecosystems.

The rate and process of litter decomposition greatly influence the soil formation and the availability of carbon, nitrogen and other nutrients to plants and microorganisms in forest ecosystems. The biological activity increases gradually with soil age (Liski 1995, Bens et al. 2006). The decomposition dynamics were also affected by the initial chemical composition of the litter (Fioretto et al. 1998). Concentrations of N, P, cellulose, lignin, lignin-C and lignin-N content were related to mass loss in decomposing Scots pine needles (Couteaux et al. 1998).

Soil enzyme activities are known to be involved in nutrient cycling, and as such, have been suggested as potential indicators of soil quality and changes in properties of the forest floor because of their essential role in soil biology and ease of measurement

and rapid response to changes in soil management (Kandeler et al. 1999). For example, dehydrogenase plays an important role in the initial oxidation of soil organic matter; protease and phosphatase is involved in breaking down proteins and in the organic phosphorus mineralization, respectively; urease is involved in nitrogen cycling (Tabatabai and Bremner 1972, Amador et al. 1997, Kramer and Green 2000, Quilchairo and Maronon 2002). Dehydrogenase and acid phosphatase activities showed different values in soil under different trees species due to differences in the leaf N, lignin and cellulose contents of the residues (Defrieri et al. 2011).

The aim of the study was to evaluate the effects of two pine species, native *Pinus sylvestris* and alien *Pinus mugo*, and stand age on the soil biochemical and microbial properties. Understanding of these differences will help to provide a more complete picture of how the effects of plant species can influence soil properties in a sensitive area, where the long lasting attempts (mainly by foresters) were intended to stop soil erosion due to deforestation and introduction of alien species with the aim to stop destructive processes.

Nowadays silvicultural policy tends to prevent future spread of *P.mugo* and step by step to replace it by *P. sylvestris*. Scientifically adjusted knowledge would also help decision makers in this situation. We studied the individual trees and their influence on belowground ecosystem. While the decomposition of pine needles has been widely studied in laboratory and field conditions (Leege and Murphy 2001, McTiernan et al. 2003); we have no information about comparative study which would test the decomposition rates of *P. sylvestris* and *P. mugo* and their impact on soil properties at the same time.

Understanding the potential influence of needle litter on soil is essential to understanding how different pine species affect ecosystem properties.

Materials and methods

Description of sites

The Curonian Spit stretches in a gentle curve between the Curonian lagoon and the Baltic Sea. Most of the first dune ridges from the sea are artificial. The main dune area is overgrown with trees or has been afforested. The drifting of dunes began in the 16th century due to deforestation of the spit caused by overgrazing and timber harvesting. To stop this process, starting in 1825, re-vegetation began with planting of a non-native species – mountain pine (*Pinus mugo*). It is a native tree of mountain areas in the Central and Southern Europe. Nowadays forest covers 89% of the spit, it consists mostly of Scotch pine

(*Pinus sylvestris*) – 53% and mountain pine – 27%. In favourable conditions, the mountain pine reaches 10 m in height, but on the dunes it is just 1 m high. Even being blown over with sand it stays alive for several years (Motiekaityte 2000). The mean annual temperature in the region is 7.4 °C, and the mean annual precipitation is 771 mm. Pine stands grew on a *Haplic Arenosol* (FAO 2006) with a thin organic layer and surrounded by *Pinetum vacciniosum* forest type.

Sampling procedure

The paired-site design was used in the study. Samples of needle litter from forest floor and soil were collected in pine forest, in the Curonian spit, in about 40 km long transect of dunes. There were 36 sites, grouped into two pairs, where pure stands of the *Pinus sylvestris* and *P. mugo* of the same age were growing on the same soil in order to minimize the heterogeneity among them. There were chosen three age classes of trees. The age of the youngest-generation pine ranged from 14 to 20 years, mature-generation trees from 50 to 65 years, and age of the older generation from 110 to 120 years, which represented 2nd, 5th and 11th age class (by forest inventory description), and accordingly are equivalents for juvenile age, middle age, mature age. Each age group is represented by six samples in pair for *P. sylvestris* and *P. mugo* from each selected site of about 10–10 m area. For respiration measurement needle samples from the forest floor were taken in September 2010 in each age's class, bulked to composite samples and air-dried. All samples for general soil chemical properties and microbial analysis and fresh needles shake from the trees were obtained in July 2011.

The soil contained thin organic layer, so the samples were not separated into different horizons and were sampled uppermost 10 cm layer. After the upper litter layer was removed, six to eight scoops were randomly collected in each site to obtain soil samples with a minimum of 1 kg of soil per sampling area. Soil samples were mixed thoroughly and placed in tightly sealed plastic bags and kept at 4 °C to keep them field moist for microbial analyses. All microbial determination was performed within one week of sampling. Soil moisture content was determined gravimetrically after drying soil samples at 105 °C.

