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Denoting the intensity of soil biochemical transition according to stand species composition

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Abstract. The aim of this study was to denote biochemical soil activity in pure Scots pine, Norway spruce, silver fir, European larch, European beech and oak stands as well as in mixed fir-pine, beech-pine and fir-beech forests growing on a fertile fresh mixed deciduous site. The field work was carried out in the following Forest Districts: Nowe Ramuki (Mazursko-Podlaska forest region), Płońsk, Jabłonna, Brzeziny Siedlce, Grójec (Mazowiecko-Podlaska forest region) and Skarżysko, Ostrowiec and Marcule (Małopolska forest region). In 2015–2017, sample plots were assigned and chemical as well as soil enzyme activity measurements were made in each forest stand. Samples were taken from the organic (O) and humus (A) layers and for both the acidity (in 1M KCl), content of nitrogen, carbon, sum of exchangeable alkaline cations and hydrolytic acidity were determined. The investigation of soil enzymes included the measurements of urease, asparginase, acid phosphatase and dehydrogenase activity. Coniferous trees, especially fir, spruce or larch, and mixed fir-beech and pine-beech stands were observed to have a very positive influence on the biochemical soil properties. The highest activity of dehydrogenase was observed in soils of spruce and mixed fir-beech stands, whereas it was lower in soils of beech and pine stands, and the lowest in oak stands. Oak stands were furthermore characterized by the lowest soil acidity, lowest concentration of alkaline cations, the lowest nitrogen and carbon content as well as the smallest C/N ratio. In overall, soil enzyme activity showed a significant correlation with chemical soil parameters.

Keywords: enzymatic activity, chemical properties, coniferous trees, beech, oak, mixed stand

1. Introduction

Species composition of forest tree stands is one of the factors shaping the richness of forest soil in nutrients (Błońska, Januszek 2010; Olszowska 2016). Variations are observed among tree species and falling down leaves, dead roots and other plant debris as well as root discharges,. have different effects on soil quality, quantitative and qualitative microorganism composition and the patterns of the processes of organic matter decomposition (Burns 1982; Alkrota et al. 2003; Caldwell 2005; Allison 2006). A number of studies (Côte et al. 2000; Bonifacio et al. 2008) indicate favorable effects of deciduous tree species on the development of soil microorganisms, as both the litter and dead roots are more predisposed to decomposition in deciduous stands and constitute the substrate that allows more efficient release of nutrients available to plants. It was also found that nutrients from deciduous litter leached comparatively faster into the soil (Zwoliński 2004). In forestry practice, planting deciduous tree species into Scots pine monocultures aims to improve both the quality of soils and efficiency of nutrient use, leading to greater productivity of the dominant species (Zak et al. 1994; Lucas-Borja et al. 2016).

The influence of different stand species compositions on the intensity of soil biochemical transformations has not been fully understood. Soil fertility and the nutritional status of individual tree species do not incorporate interactions between tree species in the stand. Some studies (Šnajdr et al. 2008; Olszowska 2016) point out that the content and properties of soil organic matter considerably influence ecosystem productivity and can shaped by either synergistic or antagonistic effects of tree species growing in the stand. Due to the high importance of soil microorganisms in soil biochemical transformations and chemical element composition, it is very important to understand the influence of stand species composition on

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enzyme activity of microorganisms in the soil (Bielińska et al. 2005; Błońska 2011a; Błońska et al. 2013).

The aim of the study was to assess the effects of various species of forest trees: *Scots pine Pinus sylvestris* L., Norway spruce *Picea abies* (L.) Karst., silver fir *Abies alba* Mill., European larch *Larix decidua* Mill., pedunculate oak *Quercus robur* L. and common beech *Fagus sylvatica* L., as well as those of mixed stands: fir-Scots pine, Scots pine-beech and fir-beech on soil physico-chemical and biochemical properties, and in particular on enzyme activity of dehydrogenase, urease, asparaginase and acid phosphatase.

