Slit seeded grass-legume mixture improves coal mine reclamation

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ABSTRACT

Slit seeding (sowing into shallow cuts to the soil surface) was evaluated on coal mine reclamation in the North Bohemian Brown Coal Basin (Czech Republic) between 2001 and 2008, on plots both with and without biodegradable waste enrichment. Prior to experimentation (in 2000, once) were applied dehydrated, anaerobically digested wastewater sludge, 200 (41) t/ha fresh mass (dry mass), together with paper mill waste, 400 (131) t/ha, and bark substrate Envima, 400 (145) t/ha. Spontaneous herbaceous cover was enhanced each end of April from 2001 to 2003 by slit seeded grass-legume mixture containing *Festuca pratensis* L. (46%); *Lotus corniculatus* L. (22%); *Coronilla varia* L. (18%); *Festuca rubra* L. (9%); and *Medicago lupulina* L. (5%). All slit seeded species proliferated better on surface strip mine deposits without biodegradable waste enrichment and brought beneficial changes in a number of parameters over the ensuing five years due to lower competition with native expanding eutrophic species including *Calamagrostis epigejos* L.; *Cardaria draba* L.; *Urtica dioica* L.; and *Galium aparine* L. Available organic carbon (extracted from field-moist soil by 0.5 mol/L K_2SO_4) showed a notable tendency toward values 10–66 mg C/kg DM (dry mass), microbial biomass 302–1131 mg C/kg DM, basal respiration 3.9–5.8 mg C/kg DM/h, and metabolic quotient (basal respiration/microbial biomass carbon) × 1000 = 5.2–7.9.

Keywords: *Festuca pratensis* L.; *Lotus corniculatus* L.; *Coronilla varia* L.; wastewater sludge; paper mill waste; bark substrate; soil organic carbon; microbial biomass; respiration

Harris (2003, 2009), Růžek et al. (2003), Jasper (2007), Chodak et al. (2009), among other authors emphasized the beneficial role of soil microorganisms, especially mycorrhizal fungi and diazotrophs, particularly rhizobia, and recommended soil management in restoration to enhance the survival of soil biological components. The origin and quantity of carbon that enters the soil determines the necessary stabilization period of soil. The use of bark and combined paper sludge as a suitable plant growth substrate was investigated by Beauchamp et al. (2006). They found the most effective soil cover to consist of 2.5 cm of combined paper sludge on 2.5 cm black bark, seeded with birdsfoot trefoil. Birdsfoot trefoil in combination with smooth brome grass *Bromus inermis* L. gave good vegetation growth and yields. Tripathy et al. (2008), in a laboratory incubation study, found paper mill sludge combined with decomposed cow manure to produce no negative effects on soil microbial parameters even at the highest application rate. In connection with reclamation, crown vetch *Coronilla varia* L., meadow fescue *Festuca pratensis* L., and red fescue *Festuca rubra* L. are mentioned less often.

Gradual enrichment of spontaneous herbaceous cover by slit seeding (sowing into shallow cuts in the soil surface) of meadow fescue, birdsfoot trefoil, crownvetch, red fescue, and black medick in consecutive spring seasons, has not been exploited

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in reclamation. The main objectives of the present study were to compare microbial biomass and related microbiological parameters in soil treated with organic waste to soil that was untreated, as well as to compare plant growth on these soils, in both cases five years after a slit seeding program (each end of April from 2001 to 2003).

MATERIAL AND METHODS

Eight years after cessation of quarrying and technical reclamation in 2000, soil microbial parameters and herbaceous cover (Table 1) were assessed in three former strip mines (Slatinice, Most, and ČSA) at North Bohemian Brown Coal Basin. Thirty experimental plots (25 m × 25 m) were established and marked at the beginning of reclamation in spring 2001. Ten plots were located in each of three mines, five on areas treated with organic waste and five on untreated areas (Table 2). Soil sampling for microbiological analysis was conducted annually from 2001 to 2008 on the diagonals of the 30 squares. Botanical assessment in 2008 was based on a 20 m diameter circle, inscribed into the marked square plots. The total area for microbiological and botanical observations was 18 750 m^2. All experimental plots are located in the north-western Czech Republic at a mean elevation of 240 m a.s.l. The average annual precipitation is 499 mm, and the mean annual temperature is 8.2°C (Meteorological Station Kopisty, 50°55'N,