Soil and needle chemical analysis

Soil pH was measured in a soil–water suspension (volume ratio 1:5) using a WTW Inolab pH meter. The total soil nitrogen and organic carbon were determined by dry combustion method (DIN/ ISO 13878). Needle samples dried and ground by a mill with 1 mm sieve were used for chemical analyses. Carbon content was deter-

mined using an automatic spectrophotometer UV/VIS Carry 50 (Varian, Germany) at the wavelength of 590 nm using glucose as a standard after wet combustion. N determined by Dumas method. Concentrations of water-soluble carbohydrates (WSC) was evaluated by Anthron method, crude fibre (CF) by using near infrared spectrometer NIRS – 6500 (Perstorp Analytical, Silver Spring, Maryland, USA), fat determined by Soxhlet apparatus using gravimetric method. Lignin (acid detergent lignin) (ADL) was estimated by cell wall detergent fractionation method using the van Soest methodology of fibre fraction. Ash was determined by combustion and digestibility of the dry matter in vitro using pepsin-cellulose method. Major elements (Na, K, Ca and Mg) were measured using a Perkin Elmer Instrument model AAnalyst 200 flame atomic absorption spectrometer, P was determined by a spectrophotometer.

Soil enzymatic activities

Fresh soil samples were used for all enzyme assays. Dehydrogenase (EC 1.1) activity was determined using the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF) method and results were expressed, $\mu\text{g TPF g}^{-1} \text{dw } 24 \text{ h}^{-1}$. The urease (EC 3.5.1.5) activity estimation was based on the colorimetric determination of ammonium formation after enzymatic urea hydrolysis by buffered method. The released ammonium was determined spectrophotometrically at 630 nm. Results were expressed as $\mu\text{N g}^{-1} \text{dry soil}$. Protease activity (EC 3.4.21-24) was estimated by determination of tyrosine released after incubation of sodium caseinate as substrate and Tris buffer at 50°C for 2 h and the residual casein was precipitated with 10% trichloroacetic acid and filtrate was reacted with Na_2CO_3 and Folin-Ciocalteu reagent. The tyrosine concentration was measured colorimetrically at 700 nm after 1h incubation at room temperature. For alkaline phosphatase activity (EC 3.1.3.1) soil sample (1.0 g) was reacted with substrate (50 mM sodium p-nitrophenyl phosphate). The yellow colour intensity of the released p-nitrophenol was measured at 400 nm using a spectrophotometer after incubation at 37°C for 1 h.

The potential ammonium oxidation (potential nitrification) rate in soil was assayed as accumulated nitrite according to the rapid test by ammonium oxidation technique described in the draft standard ISO/DIS 15685 (1999) for testing of the potential nitrification in soil. Results are expressed as $\mu\text{g of NO}_2 \text{ g}^{-1} \text{dw}$. Standard curves were obtained for each analytical run using serially diluted nitrite standards (NaNO_2).

Enzyme activities were assayed according to the procedure as described by Schinner et al. (1995). All spectrophotometrical analyses were made by a Perkin Elmer Lambda 25 UV/VIS spectrophotometer. All anal-

yses were carried out in triplicate and values were calculated on the basis of oven-dry weight of soil.

Respiration from litter, microcosm decomposition study

For respiration or CO₂ production measurement needle samples were taken in 2010. A beaker with 1 g of needle litter was placed in a 1 l air-tight jar with 10 ml H₂O at the bottom and with a CO₂ trap (10 ml of 0.5 M NaOH) and incubated in total darkness at 17 °C. The needle was moistened by soil suspension. Soil suspensions were prepared by shaking 10 g of soil with 100 ml of distilled water for 15 min. Portions (3 ml) were extracted from the suspensions and applied to each beaker in order to inoculate litter samples by microorganisms. The traps were replaced at 21 day intervals. The CO₂ released was determined by titration with HCl (0.25 M), combined with 1 ml of BaCl₂ (1.0 M) and five drops of phenolphthalein solution (0.1% in 60% ethanol), until the solution had changed from pink to cloudy white. The measurement was performed in triplicate on each litter sample. Respiration was expressed as μg C- CO₂ g⁻¹ litter and measured in a closed environment after each 21 days. The incubation lasted for 105 days. The daily decay rate (K) of litter for the entire study was calculated through the negative exponential decay model:

$$W_t/W_0 = \exp (-kt);$$

where W₀ is initial weight (1 g of dry litter) and W_t is weight remaining after time t (the end of experiment, 105 days).

