

## The effect of spruce stand thinning on biological activity in soil

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**ABSTRACT:** The effect of thinning of young spruce stands by 500 trees/ha on biological activity in the soil profile was studied in the mountainous area of the Moravian-Silesian Beskids. The biological activity of soil was determined under optimal laboratory conditions by tests of soil respiration, catalase activity and intensity of cellulose decomposition. No statistically significant differences were found between the individual biological tests when the two experimental stands were compared ( $P$ -level 0.05). All biological activities within each stand were correlated, and significant correlations were found between biological activities in the soil and ammonium nitrogen content.

**Keywords:** spruce; stand density; soil respiration; catalase activity; cellulose decomposition; nitrogen

Industrial emissions and subsequent acidification often result in slower decomposition of acid fallen spruce needles and development of a thick layer of surface humus under spruce monocultures of mountainous areas. Similar conditions developed in some parts of the Moravian-Silesian Beskids Mts. For the purposes of soil acidity elimination, nutrition improvement and support of decomposition processes, extensive areas of the Czech Republic, including the Moravian-Silesian Beskids, were ameliorated by liming. The positive effect of liming on base saturation is generally accompanied by a negative effect on nitrogen dynamics in acid soils under spruce monocultures (FORMÁNEK, VRANOVÁ 2003) and other measures need to be sought to achieve a sufficient rate of organic matter decomposition.

The growth density plays an important role in the microclimate and water balance of forest stands (KANTOR, KLÍMA 1997). Thinning of coniferous trees is one of the possibilities to achieve more intensive soil activity, increased supply of ammonium nitrogen and reduction of its oxidation into nitrate forms (THIBODEAU et al. 2000), which is not preferred in spruce nutrition (AARNES et al. 1995). For that reason we performed a series of laboratory tests aimed at finding how the biological activity would differ in two spruce stands of different density. The selected indicators of biological activity of the soil included laboratory tests of basal and substrate induced soil respiration, catalase activity in the soil and intensity of cellulose decomposition.

On the basis of the soil respiration tests where temperature and moisture are not limiting factors, we are able to detect the level of mineralization (SPARLING 1997). Soil respiration induced by substrate addition is a measure of microbial biomass (ANDERSON, DOMSCH 1986) and after

addition of easily accessible sources of C and N we can assess which of the sources represents a limiting factor for the microbial community (SMETHURST et al. 1998). In general, the intensity of heterotrophic soil respiration under favourable temperature and moisture conditions is a criterion of substrate quality, nitrogen availability, and C-protection by clays (MARY et al. 1996).

The soil catalase activity, decomposing  $H_2O_2$  derived from the activity of flavine oxidases (and some other enzymes) during microbial metabolism, is connected with the concentration of substrate and the level of its use by micro-organisms (SARGANOVA et al. 1997), and is one of the best approaches to estimate the load of heavy metals and stress factors in the soil (GRIGORIAN 1983). The catalase activity is affected by the plant cover (BRZEZINSKA et al. 2001) and its intensity decreases under water stress (HU et al. 1999) and correlates with CEC, total N, available P and K, organic matter content, and the activity of phosphatase and urease (ARCAK et al. 1997).

A laboratory test of cellulose decomposition is another measure for the specification of organic matter decomposition. Under favourable temperature and moisture conditions set up for laboratory tests, cellulose decomposition can be affected by soil pH (PAN et al. 1998), aeration, and also by the presence of available nitrogen and carbohydrates (KNAPP et al. 1983). According to TODOROVA (1972) the decomposition of cellulose positively correlates with the contents of  $P_2O_5$  and  $NO_3^-$  and is inhibited by the presence of heavy metals and  $SO_4^{2-}$  (VOROBICHNIK 2002).

