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Leaf litterfall decomposition of pedunculate (*Quercus robur* L.) and sessile (*Q. petraea* [Matt.] Liebl.) oaks and their hybrids and its impact on soil microbiota

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Abstract: Trakas Forest is the only natural habitat of sessile oak in Lithuania. Sessile oak stand here is growing about 60–70 km from the nearest natural sessile oak stands in Poland. The purpose of this study was to determine whether autumn leaf litterfall of pedunculate and sessile oaks and their hybrids have different biochemical composition and decomposition rate and, consequently, different impacts on microbial condition of rhizosphere. For this purpose in autumn leaf litterfall C, N, P, K, Ca, Mg, lignin, ash, fat, crude fibre and water-soluble carbohydrates contents and stocks, lignin/N, lignin/P, C/N, C/P, N/P ratios, the decomposition rate and CO_2 emissions were determined. In rhizosphere of studied oak species N, C concentration, pHH_2O, C/N ratio, and dehydrogenase, urease, phosphatase, bacteria and micromycetes amount were estimated as well. The litterfall of pedunculate oak was distinguished by a higher content of lignin, higher lignin/N ratio, lower decay rate and lower carbon release, which determines decreased activity of micromycetes in the rhizosphere. Metabolic activity of microorganisms differed insignificantly among tree species rhizospheres. However, the potential for the use of carbon compound substrates and biodiversity index have a tendency to be higher in the soil under sessile oak. Lower decomposition rate of leaf litterfall and organic compounds in the rhizosphere under pedunculate oak allowed to suppose that the conditions for natural regeneration were more suitable in stands where sessile and hybrid oaks dominate.

Keywords: biochemical properties, enzymatic activity, metabolic activity, microorganisms, microbial communities.

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Introduction

Sessile oak (*Quercus petraea* [Matt.] Liebl.) in Trakas Forest in the south-western part of Lithuania was discovered in a 70 ha area by S. Tuminauskas in 1954 (Tuminauskas, 1957). In Trakas Forest, sessile oak grows at the eastern edge of the distribution area. This stand is growing about 60–70 km from the nearest natural sessile oak stands in Poland (Navasaitis et al., 2003). Pedunculate oak (*Q. robur* L.) comprised up to 44%, sessile oak 18%, and trees possessing features of hybrids 38% of the total number of oaks in Trakas Forest (Baliuckas, 2000). Oak undergrowth consists of only 5% sessile oak, 20% hybrids and the remaining 75% pedunculate oak (Jurkšienė et al., 2012). A fairly high degree of hybridization (15–55%) was determined by Carlisle and Brown (1965), Wigston (1975) and Kissling (1980a; 1980b; 1983) in Switzerland, Rushton (1978; 1979; 1983), Minihan and Rushton (1984) in the United Kingdom and Jensen et al. (2009) in Denmark. However, lower rates of hybridization were obtained in similar studies made in Central Europe (Aas, 1993; Steinhoff, 1998; Streiff et al., 1999).

Tree foliar litterfall is very important for the initial biological cycle process in forest stands (Kaiser et al., 2011; Strickland et al., 2013). On decomposition of forest floor the nutrients, mainly organic carbon and nitrogen, return to the soil's available nutrient pool, where they are reused by plants (Osman, 2013). Therefore, it is known that the chemical composition of mineral topsoil can be tightly related to the chemical composition of the forest floor by the nutrient resorption during senescence (Hobbie et al., 2006).

Leaf litter of different oak species is highly lignified (Sinsabaugh et al., 2002) and decomposes slowly, resulting in slower nutrient release (Hobbie et al., 2006). Therefore, a high oak quantity in forests may increase carbon storage in the forest floor through lower rates of litter decomposition and potential nutrient availability from delayed litter mineralization (Piatek et al., 2009). Chávez-Vergara et al. (2016) propose that *Quercus* species influence the organic nutrient mineralization by determining the composition and activity of the forest floor microbial communities.

Soil enzymatic activities are important for the biological decomposition cycle process and were suggested as potential indicators of soil quality, especially for changes of forest floor properties (Bolton et al., 1985; Kandeler et al., 1996; Yang et al., 2006; Hussain et al., 2009; Das & Varma, 2011; Utobo & Tewari, 2015). Numerous studies confirmed that dehydrogenase is involved in the initial oxidation of soil organic matter and phosphatase predicts organic phosphorus mineralization, while urease is involved in nitrogen cycling (Tabatabai & Bremner, 1972; Amador et al., 1997; Krämer & Green, 2000; Quilchano & Maranon, 2002). Consequently, the dehydrogenase activities in soil are biological indicators of overall microbial respiratory activity of soils and are used by microorganisms in the soil to break down organic matter (Bolton et al., 1985; Utobo & Tewari, 2015). Other soil enzymes urease and phosphatase have a crucial role in N and P cycle respectively (Karaca et al., 2011). In addition, the activity of phosphatases was found to correlate with organic matter in various studies (Guan, 1989; Jordan & Kremer, 1994; Aon et al., 2001) according to Das and Varma (2011), it is crucial for predicting their interactions as their activities may, in turn, regulate nutrient uptake and plant growth.