Fungi counts

Before analyses, consolidated soil samples were passed through a 2 mm sieve. Conventional dilution spread-plating was performed to assess the culturable fungal colony forming units (cfu). Total fungi were enumerated by plating the dilution series onto malt extract agar. All inoculated media were incubated at room temperature (22 ± 3 °C) for ca. 4-5 days. All microbial enumerations were carried out in triplicate. Data are reported as 10⁴ CFU g⁻¹ dry soils.

Statistical analyses

Data are reported as mean ± standard error of the mean and were examined using analysis of variance (ANOVA) procedures. We used a two-way analysis of variance (ANOVA) to determine the influence of pine species and age on enzyme activities and amount of microbes. In the soil, the significance of differences in soil properties with stand and age was assessed separately for each stand by a one-way analysis of variance. For the microbial properties, an arsine square-root transformation was done. Significant differences among treatment means were assessed by Fisher's least significant difference test (LSD, P < 0.05) and using an F-test. Statistical computations were performed with software ANOVA adapted by P. Tarakanovas in the Visual Basic of Application as macro program to run in the EXCEL (Tarakanovas and Raudonius 2003). SAS STEPDISC procedure was used for selecting variables best discriminating pine species (SAS institute, Inc. 2004). The method of principal components in factor analysis was applied to analyse the data of soil and pine needles components by the FACTOR procedure. The dendrogram of cluster analysis (method= average) was generated in order to present the relationships among pine species in different age groups. SAS CLUSTER and TREE procedures were used for data processing.

Results

Chemical properties of soil and litter

Table 1 presents the data of soil properties. All soil under *Pinus sylvestris* had higher soil C concentration than under *P. mugo*. In addition, the C concentration (%) in the soil under the oldest pines was significantly higher (P < 0.05) than under soils with the younger pines (Table 1), in the oldest *P. sylvestris* stands, as much as 40% higher amount of C than that in *P. mugo* soil. Also the total N content in the soil under *P. sylvestris* was higher than under *P. mugo* by 61%, with the highest value in the oldest stand. The C/N ratio was lower in younger soils for both pine species, and in 11th age class

Age, pine	C	N	C/N	P	pH
Ps					
2 c	2.35 (0.86)a	0.059 (0.01)ab	38.13 (7.16)ab	0.012(0.006)ab	3.83 (0.14)b
5 c	3.72 (1.32)abc	0.062 (0.01)ab	57.07 (13.47)b	0.009 (0.002)ab	3.82 (0.13)ab
11 c	10.30 (2.71)c	0.208 (0.06)b	51.63 (8.33)ab	0.016 (0.003)b	3.77 (0.14)ab
p-level	0.065	0.087	0.391	0.531	0.926
Pm					
2 c	2.10 (0.62)a	0.063 (0.02)ab	33.79 (2.92)a	0.012 (0.007)a	4.03 (0.16)c
5 c	2.61 (0.69)a	0.051 (0.01)ab	49.54 (7.83)abc	0.009 (0.003)a	3.87 (0.13)abc
11 c	7.36 (0.64)b	0.091 (0.02)b	89.28 (16.15)c	0.005 (0.002)a	3.71 (0.09)a
p-level	0.0017	0.389	0.072	0.686	0.050

Table 1. ANOVA analysis for soil chemistry of study stands. Values are % of the dry mass

In each column, means with different letters are significantly different (P<0.05). Standard errors of the means are in parentheses, n=3. Ps – *P. sylvestris*, P.m – *P. mugo*; (2c, 5c 11c – 2nd, 5th and 11th age class of stand)

soil under *P. mugo* reached the highest ratio. P amount was low and similar in all sites, except for the 11th age class soil under *P. sylvestris* where it reached the highest value. The pH values of all soil samples were in the range from 3.36 to 4.48, thus being very acidic. pH-values were also affected by a tree species, with the pH of the *P. sylvestris* being slightly lower than that of *P. mugo* (Table 1), and with tendency to decrease with stand age.

Pronounced differences in litter quality were detected for fresh fallen needle (Table 2).

Table 2. Chemical characteristics of fresh needle litter collected from two pine species from 5th age class stands, values are % of the dry mass. Ps – *P. sylvestris*, P.m – *P. mugo*

Pine	pH	CF	Ash	Lignin	Fat	WSC	N	C	P	K	Mg	Ca	Na
Ps	4.15	43.8	2.35	32.1	10.5	3.51	1.09	67.8	0.126	0.45	0.072	0.374	0.33
Pm	4.54	51.4	1.47	43.4	6.81	2.29	0.68	67.8	0.078	0.31	0.062	0.343	0.23

Ps – *P. sylvestris*, P.m – *P. mugo*. CF – crude fibre, WSC – water-soluble carbohydrates

Higher values of the chemical elements were found in *P. sylvestris* needles, and only crude fibre and lignin contents were higher in *P. mugo* needles. There were no differences in C concentration in the decomposed needle litter collected on the forest floor between both species (Table 3).

