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Differences in chemical composition of needle and leaf litter from exotic and native tree species stands

Abstract: The potential differentiations in litter chemistry among native and non-native trees are poorly understood. We compared the chemical composition of leaf litterfall of 11 exotic tree species, e.g. coniferous: *Abies cephalonica, A. grandis, A. procera, Chamaecyparis pisifera, Pinus peuce, Pseudotsuga menziesii, Thuja plicata,* and deciduous: *Acer rubrum, A. saccharum, Betula alleghaniensis* and *Quercus rubra,* with that of a native European conifer, *Pinus sylvestris* (as reference to coniferous species) and a mixture of native European *Quercus robur, Carpinus betulus, Tilia cordata, T. platyphyllos* and *Corylus avellana* leaves (as a reference mixture of deciduous species). We found significant differences among the species studied in nitrogen and carbon content in needles/leaves, C/N ratio, as well as total soluble phenolic compounds (TPh) and total nonstructural carbohydrates (TNC) content, including soluble carbohydrates and starch. However, we found no clear differentiation of exotic from native tree species in the analyzed elements and metabolites. Among the exotic coniferous tree species, *P. menziesii* stood out among the species studied – fallen needles of this species were characterized by relatively high TPh and TNC content. The relationships between TPh and TNC content in leaf and needle litter among tree species were similar among two consecutive years. For deciduous tree species, the tendency of higher TPh content and C/N ratio in leaves falling earlier (September; leaves of sun-type) than later (November; leaves of shade-type) was more distinct than in coniferous tree species. Generally, we cannot see any special differences in the levels or mutual quantitative relationships of the chemical compounds studied in fallen needles/leaves of exotic tree species in comparison with native tree species.

Additional key words: carbohydrates, carbon, native and exotic species, nitrogen, phenolics

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Introduction

Individual species of forest trees clearly affect the biodiversity of soil organisms (Hobbie et al. 2006; Jagodziński et al. 2011; Kasprowicz et al. 2011; Skorupski et al. 2011; Trocha et al. 2012) and communities (Knight et al. 2008) – one of the most essential factors having an influence on organic matter decomposition and nutrient cycling (Withington et al. 2006; Dauer et al. 2007; Mueller et al. 2012). Organisms which inhabit soil are both the source and direct acceptor of basic biogenic elements such as carbon, nitrogen, phosphorus and sulfur (Marshall and Lousier 2000; Lavelle and Spain 2001). Individual tree species can influence species diversity and biomass of organisms inhabiting the soil beneath the crowns of a forest stand, including a large extent by the composition of litter coming from the needle and leaf fall (Binkley and Giardina 1998; Reich et al. 2005).

The stands composed of exotic tree species may lead to distinct modifications of many key processes in forest ecosystems in comparison with stands composed of native tree species. For example, changes in the species composition of nematodes (Forge and Simard 2001) and bacteria (Priha et al. 2001) can be one of such consequences. On account of their role in soil processes, mainly through the decomposition of organic matter (Hobbie et al. 2006, 2007), their number and taxonomical diversification is of primary importance for ecosystem function. The other considerable consequence of exotic vs. native tree species influence on forest ecosystem is their significant differentiation of herbaceous layer regarding species number under the forest canopy (Knight et al. 2008) and mycorrhizas diversity (Dickie et al. 2006; Trocha et al. 2012).

In forest ecosystems, trees through changing physical and chemical properties of the soil (Binkley and Giardina 1998; Hobbie et al. 2010) affect entire ecosystem functioning through structural and functional changes of soil. Natural forests are often comprised of multiple species with trees of different ages. One of the most important components enabling characterization of the influence of differing tree species on ecosystems is soil chemistry and its role in macro- and micro-element cycling and consequences on soil processes (Dauer et al. 2007; Mueller et al. 2012). Soil processes are closely associated with the chemical composition of falling leaves/needles, like nitrogen, phenols and lignins content, and C/N ratio, etc. (Aerts 1997; Hobbie et al. 2006). Thus, the objective of our study was to investigate the differences in chemistry of fallen needles/leaves of exotic and native tree species. We have chosen the macro-elements and metabolites that influence rates of litter decomposition. These substances, both directly and indirectly,

with participation of fungi, bacteria and representatives of various small animals, are involved in litter decomposition. At present there is insufficient information on this issue for native tree species, and even less so for exotic tree species. For example, in exotic trees, differences from those typical for their climate and soil conditions may affect the balance between litter leaves and biotic factors causing their decomposition. This may be due to changes in both components of the system: litter leaves (structure and chemistry) and the organisms causing the decomposition of fallen leaves (species composition, growth and development).

Results of our study should broaden knowledge of the influence of chemical composition of needle and leaf fall from exotic and native tree species stands. Therefore, in the study we considered carbon (C), nitrogen (N) and metabolites (non-structural carbohydrates), which are beneficial for growth and development of organisms involved in litter decomposition. On the other hand, we analyzed phenolic compounds content and C/N ratio, being characteristic for moving the metabolism into carbon defensive compounds synthesis that is factors hinder functioning and limiting growth and development of these soil organisms.