### METHODS

Research plots are in the ridge part of the Moravian-Silesian Beskids at an altitude of 908 m. Their geographical

position is given by the following co-ordinates: 49°30' 17" N latitude and 18°32' 28" E longitude. The parent rock is built of flysch layers with the predominance of Godula sandstone. Soil type is humo-ferric podzol. The region is moderately cold, wet and rich in precipitation. Mean air temperature is 4.9°C, mean annual precipitation amount 1,100 mm and mean relative air humidity 80%. Southern winds are prevailing (JANOUS et al. 2000). The research stand of Norway spruce (*Picea abies* [L.] Karst.) is situated on a southern 13° slope. The stand age was 21 years and Norway spruce occurring there was in the 2<sup>nd</sup> generation. According to the stand density the research areas was divided into two stands (FD and FS). The density in FD stand was 2,600 trees/ha and in FS stand 2,100 trees/ha. In 1983, 1985 and 1987 the area was limed by dolomitic limestone at a total rate of 9 t/ha. The input of mineral nitrogen and sulphur in throughfall in 1999 amounted to 12.34 kg/ha/year, or 21.34 kg/ha/year on FD stand. On FS stand the soil was added 8.76 kg N<sub>min</sub>/ha/year and 12.92 kg S/ha/year (FORMÁNEK 2000).

One representative soil profile was dug in each stand at a place where the conditions represented the growth density of the stand. Horizons O<sub>p</sub>, O<sub>f+h</sub>, A<sub>e</sub>, E<sub>p</sub>, B<sub>h</sub>, B<sub>s</sub> and C<sub>d</sub> were distinguished in the soil profiles. Soil samples were taken from all horizons of the soil profile on a single date in May, August and October in 1999. As the transitory A<sub>e</sub> layer was lower in the soil profile of the FD stand samples from A<sub>e</sub> and E<sub>p</sub> horizons were not separated (KLIMO et al. 1998). The soil samples were stored at 4°C. After 12 hours the samples were homogenised and sieved through a 10mm mesh. The soil samples were stored at 4°C again and laboratory tested after 24 hours. Modified indophenol method was used for the determination of ammonium nitrogen concentration after soil extraction by 1% K<sub>2</sub>SO<sub>4</sub> (ZBÍRAL et al. 1997), the concentration of nitrate nitrogen in soil was measured by capillary electrophoresis after extraction by demineralised water (KUBÁŇ et al. 1999). Soil catalase activity was determined manganometrically (TOMÍČEK 1947). Carbon dioxide evolved from soil was absorbed in NaOH, and the amount of carbonates was determined titrimetrically after the addition of BaCl<sub>2</sub>. Cellulose decomposition was examined in Petri dishes. All biological activity tests were performed at room temperature. Total C and N were analysed by LECO analyser while pH/H<sub>2</sub>O and pH/KCl were determined potentiometrically.

Data sets obtained from the FD (dense) and FS (thinner) stands were compared separately for each property (biological activity, concentration of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N, pH/H<sub>2</sub>O and pH/KCl) by *t*-tests. Correlations between the measured soil properties were assessed separately for each site. All the values included in the text and in the tables are arithmetic means ± 95% C.I. while the values used in the diagrams are arithmetic means ± 95% S.D.

## RESULTS

The highest percentage of C<sub>t</sub> and N<sub>t</sub> and C/N ratio was in the litter layer in both stands (Table 1). The C/N ratio

exceeded the value of 20 and was most favourable in the B<sub>h</sub> horizon. The soil reaction determined three times in the course of the season was acid in both stands with the lowest values were found in the A<sub>e</sub> and E<sub>p</sub> horizons (Table 2), which is above all a consequence of the base cation content in the soil-forming rock and its consumption by trees. The concentration of ammonium nitrogen reached the highest value in the surface humus layer and different concentrations of nitrates were determined in the individual soil horizons due to high mobility (Table 3).

Laboratory tests of basal soil respiration (BaR) showed the highest degree of CO<sub>2</sub> release from the litter. The intensity of basal respiration decreased as the profile depth increased, with the highest drop between O<sub>f</sub> and A<sub>e</sub>/E<sub>p</sub> or A<sub>e</sub> horizons (Fig. 1). The reaction of soil microflora to glucose addition (GIR) as a source of easily utilisable carbon reached the highest values in the FD stand in B<sub>h</sub> samples where the intensity of CO<sub>2</sub> evolution reached five times the value of basal respiration (Table 4a). In the FS stand the highest increase in respiration intensity after the addition of glucose was determined in the B<sub>h</sub> horizon with nearly 4 times increase, in comparison with basal respiration (Table 4b). The lowest reaction of microbial community to the addition of an easily available source of carbon was measured in the surface humus layer. The reaction of microbial community after the addition of an easily available source of carbon and nitrogen (soya flour) was highest in the B<sub>h</sub> horizon again in both stands, with over 6 times increase in comparison with the level of basal