Table 3. ANOVA analysis of needle litter chemical characteristics. The samples analysed were collected in pine stands of both species at different age (presented in age classes). The values are percents of dry mass

Age, pine	C	N	Lignin	C/N
Ps				
2 c	64.60 (0.15)ab	0.55 (0.05)a	35.73 (1.39)b	119.8 (10.6)b
5 c	65.13 (0.03)b	0.76 (0.11)a	34.03 (1.47)a	89.7 (14.4)ab
11 c	64.43 (0.48)ab	0.74 (0.12)a	33.33 (1.18)a	92.4 (15.8)ab
p-level	0.357	0.453	0.026	0.432
Pm				
2 c	65.76 (0.41)abc	0.43 (0.12)ab	41.00 (1.64)b	173.4 (43.2)b
5 c	64.86 (0.89)b	0.66 (0.17)b	36.96 (3.39)ab	112.4 (31.4)ab
11 c	63.86 (0.14)a	0.54 (0.04) ab	38.86 (2.52)ab	118.1 (8.9)ab
p-level	0.082	0.472	0.609	0.413

In each column, means with different letters are significantly different ($P < 0.05$). Standard errors of the means are in parentheses, $n = 3$. Ps – *P. sylvestris*, P.m – *P. mugo*; (2c, 5c 11c – 2nd, 5th and 11th age class of stand)

However, a tendency was revealed that litter N concentration was by as much as 25.9% higher in *P. sylvestris* needles, while C/N ratio was lower in *P. sylvestris* needles by 33.8%. Lignin content was significantly ($P=0.01$) higher in *P. mugo* needles compared with *P. sylvestris*. Among the age classes, the needles from younger stand had the lower N concentration, and higher C/N ratio, also lignin content. Initial lignin concentration in fresh and decomposed needles of *P. mugo* was by 35.2 and 13.3%, respectively higher compared with lignin content in *P. sylvestris* needles.

Soil enzyme activities

The stand age showed positive impact on all soil microbial properties (Figure 1; Table 4). The activity of alkaline phosphatase, urease and protease increased significantly in the oldest stand almost by 3, 2.5 and 1 times, compared with the youngest stand, respectively. Lesser impact was found in the case with dehydrogenase and potential nitrification, by about 50% higher in the oldest pine stand (Figure 1).

The results summarized in Table 4, show that pine species was an important factor for enzyme activities. In general, the activities of all enzymes were higher in the soil under *P. sylvestris*, than under *P. mugo* (Figure 1).

Table 4. Results of ANOVA for the effects of pine species and stand age on soil microbial properties

Variable	Source of variable	df	F	P
Dehydrogenase (μg triphenyl formazan g^{-1} of soil for 24 h)	Age	2	8.93**	0.000
	Pinus	1	12.29**	0.000
	AxP	2	0.44	0.647
Urease (μg ammonium g^{-1} of soil for 1 h)	Age	2	57.05**	0.000
	Pinus	1	3.98	0.051
	AxP	2	12.01**	0.000
Protease (μg tyrosine g^{-1} soil h^{-1})	Age	2	3.06	0.059
	Pinus	1	0.32	0.577
	AxP	2	0.62	0.544
Alkaline phosphatase (μg p-nitrophenol g^{-1} soil h^{-1})	Age	2	11.65**	0.000
	Pinus	1	1.13	0.305
	AxP	2	0.69	0.516
Potential nitrification (μg N-NO ₂ g^{-1} soil)	Age	2	2.86	0.088
	Pinus	1	2.02	0.175
	AxP	2	0.74	0.494
Fungi (CFU g^{-1} soil)	Age	2	4.32**	0.016
	Pinus	1	8.55**	0.004
	AxP	2	0.46	0.633

** – significant at $P < 0.05$ and 0.01

Litter decomposition rate

Litter of different pine species and different age showed variations in their respiration intensity (Figure 2). The total release of CO₂ from the litter during 105 days ranged from 17.37 (*P. mugo* sample at juvenile age) to 28.74 μg C-CO₂ g^{-1} (*P. sylvestris* sample at middle age). Respiration was highest in *P. sylvestris* needle of 5th age class, and lowest in *P. mugo* stand of 2nd age class.