Table 1. More frequent species on slit seeded (sowing into shallow cuts to the soil surface) coal mine reclamations in the North Bohemian Brown Coal Basin (Czech Republic)

Species		Part by per cent on plots with enrichment[1]	Part by per cent on plots without enrichment
Meadow fescue	*Festuca pratensis*	10.4[2]	15.3[2]
Bushgrass	*Calamagrostis epigejos*	13.4[3]	7.6[3]
Birdsfoot trefoil	*Lotus corniculatus*	7.6[2]	19.1[2]
Crown Vetch	*Coronilla varia*	6.1[2]	15.3[2]
Whitetop	*Cardaria draba*	4.8[3]	0.4[3]
Rough-stalked meadow-grass	*Poa trivialis*	4.6[3]	0.0[3]
Stinging nettle	*Urtica dioica*	4.5[3]	0.0[3]
Cursed Thistle	*Cirsium arvense*	3.4[3]	0.8[3]
Tansy	*Tanacetum vulgare*	3.3[3]	0.8[3]
Kentucky bluegrass	*Poa pratensis*	3.1[3]	7.6[3]
Small tumbleweed mustard	*Sisymbrium loeselii*	3.1[3]	0.4[3]
Mouse-ear-chickweed	*Cerastium glutinosum*	3.1[3]	0.4[3]
False oat-grass	*Arrhenatherum elatius*	3.1 [3]	0.4[3]
Red fescue	*Festuca rubra*	3.1[2]	7.6[2]
Cleavers	*Galium aparine*	3.1[3]	0.0[3]
Sainfoin	*Onobrychis viciifolia*	3.0[3]	7.6[3]
Common yarrow	*Achillea millefolium*	3.0[3]	3.8[3]
Germander Speedwell	*Veronica chamaedrys*	3.0[3]	3.8[3]
Cinquefoil	*Potentilla reptans*	3.0[3]	0.0[3]
Dandelion	*Taraxacum officinale*	1.8[3]	4.2[3]
Creeping Soft Grass	*Holcus mollis*	1.6[3]	0.4[3]
Black Medick	*Medicago lupulina*	1.6[2]	4.2[2]
Corn Speedwell	*Veronica arvensis*	1.6[3]	0.0[3]
Dewberry	*Rubus caesius*	1.6[3]	0.4[3]
Purple Deadnettle	*Lamium purpureum*	1.5[3]	0.0[3]
Spear Thistle	*Cirsium vulgare*	1.5[3]	0.0[3]

[1]paper mill waste (400 t/ha FM), together with wastewater sludge (200 t/ha FM), and bark substrate Envima (400 t/ha FM) a once in 2000; [2]slit seeding each end of April from 2001 to 2003; [3]spontaneous establishment; FM – fresh mass

Table 2. Characteristic of reclaimed land at former strip mines of the Most Coal Company (North Bohemian Brown Coal Basin, Czech Republic)

Slatinice mine	Most mine	ČSA mine
The soil was clayey Anthrosol with slack admixture	The soil was sandy-clay Anthrosol	The soil was clayey Anthrosol
Ten plots (10 × 625 m^2) were selected in 2001	Ten plots (10 × 625 m^2) were selected in 2001	Ten plots (10 × 625 m^2) were selected in 2001
5 plots treated with bark substrate Envima[1] a once in 2000 and 5 plots untreated	5 plots treated with wastewater sludge, together with paper mill waste[2] a once in 2000 and 5 plots untreated	5 plots treated with bark substrate Envima a once in 2000 and 5 plots untreated
Initial soil chemical and textural properties (untreated plots) in the surface layer 0–200 mm		
A400/A600 6.6	A400/A600 6.8	A400/A600 5.3
C_{org} 2.7%	C_{org} 0.3%	C_{org} 2.4%
Nt 0.27%	N_t 0.04%	N_t 0.22%
pH (H_2O) 5.4	pH(H_2O) 7.8	pH(H_2O) 7.8
sand 12.6%[3]	sand 30.5%	sand 20.4%
silt 43.8%[3]	silt 38.3%	silt 46.1%
clay 43.6%[3]	clay 31.2%	clay 33.5%
Slit seeded grass-legume mixture[4] each end of April from 2001 to 2003	Slit seeded grass-legume mixture each end of April from 2001 to 2003	Slit seeded grass-legume mixture each end of April from 2001 to 2003
Mowed, and the crushed plant biomass (all produced) was left as mulch each first half of June and each first half of August from 2001 to 2008	Mowed, and the crushed plant biomass (all produced) was left as mulch each first half of June and each first half of August from 2001 to 2008	Mowed, and the crushed plant biomass (all produced) was left as mulch each first half of June and each first half of August from 2001 to 2008