2. Area and research methodology

The study was carried out in the following forest districts: Nowe Ramuki, Jabłonna, Brzeziny, Płońsk, Siedlce, Grójec, Skarżysko, Ostrowiec Świętokrzyski and Marcule. Twenty-seven forest sites were selected that had 90- to 120-year-old tree stands (Table 1).

In the spring and autumn of the years 2015 to 2017, soil samples for chemical analyses and enzyme activity assays were taken from the organic and humus soil layers (O, A, respectively), at 10 locations evenly distributed within each study plot. Evaluations of soil chemical properties and enzyme activity were performed after sifting air-dry soil samples through a 2 mm sieve. The chemical analyses, conducted using conventional methods (Ostrowska et al. 1991), included the assessments of the following: soil reaction in 1 M KCl (using the potentiometric method), total nitrogen (by means of the Kjeldahl digestion method), carbon content (with the use of Leco SC-132 analyzer), the sum of exchangeable base cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) (after soil extraction with 1 M ammonium acetate, with the use of atomic absorption procedure), soil hydrolytic acidity (using

Table 1. Characteristics of research plots

Forest district	Forest region[1]	Species composition	Tree species	Forest site type	Soil type
Nowe Ramuki	II Mazursko-Podlaska	6So, 3Md, 1Św 8So, 1Brz, 1Db	So	LMśw	RDbr RDb
Jabłonna	IV Mazowiecko-Podlaska	6Św, 3So, 1Św	Św	LMśw	RDbr RDw
Płońsk	IV Mazowiecko-Podlaska	8Św, 2So 7Św, 3So		LMśw	RDbr RDbr
Brzeziny	IV Mazowiecko-Podlaska	7Jd, 2So, 1Db 8Jd, 1Db, 1Db	Jd	LMśw	RDw
Skarżysko	VI Małopolska	6Jd, 2Jd, 2Db 7Jd, 3Jd		LMśw	RDw
Ostrowiec Świętokrzyski	VI Małopolska	10Md 8Md, 1Bk, 1Db	Md	LMśw	BRwy
Marcule	VI Małopolska	9Md, 1Bk		LMśw	RDbr
Grójec	IV Mazowiecko-Podlaska	8Db, 2So	Db	LMśw	RDbr
Siedlce	IV Mazowiecko-Podlaska	9Db, 1So		LMśw	RDw
Brzeziny	IV Mazowiecko-Podlaska	8Bk, 1Bk, 1Db	Bk	LMśw	BRwy
Brzeziny	IV Mazowiecko-Podlaska	5So, 3Bk, 2Db	So-Bk	LMśw	RDbr BRk
Brzeziny	IV Mazowiecko-Podlaska	4Jd, 3So, 3Db	Jd-So	LMśw	BRwy BRwy
Skarżysko	VI Małopolska	6Jd, 2Bk, 2So 9Bk, 1Jd	Jd-Bk	LMśw	RDw RDbr

[1] According to Zielony and Kliczkowska (2012)

RDbr – cambic brunic arenosol, BRwy – dystric cambisol, RDw – cambic arenosol, RDb – albic brunic arenosol, LMśw – fresh mixed broadleaved forest, So – Scots pine, Św – Norway spruce, Jd – silver fir, Md – European larch, Db – oak sp., Bk – European beech, So-Bk – beech-pine, Jd-So – fir-pine, Jd-Bk – fir-beech

Kappen's method) as well as soil sorption capacity (T) and base saturation rate (V%).

Enzyme activity studies comprised the assessments of the activity of urease and asparaginase (mg N-NH4/10g soil; using Tabatabai and Bremner assay), acid phosphatase (mg PNP/100 g soil; Tabatabai and Bremner assay), as well as dehydrogenase (μmol triphenyl formazanate (TPF)/100 g soil (with the use of Lenhard's method according to the Casida procedure; Alef, Nanniperi 1995; Tabatabai, Bremner 1969).