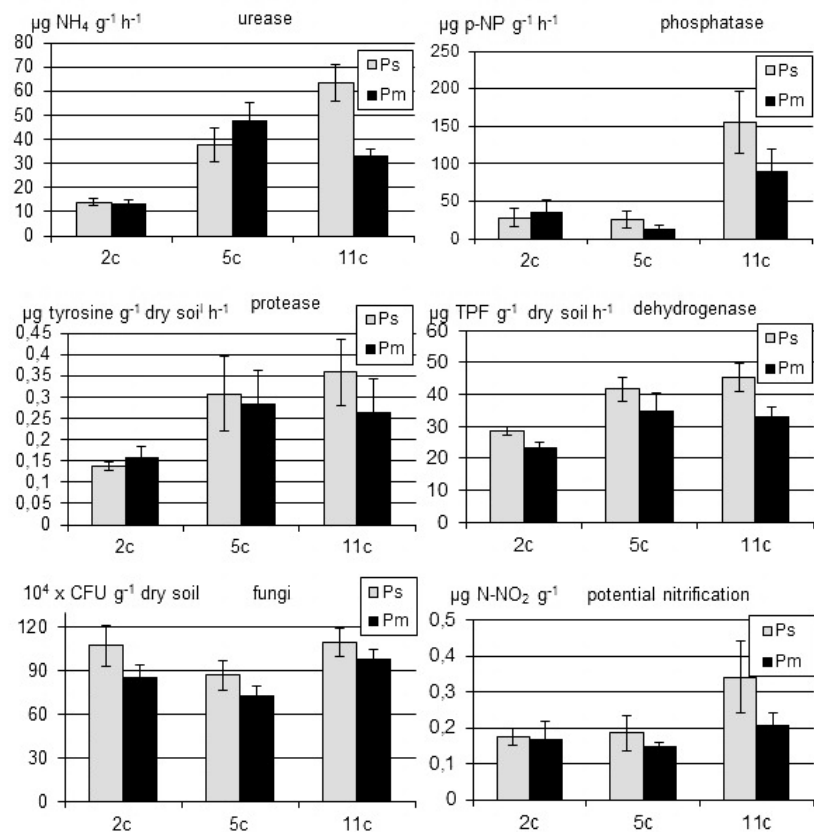


Figure 1. Mean (\pm SE) of urease, dehydrogenase, phosphatase, protease, potential nitrification activity and fungi amount in the soil of the pine species investigated at each age classes. Ps – *P. sylvestris*, Pm – *P. mugo*; (2c, 5c 11c – 2nd, 5th and 11th age class of stand)

The decomposition rate constant (K_w), calculated from mass losses was 0.001647 and 0.001474 for *P. sylvestris* and *P. mugo*, respectively, (Figure 2), with being higher by 11.7% in average for *P. sylvestris*. In addition, for all *P. mugo* variants the first-order decomposition rate constant (K_w) varied from each other weakly. However, for *P. sylvestris* the decomposition rate constant (K_w) differed among needles from different age classes, with higher values for needles from the oldest trees (K_w – 0.001855). The best relationships were established between the decomposition rate and N, $r = -0.88$. Litter C/N ratio and C amount showed the weak correlation ($r = -0.48$) and no relationship with decomposition rate.

Also we analysed by comparing fresh leaf litter (collected from trees after its shaking) and decomposed litter from the forest floor. The results showed the same tendency – the decomposition rate constant (K_w), calculated from mass losses was 0.0025 and 0.0018 for *P. sylvestris* and *P. mugo*, respectively.

Soil fungi

The density of fungi varied across the soils from the different pine species. The amount of fungi differed significantly ($p=0.004$) between the soil under *P. sylvestris* and under *P. mugo* and the higher amount in average reached in the site under Scots pine, by 101.4×10^4 cfu g^{-1} soil (Figure 1). The amount of fungi

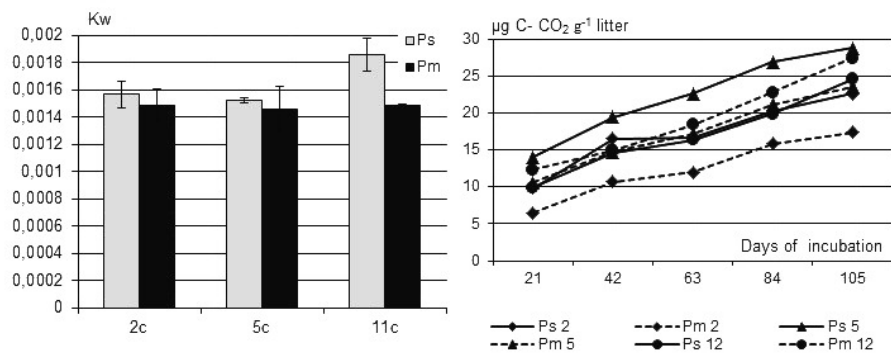


Figure 2. The needle decomposition rate constant (K_w), calculated from leaf mass loss. Mean values \pm SE are shown per each measurement. Carbon release as CO_2 measurement during 105 d incubations of different pine species and at each age classes. Ps – *P. sylvestris*, P.m – *P. mugo*; (2c, 5c, 11c – 2nd, 5th and 11th age class of stand)

in the soil under different age classes also differed significantly between two species, being lowest in the soil under *P. mugo* of 5th age class, and the highest soil fungi population occurred in the oldest *P. sylvestris* stand, 73.2 x10⁴ and 109.7 x10⁴ cfu g⁻¹ soils, respectively.