Materials and methods

Study site

The study was conducted in the Rogów Arboretum of the Warsaw University of Life Sciences (SGGW), Poland (51°49'N, 19°53'E; elevation 189 m a.s.l.). Trees used in this study included seven conifers: *Abies cephalonica*, *A. grandis*, *A. procera*, *Chamaecyparis pisifera*, *Pinus peuce*, *Pseudotsuga menziesii* and *Thuja plicata*, and four deciduous angiosperm species: *Acer rubrum*, *A. saccharum*, *Betula alleghaniensis* and *Quercus rubra*. The species studied represented a relatively wide range of geographic origins within the northern temperate climatic zones worldwide and have been introduced into Poland and tested at the field site for possible use as forest trees. All studied trees were between 36-61 years old (Table 1). The trees were growing in permanent, replicated single-species plots (400 m^2 each), two plots per species.

The climate of the region is transitional between maritime and continental. According to long-term meteorological observations (55 years) from the closest meteorological station in Strzelna (ca. 1.5 km from the research area), mean annual temperature is 7.2°C (January: –3.2°C, July: 17.3°C), mean annual precipitation is 596 mm (404–832 mm, ca. 70% of annual precipitation is in the growing season), and mean growing season length (calculated as the number of days with mean temperature =5°C) is 212 days (Jagodziński and Banaszczak 2010). Prior to the ex-

periment, the site was a mature pine-dominated (*Pinus sylvestris* L.) forest. The soils were developed on a postglacial formation, in the region of a ground moraine. They represent the grey-brown podzolic type with horizons O-A-Eet-Bt-C. The pH is 5.0 in the humus layer, 4.2 in the topsoil and 4.8 in the eluvial horizon.

The litter input was measured using litter traps (6 per plot; 60 cm × 60 cm × 20 cm; 0.36 m²) arranged on the forest floor (12 litter traps per species). The litter traps were fitted with a fine-mesh nylon sheet to provide for free drainage of rainfall. Litter traps were placed inside each experimental plot. The litter was collected on the last day of each month (September, October, November) for two consecutive years (2008 and 2009). Litter from each trap was divided into leaves/needles of the studied species, leaves/needles of accompanying species (e.g. shrubs) and woody litter. The samples were oven dried to a constant mass (65°C) in a drier with forced air circulation (ULE 600; Memmert GmbH+Co.KG, Germany) and then weighed. Dry foliage tissue of the analyzed species was pulverized in a Culatti Mikro-Feinmühle mill (IKE Labortechnik Staufen, Germany) and subjected to chemical analysis.

To compare the data obtained for exotic tree species, we collected needles of *Pinus sylvestris* (as reference to coniferous species) and a mixture of *Quercus robur, Carpinus betulus, Tilia cordata, T. platyphyllos* and *Corylus avellana* leaves (as reference to deciduous species). All the species listed are native to Poland and grow in the area of Rogów Arboretum very close to research stands with exotic tree species. The litter of these species was collected in September, October and November 2008 and 2009 using the same method as for exotic tree species (*Pinus sylvestris* stand – 2 plots, mixed stand – 2 plots).

Chemical analyses

In the pulverized (Mikro-Feinmühle-Culatti mill, IKA Labortechnik Staufen, Germany), dried (65°C) plant material we determined carbon (C) and nitrogen (N) content, total soluble phenolic compounds (TPh) and total nonstructural carbohydrates (TNC) content as the sum of soluble carbohydrates (SC) and starch (ST). C/N ratio was also calculated.

For determination of carbon and nitrogen content, the pulverized needles/leaves material was subjected to analysis in the Elemental Combustion System CHNS-O 4010 (Costech Instruments, Italy/USA;). Carbon and nitrogen content is given in % of needle/leaf dry mass (% d.m.).

Total nonstructural carbohydrates were determined by a modification of the method described by Haissig and Dickson (1979) and Hansen and Møller (1975). Soluble sugars were extracted from oven-dried tissue powder in a mixture of methanol, chloroform, and water (12:5:3 by volume). The tissue residuals were used for determination of starch content. Soluble carbohydrates were determined colorimetrically with anthrone reagent at λ=625 nm within 30 min. Starch in the tissue residual was gelatinized and converted to glucose with amyloglucosidase. Glucose concentrations were measured using the peroxidase-glucose oxidase-o-dianisidine dihydrochloride reagent. Absorbance was measured at λ=450 nm after a 30 min. incubation at 37°C against glucose standards. Concentrations of soluble sugars and starch are expressed as a percentage of needle/leaf dry mass (% d.m.).