The results of the effects of the forest site on chemical and biological soil properties were statistically analyzed using multi-way ANOVA. The relationships between soil biological activity and soil chemical properties as well as those between individual biochemical parameters were tested using Pearson's correlation coefficients (95% confidence level; $p < 0.05$). All the statistical tests were carried out using Statistica 10 software (Statsoft 2010).

3. Results

3.1. Chemical properties of the soil

A wide differentiation of chemical parameters was found in both analyzed layers (O and A) of the soils examined (Table 2). The results of pH measurements in 1 M KCl indicated that irrespective of the tree species, soil reaction

Table 2. Chemical properties of soils under different species of trees

Organic horizon (O)	Tree species																	
	So		Św		Jd		Md		Db		Bk		So-Bk		Jd-So		Jd-Bk	
	x	±a	x	±a	x	±a	x	±a	x	±a	x	±a	x	±a	x	±a	x	±a
pH w KCl	3.0	0.1	3.0	0.1	3.2	0.08	3.55	0.17	3.8	0.03	3.3	0.07	3.1	-	3.1	0.1	3.7	0.3
$C_{org.}$ [%]	21.3	4.3	28.7	1.7	21.7	2.19	16.06	1.50	4.8	0.62	10.8	1.19	22.1	4.1	20.1	3.0	19.4	2.5
N [%]	0.9	0.2	1.2	0.1	1.1	0.10	0.80	0.07	0.3	0.03	0.6	0.05	1.0	0.2	0.9	0.1	0.9	0.1
C/N	23.2	1.8	23.1	0.6	20.2	0.35	20.1	0.76	16.2	0.22	19.1	0.48	22.8	0.7	22.5	0.1	21.7	0.3
H [cmol/kg]	59.4	13.6	83.1	4.6	59.2	4.85	43.01	4.85	12.4	1.26	32.5	4.41	61.8	11.9	57.7	10.9	46.9	7.7
S [cmol/kg]	8.3	1.6	11.3	1.6	10.1	0.96	14.54	1.23	4.1	0.35	4.8	0.27	10.5	3.4	9.7	0.2	19.5	3.3
T [cmol/kg]	67.7	15.2	94.4	4.0	69.3	4.65	57.55	3.89	16.4	1.32	37.4	4.48	72.2	15.4	67.5	11.0	66.4	4.4
V [%]	14.7	0.9	13.9	2.3	18.7	2.85	39.45	8.57	35.0	4.94	15.9	2.34	16.5	2.4	17.4	3.0	44.0	14.2
Humus horizon (A)	Tree species																	
	So		Św		Jd		Md		Db		Bk		So-Bk		Jd-So		Jd-Bk	
	x	±a	x	±a	x	±a	x	±a	x	±a	x	±a	x	±a	x	±a	x	±a
pH w KCl	3.3	0.1	3.1	0.0	3.1	0.04	3.4	0.12	3.7	0.02	3.4	0.04	3.2	0.2	3.1	0.1	3.3	0.2
$C_{org.}$ [%]	2.2	0.2	3.0	0.4	2.4	0.29	2.2	0.14	1.8	0.20	2.4	0.12	2.2	0.2	2.5	0.3	2.8	0.4
N [%]	0.1	0.0	0.1	0.0	0.1	0.02	0.1	0.01	0.1	0.01	0.1	0.01	0.1	0.0	0.1	0.0	0.2	0.0
C/N	20.7	1.7	20.3	1.2	19.0	0.67	16.8	0.95	16.1	0.88	20.8	0.28	20.2	3.5	18.8	0.2	18.2	0.1
H [cmol/kg]	11.8	1.0	17.3	1.8	12.2	1.36	11.9	1.12	8.0	0.49	10.6	0.52	12.1	0.0	11.9	0.8	13.3	0.1
S [cmol/kg]	0.6	0.1	0.8	0.1	0.7	0.09	1.9	0.65	0.7	0.22	0.5	0.08	1.0	0.2	0.9	0.1	2.1	0.8
T [cmol/kg]	12.4	1.0	18.1	1.7	13.0	1.36	13.8	0.96	8.7	0.46	11.1	0.55	13.1	0.2	12.8	0.9	15.4	0.8
V [%]	5.0	0.6	4.7	0.8	6.3	1.09	18.7	8.01	9.3	3.10	4.3	0.75	8.0	1.6	7.3	0.3	16.0	6.4