Joint analysis of all soil and needle components

Soil and needle components were the most distinguishing between species at mature age. Average distance of mature Scots pine cluster from the rest was twice as large as for mature *P. mugo* (Figure 3).

ed properties of soil and needle components (fungi, bacteria, pH, urease, dehydrogenase, soil C, soil N, needle N, needle C/N) is evident. All the characteristics, except ph and needle C/N, correlated positively with Factor1 estimates. Fungi, soil C, soil N and needle C_N correlate positively and significant with Factor2, the rest negatively. And only pH, dehydrogenase and N of all used characteristics had negative correlation with Factor3 estimates.

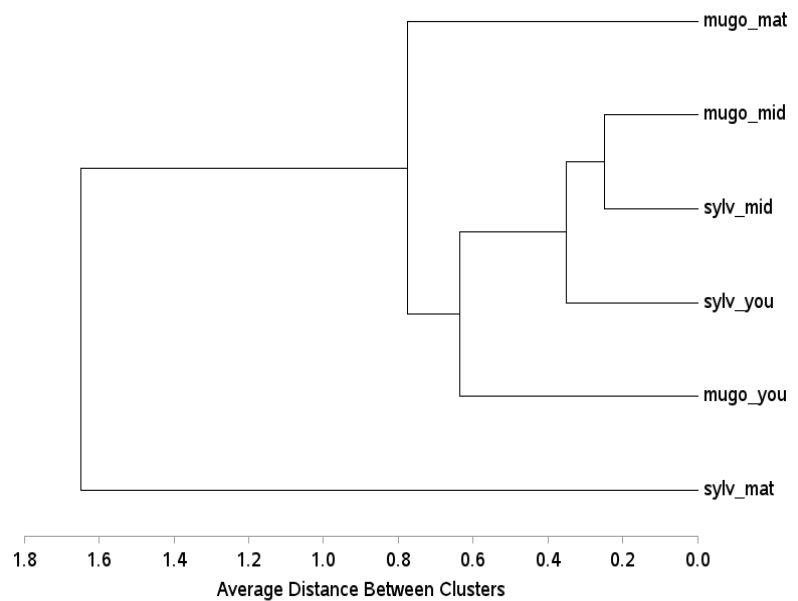


Figure 3. Dendrogram showing the relationships between two pine species in all characteristics at different stand age (abbreviations: you- juvenile age, mid – middle age, mat – mature age, which correspond 2nd, 5th and 11th age class of stand) based on the trait average

The least difference was between *P. mugo* and *P. sylvestris* at middle age. *P. sylvestris* at juvenile age was the closest to the cluster of both species stands at middle age. *P. mugo* at juvenile age was at the distance 0.64 from the mentioned group, while the distance of mature *P. mugo* was 0.78. Factorial analysis of characteristics best discriminating pine species proves that differences between two species are substantial (Figure 4).

Nine of eighteen characteristics were significantly contributing to differences between species, though variance explained by three factors did not exceed 10 percent. Even if portion of variance explained is not large, the separation of both pine species by estimat-

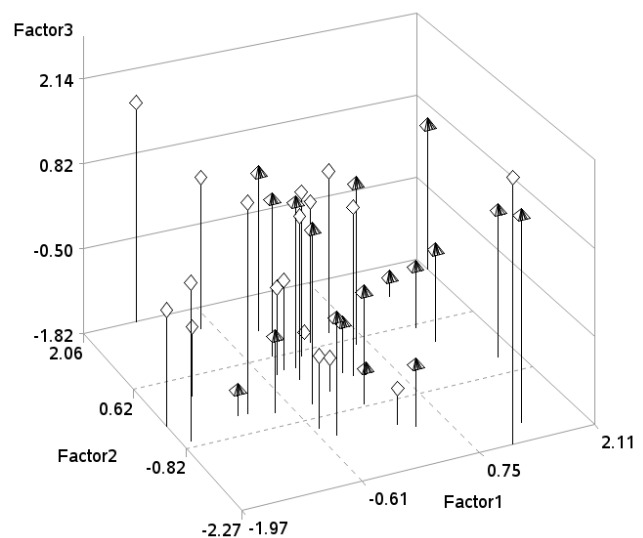


Figure 4. Plotting of estimated properties of soil and needle components (fungi, bacteria, ph, urease, dehydrogenase, soil C, soil N, needle N, needle C/N) in tridimensional perspective on factorial axis. Different shapes indicate pine species: diamonds indicate *P. mugo* and pyramids – *P. sylvestris*. Variance explained by Factor1, Factor2 and Factor3 was accordingly 3.18, 1.70 and 1.25

Discussion

All sampling sites were located within transect of the Curonian spit. The sampling sites were chosen relatively similar in pine age and soil. Therefore, our data should be interpreted assuming that the major differences in the revealed properties can be attributed to the effect of pine species and age.