[1]Envima (400 t/ha fresh mass); [2]paper mill waste (400 t/ha fresh mass), together with wastewater sludge (200 t/ha fresh mass); [3]ISO 11277; [4]included meadow fescue (13 kg/ha), birdsfoot trefoil (6 kg/ha), crownvetch, (5 kg/ha), red fescue (2.5 kg/ha), and black medick (1.5 kg/ha)

13°64'E). Prior to the study, in 2000, was made a single application of 400 (145) t/ha fresh mass (dry mass) of bark substrate Envima to a 400 ha area or even 200 (41) t/ha fresh mass (dry mass) of dehydrated anaerobically digested wastewater sludge, together with paper mill waste, 400 (131) t/ha fresh mass (dry mass) once to a 210 ha area. The total reclaimed land of 3500 ha, of which 2890 ha was untreated; 400 ha treated with bark substrate Envima and 210 ha treated with wastewater sludge, together with paper mill waste once in 2000, was mowed, and the crushed plant biomass was left as mulch each first half of June and each first half of August from 2001 to 2008. The area surrounding the designated sites is similar in terms of both botanical and microbiological parameters. In 30 selected areas, the initial vegetation cover formed by spontaneously spreading plant species was gradually enriched by slit seeding into shallow cuts in the soil surface a grass-legume mixture each end of April from 2001 to 2003. The mixture included meadow fescue *Festuca pratensis* L. (46%, 13 kg/ha), birdsfoot trefoil *Lotus corniculatus* L. (22%, 6 kg/ha), crownvetch *Coronilla varia* L. (18%, 5 kg/ha), red fescue *Festuca rubra* L. (9%, 2.5 kg/ha), and black medick *Medicago lupulina* L. (5%, 1.5 kg/ha).

Soil samples (characteristics in Table 3) were collected using the Eijkelkamp Agrisearch sampler equipment as a mixture of 10 sub-samples from the profile (0–200 mm) annually in October. Only the central area of each 25 m × 25 m experimental plot was used for soil samples collection. Soil samples were transported in a cooling box (temperature 6–12°C), sieved (mesh 2 mm) and stored in a refrigerator (4–6°C) for two weeks. Prior to biological analyses the samples were pre-incubated at room temperature (22 ± 2°C) overnight.

The following tests were used for soil characterization:

– pH(H_2O): 15 mL of deionized water (DW) and 3 g of a field-moist soil were shaken (60 min, 250 swing/min), and pH was determined with amplified electrode by Hanna.

Table 3. Basic parameters of slit seeded coal mine reclamations

Locality	Years	pH (H_2O)	C_{org} (%)	N_t (%)	C-K_2SO_4 (mg/kg DM)[3]	A 400/A 600 ratio[4]
Mine Slatinice without enrichment	2001–2004	5.6^A	2.85^B	0.28^B	143.0^C	6.8^A
	2005–2008	5.9^A	3.35^B	0.30^B	65.5^B	6.8^A
Mine Slatinice[1]	2001–2004	5.4^A	3.69^B	0.31^B	137.2^C	6.9^A
	2005–2008	6.1^A	3.85^B	0.32^B	115.1^C	6.2^A
Mine Most without enrichment	2001–2004	7.7^B	0.33^A	0.04^A	27.6^A	6.9^A
	2005–2008	7.6^B	0.83^A	0.07^A	10.2^A	7.4^A
Mine Most[2]	2001–2004	7.3^B	4.22^B	0.38^C	143.7^C	7.1^A
	2005–2008	7.4^B	4.64^B	0.40^C	126.4^C	7.6^A
Mine ČSA without enrichment	2001–2004	7.7^B	2.61^B	0.23^B	47.3^B	5.4^A
	2005–2008	7.2^B	2.84^B	0.25^B	46.9^B	5.9^A
Mine ČSA[1]	2001–2004	7.5^B	4.67^B	0.39^C	60.3^B	6.1^A
	2005–2008	7.6^B	4.82^B	0.41^C	36.1^B	6.0^A