x – average value. ±a – standard error. S – sum of exchangeable bases. T – hydrolytic sorption capacit. V – degree of base saturation. H – hydrolytic acidity So – Scots pine. Św – Norway spruce. Jd – silver fir. Md – European larch. Db – oak sp.. Bk – European beech. So-Bk – beech-pine. Jd-So – fir-pine. Jd-Bk – fir-beech was acidic. There were significant differences observed in soil pH values between oak soil and Scots pine and Norway spruce soils in O layer (H = 23.7, p = 0.003) and between oak soil and Norway spruce and fir soils in A layer (H = 25.83, p = 0.001). The differences in pH values observed between larch soil and beech soil were not statistically significant. Chemical analysis showed a significant relationship between the content of organic carbon and the site where soil samples were taken. In the O layer of the studied soils, statistically higher organic carbon values were recorded in the soil of Norway spruce and fir as compared to that of oak (H = 25.48, p = 0.0013). The differences observed in A layer were not significant. Similarly, in the case of carbon content, in the soil organic layer (O), significantly more nitrogen was observed in the samples taken from under Norway spruce and fir trees when compared to those collected under oak (H = 24.97, p = 0.0016). In the humus layer (A) of the studied soils, the differences between nitrogen contents were not statistically significant. In O layer, C/N ratio was significantly broader (H = 24.99; p = 0.0016) in the soils of Scots pine and Norway spruce as compared to oak soil. In the A layer, the lowest C/N ratio was observed in the soils of larch and oak, and the highest in the soils of Scots pine, Norway spruce and beech. However, the differences were not significant. The base cation content in the soils of larch as well as those of fir-beech mixed stands were significantly higher (H = 26.83, p = 0.0008) as compared to the soils of oak and beech in both tested soil layers. Furthermore, in both tested soil layers, a significantly higher hydrolytic activity was observed (H = 25.28, p = 0.0014) in Norway spruce, fir and Scots pine soils when compared to the soil of oak. Significantly higher (H = 25.99, p = 0.001) soil sorption capacity was observed in the soils of fir and Norway spruce as compared to those of oak and beech, and this regularity occurred in both soil layers analyzed. In larch and oak soils as well as in the soil collected in fir-beech mixed stand, the share of base cations was significantly higher (H = 23.7; p = 0026) than in the soils of Scots pine, Norway spruce and beech (significant differences were found only in O layer).