Table 1. Total carbon, nitrogen and C/N ratio in soil samples

a. FD stand			
Horizon	C <sub>t</sub> (%)	N <sub>t</sub> (%)	C/N
O <sub>l</sub>	36.40	1.309	27.80
O <sub>f+h</sub>	22.40	0.957	23.41
A <sub>e</sub> /E <sub>p</sub>	5.20	0.200	26.00
B <sub>h</sub>	3.81	0.174	21.90
B <sub>s</sub>	2.39	0.101	23.66
C <sub>d</sub>	1.00	0.040	25.00
b. FS stand			
Horizon	C <sub>t</sub> (%)	N <sub>t</sub> (%)	C/N
O <sub>l</sub>	33.80	1.250	27.04
O <sub>f+h</sub>	15.90	0.720	22.08
A <sub>e</sub>	6.50	0.286	22.73
E <sub>p</sub>	3.31	0.134	24.70
B <sub>h</sub>	3.11	0.142	21.90
B <sub>s</sub>	3.50	0.166	21.08
C <sub>d</sub>	2.10	0.093	22.58

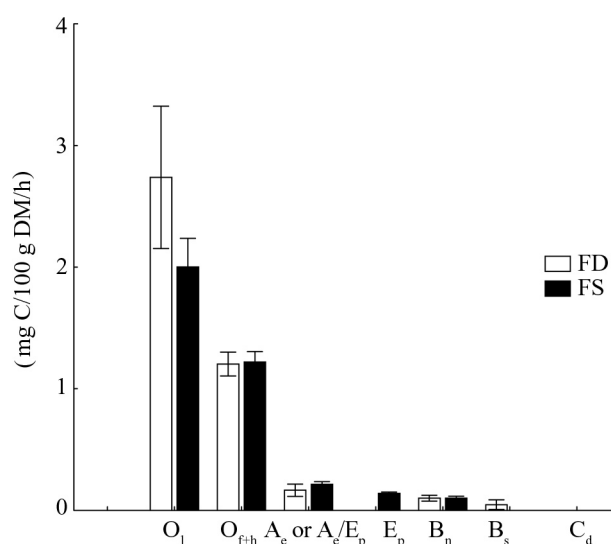


Fig. 1. Laboratory tests of basal soil respiration

respiration and an over 4 times increase was also observed in samples from  $A_e/E_p$  (FD stand) or  $E_p$  horizon (FS stand). The levels of basal respiration and glucose or soya flour induced soil respiration are shown in Figs. 2 and 3.

The activity of soil catalase decreased across the profile, with the highest values in the surface humus (Fig. 4). Differences between the two stands were mainly demonstrated in the surface humus layer with higher values in the FS stand – by 6.14 ( $O_1$ ) and 6.9 mg  $H_2O_2/g$  DM/15

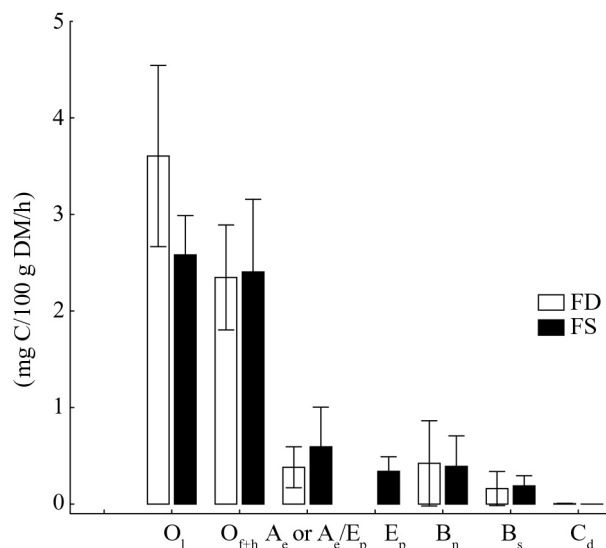


Fig. 2. Laboratory tests of soil respiration induced by glucose addition

min( $O_{f+h}$ ). The intensity of cellulose decomposition also reached the highest values in the surface humus, with zero values measured in the  $B_h$ ,  $B_s$  and  $C_d$  horizons (Fig. 5). More decomposed cellulose in  $O_1$  (by 0.47 mg/week) was found in the FS stand.