Our prediction that the different pine species influenced the soil chemical and biological properties was supported by the results. Our study showed that even pine species affect decomposition rate, soil fungi abundance and enzymatic activity in a distinct manner.

Litter fall and decomposition are important functions in the case of recently afforested soils with low organic matter or without it, particularly in dunes, for organic matter accumulations (Sariyildiz et al. 2005) and for other associated ecological benefits. When a plant has rapidly decomposing litter due to the differences in the litter chemistry (Ashton 2005), it tends to raise rates of nutrient cycling within the young ecosystem. Despite the fact that in many cases it has been noted that litter from alien plants decomposes and nutrients are released more quickly compared with native plant litter (Ehrenfeld 2003, Ashton 2005, Liao et al. 2008), in our study we found an opposite effect. Due to variations in needle chemical properties of pine species (Tables 2, 3), differences in needle decomposition rates were expected. Similar differences in chemical litter composition of the two pine species were observed in other studies (Nardi et al. 2000).

N, C/N, and lignin concentrations were the best predictors of needle mass losses of different pine species and affected their decomposition rates and hence the nutrient cycling process in this forest ecosystem. *P. mugo* had less N-rich needles, higher lignin content, and thereby slower litter decomposing rate. Conversely, the litter from *P. sylvestris* contained relatively low levels of lignin and crude fibre, and high levels of ash, fat, N and water-soluble carbohydrates (WSC), and *P. sylvestris* exhibited significantly faster rates of decay. There was no difference between initial litter C content between species, and C/N rate was high enough for both species. This suggests that decomposition rate is determined by N and/or other nutrient content rather than by C. This result confirms the previously reported pattern that high quality litter (characterized by higher N concentration and lower C/N ratio) can decompose faster in comparison with low quality litter (Vesterdal et al. 2008). For needles of *P. sylvestris* with higher N content the mass loss and respiration rate were more than 11.7 and 11.1% higher respectively, than for needles of *P. mugo* with lower N content. The importance of the initial N con-

tent for the decomposition rate was noted also previously (Vestgarden 2001). Also litter, containing relatively low levels of lignin and high levels of carbohydrates, exhibited significantly faster rates of decay than the highly lignified litter (Hansson et al. 2011) and may be as a good predictor for mass losses from litter (Sariyildiz and Anderson 2003). Moreover, despite the different needle fall time of the two pine species, the comparison of chemistry, in both cases showed *P. sylvestris* to have higher N concentration and lower C/N ratio in soil (Table 1). C concentration was slightly higher in the soil under *P. sylvestris*. This can be explained by differences in needle longevity, as *P. sylvestris* needle longevity is usually around 3-5 years, compared with more than 5 years for *P. mugo* needles. As a result, increased needle litter input is the prime factor, together with needle properties for accumulation of organic matter.

The age of tree stand may also alter ecosystem. Effects of stand age on the microbial properties also was detected in previous studies (Bauhus et al. 1998). Tree species impact on soil biological properties was small and mostly not significant on young soils. This is common that organic layer accumulation after pine establishment is a long-term process, particularly after *P. mugo* establishment more than 100 years is required to increase the organic layer by more than 10 cm (Dirnböck et al. 2008). We may assume that for pine stands in new afforested sites, the period of about 100-120 years is enough to exhibit clear differences in soil properties. Our results indicate that the slightly increased respiration of the *P. sylvestris* and the values of enzyme activities in general were greater for the most developed soils. It agrees with other studies that the microbial communities in less developed soils are less efficient at mineralizing C (Hopkins et al. 2007), nitrification rates generally increase with age (Barford and Lajtha 1992). Moreover, more large differences between the investigated microbial indicators were determined by the oldest *P. sylvestris* stand compared with *P. mugo* stand.

When areas without organic soil layer are reclaimed for forestry, soil microbes are responsible for establishing biogeochemical cycles and for energy transfer, and are involved in forming soil structure (Diaz-Ravina et al. 1993). Soil and litter resource quality also directly affect the abundance, composition, and activity of the decomposer community. Older stand have higher content of organic matter and create conditions for habitat heterogeneity, which in the oldest stand seemed to be responsible for the more abundant soil fungi population (Figure 1). The overall amount of fungi was higher in soil under *Pinus sylvestris*.