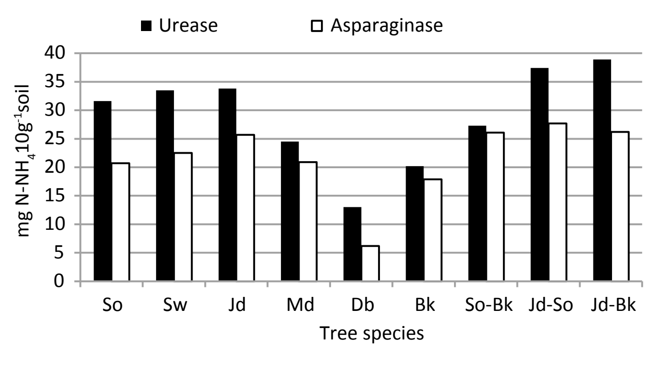
3.2. Soil enzyme activities

Urease activity was significantly higher (H = 25.7, p = 0.0012) in the soils of fir and fir-beech mixed stand as compared to the soil of oak (statistically significant differences were found only in the organic layer) (Fig. 1). In both tested soil layers, asparaginase activity was significantly higher in the soils of beech and fir when compared to the soil of oak (O layer: H = 18, p = 0.02; A layer: H = 22, p = 0.004) (Fig. 1 and 2). There were no significant differences between soils of the tree species examined as regard acid phosphatase activity in the layer A (Fig. 4). Nonetheless, the activity of this enzyme was significantly higher (H = 27.3, p = 0.006) in the layer O of the soils of spruce, larch and Scots pine, as well as those of fir-pine, fir-beech and Scots pine-beech mixed stands when compared to the soil of oak (Fig. 3). Similar to the activities of the aforesaid enzymes, dehydrogenase activity was significantly higher (H = 18, p = 0.02) only in the O layer of the soils of Norway spruce as compared to those of oak, beech and fir-Scots pine mixed stands (Fig. 3).

In the plots of the tree species under the study, the values of soil chemical parameters analyzed were significantly correlated with those obtained for biochemical parameters of the soils (Tab. 3).

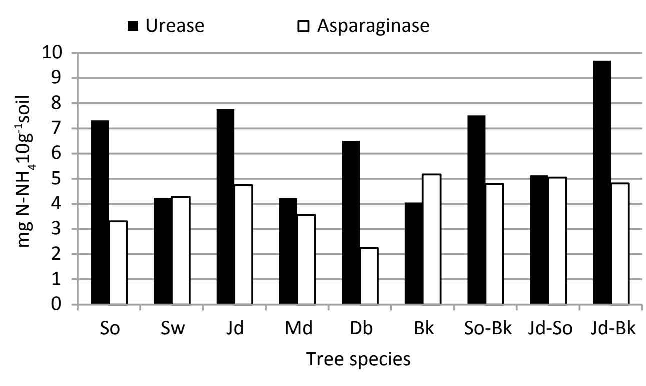
4. Summary

The analysis of the effects of forest tree species on soil biochemical properties showed an exceptionally beneficial



Denotes as in table 2

Figure 1. Urease and asparaginase activity in O level under different species of trees



Denotes as in table 2

Figure 2. Urease and asparaginase activity in A level under different species of trees

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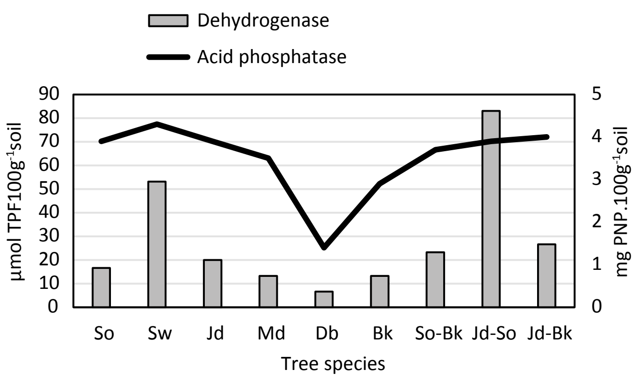
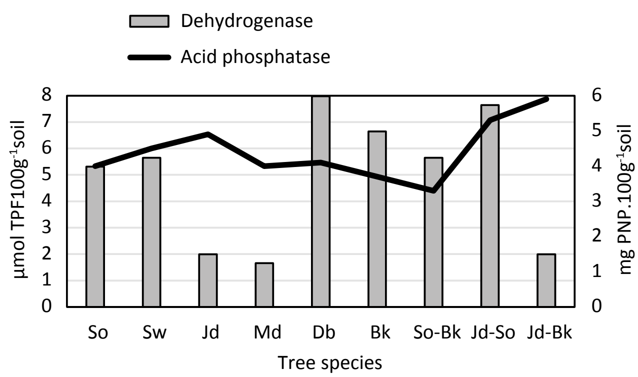
effect of coniferous tree species, especially fir, larch and Norway spruce, and also in the cases when conifers grew in mixed stands, i.e. fir, beech as well as Scots pine-beech. In comparison, the soil of oak trees was characterized by the lowest acidity, base cation concentrations, carbon and nitrogen contents as well as the narrowest C/N ratio. Significantly higher values of chemical parameters examined were observed in the soils of fir, larch and Norway spruce as well as in those of mixed stands. In the present study, the highest activity of dehydrogenase enzymes, recognized as an indicator of metabolic processes of soil microorganisms (Nannipieri et al. 2002; Piotrowska 2011), was observed in the soil of Norway spruce and mixed fir-beech stands, whereas it was lower in the soil of beech and Scots pine stands, and the lowest in the soil of oak stand. Trees have an influence on soil properties not only through their lifeless parts forming the litter, but also through their root systems. Grygoruk (2016) showed the highest biomass of fine roots in beech and oak stands, and under the conditions of the present study, the lowest activities of the soil enzymes examined were ob-