Table 1. Characterization of study plots: plot area, tree age (range and mean), mean stand density, mean tree diameter at breast height (DBH) and mean tree height (±SE; standard error calculated for each pair of plots)

Species	Plot area (m²)	Age (range) (years)	Mean age (years)	Mean stand density (trees ha⁻¹)	Mean DBH (cm)	Mean height (m)
Coniferous tree species						
Abies cephalonica Loundon	413	61	61 ± 0	729 ± 54	28.2 ± 0.1	19.6 ± 2.3
Abies grandis (Dougl ex D Don) Lindl	408	49–61	55 ± 6	502 ± 12	31.7 ± 0.6	24.4 ± 0.1
Abies procera Rehd	400	38–42	40 ± 2	1413 ± 138	19.9 ± 0.6	13.2 ± 0.5
Chamaecyparis pisifera (Siebold & Zucc) Endl	400	61	61 ± 0	1163 ± 113	22.3 ± 0.1	18.1 ± 0.5
Pinus peuce Griseb	400	40–48	44 ± 6	1588 ± 313	19.0 ± 2.3	15.9 ± 0.8
Pseudotsuga menziesii (Mirbel) Franco	400	47–49	48 ± 1	750 ± 50	28.4 ± 0.1	21.5 ± 0.1
Thuja plicata Donn ex D Don	400	44	44 ± 0	1625 ± 162	15.5 ± 0.5	17.3 ± 1.1
Deciduous tree species						
Acer rubrum L	400	39	39 ± 0	1064 ± 13	18.0 ± 0.2	20.7 ± 0.0
Acer saccharum Marsh	203	37	37 ± 0	1062 ± 123	14.5 ± 0.8	14.1 ± 0.9
Betula alleghaniensis Britton	200	36	36 ± 0	1151 ± 0	15.7 ± 0.2	13.7 ± 0.2
Quercus rubra L	400	52–61	57 ± 5	413 ± 88	32.3 ± 1.0	23.8 ± 2.5

To determine the total soluble phenolic compounds, samples of 0.1 g were extracted first in 95% ethanol for 15 min. and then in 80% ethanol for 10 min. Total phenolic concentration was determined spectrophotometrically using Folin and Ciocalteu's Phenol Reagent (SIGMA F-9252) in accordance with the method described by Johnson and Shaal (1957) and modified by Singelton and Rossi (1965). The absorbance was measured at $\lambda = 660$ nm. The content of phenolic compounds has been expressed in micromoles of chlorogenic acid per gram of dry mass. All the absorbances were determined with a spectrophotometer (UV–1700 PharmaSpec, UV-Visible Spectrophotometer, Shimadzu).

Statistical analyses

All statistical analyses were conducted using JMP 8.0 (SAS Institute Inc., Cary, NC, USA; http://www.sas.com/). To compare the influence of tree species and litter collection period (year and month) on the variables studied, analyses of variance (ANOVAs) were conducted. ANOVAs were run separately for coniferous and deciduous tree species. Prior the statistical analyses all percentage values were arc-sin transformed as given by the Bliss formula (Snedecor and Cochran 1976). Additionally, when critical differences were noted among species and litter collection period (e.g. among months), multiple comparisons were carried out based on Tukey's test for equal sample sizes, while the differences among years were analyzed according to Student's t-test. Same letters in the Table 2 and 3 indicate a lack of statistically significant differences between analyzed variables according to Tukey's a posteriori test ($P<0.05$). Moreover, in order to determine the significance of differences between native and exotic tree species, the test of contrasts was conducted (Tables 2 and 3).

Results

The differences in chemical composition of fallen needles of coniferous species (Table 2) and leaves of deciduous (Table 3) tree species were analyzed separately. Generally (excluding some carbohydrate fractions), we found statistically significant differences among tree species in chemical composition of leaf litter.

The mean content (for three months) of phenolic compounds in the fallen needles of the species studied was significantly ($P<0.0001$) diversified (Table 2, Fig. 1 A). Out of 7 coniferous exotic tree species, needles of one of them were characterized by significantly higher (*P. menziesii*) and two other (*P. peuce* and *T. plicata*) significantly lower TPh content than needles of *P. sylvestris* (a species native to Europe). The content of phenolic compounds in needles of the 4 remaining exotic tree species did not differ statistically from that of *P. sylvestris*. Also the C/N ratio in needles (Fig. 1 C) depended on the species, but only three species were significantly different from *P. sylvestris*. The significantly higher C/N ratio was observed only for *P. peuce* needles, whereas a significantly lower C/N ratio was noticed for *A. cephalonica* and *A. procera* (in comparison with values obtained for *P. sylvestris* needles).

In conclusion, one must conclude that the differences between coniferous exotic tree species and the native *P. sylvestris* were small. The only significant difference was higher carbon content in the needles of exotic tree species (on average for both years; Table 2), but this significance was a result of statistically significant difference found in only for the plant material collected in 2009.

In deciduous tree species, the fallen leaves of the two maples studied (*A. rubrum* and *A. saccharum*) were characterized by a higher content of phenolic compounds (means of three months) in comparison with the leaf mixture of native deciduous tree species (Table 3). Therefore, the test of contrasts showed that significantly higher TPh content in leaves was found for exotic tree species (Table 3). This significant difference ($P<0.05$) was found, but only in 2009. Even though the C/N ratio was relatively high in both maple species, it was not significantly different from the C/N ratio determined for the mixture of native deciduous tree species.