This work could be distinguished from similar studies, as the research object includes sessile oak habitat at the edge of species natural distribution. We hypothesized that the higher species decomposition rate of litterfall under different oak species, the less lignin concentration and lignin/N ratio as well create better conditions to natural regeneration of undergrowth (could influence natural regeneration and expansion of sessile and hybrid oak). The purpose of this study was to determine whether autumn leaf litterfall of pedunculate and sessile oaks and their hybrids have different biochemical composition and decomposition rate and, consequently, different impacts on microbial condition of rhizosphere.

Materials and methods

Study site and sampling

Autumn leaf litterfall (hereafter – leaf litter) and soil samples were collected in Trakas Forest (54°14'11"N, 23°45'30"E, 190 m a.s.l.), 2 km west from Seirijai in Alytus district, south-west Lithuania (Fig. 1). This forest has had the status of a strict nature botanical-zoological reserve in Meteliai Regional Park since 1960. Pure pedunculate and sessile oak stands occupy only 9% of the total Trakas Forest area and in 49% of the forest area they are an admixture mainly with Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* (L.) H. Karst.), silver birch (*Betula pendula* Roth.), etc. (Tuminauskas, 1957; Patalauskaitė, 2008). The main forest site type according to Lithuanian classification is mesoeutrophic mineral soils of normal moisture (Nc), while the soil according to World Reference Base for Soil Resources was classified as Luvisols (sandy loam over sandy clay loam) (LR AM/LMI/MVT, 2006; WRB, 2014). The forest belongs to the *Querco-Fagetea* class, *Fagetalia sylvaticae* Pawłowski 1928 order, *Carpinion betuli* alliance, *Tilio-Carpinetum betuli* Traczyk 1962 association and the *calamagrostetosum* subassociation (Patalauskaitė, 2007).

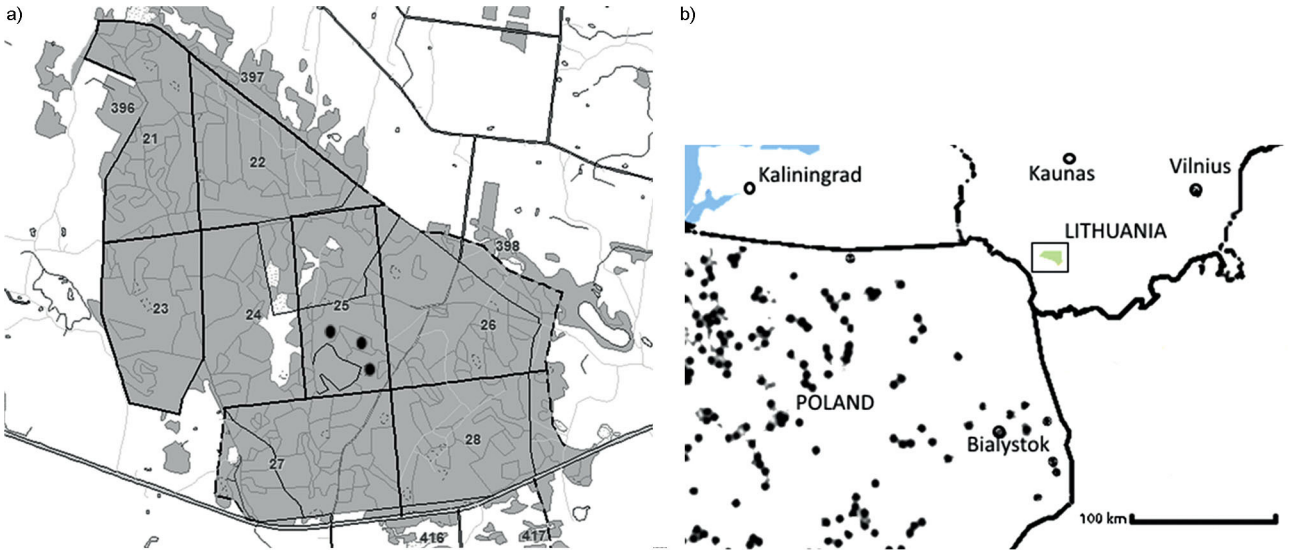

Fig. 1. Trakas Forest in Alytus district, Veisiejai Forest Enterprise, Seirijai Forest District in Lithuania (54°14'11"N, 23°45'30"E, 190 m a.s.l.): a) Trakas Forest; numbers and lines indicate forest blocks, larger black circles – the three sampling sites; b) rectangular shows the location of Trakas Forest in Lithuania and smaller black circles – the nearest natural sessile oak stands in Poland

From the data of the Lazdijai hydro-meteorological station which is located at a distance of 16 km from Trakas Forest, the mean annual temperature in this region was 6.5–7 °C and annual precipitation 650–700 mm (Galvonaitė et al., 2013). During soil and leaf litter summer sampling in June 2011, the mean temperature was 17.8 °C (min – 5°C, max – 30°C) and precipitation was 70 mm (LHMT/AM, 2011a). During autumn sampling in November 2011 and 2012, mean monthly temperature was 3.0–4.6 °C (min – –6 and –1°C, max – 10 and 11°C respectively), while the amount of precipitation in 2011 was 34 mm or two times lower than in 2012 (LHMT/AM, 2011b; LHMT/AM, 2012).