Mutual comparisons of biological activities, concentrations of mineral forms of nitrogen and values of active and exchange soil reactions showed statistically significant

Table 2. Active (pH/ $H_2O$ ) and exchangeable (pH/KCl) soil reaction

a. FD stand

Horizon	pH/ $H_2O$	pH/KCl
$O_1$	4.72 ± 0.97	4.16 ± 0.74
$O_{f+h}$	4.85 ± 0.44	4.25 ± 0.10
$A_e/E_p$	4.06 ± 0.83	3.21 ± 0.20
$B_h$	4.07 ± 0.28	3.37 ± 0.67
$B_s$	4.42 ± 0.57	4.00 ± 0.45
$C_d$	4.56 ± 0.78	4.18 ± 0.07

b. FS stand

Horizon	pH/ $H_2O$	pH/KCl
$O_1$	4.79 ± 0.83	4.44 ± 1.39
$O_{f+h}$	4.56 ± 0.47	4.14 ± 0.93
$A_e$	3.84 ± 0.78	3.00 ± 0.33
$E_p$	3.86 ± 0.52	2.96 ± 0.39
$B_h$	3.99 ± 0.19	3.37 ± 0.34
$B_s$	4.37 ± 0.16	3.69 ± 0.02
$C_d$	4.66 ± 0.13	4.07 ± 0.18

Table 3. Ammonium and nitrate nitrogen contents (given in mg/kg DM)

a. FD stand

Horizon	$NH_4^+-N$	$NO_3^- -N$
$O_1$	15.78 ± 18.36	5.20 ± 22.36
$O_{f+h}$	13.12 ± 15.67	0.54 ± 9.26
$A_e/E_p$	3.77 ± 2.7	3.35 ± 9.36
$B_h$	0.78 ± 3.33	5.70 ± 19.19
$B_s$	0.15 ± 0.63	1.87 ± 5.05
$C_d$	1.06 ± 4.58	5.19 ± 15.77

b. FS stand

Horizon	$NH_4^+-N$	$NO_3^- -N$
$O_1$	12.22 ± 34.55	4.93 ± 13.91
$O_{f+h}$	6.72 ± 5.05	2.42 ± 10.42
$A_e$	2.92 ± 0.76	4.43 ± 9.55
$E_p$	2.32 ± 2.18	0
$B_h$	0.45 ± 1.95	2.39 ± 5.80
$B_s$	0.74 ± 3.18	5.16 ± 10.97
$C_d$	1.46 ± 6.29	1.29 ± 2.07

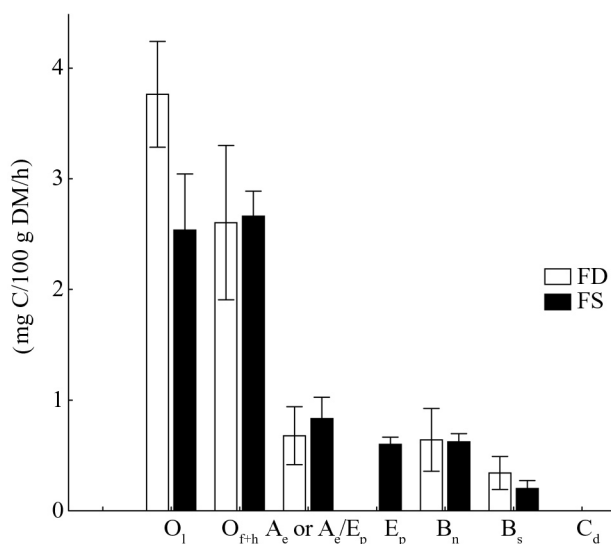


Fig. 3. Laboratory tests of soil respiration induced by soya flour addition

( $P < 0.05$ ) positive correlations in the soil profiles, in the FD and FS stands between the intensity of cellulose decomposition, catalase activity, basal and glucose/soya flour induced respiration and ammonium nitrogen concentration (Table 5). While the FD stand (more dense growth) showed higher correlations between the tested biological activities and the concentration of ammonium nitrogen, the FS stand (thinner growth) also showed statistically significant correlations between the biological activities