The phenolic compounds content in leaves of two remaining species (*B. alleghaniensis* and *Q. rubra*) was low and not significantly different from native deciduous tree species. The former tree species were characterized also by a significantly higher C/N ratio in comparison with other deciduous tree species studied, including native tree species. Relationships between the level of phenolic compounds content (Fig. 2 A) and C/N ratio (Fig. 2 C) were more consistent in deciduous than coniferous species. The only exception was *Q. rubra*, in which phenolic compounds content in leaves was not significantly different from the value found in native deciduous tree species, although C/N ratio was significantly higher in leaves of native species in comparison with *Q. rubra* (Table 3).

In the case of deciduous tree species, we found an unambiguous tendency of higher content of phenolic compounds and C/N ratio in leaves which fall earlier (September) than in late autumn (November), and the tendency was more distinct in this group than in the coniferous tree species. This pattern is caused by earlier leaf fall of sun-type leaves, from the outer and upper parts of the crowns, i.e. more strongly sunlit portions of crowns, than leaves of the shade type. Likewise, we observed a lower pattern of nonstructural carbohydrates contents in leaves collected in November than in September, particularly in case of deciduous (Fig. 2 B, D, F) than coniferous trees (Fig. 1 B, D, F).

Table 2. Influence of tree species (S), month of litterfall collection (M – September, October and November) and year of harvest (Y), on litterfall biomass (kg d.m. ha⁻¹), content of carbon (C, % d.m.), nitrogen (N, % d.m.), carbon to nitrogen ratio (C/N), content of total phenols (TPh, expressed as µM of chlorogenic acid g⁻¹ d.m.), total non–structural carbohydrates (TNC, % d.m.), in it soluble carbohydrates (SC, % d.m.) and starch (%d.m.), in fallen needles of seven exotic tree species and one native species *Pinus sylvestris* (mean values, in brackets show ±SE). Analysis of variance (ANOVA) was used to assess the influence of species, month and year of harvest on the traits measured, followed by means separation using Tukey's test for species, month and year. The significance of differences between native and exotic tree species was identified by the test of contrasts, where: * – P<0.05

Treatment		Litterfall biomass	Carbon	Nitrogen	C/N	TPh	TNC	SC	Starch
Species	*A. cephalonica*	136 d (25)	51.1 bc (0.3)	0.94 ab (0.03)	54.8 de (1.8)	111 cd (9)	2.94 c (0.35)	2.52 c (0.35)	0.42 a (0.01)
	A. grandis	606 abc (217)	52.1 ab (0.3)	0.83 c (0.03)	63.5 cd (2.0)	162 b (11)	4.75 ab (0.29)	4.32 ab (0.29)	0.43 a (0.01)
	A. procera	553 abcd (97)	51.8 abc (0.4)	0.99 a (0.04)	53.4 e (2.4)	111 cd (10)	2.58 c (0.18)	2.13 c (0.19)	0.45 a (0.02)
	C. pisifera	632 abc (200)	52.8 a (0.2)	0.86 bc (0.05)	63.7 cd (3.9)	123 cd (10)	2.68 c (0.39)	2.24 c (0.37)	0.44 a (0.04)
	P. peuce	927 ab (214)	50.7 c (0.2)	0.59 e (0.01)	85.7 a (1.9)	68 e (5)	2.20 c (0.37)	1.78 c (0.37)	0.42 a (0.01)
	P. menziesii	466 bcd (116)	50.9 bc (0.3)	0.80 cd (0.06)	67.8 c (5.3)	206 a (12)	5.30 a (0.38)	4.87 a (0.38)	0.43 a (0.01)
	T. plicata	1002 a (290)	52.3 ab (0.6)	0.71 de (0.05)	76.3 ab (4.5)	97 de (11)	2.90 c (0.52)	2.43 c (0.50)	0.46 a (0.09)
	P. sylvestris	389 cd (86)	50.9 bc* (0.7)	0.81 cd (0.10)	70.2 bc (5.7)	133 bc (13)	3.44 bc (0.53)	2.99 bc (0.52)	0.45 a (0.02)
Month	September	501 b (114)	52.0 a (0.3)	0.88 a (0.03)	62.5 b (2.6)	137 a (9)	3.57 a (0.27)	3.10 a (0.27)	0.47 a (0.03)
	October	828 a (141)	51.5 ab (0.3)	0.79 b (0.05)	70.7 a (3.4)	123 ab (10)	3.30 a (0.30)	2.89 a (0.30)	0.41 a (0.01)
	November	438 b (64)	51.2 b (0.2)	0.79 b (0.03)	67.5 a (2.4)	120 b (9)	3.17 a (0.31)	2.74 a (0.31)	0.44 a (0.01)
Year	2008	739 a (109)	51.2 b (0.2)	0.79 b (0.03)	68.2 a (2.3)	116 b (7)	3.30 a (0.24)	2.90 a (0.24)	0.41 b (0.01)
	2009	439 b (68)	52.0 a (0.2)	0.85 a (0.03)	65.5 b (2.4)	137 a (8)	3.39 a (0.24)	2.92 a (0.24)	0.47 a (0.02)