[1]enrichment by bark substrate Envima (400 t/ha FM) once in 2000; FM – fresh mass; DM – dry mass of soil (105°C; 24 h); [2]enrichment by paper mill waste (400 t/ha FM) together with anaerobically digested wastewater sludge (200 t/ha FM); [3]available organic carbon extracted from field-moist soil by 0.5 mol/L K_2SO_4; [4]humus quality using ratio of absorbance of soil sodium pyrophosphate and sodium hydroxide extract (pH = 12.00) at wavelengths of 400 and 600 nm; [ABC]different characters indicate a significant difference (One–way ANOVA [Analysis of variance; Multiple range tests]; Scheffe's test; $P \leq 0.05$)

– Soil organic carbon C_{org}-MW (Islam and Weil 1998) was determined by microwave irradiation with colorimetric determination at 590 nm after overnight sedimentation. A field-moist soil sample (dry mass [DM] equivalent) from 0.03 g (high organic level) to 0.40 g (low organic level) was shaken in a 25 mL Erlenmeyer flask with 2 mL 0.34 mol/L $K_2Cr_2O_7$, followed by injection of 2 mL H_2SO_4 (96%), microwave digestion [1000 J/mL = 600 W, 60 s, 36 mL (9 × 4 mL)] and, after cooling, dilution with 20 mL DW. After overnight sedimentation, 5 mL of supernatant were diluted with 20 mL DW immediately before colorimetric C determination at 590 nm.

– Total Kjeldahl nitrogen (N_t) – European Standard EN 13342.

– Available organic carbon was extracted from field-moist soil samples by 0.5 mol/L K_2SO_4 (Růžek et al. 2009): non-sterilized (C-K_2SO_4), and heated (C-K_2SO_4-RHD). In all cases an equivalent of 7.14 g DM was placed in a 50 mL polypropylene vial (DigiTUBEs, SCP Science) and moistened with 2 mL DW. Nonsterilized soil samples (C-K_2SO_4) were immediately inverted and horizontally shaken (60 min, 250 rpm) with 20 mL of reagent at room temperature (22 ± 2°C). A similar procedure was followed with other samples (C-K_2SO_4-RHD) after heating in forced-air oven (24 h at 64°C). This was followed by sedimentation (20 min), centrifugation (2 mL, 3 min; 13 700 × g), and soil extract digestion in microwave oven Panasonic NE-9051 (800 J/mL; 250 W, 77 s, 24 mL), in the mixture (M): 400 mg $K_2Cr_2O_7$ in 10 mL DW, 50 mL H_2SO_4 (96%) and 20 mL conc. H_3PO_4 (12 × soil extract or blank [1 mL] and M [1 mL]). The samples were diluted with 10 mL DW immediately before colorimetric C determination at 590 nm.

– Microbial biomass carbon (MBC) was determined by re-hydration technique (Blagodatskiy et al. 1987) and calculated as:
MBC = (C-K_2SO_4-RHD – C-K_2SO_4)/0.250.

– Basal respiration (BR; mg C/kg DM/h): CO_2 is released by the soil sample after addition DW. 4.5 g (high organic level) up to 10 g (low organic level) of field-moist soil weighed into a 50 mL polypropylene vial (DigiTUBEs, SCP Science, Quebec, Canada), moistened with 1 mL DW, and placed in a rectangular waterproof polypropylene container (JETS 12; 1200 mL, Wipperfürth, Germany) with lid (Jokey Plastik). Support for two polypropylene vials side by side in an oblique position, the first with soil, the other with CO_2 absorbent (2.5 mL NaOH; 1 mol/L), provides a modified foam sponge

(Spontex 5 Megamax by SÖKE [105 × 75 × 35 mm]). The container was sealed and placed in controlled incubator (29°C) 20 h. Before titration with 0.1 mol/L HCl was added to 2.5 mL sodium hydroxide, 2 mL $BaCl_2$ (1 mol/L; 24.4 g/100 mL), and 5 drops phenolphthalein (1 mg in 1 mL of ethanol). The captured CO_2-C was calculated from the consumption of HCl (0.1 mol/L): 0.1 mL HCl consumed during titration corresponds to 0.06 mg CO_2-C.