The area of investigated oak stand was about 20 ha. Oak species are mixed in different proportions in this stand, so three sites were selected where both oak species and their hybrids were present. The samples of leaf litter (soil organic OL horizon) and mineral soil (at 0–15 cm in depth) were collected at three sites in Trakas Forest where pedunculate and sessile oaks and their hybrids were presented together (Fig. 1): first site – 54° 14' 13" N, 23° 45' 20" E, 172 m a.s.l.; second site – 54° 14' 12" N, 23° 45' 38" E, 184 m a.s.l.; third site – 54° 14' 07" N, 23° 45' 38" E, 176 m a.s.l. The average distance between all three studied sites was around 200 m, while in each site of each oak sampling area was about 20x20 m^2. The composite samples of leaf litter were collected in November 2011 and 2012. Mineral soil samples at 0–15 cm in depth at each site were collected in June 2011. November 2011 and 2012 composite samples in each of three sites were taken so as to form a triangle around the tree stem (the distance between points is more than 5 m) under pedunculate oak, sessile oak and their hybrids.

For leaf litter sampling hybrids of pedunculate and sessile oaks were identified by their morphological leaf characteristics (Jurkšienė et al., 2014). With the same sampling design for the calculation of leaf litter mass, the samples were collected with a 0.1 m^2 metallic circular frame in November 2013. In both cases, nine composite samples (from three repeats at each point) were collected in total. In order to determine the dry mass, the samples of leaf litter were oven-dried at 105 ± 5 °C and weighed (ISO 11465:1993).

Composite mineral soil samples with a minimum of 1 kg of soil were mixed thoroughly and placed in tightly sealed plastic bags and kept at 4 °C to retain field moisture for microbial analyses. All microbial determination was performed within one week of sampling (Öhlinger, 1996). Soil moisture content was determined gravimetrically after drying soil samples at 105 °C.

Leaf litter and soil biochemical analysis

Leaf litter and soil biochemical analyses were performed in the Chemical Research Laboratory of the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry (Akademija, Lithuania) as described previously (Janušauskaitė et al., 2013). The concentration of total organic carbon (C) and total phosphorus (P) in leaf litter was determined using an automatic UV/VIS Carry 50 spectrophotometer (Varian, Germany). Total nitrogen (N) concentration in leaf litter was determined by the Dumas method. Other major elements such as potassium (K), calcium (Ca) and magnesium (Mg) were measured using a Perkin Elmer Instrument Analyst 200 flame atomic absorption spectrometer. Lignin (acid detergent lignin) was

estimated using the cell wall detergent fractionation method according to the Van Soest methodology of fibre fractionation (Van Soest et al., 1991). Ash was determined by combustion and digestibility of the dry matter in vitro using the pepsin-cellulase method. Fat was determined by Soxhlet apparatus using the gravimetric method. Crude fibre (CF) was defined using an NIRS-6500 near infrared spectrometer (Perstorp Analytical, Silver Spring, Maryland, USA). Concentrations of water-soluble carbohydrates (WSC) were evaluated using the Anthrone method, while pH_{H2O} in soil and leaves was measured using a WTW Inolab pH meter. The total soil N and C were determined using the dry combustion method (DIN/ISO 13878 1998). In addition, the ratios of concentration Lignin/N, Lignin/P, C/N, C/P, N/P were calculated.

Biochemical characteristics of stocks (g m^{-2}) in leaf litter of the studied oaks were calculated by multiplying the dry mass of leaf litter (kg m^{-2}) by the concentration (g kg^{-1}) of the biochemical parameters.

Leaf litter decomposition study

Leaf litter decomposition studies and soil enzymatic, biological and metabolic activities were performed in the Department of Plant Pathology and Protection of the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry (Akademija, Lithuania) as described previously (Janušauskaitė et al., 2013). For respiration measurements of CO_2 emission, beakers (n = 3) with 1 g leaf litter samples were placed in a 1 l air-tight jar with 10 ml H_2O at the bottom and with a CO_2 trap (10 ml of 0.5 M NaOH) and incubated in total darkness at 17 °C. The samples were moistened with mineral 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 with microorganisms. The traps were replaced at 21 day intervals. The CO_2 released was determined by titration with HCl (0.25 M), combined with 1 ml of $BaCl_2$ (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 for each litter sample. Respiration was expressed as μg CO_2-C g^{-1} leaf litter and measured in a closed environment after each 21 days. The incubation lasted for 105 days in 2011 and 125 days in 2012 (Ayres et al., 2006).

In addition, the mean decomposition constant (k_W) of leaf litter for the mentioned incubation periods was calculated using the negative exponential decay model (Olson, 1963):

$$W_t = W_0 e^{-kt} \quad (1)$$

where W_0 is initial weight (1 g of dry litter) and W_t is the weight remaining after time period t (the end of the experiment, 105 or 125 days).

Soil enzymatic activity

Fresh mineral soil samples were used for all enzyme analyses. Dehydrogenase (EC 1.1) was determined using the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF) method and results were expressed as μg TPF g^{-1} soil dry weight (DW) 24 h^{-1} (Öhlinger, 1996). Urease (EC 3.5.1.5) estimation was based on the colorimetric determination of ammonium formation after enzymatic urea hydrolysis by the buffered method (Kandeler, 1996). The released ammonium was determined spectrophotometrically at 630 nm. The results were expressed in μg NH_4 g^{-1} DW soil. For alkaline phosphatase (EC 3.1.3.1), soil samples (1.0 g) were reacted with substrate (50 mM sodium p-nitrophenyl phosphate) (Margesin, 1996). 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. All analyses were carried out in triplicate and expressed per g of dry mass of soil.