ANOVA

	df	Litterfall biomass F	P	Carbon F	P	Nitrogen F	P	C/N F	P	TPh F	P	TNC F	P	SC F	P	Starch F	P
Species (S)	7	7.19	<0.0001	6.81	<0.0001	28.90	<0.0001	26.88	<0.0001	34.65	<0.0001	9.83	<0.0001	10.75	<0.0001	0.16	ns
Month (M)	2	10.70	0.0001	5.00	0.0108	13.17	<0.0001	12.56	<0.0001	4.39	0.0177	0.98	ns	0.93	ns	1.96	ns
Year (Y)	1	16.50	0.0002	14.05	0.0005	12.69	0.0009	5.01	0.0299	17.65	0.0001	0.30	ns	0.07	ns	7.09	0.0105
S x M	14	5.04	<0.0001	2.39	0.0130	7.37	<0.0001	4.86	<0.0001	2.98	0.0024	1.84	ns	1.92	0.0478	0.59	ns
S x Y	7	6.80	<0.0001	2.46	0.0307	8.51	<0.0001	5.56	0.0001	1.22	ns	1.83	ns	1.82	ns	1.62	ns
M x Y	2	14.43	<0.0001	0.18	ns	19.70	<0.0001	17.28	<0.0001	5.10	0.0098	0.83	ns	0.88	ns	0.27	ns
S x M x Y	14	1.27	ns	3.76	0.0003	5.67	<0.0001	4.30	<0.0001	3.72	0.0003	1.64	ns	1.67	ns	1.71	ns
df error		48		47		47		47		48		48		48		48	

Table 3. Influence of tree species (S), month of litterfall collection (M – September, October and November) and year of harvest (Y), on litterfall biomass (kg d.m. ha⁻¹), content of carbon (C, % d.m.), nitrogen (N, % d.m.), carbon to nitrogen ratio (C/N), content of total phenols (TPh, expressed as μM of chlorogenic acid g⁻¹ d.m.), total non-structural carbohydrates (TNC, % d.m.), in it soluble carbohydrates (SC, % d.m.) and starch (% d.m.), in fallen leaves of four exotic tree species and mixture of a few native species (mean values, in brackets show ±SE). Analysis of variance (ANOVA) was used to assess the influence of species, month and year of harvest on the traits measured, followed by means separation using Tukey's test for species, month and year. The significance of differences between native and exotic tree species was identified by the test of contrasts, where: * – $P<0.05$, ** – $P<0.01$

Treatment		Litterfall biomass	Carbon	Nitrogen	C/N	TPh	TNC	SC	Starch
Species	A. rubrum	1017 a (270)	48.4 ab (0.5)	0.93 b (0.11)	58.3 a (5.2)	170 a (33)	4.01 a (0.59)	2.62 a (0.50)	1.39 a (0.16)
	A. saccharum	798 ab (252)	47.2 b (0.3)	0.89 b (0.06)	55.0 a (3.0)	225 a (42)	3.90 a (0.36)	2.96 a (0.35)	0.95 b (0.09)
	B. alleghaniensis	926 a (232)	49.3 a (0.8)	1.30 a (0.11)	40.5 b (3.1)	64 b (13)	2.42 b (0.33)	1.97 a (0.34)	0.45 c (0.01)
	Q. rubra	1052 a (370)	49.9 a (0.3)	1.25 a (0.12)	43.8 b (3.8)	90 b (19)	2.98 ab (0.41)	2.52 a (0.41)	0.46 c (0.01)
	Native species	369 b (225)	48.9 ab (0.8)	1.03 b (0.11)	55.8 a (7.6)	93 b* (14)	2.69 ab (0.35)	2.11 a (0.34)	0.59 c** (0.07)
Month	September	835 b (161)	49.7 a (0.6)	1.00 b (0.10)	57.3 a (4.5)	215 a (27)	4.26 a (0.28)	3.44 a (0.26)	0.82 a (0.12)
	October	1489 a (242)	48.6 ab (0.5)	0.92 b (0.05)	55.7 a (3.2)	84 b (10)	3.09 b (0.31)	2.25 b (0.26)	0.84 a (0.11)
	November	174 c (103)	47.9 b (0.3)	1.35 a (0.08)	38.0 b (2.5)	79 b (16)	2.17 c (0.26)	1.57 c (0.22)	0.60 b (0.07)
Year	2008	966 a (211)	48.4 a (0.4)	1.07 a (0.05)	48.6 b (2.5)	114 b (16)	3.68 a (0.30)	2.88 a (0.26)	0.80 a (0.08)
	2009	699 b (125)	49.0 a (0.4)	1.10 a (0.09)	52.5 a (3.8)	140 a (23)	2.72 b (0.23)	2.00 b (0.20)	0.72 a (0.09)