Table 4. Ratio between soil respiration induced by glucose (GIR) or soya flour (SIR) addition and basal soil respiration (BaR)

a. FD stand

Horizon	GIR/BaR	SIR/BaR
O <sub>1</sub>	1.43 ± 1.90	1.46 ± 1.42
O <sub>f+h</sub>	1.93 ± 0.82	2.14 ± 1.08
A <sub>e</sub> /E <sub>p</sub>	2.42 ± 3.75	4.28 ± 5.14
B <sub>h</sub>	4.83 ± 0.79	6.23 ± 5.46
B <sub>s</sub>	2.24 ± 0.51	3.16 ± 7.84
C <sub>d</sub>	0	0

b. FS stand

Horizon	GIR/BaR	SIR/BaR
O <sub>1</sub>	1.31 ± 0.94	1.28 ± 0.78
O <sub>f+h</sub>	1.97 ± 1.57	2.19 ± 0.56
A <sub>e</sub>	2.94 ± 6.18	3.98 ± 3.67
E <sub>p</sub>	2.51 ± 3.51	4.32 ± 1.17
B <sub>h</sub>	3.78 ± 7.31	6.40 ± 4.97
B <sub>s</sub>	2.20 ± 7.70	1.32 ± 3.77
C <sub>d</sub>	0	0

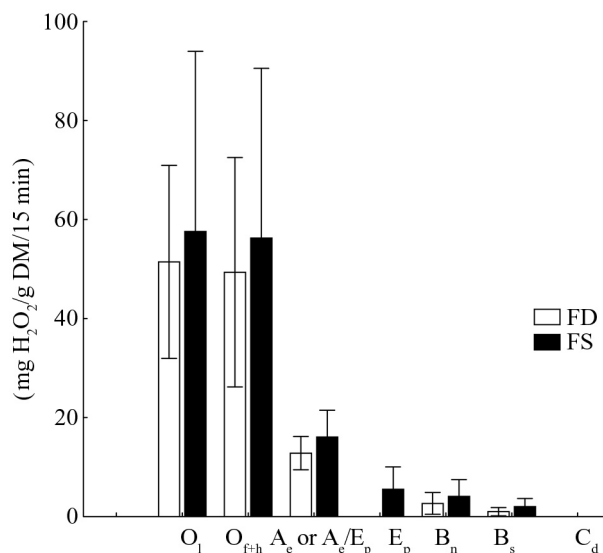


Fig. 4. Tests of catalase activity in soil

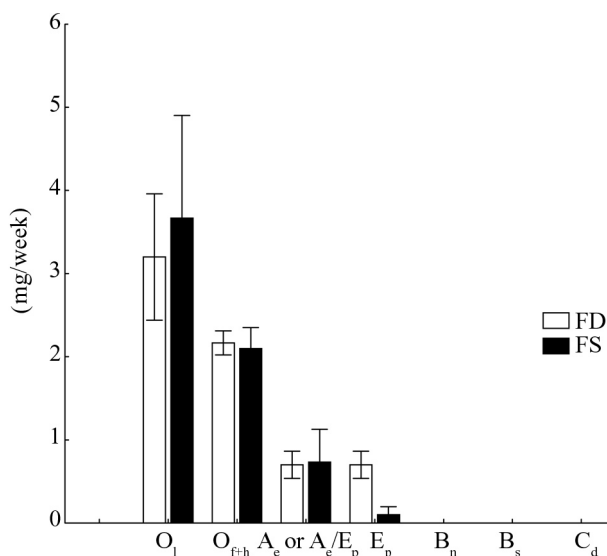


Fig. 5. Tests of cellulose decomposition

and the exchangeable or active soil reaction. The FS stand showed even a statistically significant correlation between the actual concentration of nitrate nitrogen and the activity of soil catalase. The differences between the individual biological activities in the soil samples taken from the FD and FS stands were statistically insignificant ( $P$ -level 0.05).

## DISCUSSION

The laboratory tests of biological activities demonstrate that different supply of light, water and nutrients, following the thinning of young spruce stands by 500 trees per ha had no statistically significant effect on the activity of soil microflora in the surface and deep layers of the soil. Before the commencement of the study there was a hypothesis that the forest stand thinning would change the amount of organic matter entering the soil and the related amount of water-soluble fraction. The present biological activity tests were performed in the optimal laboratory