Soil biological activity

Composite soil samples were passed through a 2 mm sieve before the analyses. Conventional dilution spread-plating was performed in order to assess the cultural fungal and bacterial colony forming units (CFU) (Trolldenier, 1996). Total abundance of fungi was enumerated by plating the dilution series onto malt extract agar and heterotrophic bacteria on soy agar medium. All inoculated media was incubated at room temperature (22 ± 3 °C) for ca. 3, 5, 10 and 40 days. All microbial enumerations were carried out in triplicate. Data is reported in 10^4 CFU g^{-1} DW soil.

Determination of microbial communities using Biolog EcoPlates

Biolog EcoPlates (Biolog Inc., Hayward CA., USA) (n = 3 under each studied oak) were used to determine soil microbial functional diversity based on the utilization of 31 carbon substrates. Analysis was carried out as described by Insam & Goberna (2008) according to protocol 1.

AWCD (average well colour development) was calculated from each plate at each reading time (24 hours, 48 hours, 72 hours) with a microplate reader, using Biolog MicroLog™ 3E. Diversity parameters were calculated using the following two equations (Insam & Goberna, 2008):

$$Sh = -\sum p_i(\ln p_i) \quad (2)$$

where *Sh* is Shannon's diversity index and p_i is the ratio of the corrected absorbance value of each well to the sum of the absorbance value of all wells;

$$E = Sh/\log R' \quad (3)$$

where *E* is substrate evenness, *Sh* is Shannon's diversity index and *R* (substrate richness) is the number of different substrates used by the community (counting all positive optical density readings).

Statistical analysis

Data are reported as means with the standard error and were examined using analysis of variance (one-way ANOVA) procedures with the STATISTICA 8.0 program. The purpose of ANOVA was to test the significant differences of obtained data for pedunculate oak (P), sessile oak (S) and their hybrids (H). In cases when data did not hold the requirement for normal distribution, Mann-Whitney test was applied.

Results and discussion

Biochemical properties of leaf litter an soil

First of all, it is purposive to show how the biochemical composition of the leaf litter of the studied oak species and their hybrids differed. As can be seen in Table 1, the mean pH_{H2O} parameter and mean concentrations of C, P, K, Ca, Mg, ash, fat, CF and WSC did not significantly differ ($p > 0.05$) in the leaf litter of the studied oak species and their hybrids. Only mean concentrations of total N were significantly (12–13%) higher ($p < 0.05$) in the leaf litter of hybrid oak than in pedunculate oak. Also, mean lignin concentration was 11% higher in the leaf litter of pedunculate oak compared with sessile oak. Mean lignin concentrations in the leaf litter of hybrids were intermediate, with no significant differences from the leaf litter of other oaks.

Only the mean concentrations of lignin/N and N/P ratios differed significantly ($p < 0.05$). As can be seen in Table 1, mean lignin/N ratio was the highest by 15–18% in pedunculate oak leaf litter, which contained the lowest concentration of N. Mean N/P ratios were the highest, about 42–47%, in sessile oak leaf litter compared to pedunculate and hybrid oaks.

Lignin/N, lignin/P, C/N, C/P and N/P ratios reflect immobilization of C, N and P organic compounds, or the opposite intensity of mineralization of organic matter (Gosz et al., 1973; Edmonds et al., 1980; Koerselman & Meuleman, 1996; Moore et al., 2006; Piatek et al., 2009). According to Hobbie et al. (2006), the decomposition of leaf litter of different tree species depends not only on lignin content, but also the lignin/N ratio. A critical maximal value of the lignin/N ratio in the range 23–25 indicates the beginning of recalcitrant lignin decomposition (Osono & Takeda, 2004). However, as can be seen in Table 1, mean lignin/N ratios (33–40) in the leaf litter of all studied oak species reflected the slow decomposition intensity of lignin in autumn. We did not find any published data on estimation of lignin/P ratios in leaf litter. The C/N ratio for forest foliar litterfall can range from about 23–35 (Edmonds, 1979; 1980) to about 80 (Hart et al., 1992). According to our studies, mean C/N ratios in the studied oak leaf litter varied from 49 to 56. According to the studies of Rustad & Cronan (1988) and Gosz et al. (1973), critical maximal C/P ratio for P immobilization ranged from 350 to 480. In our study, only the C/P ratio in sessile oak leaf litter was in general higher (452–705) than the mentioned critical ratio. The above-mentioned data on C/N and C/P ratios also reflect the slow decomposition rate of organic compounds in leaf litter. A litter N/P concentration ratio < 16 indicates mineralization of organic P and a ratio < 14 indicates N limitation for the plants, while an N/P ratio > 16 shows mineral P limitation for plants (Koerselman & Meuleman, 1996; Moore et al., 2006) According to our research results (N/P varied from 7.4 to 10.9), leaf litter characterized from the studied oaks reflects N immobilization and mineralization of P. Limitation of N was the largest in hybrid oak leaf