ANOVA	df	Litterfall biomass F	P	Carbon F	P	Nitrogen F	P	C/N F	P	TPh F	P	TNC F	P	SC F	P	Starch F	P
Species (S)	4	6.43	0.0007	5.89	0.0013	17.61	<0.0001	16.78	<0.0001	27.19	<0.0001	5.12	0.0030	2.48	0.0660	26.75	<0.0001
Month (M)	2	60.39	<0.0001	6.41	0.0049	42.80	<0.0001	51.73	<0.0001	72.02	<0.0001	19.70	<0.0001	22.03	<0.0001	4.72	0.0168
Year (Y)	1	7.47	0.0104	2.12	ns	0.01	ns	5.99	0.0207	5.18	0.0304	13.29	0.0010	15.73	0.0004	3.17	ns
S x M	8	14.23	<0.0001	1.84	ns	13.77	<0.0001	12.71	<0.0001	4.71	0.0009	0.71	ns	0.70	ns	3.03	0.0134
S x Y	4	0.68	ns	5.67	0.0017	7.91	0.0002	18.41	<0.0001	1.50	ns	1.85	ns	2.95	0.0370	0.53	ns
ghtM x Y	2	12.28	0.0001	0.01	ns	4.45	0.0207	6.39	0.0050	9.04	0.0009	0.41	ns	0.86	ns	0.48	ns
S x M x Y	8	6.13	0.0001	2.91	0.0165	3.31	0.0082	4.53	0.0012	3.83	0.0035	1.27	ns	1.64	ns	2.19	ns
df error		30		29		29		29		29		29		29		29	

The study conducted throughout two consecutive years also showed a significant influence of the year when litter was collected, both on phenolic compounds content and C/N ratio (Tables 2 and 3). However, we found no statistically significant interaction of species × year. Thus, in both years the relationships among tree species studied were similar in their influence on the level of defensive compounds.

As in case of defensive compounds (TPh) and C/N ratio, we found a statistically significant influence of tree species on nitrogen and total nonstructural carbohydrate content in fallen leaves, substances that are beneficial both for growth and development of needles (Table 2) and leaf-decaying organisms (Table 3). The majority of coniferous species studied were characterized by similar nitrogen content in needles, as in *P.*
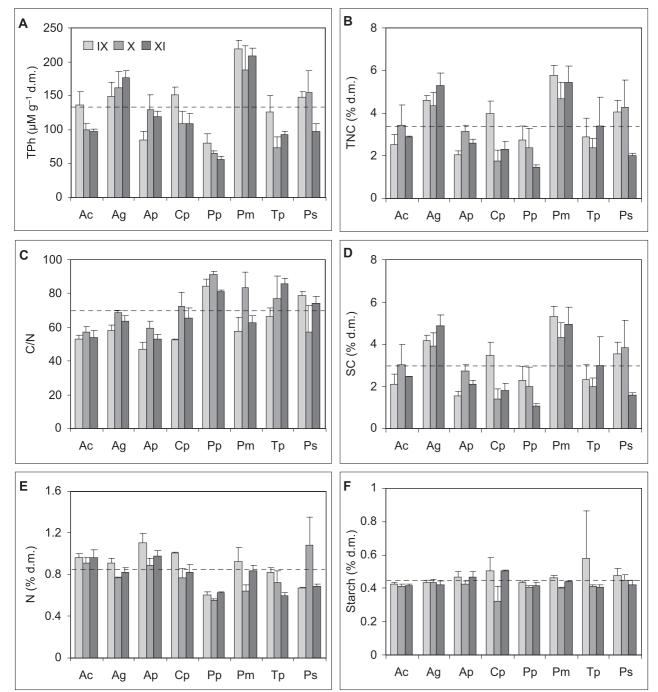

Fig. 1. Content of total phenolic compounds (A), nitrogen (E), total non–structural carbohydrates (B), in it soluble carbohydrates (D) and starch (F), and carbon to nitrogen ratio (C), in fallen needles of seven exotic tree species: *Abies cephalonica* (Ac), *A. grandis* (Ag), *A. procera* (Ap), *Chamaecyparis pisifera* (Cp), *Pinus peuce* (Pp), *Pseudotsuga menziesii* (Pm) and *Thuja plicata* (Tp), and one native species *Pinus sylvestris* (Ps), collected separately in three subsequent months (IX–XI), in two years (2008 and 2009, mean values). A dotted line shown mean value for native species (for three months). Vertical segments shown on graphs are standard error (SE)

sylvestris. Only fallen needles of *A. procera* had significantly higher nitrogen content and *P. peuce* a significantly lower in comparison with *P. sylvestris* needles. Both the mentioned exotic species were not significantly different from *P. sylvestris* in total nonstructural carbohydrate contents. Only *P. menziesii* needles were significantly different than *P. sylvestris* needles in TNC content. The former species had ca. 1.5-fold higher TNC content in needles than *P. sylvestris*.