Table 5. Correlation between soil characteristics measured in whole soil profile (CA – catalase activity, CE – cellulose decomposition, BR – basal respiration, SIR – respiration induced by soya flour, GIR – respiration induced by glucose,  $\text{NH}_4^+\text{-N}$  – actual concentration of ammonium nitrogen,  $\text{NO}_3^-\text{-N}$  – actual concentration of nitrate nitrogen,  $\text{pH}/\text{H}_2\text{O}$  – active soil reaction,  $\text{pH}/\text{KCl}$  – exchangeable soil reaction),  $r$  – values are given for  $P < 0.05$

a. FD stand

	CA	CE	BaR	SIR	GIR	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	$\text{pH}/\text{H}_2\text{O}$	$\text{pH}/\text{KCl}$
CA		0.90	0.70	0.79	0.74	0.69		0.54	0.51
CE	0.90		0.93	0.94	0.92	0.89		0.48	
BaR	0.70	0.93		0.96	0.98	0.94			
SIR	0.79	0.94	0.96		0.96	0.92			
GIR	0.74	0.92	0.98	0.96		0.94			
$\text{NH}_4^+\text{-N}$	0.69	0.89	0.94	0.92	0.94				
$\text{NO}_3^-\text{-N}$									
$\text{pH}/\text{H}_2\text{O}$	0.54	0.48							0.81
$\text{pH}/\text{KCl}$	0.51							0.81	

b. FS stand

CA		0.65	0.61	0.71	0.72	0.69	0.48	0.47	0.66
CE	0.65		0.89	0.90	0.86	0.63			0.54
BaR	0.61	0.89		0.93	0.98	0.64		0.50	0.47
SIR	0.71	0.90	0.93		0.94	0.54		0.37	0.44
GIR	0.72	0.86	0.98	0.94		0.70		0.50	0.52
$\text{NH}_4^+\text{-N}$	0.69	0.63	0.64	0.54	0.70			0.45	0.56
$\text{NO}_3^-\text{-N}$	0.48								
$\text{pH}/\text{H}_2\text{O}$	0.47		0.50		0.50	0.45			0.86
$\text{pH}/\text{KCl}$	0.66	0.54	0.47	0.44	0.52	0.56		0.86	

conditions with broken food webs and exclusion of the effects of root exudation. The soil samples were oxidised by mixing before the analysis started and potential toxic volatilised products were removed (STAMTSEVICH 1972).

Despite of the different inputs of nitrogen and sulphur in throughfall (FORMÁNEK 2000) no differences were found in soil reaction and ammonium or nitrate nitrogen content in the soil of the studied stands. That is probably an important reason for similar results of the biological activity tests. Basal respiration is a simple indicator of the biological activity of soil and is closely connected with nitrogen mineralisation (MARY et al. 1996). Under optimal conditions set up in our experiments the soil respiration was not affected by the principal limiting factors, i.e. temperature and moisture (ANDREWS et al. 2000; BUCHMANN 2000) and significant correlation with the content of the ammonium nitrogen unused by the micro-organisms was found (ALEXANDER 1977). The intensity of respiration was probably affected by labile C, more easily and increasingly released under the optimal conditions (VANCE, CHAPIN 2001). The low reaction of microbial community to the addition of easily utilisable sources of C or C and N in the surface humus showed that the quantity of these sources was not a limiting factor of the activity of the functional microbial groups (PAUL, CLARK 1989).

The different composition of the herb layer did not affect soil catalase in the litter (POPOVA, PEREVOZNIKOVA 1996), which is reflected by the quantity of microbial biomass (GUWY et al. 1999) and its level closely correlates with the activity of cellulolytic micro-organisms (SARGANOVA et al. 1997). The C/N ratio was similar in both stands, which created no preconditions for different intensity of decompositions (LARIONOVA et al. 1995). This was established by the tests of cellulose decomposition with a correlation to the content of available ammonium nitrogen in the soil (MELILLO et al. 1982; BEYER 1992; ENTRY, BACKMAN 1995), like in the case of soil respiration. Cellulose decomposition can also be related to the content of available phosphorus in the soil (BALIGAR et al. 1997) that could be different in both stands, however this fact is not supported by the results reported for example by PANDE (1999). The correlation between cellulose decomposition and soil pH (ANDERSON, DOMSCH 1993) was evident in our study, however we have no data concerning the heavy metal content in the mineral soil and in the surface humus. The stand characteristics, with the exception of growth density, were also very similar, which could also significantly affect cellulose decomposition, as reported by KURKA et al. (2001). The reduced inputs of sulphur in the throughfall in thinned stand (FORMÁNEK 2000) had no effect on the intensity of cellulose decom-

position and H<sub>2</sub>O<sub>2</sub> break up, which does not correspond to the results reported by GRUNDA (2000).