Table 1. Mean pH and mean concentrations of biochemical parameters (expressed on a DW basis, g kg^{-1}) and some of their ratios in autumn leaf litterfall of pedunculate (P) and sessile (S) oak and their hybrids (H) in Trakas Forest, Lithuania

Parameter	P	S	H
C	622.2 ± 15.7a	611.0 ± 14.6a	613.7 ± 15.4a
N	11.2 ± 0.3a	11.8 ± 0.6ab	12.6 ± 0.4b
P	1.51 ± 0.20a	1.10 ± 0.06a	1.71 ± 0.32a
K	6.42 ± 0.97a	5.75 ± 1.18a	6.84 ± 2.25a
Ca	14.71 ± 8.19a	15.51 ± 6.33a	14.17 ± 7.29a
Mg	3.39 ± 1.20a	3.11 ± 0.91a	3.35 ± 1.11a
pH_{H_2O}	4.56 ± 0.30a	4.58 ± 0.37a	4.66 ± 0.30a
Lignin	435.9 ± 8.8b	391.5 ± 12.a	411.6 ± 8.8ab
Ash	55.2 ± 5.9a	52.9 ± 3.2a	53.6 ± 3.3a
Fat	28.0 ± 2.2a	33.2 ± 2.4a	25.6 ± 3.4a
Crude fibre	373.0 ± 2.0a	410.0 ± 16.0a	413.0 ± 17.0a
Water-soluble carbohydrates	21.3 ± 1.5a	17.4 ± 4.1a	15.0 ± 1.4a
Lignin/N	39.5 ± 1.8b	34.5 ± 2.1ab	33.3 ± 1.3a
Lignin/P	303.5 ± 72.7a	368.6 ± 79.4a	239.7 ± 20.3a
C/N	55.8 ± 2.5a	52.9 ± 3.5a	48.9 ± 2.3a
C/P	436.3 ± 108.9a	578.5 ± 126.5a	359.9 ± 26.8a
N/P	7.7 ± 1.4ab	10.9 ± 0.7b	7.4 ± 0.1a

Notes: Results are expressed as the mean ± SE (n=9). Different letters in the same row indicate significant differences at $p < 0.05$.

Table 2. Mean stocks of biochemical parameters (expressed on a DW basis, g m^{-2}) and some of their ratios in autumn leaf litterfall of pedunculate (P) and sessile (S) oak and their hybrids (H) in Trakas Forest, Lithuania

Parameter	P	S	H
C	237.2 ± 5.9a	241.6 ± 5.8a	311.7 ± 7.8b
N	4.3 ± 0.1a	4.6 ± 0.2a	6.4 ± 0.2b
P	0.57 ± 0.08a	0.43 ± 0.03a	0.87 ± 0.12a
K	2.45 ± 0.38a	2.27 ± 0.60a	3.47 ± 0.86a
Ca	5.61 ± 3.24a	6.13 ± 3.21a	7.20 ± 2.78a
Mg	1.29 ± 0.47a	1.23 ± 0.46a	1.70 ± 0.42a
Lignin	166.2 ± 3.4a	154.8 ± 4.9a	209.0 ± 4.5b
Ash	21.0 ± 2.3a	20.9 ± 1.3a	27.2 ± 1.7a
Fat	10.7 ± 0.8a	13.1 ± 0.9a	13.0 ± 1.7a
Crude fibre	142.2 ± 0.8a	162.1 ± 6.3a	209.7 ± 8.6b
Water-soluble carbohydrates	8.1 ± 0.6a	6.9 ± 1.6a	7.6 ± 0.7a

Notes: see in Table 1.

litter and the smallest in sessile oak (strongest mineralization of P).

The mean dry mass of pedunculate oak leaf litter was 381±90, hybrid oak – 508±141 and sessile oak – 395±92 g m^{-2}. The mean mass of hybrid oak leaf litter was about 21–25%, but not significantly ($p > 0.05$) higher than pedunculate and sessile oaks. However, the calculated mean stocks of N, C, lignin and CF were significantly (21–33%) higher in the leaf litter of hybrids compared with pedunculate and sessile oaks (Table 2). Mean mass lignin/N ratio

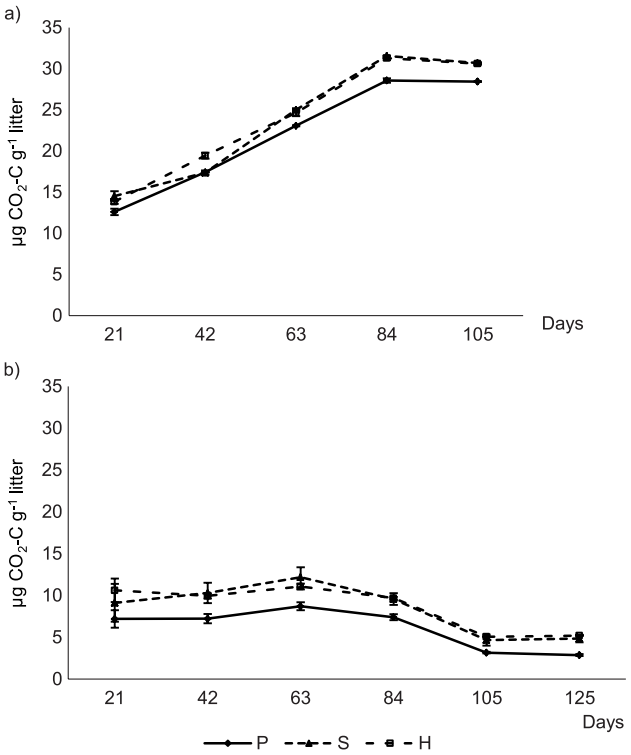

Fig. 2. Mean carbon release as CO_2 (μg CO_2-C g^{-1} ± SE) during incubation period from the autumn leaf litterfall of pedunculate (P) and sessile (S) oaks and their hybrids (H): a) in 2011 for 105 and b) in 2012 for 125 days period