In case of deciduous tree species, the differences among species in nitrogen content in fallen leaves were much less visible than in coniferous tree species. Leaves of two species (e.g. *B. alleghaniensis* and *Q. rubra*) had statistically significant, but only ca. 20% higher nitrogen content than leaves of native tree species (Table 3). Leaves of none of exotic tree species studied differed significantly in TNC content compared to native tree species. As revealed by the test of
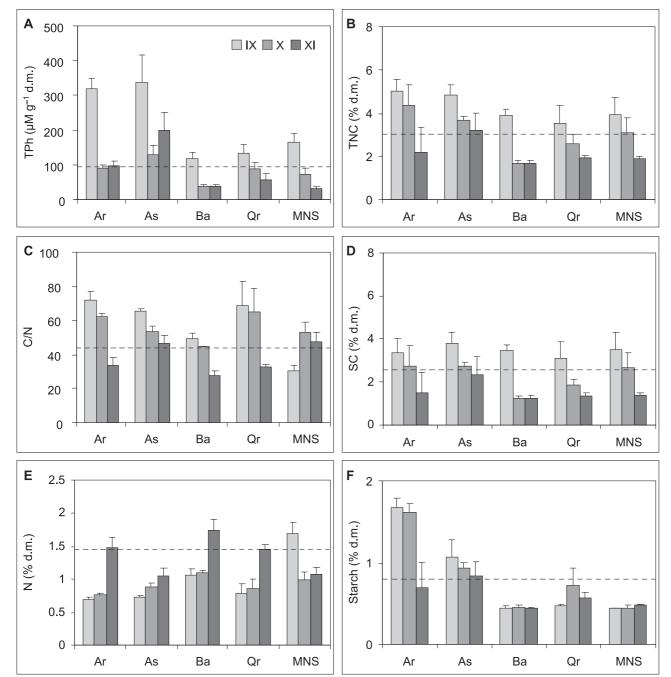

Fig. 2. Content of total phenolic compounds (A), nitrogen (E), total non–structural carbohydrates (B), in it soluble carbohydrates (D) and starch (F), and carbon to nitrogen ratio (C), in fallen leaves of four exotic tree species: *Acer rubrum* (Ar), *A. saccharum* (As), *Betula alleghaniensis* (Ba) and *Quercus rubra* (Qr), and mixture of a few native species (MNS): *Quercus robur*, *Carpinus betulus*, *Tilia cordata*, *T. platyphyllos* and *Corylus avellana*, collected separately in three subsequent months (IX–XI), in two years (2008 and 2009, mean values). A dotted line shown mean value for native species (for three months). Vertical segments shown on graphs are standard error (SE)

contrasts, these differences were due to higher content of starch in the leaves of the exotic tree species in comparison with native tree species. This concerned both the average values of both years and each year analyzed separately.

We found statistically significant differences among months in nitrogen content in needles of coniferous tree species (Table 2) and both in nitrogen and TNC content in leaves of deciduous tree species (Table 3). In case of nitrogen, a statistically significant interaction of species × month was found. For the majority of species studied, nitrogen content in needles falling later (November) was lower than in needles falling in September (Fig. 1 E). Similarly, needles falling later contained less nonstructural carbohydrates than needles collected in September (Fig. 1 B).

In deciduous trees, the influence of tree species on the relationship of N and TNC content and month of leaves collection was less marked than in coniferous tree species. The influence of the month when fallen leaves were collected on the content of nonstructural carbohydrates had a decreasing tendency, which is similar to coniferous tree species. All of the deciduous tree species studied (also native species) had lower TNC content in leaves falling later in the growing season (November; Fig. 2 B). In the case of nitrogen content, all the exotic tree species had higher nitrogen content in leaves falling later (Fig. 2 E). This trend differed from that of the coniferous species. By comparison in deciduous trees, nitrogen resorption was not observed. Thus, and for these relationships, our results indicate a greater difference between exotic and native tree species, in the case of defensive compounds than for substances such as non-structural sugars and nitrogen.

Discussion

We found that tree species influenced chemical composition of leaf litter, in these so important components as nitrogen, carbon, phenolics and nonstructural carbohydrates. This indicates a range of potential species differences in the leaf litter decomposition by micro- and macro-organisms, including potential inhibitory effects, and on the other hand, potential improvement of conditions and functioning of these soil organisms by leaf litter substances beneficial for their growth and development.

The content of phenolic compounds in needles of most exotic tree the species we examined did not differ statistically from that of *P. sylvestris*. However, higher content of phenols in *P. menziesii* needles in comparison with *P. sylvestris* needles may cause slower needle decomposition in *P. menziesii* and is consistent with higher lignin contents (as the polymerization products of phenols) in this species (Hobbie et al. 2006). Also the C/N ratio was significantly different from *P. sylvestris* only for three species, and significantly higher C/N ratio was observed only for *P. peuce*. A shift in plant metabolism toward substances rich in carbon is often combined with an increase of the synthesis of defensive compounds such as phenols and terpenoids (Coviella et al. 2002; Kaplan et al. 2008; Karolewski et al. 2011). On the other hand, this relationship is complicated, because it is know that fallen leaves are decreased nitrogen by retranslocation (Oleksyn et al. 2003; Covelo and Gallardo 2004).