Denotes as in Table 2

Figure 3. Dehydrogenase and acid phosphatase activity in the O level under different species of trees



Denotes as in Table 2

Figure 4. Dehydrogenase and acid phosphatase activity in the A level under different species of trees

Table 3. Correlation (r_{yx}) between chemical (y) and biological (x) parameters of soil

y	x			
	Urease	Asparaginase	Dehydrogenases	Acid phosphatase
C [%]	0.80167***	0.77566***	0.36731*	0.88436***
pH KCl	-0.59116***	-0.6157***	-0.31786*	-0.6582***
N [%]	0.81466***	0.782553***	0.35029*	0.87804***
C/N	0.65078***	0.73944***	0.355705*	0.79491***
Hydrolytic acidity (H_h)	0.78044***	0.74014***	0.38399*	0.86928***
Sum of exchangeable bases (S)	0.47484**	0.46866**	0.19099	0.59145**
Hydrolytic sorption capacity (T)	0.80256***	0.764425***	0.387206**	0.90519***
Degree of base saturation V [%]	-0.41566**	-0.4414**	-0.22881	-0.37992*

*** p<0,001 ** p<0,01 * p<0,05

served in the soils of these tree species. The results obtained in the present study confirmed favorable effects of fir–beech, fir–Scots pine and Scots pine–beech mixed stands on soil biochemical activity. On the other hand, Błońska and Januszek (2010) reported positive effects of oak as an admixture in Scots pine stands, resulting in the increased biochemical activity of the soil. As a result of this influence, nitrogen -rich soil layers with accumulated humus were formed. In the present study, fir, larch and Norway spruce as well as mixed fir–beech stands were the most advantageous in terms of the intensity of enzyme activity. The observed enzyme activities were strongly related to soil organic matter contents, as evidenced by higher values of enzyme activity in the soil organic layer when compared to the soil humus layer, irrespective of stand species composition. Numerous literature data (Landgra et al. 2000; Leirós et al. 2000; Zwoliński 2008; Olszowska 2010; Kotroczó et al. 2014) confirmed strong relationships between soil enzyme activity and the contents of organic carbon (considered as primary energy substrate for microorganisms).