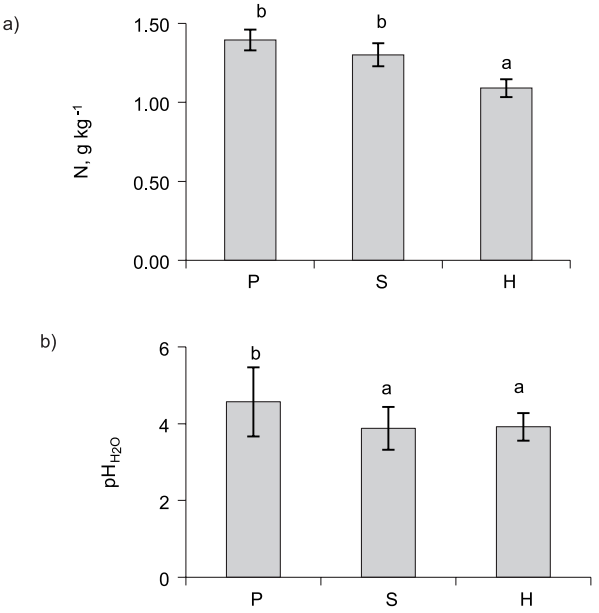

Fig. 3. Some chemical characteristics (mean for 2011–2012 ± SE; n = 9) of mineral top soil (0–15 cm) under pedunculate (P) and sessile (S) oaks and their hybrids (H): a) total nitrogen (N); b) pH_{H2O}. Means with different letters are significantly different ($p \leq 0.05$)

significantly differed ($p < 0.05$) only for pedunculate and hybrid oaks. Significant differences of stocks of other chemical characteristics of leaf litter of different oak species and their hybrids were not found.

Biochemical characteristics of leaf litter can affect the chemical composition of mineral top soil (Baldrian & Štursova, 2011). In our study we found that mineral topsoil acidity under pedunculate oak was statistically less ($p < 0.05$) (pH value was on average higher by 0.1–0.2 pHH_2O), and mean N concentrations in this soil were 8–27% higher than under pedunculate and sessile oaks (Fig. 3). However, low mean C/N ratios (19–24) indicated a high rate of humification and slow mineralization of organic N in mineral topsoil under all studied oaks. A. Hagen-Thorn et al. (2004) found that soil C, N and C/N ratios did not differ significantly among six European tree species.

Foliar litter decomposition rate

The decomposition rate of leaf litter evaluated according to k_W was 0.0017 for to pedunculate oak, 0.0020 to sessile oak and 0.0019 to hybrid oak. Mean k_W for sessile and hybrid oaks was at the same level while it was 11–15% lower for pedunculate oak ($p < 0.05$).

As can be seen from Fig. 2, the respiration of the leaf litter from the studied oaks in 2011 and 2012 was significantly different, due to the difference in precipitation amount in given years. The amount of

precipitation in 2011 was two times lower than in 2012 (see chapter "Study site and sampling"). However, mean total carbon release calculated as CO_2 from leaf litter of sessile and hybrid oaks did not differ ($p > 0.05$) in both years. Meanwhile, compared with the above-mentioned oaks, mean total carbon release from the litter of pedunculate oak in 2011 was 7–10% lower after 63–105 days of incubation. In 2012, these differences were more evident: mean total carbon release after 42–84 days was on average 29–42%, and after 105–125 days was 47–80% lower.

A more intense k_W of the leaf litter of sessile oak compared with decomposition of leaf litter of pedunculate oak has been also obtained by laboratory tests carried out by Straigytė et al. (2006). As in our studies, the significantly lower lignin concentration in sessile oak leaf litter reflected a higher k_W and lower total release of CO_2.

Biological activity of mineral soil

As can be seen in Fig. 4, soil enzyme activity of the studied oak species and their hybrids in general was similar ($p > 0.05$) during all periods of the study. For example, dehydrogenase in the autumn of 2011 was significantly less ($p < 0.05$), only about 30–50%, in the soil under sessile oak than under pedunculate and hybrid oaks. However, mean activity