For this study we assumed that enzyme activities could serve as sensitive indicators for the measuring

of impact of pine species on soil formation and quality. Changes in enzyme activities during litter decomposition provide information on the changes in soil quality and functional microbial succession (Nannipieri et al. 2003). Instead, the higher activity was in soil under *P. sylvestris*, which agrees with other studies (Nardi et al. 2000). Also, the phosphatase activity values in the present study are within the range of those reported for acidic soils from coniferous forests (Pang and Kolenko 1986). Enzyme activity for each age class was found to increase proportionally with stand ages. There were only occasional differences in enzyme activity in younger stands, and significant differences in older ones. Longer period of tree growth results in higher organic matter accumulation in the soil. Lower enzyme activity in soil from young stands may be due to lower organic C as was noted previously that soil organic matter amount influences enzyme activity (Nannipieri et al. 2003). In addition, it was found that changes of land use associated with organic matter content can alter the soil enzyme activities (Kizilkaya and Dengiz 2010). Microbial succession with shifting enzymatic capabilities enhances decomposition (Hättenschwiler et al. 2005), which is tied to organic matter content in soil.

The pH values of the pine soil investigated in our study were generally low. Conifers may increase soil acidity due to needle litter which has low pH (Hagen-Thorn et al. 2004, Oostra et al. 2006). Lower pH of the soil under *P. sylvestris* is linked to lower needle pH of 4.15, compared to *P. mugo* needle pH of 4.54. A decrease in pH with soil development is a common phenomenon and has been observed at other reclaimed sites (Graham and Haynes 2004) as well as during primary succession on sand dunes (Emmer and Sevink 1994).

Conclusions

The variability of needle litter decomposition and enzyme activities, as occurs among different pine species, is accompanied by differences in needle litter chemistry. Needle from native pine *P. sylvestris* has higher decomposition rate and nutrient contents, than litter from alien *P. mugo* species, potentially leading to increased rates of nutrient cycling, productivity in the forest ecosystem and soil formation intensity.

The results of a study also bear geographic relevance as there was no such study performed before, and future investigations might be focused on micro-organism activity shift associated with plant species.

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РАЗЛОЖЕНИЕ ПОДСТИЛКИ МЕСТНОГО *PINUS SYLVESTRIS* L. И ЧУЖЕРОДНОГО *PINUS MUGO* ВИДОВ СОСНЫ РАЗНЫХ ВОЗРАСТОВ, И ВОЗДЕЙСТВИЕ НА СВОЙСТВА ПОЧВЫ И АКТИВНОСТЬ ФЕРМЕНТОВ В ПЕСЧАНЫХ ДЮНАХ**Д. Янушаускайте, В. Балюцкас, З. Дабкявичюс***Резюме*

На блуждающих дюнах Куршской косы (Западная Литва) произрастают в основном сосны местного происхождения *Pinus sylvestris* L. и интродуцированные *Pinus mugo*. Посадка интродуцированных видов вызвала необходимость исследований, направленных на выявление эффекта воздействия каждого вида на почвенную экосистему. Было определено количество почвенных грибов, активность микробных ферментов, скорость разложения хвои, интенсивность дыхания, химический состав почвы и хвои на местах произрастания рядом растущих местных и интродуцированных сосен. Объекты исследования были подобраны в древостоях разного возраста, произрастающих в нескольких местах. Всего для исследований было подобрано 18 объектов, где произрастают сосняки трех классов возраста, и по возрасту распределяются от 14 до 120 лет. Хвоя *P. sylvestris* разлагалась быстрее хвои *P. mugo*. Содержание азота в хвое *P. mugo* было ниже, а соотношение углерода к азоту (C/N) и содержание лигнина было выше по сравнению с *P. sylvestris*. Потеря массы хвои положительно коррелировала с C/N и с количеством грибов в почве. Все образцы почвы из под *P. sylvestris* имели более высокую концентрацию почвенного углерода и азота. Анализ образцов почвы показал, что в большинстве случаев обилие грибов в почве под *P. sylvestris* превосходило *P. mugo*. Активность ферментов почвы была ниже под *P. mugo*. Активность инвертазы, дегидрогеназы, щелочной фосфатазы, протеазы, и потенциальная активность нитрификации после посадки леса постепенно увеличивалась с возрастом древостоев. Полученные результаты указывают на различия лесной подстилки и скорости разложения, в связи с чем могут произойти изменения микробной активности и обмена питательных веществ в экосистеме леса.

Ключевые слова: скорость разложения лесной подстилки, активность ферментов, сосна обыкновенная, сосна горная, качество лесной подстилки