Physical and chemical properties of the soil are strongly interrelated and significantly affect soil organisms and enzyme activity (Gil-Sotres et al. 2005; Mueller et al. 2012). A number of studies have shown a significant correlation between soil biological activity and its fertility (e.g. Myśków et al. 1996; Trasar-Cepeda et al. 2000, 2008; Zwoliński 2004; Olszowska et al. 2005, 2007). This was confirmed by the results obtained in the present study, which indicated obvious dependence of enzyme activity upon soil chemical properties. Biochemical features of the soils examined were significantly correlated with at least several parameters of soil fertility, such as the contents of organic carbon and nitrogen, the sum of base cations, hydrolytic acidity and sorption capacity. Apart from soil chemical properties, other factors, such as soil granulometric composition, quality of organic matter that was shaped by tree stand species composition and climatic conditions influenced the studied biochemical parameters (Bauchus et al. 1998; Côte et al 2000; Chaer et al. 2009). All the tested biochemical parameters are related, albeit in various aspects, to decomposition of organic matter, i.e. the process guaranteeing the maintenance of essential soil nutrients for plants. Soil properties, such as, as among others, chemical composition, microbiological status and enzyme activity, are considered to be reliable indicators of soil site fertility (Nannipieri et al 2002; Olszowska et al. 2005; Błońska 2011b; Piotrowska 2010, 2011). The type of organic matter accumulated in the soils of forest stands depends on stand species composition, and also on characteristics of the soil on which the stands grow, especially its granulometric composition and the presence or absence of calcium carbonate. Fir or Norway spruce stands and Scots pine-beech, fir-beech and fir pine mixed forests provide more advantageous conditions for microbial decomposition of organic matter, whereas oak and beech inhibit decomposition processes. Błońska (2015) stated that beech effects varied depending on the richness of the bedrock in calcium. If the amount of calcium in the bedrock are high, beech causes calcium allocation to the soil layers accumulating humus, and this positively affects biological activity. When the bedrock is poor in calcium, the influence of beech is less favorable, and organic matter decomposes at a slower rate. The results of the present study showed that diversity of soil biochemical properties depended not only on stand species composition, but also on homogeneity of the humus accumulation layer. In the biosphere, the soils constitute the largest terrestrial deposit of organic carbon, and accumulate more carbon resources than plants together with the atmosphere. Due to large forested areas, forest soils play an important role in the global carbon cycle (Jastrow et al. 2007; Mueller et al. 2012). The accumulation of organic carbon is also a key process for forest ecosystems as well as it is particularly important in the element cycles and improvement of soil fertility. The current species composition of forests is for the most part a result of human economic activity. The development of agriculture has led to deforestation and changing forest lands with fertile soils into farming lands. As a result, there are forests surviving on poor soils, such as, for example, Scots pine forests. In Poland, Scots pine occupies the weakest dystrophic and oligotrophic sites. On such a poor substrate, it is extremely important to ensure the appropriate share of admixture species, which enrich soil surface layer with plant material falling down into the litter, thus contributing to the acceleration of the decomposition rate of organic matter and to the efficient circulation of nutrients. The present study showed that fir, larch and Norway spruce as well as mixed fir–beech and Scots pine–beech stands favorably affect soil biochemical properties.

The relatively low use of biochemical tests in forest soil diagnosis results from the lack of standardized analytical methods that enable interpretation of the obtained results (Sariyildiz et al. 2005; Zornoza et al. 2007). Diversified structure of forest soils, as well as the influence of a number of environmental factors on soil enzymes, make it impossible to establish the standards for biochemical parameters of individual soil types or forest sites, as is the case with chemical parameters (Nielsen et al 2002; Moffat 2003). Then again, biochemical indicators can be very useful in comparative studies to assess the quality of the soils or their response to external factors, both natural and anthropogenic. This was shown by the studies carried out in forest stands comprising various tree species. The results so far obtained favor the use of biochemical indicators in research on forest soils, especially when assessing impact of stress factors on forests (e.g. industrial pollution, fires, extreme weather events)

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along with those of climate change and silvicultural operations as well as in projecting further development of forests.

5. Conclusions

1. A clearly lower nutrient status, expressed as lower content of organic carbon, nitrogen and the base cations, as well as lower sorption capacity were observed in the soils under oak and beech, when compared to the soils under coniferous trees and mixed stands.

2. Higher activity of urease, asparaginase, acid phosphatase and dehydrogenases observed in soils of mixed forests when compared to single-species stands may indicate that inserting deciduous tree species into forest stands will have a positive effect on the quality and fertility of soils, contributing to higher productivity of the dominant tree species in the stand.

3. The activity of the studied enzymes is significantly correlated with the contents of organic carbon and nitrogen in the soil as well as the sum of base cations, hydrolytic acidity and sorption capacity. A strong relationship between these parameters and soil fertility indicates the possibility of using biochemical activity parameters as reliable fertility indicators of forest soils.

4. Positive interaction of fir and beech as admixture species in Scots pine stands results in increased microbial activity of the soil.

Conflict of interest

No potential conflicts are declared.

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