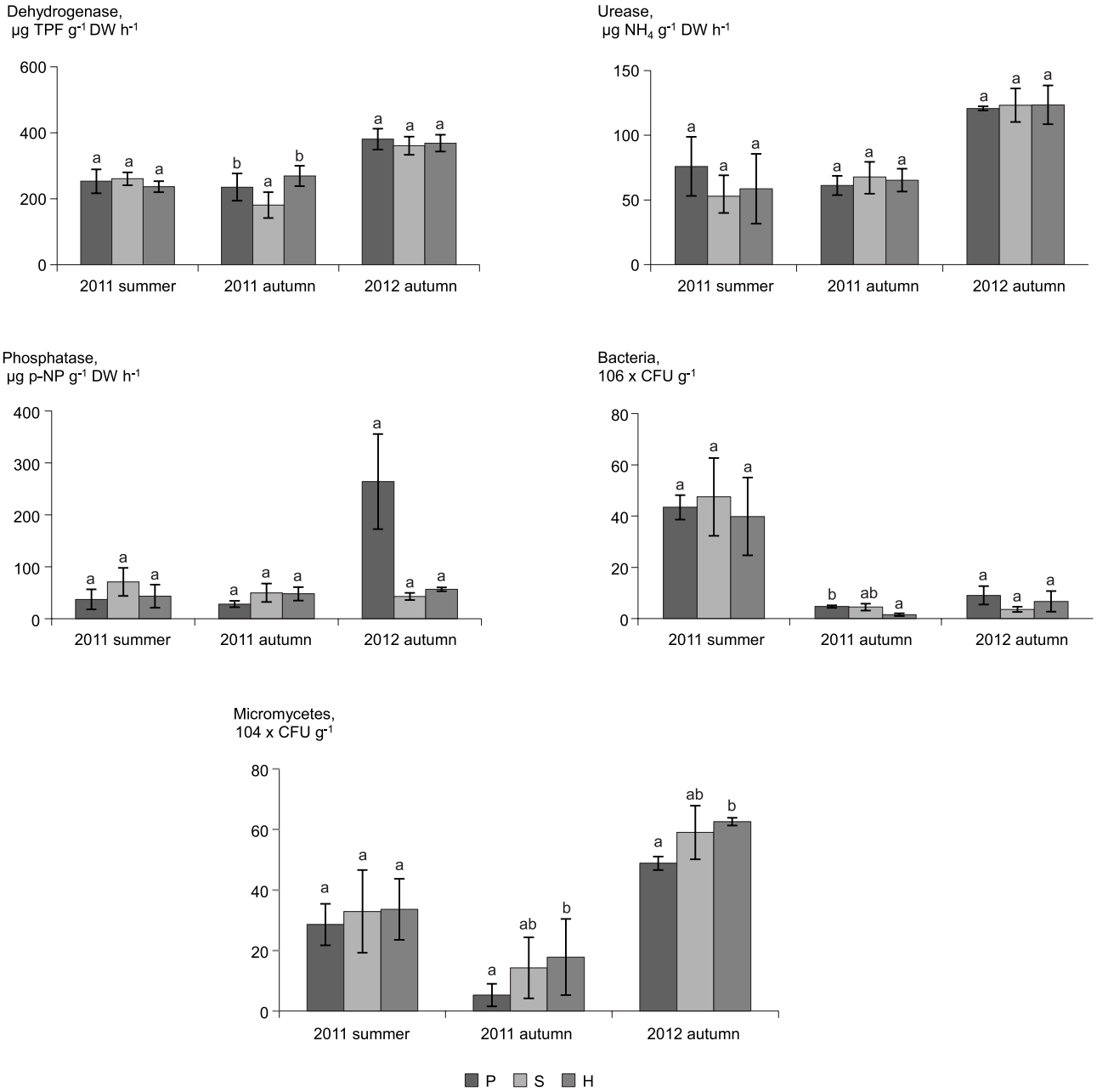

Fig. 4. Mean activity (± SE) in mineral top soil (at 0–15 cm) under pedunculate (P) and sessile (S) oaks and their hybrids (H). Means with different letters are significantly different ($p < 0.05$) for separate study periods (spatial analysise)

of dehydrogenase and urease obtained in autumn 2012 was 1.2–2.3 times higher than in autumn 2011 ($p < 0.05$). This could be explained by the fact that the environmental conditions in 2012 were more favourable (mainly twice higher amount of precipitation) for soil microbiological activity (Criquet et al., 2002; 2004; Sardans & Peñuelas, 2005).

Mean activity of phosphatase enzymes did not significantly differ ($p > 0.05$) in the soil under all studied oaks (Fig. 4). The activity of phosphatase enzymes found in the soil under pedunculate oak in 2012 was, on average, 7–9 times higher ($p > 0.05$) compared with the soil under sessile and hybrid oaks, but even this was not essential because of the large variation in the data obtained.

The density of soil heterotrophic bacteria was significantly (9–26 times) higher ($p < 0.001$) in the soil collected from the study area in summer compared to autumn soil samples (Fig. 4). That shows that heterotrophic bacteria are much more active in the summer (Morris, 1999; Sardans & Peñuelas 2005; Šnajdr et al., 2008; Baldrian et al., 2013). In addition, the mean density of bacteria found in soil under pedunculate oaks in autumn 2011 was three times higher ($p < 0.05$) than in the soil under hybrid oaks.

In general, in all cases, the highest density of micromycetes was found in the soil under hybrid oak and the lowest in the soil of pedunculate oak (Fig. 4). No significant differences ($p > 0.05$) were found in the summer of 2011. In the autumn both of 2011 and 2012, the mean density of micromycetes in soil under hybrid oak was 1.3–2.6 times higher than under pedunculate oak. However, the abundance of soil micromycetes under sessile oak was intermediate and did not differ ($p > 0.05$) from the soil under pedunculate and hybrid oaks as well. This shows that, compared with other oaks, the abundance of fungi that decompose stable organic compounds was lower in mineral soil under pedunculate oak (Rodriguez et al., 1996; Setälä & McLean, 2004).