In deciduous tree species the fallen leaves of the two exotic maples studied (*A. rubrum* and *A. saccharum*) were characterized by a significantly higher content of phenolic compounds then the leaf mixture of native deciduous tree species. The increased content of phenols in leaves is responsible for lower litter decomposition rates. It is caused by a direct influence of tissue lignification (Sarcanen 1971; Camm and Towers 1973), as well as indirect negative influence on organisms decomposing the litter. This finding leads to conclusion that the decay of fallen maples leaves should be relatively slow. However, this is in contrast to our previous findings of two other maple species (e.g. *A. platanoides* and *A. pseudoplatanus*), whose litter decomposition we estimated as relatively fast compared to other deciduous tree species (Hobbie et al. 2006).

Close relationship with deciduous species we found, along with the date (month) fall foliage. It is known that the sun-type leaves fall earlier, and a shaded at later terms (Coupland et al. 2005; Sanz-Pérez et al. 2009). Moreover, leaves that fell earlier had higher content of phenols. This temporal pattern might be explained by higher investment in defensive compounds of sun type leaves and needles to protect them against foliofagous and pathogenic fungi (Henriksson et al. 2003: Żmuda et al. 2008; Karolewski et al. 2011). Simultaneously these substances serve as antioxidants, protecting leaves from the effect of the UV radiation (Tegelberg et al. 2001 and cited articles). A much greater decrease in content of phenolic compounds in the consecutive months of the litter collection for deciduous than coniferous tree species, can be caused by foliar leaching of these substances. For example, Bogatyrev et al. (1983) studied the leaching of phenolic compounds from the leaf litter of 8 tree species under the influence of freezing and defrosting. The authors stated that the leaching of phenolic compounds from Scots pine needles was minimal; however, in all 7 deciduous tree species examined, leaching was much greater.

Our results indicated that both leaves and needles falling later (shaded type), characterized by a lower level of the TNC. However, coniferous species differed significantly from the deciduous in the case of

nitrogen. The all of exotic tree species had higher nitrogen content in leaves falling later and the opposite i.e. lower in needles. The differences in the influence of the month in which needles/leaves were collected on nitrogen content among deciduous and coniferous tree species may be a result of group differences in resorption and retranslocation of nitrogen, a common strategy employed by plants to overcome limitations of N (Nieminen and Helmisaari 1996; Oleksyn et al. 2003).

Generally our results show that fallen needles and leaves of exotic tree species differ more from native tree species in terms of the content of defensive compounds (of phenols) protecting them against the negative influence of biotic factors causing their decomposition (insects, fungi, bacteria, etc.), than in content of the substances (nitrogen and nonstructural carbohydrates) beneficial for the growth and development of the organisms mentioned. However, a distinct influence of tree species on both groups of compounds listed earlier and nitrogen content was found. Both in case of coniferous as well as the deciduous trees, almost half of the exotic species studied did not differ in the foliar content of phenolic compounds compared to native tree species. The remaining species (e.g. maples) leaves contained more phenolic compounds. The TPh level in needles, depending on the tree species, was both higher and lower than in the native Scots pine needles. The differences in the TNC content among the exotic and native tree species were minimal and we only found them in *P. menziesii* of the deciduous and coniferous trees studied.

The maple species studied deserve particular attention. The high phenolic compounds content and high C/N ratio in fallen leaves coupled with low nitrogen content suggest that decomposition of maple leaves should be slower than that of leaves of other tree species. However, the lack of confirmation of slow leaf decomposition rates of other maple species, suggests that we are dealing with high variability among species of the *Acer* genus or that maple leaf litter decomposition rates are not dependent on the substances we studied. Among the coniferous trees studied, *P. menziesii* is particularly interesting. The fallen needles of Douglas fir are characterized by relatively high contents of phenolic compounds. However, taking into consideration simultaneously high TNC (mainly soluble carbohydrates) and nitrogen content (not differing from other tree species studied), the decomposition rate of needles of this species may not be particularly slow, assuming the participation of soil organisms that tolerate the high content of phenols.

We did not find any unique differences in the level or mutual quantitative relationships of the chemical compounds studied in fallen needles/leaves of exotic tree species in comparison with native tree species.

Increasing the diversity of tree stands by exotic species, at least in the context studied, should not bring evident negative effects by virtue inherent differences in litter chemistry. In fact, diversity in litter quality may have a stabilizing effect on relations between macro- and micro-elements and their rates of cycling, and hence protects the greater stability of ecosystems (Bonanomi et al. 2010).

Acknowledgements

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