The above-mentioned tests of biological activities significantly correlated with the content of ammonium nitrogen in the soils of both stands. The concentration of ammonium nitrogen did not increase significantly and the concentration of nitrate nitrogen did not decrease significantly after the spruce stand thinning, as mentioned by THIBODEAU et al. (2000), and the cellulose decomposition in the soil samples taken in the thinner stand was not more intensive, either. This fact is supported by the N content in the 1<sup>st</sup> year needles measured in 1999, showing no statistically significant differences between the two experimental stands (FORMÁNEK, KULHAVÝ 2001).

## CONCLUSIONS

The thinning of young spruce stands by 500 trees per ha was found to have no significant effect (*P*-level 0.05) on the biological activity of soil and the content of mineral forms of nitrogen. Statistically significant correlations were found between the individual biological activities (soil respiration, catalase activity, cellulose decomposition) and the ammonium nitrogen concentration.

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## Vliv proředění smrkových porostů na biologickou aktivitu půdy

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**ABSTRAKT:** Byl studován vliv proředění mladého smrkového porostu o 500 jedinců/ha v hřebenové části Moravskoslezských Beskyd na biologickou aktivitu v půdním profilu. Biologická aktivita půdy byla stanovena ve všech horizontech půdních profilů laboratorními testy půdní respirace, aktivity katalázy a intenzity rozkladu celulózy. Nebyly zjištěny statisticky signifikantní rozdíly ( $\alpha = 0,05$ ) mezi jednotlivými biologickými testy při porovnání obou experimentálních ploch. Všechny biologické aktivity na každé ploše navzájem korelovaly mezi sebou a byly zjištěny významné korelace mezi biologickou aktivitou půdy a koncentrací amonného dusíku.

**Klíčová slova:** smrk; hustota porostu; respirace půdy; katalázová aktivita; rozklad celulózy; dusík

Průmyslové imise a půdní acidifikace vedou ke zpomalení rozkladu kyselého smrkového opadu a k vytvoření mohutné vrstvy nadložního humusu pod smrkovými monokulturami Moravskoslezských Beskyd i dalších horských oblastí České republiky. Jak dokazují mnohé studie, meliorace vápněním může vést k negativnímu vlivu na čistou mineralizaci dusíku a byla často důsledkem zhoršené výživy smrku po několik let.

Jednou z možností, jak ovlivnit biologickou aktivitu půdy a zpřístupňování dusíku, je změna mikroklimatických podmínek. Z tohoto důvodu jsme navrhli experiment, jehož cílem bylo stanovení biologické aktivity půdy po proředění 21letých porostů smrku na podzolech v Moravskoslezských Beskydech o 500 jedincích na hektar. Počet jedinců na hektar na hustším stanovišti byl 2 600 a na proředěném stanovišti 2 100. Na základě stu-

dia literatury jsme předpokládali, že proředění porostů povede ke zvýšení koncentrace amonného dusíku a ke snížení koncentrace nitrátového dusíku v půdě. Protože biologická aktivita půdy koreluje s obsahem amonného dusíku, předpokládali jsme její zvýšení na proředěném stanovišti.

Biologická aktivita půdy byla evaluována ve vzorcích odebraných třikrát během sezony 1999 (jaro, léto, podzim) ze všech horizontů půdních profilů na základě: laboratorních testů bazální respirace, glukózou a sójovou moukou indukované respirace, aktivity katalázy a intenzity rozkladu celulózy. Kromě biologické aktivity půdy byla stanovena aktuální koncentrace amonného a nitrátového dusíku a půdní reakce. Soubory hodnot naměřených

na obou plochách byly u každého atributu porovnány *t*-testy a v rámci každé plochy byly provedeny korelace mezi biologickými aktivitami, koncentracemi minerálního dusíku a půdní reakcí.

Statistická porovnání ukázala, že proředění mladých porostů smrku o 500 jedincích/ha nemělo signifikantní ( $\alpha = 0,05$ ) vliv na biologickou aktivitu a koncentraci minerálního dusíku v půdě. Na obou plochách byly zjištěny statisticky významné korelace ( $P < 0,05$ ) mezi intenzitou rozkladu celulózy, aktivitou půdní katalázy, intenzitou bazální nebo substrátem (glukóza/sójová mouka) indukované respirace půdy a aktuální koncentrací amonného dusíku.

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