Table 3. Mean indices (± SE) of metabolic activity of microorganisms in mineral soil (at 0–15 cm depth) under pedunculate (P) and sessile (B) oaks and their hybrids (H) in Trakas Forest, Lithuania

Species	2011	2012	Average
Carbon substrate use, AWCD			
P	0.70 ± 0.08	0.65 ± 0.21	0.68 ± 0.05
S	0.83 ± 0.11	0.74 ± 0.09*	0.80 ± 0.08
H	0.78 ± 0.07	0.60 ± 0.03*	0.71 ± 0.05
Shannon's diversity index, *Sh*			
P	2.98 ± 0.23	3.16 ± 0.12	3.05 ± 0.14
S	3.10 ± 0.20	3.29 ± 0.06	3.18 ± 0.12
H	3.11 ± 0.10	3.19 ± 0.05	3.14 ± 0.06
Evenness of substrate, E			
P	0.98 ± 0.01	0.97 ± 0.012	0.97 ± 0.006
S	0.98 ± 0.003	0.99 ± 0.006	0.98 ± 0.003
H	0.97 ± 0.01	0.98 ± 0.002	0.97 ± 0.006
Richness of substrate, R			
P	22.50 ± 3.97	25.67 ± 2.02	23.86 ± 2.34
S	25.25 ± 4.09	28.00 ± 1.15	26.43 ± 2.29
H	24.25 ± 1.80	26.00 ± 1.00	25.00 ± 1.09

*Significant differences between sessile and hybrid oaks, $p < 0.001$.

The structure of microbial communities and their functions were compared with microbial carbon biomass and Biolog samples in the soil under the studied oak species. The study of the metabolic activity of soil microorganisms in general showed that the indices of metabolic diversity of microorganisms did not differ in the mineral soil under the studied oak species ($p > 0.05$) (Table 3). Only slightly higher ($p > 0.05$) Shannon's diversity index (*Sh*), evenness of substrate (E) and richness of substrate (R) were determined in the soil under sessile oak. Meanwhile, the potential for carbon substrate use, defined by AWCD, was on average 20% higher in the soil under sessile oak and lowest ($p < 0.05$) in the soil of hybrid oak in autumn 2012. AWCD reflects the capability of soil microbiota communities to use carbon sources, so it could be an important microbiota activity indicator (Zabinski & Gannon, 1997).

Reviewing some obtained data, the lower CO_2 emission from forest leaf litter decomposition in 2012 with greater precipitation could be explained by fact that higher than normal rainfall inhabits soil CO_2 exchange range (Raich et al., 2002). On the other hand, in dry forest sites the increase of soil moisture normally increases the soil bioactivity, e.g. the activities of dehydrogenase and urease. The abundance of micromycetes could increase the increase of soil moisture, as well. However, the micromycetes mainly decompose stabile complex organic matter, therefore, in initial stage they did not affect CO_2 emission rate of the soil significantly (Treseder & Lennon, 2015).

Pedunculate and sessile oaks are taxonomically related, belong to the same genus and grow together in a restricted geographical area and this is not surprising that there were no significant differences between both species and their hybrids. However, summarizing these results, it might be noted, that the sessile oak leave litter are distinguished by lower hard-biodegradable lignin concentrations. This has led to a faster decomposition of sessile oak leave litter. Metabolic activity and diversity of microorganisms also was slightly higher in the soil under sessile oak. Hybrid oak rates little differed from the sessile oak. Therefore, we can assume that due to the higher decomposition rate of forest floor in Trakas Forest (Alytus district in Lithuania) favourable conditions for sessile and hybrid oak undergrowth spread was formed.

Conclusion

Autumn leaf litterfall mass and biochemical composition of pedunculate (*Quercus robur*) and sessile (*Q. petraea*) oaks and their hybrids differed in general insignificantly and the significant differences in mean concentrations and stocks of N, C, lignin and crude fibre did not exceed 20–30% ($p < 0.05$). However, the highest content of lignin and higher lignin/N ratio, lower decay rate and, especially, lower carbon release indicated ($p < 0.05$) the slowest decomposition of pedunculate oak litterfall compared with sessile and hybrid oaks.

The rhizosphere under the studied oaks did not differ significantly in terms of C concentration and C/N ratio, mean activities of dehydrogenase, urease, and phosphatase, abundance of bacteria, and metabolic activity of soil microorganisms. However, the lowest activity of micromycetes showed that, under pedunculate oak, the decomposition of stable organic compounds was less intensive than in the rhizosphere of sessile and hybrid oaks.

Metabolic activity (indicated as Shannon's diversity index, evenness and richness of substrate) of microorganisms differed insignificantly in the rhizosphere of the studied oaks. However, the potential for the use of different carbon compound substrates microorganisms had a tendency to be higher in the soil under sessile oak.

The decomposition rate of leaves litterfall and organic compounds in the rhizosphere under sessile and hybrid oaks were more intensive compared to pedunculate oak. Therefore, it could be supposed the conditions, mainly higher decomposition rate of litterfall for natural regeneration of undergrowth were more suitable in the stands where sessile and hybrid oaks dominate.

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