- Potential respiration (NR) – CO_2 was released after addition of 0.4 mg N-$(NH_4)_2SO_4$/g field-moist soil dissolved always in 1 mL DW and applied to 4.5 g (high organic level) up to 10 g (low organic level) of field-moist soil.
- Potential respiration (GR) – CO_2 was released after addition of 4 mg C-glucose/g field-moist soil dissolved always in 1 mL DW and applied to 4.5 g (high organic level) up to 10 g (low organic level) of field-moist soil (ISO 16072).
- Metabolic quotient (qCO_2) was calculated as (BR/MBC) × 1000. Metabolic quotient expresses C-respired per gram of microbial biomass per h (ISO 16072).
- Humus quality (A 400/A 600) was determined by the ratio of absorbance of soil sodium pyrophosphate and sodium hydroxide extract (pH = 12.00) at wavelengths of 400 and 600 nm (Schnitzer and Khan 1972, Pospíšil 1981). A field-moist soil sample equivalent from 0.10 (high organic content) up to 1.00 g DM (low organic content) was horizontally shaken (60 min, 250 swing/min) with 20 mL of reagent (0.05 mol/L $Na_4P_2O_7 \times 10\ H_2O$ with NaOH [micro-pearl form], pH = 12.00) at room temperature (22 ± 2°C). Shaking (60 min, 250 swing/min) was repeated after 24 h, followed by addition of 10 mL 0.5 mol/L $Na_2SO_4 \times 10\ H_2O$, and centrifugation (4 × 2 mL, 3 min; 13700 × g immediately before colorimetric determination, 4 × 1 mL were diluted with 20 mL of reagent.

Statistical analyses were computed by Statgraphic Centurion XV software by one-way ANOVA (Analysis of variance; Multiple range tests); Scheffe's test; $P \leq 0.05$.

RESULTS AND DISCUSSION

Plant spreading. Biodegradable waste enrichment of surface strip mine materials supports growth and spread of many native expanding eutrophic ruderal species (Table 1) including bushgrass *Calamagrostis epigejos* L. (13.4%), whitetop *Cardaria draba* L. (4.8%), stinging nettle *Urtica dioica* L. (4.5%), small tumbleweed mustard *Sisymbrium loeselii* L. (3.1%), cleavers *Galium aparine* L. (3.1%) and others. In all cases, the application of high doses of biodegradable organic wastes (400–600 t/ha) brought the ratio C_{org}-MW/N_t from 11.1 to 12.0 and from 8.3 to 11.3 in the untreated sites (Table 3). This can be explained that the slit seeded grass-legume mixture was more successful on areas without biodegradable waste due to less competition from native expanding eutrophic ruderal species. Support for that opinion can be found in other authors. Dunker and Nordmeyer (2000) investigated correlations between weed abundance and soil properties. For *Galium aparine* L. they found a positive connection with clay and total nitrogen content. A change in soil conditions, which in turn led to transformations of the vegetation, is described by Dygus (2002); this confirms the invasion of *Urtica dioica* L. and other alien species after starch sewage treatment of forest soil had been ongoing sixteen years. On plots without biodegradable waste enrichment, bushgrass proliferated (7.6%), along with the slit seeded birdsfoot trefoil (19.1%), meadow fescue (15.3%), crownvetch (15.3%), and red fescue (7.6%). For the Slatinice mine is typical that bushgrass and whitetop expanded, regardless of the slack admixture in strip mine materials along with cursed thistle *Cirsium arvense* L. and Kentucky bluegrass *Poa pratensis* L. Experimental areas were characterized by alkaline soils except the Slatinice mine, where the slack admixture on the surface reduced pH to 5.5, regardless of high doses of the bark substrate Envima. Herbaceous cover at the Slatinice Mine in the years 2001–2008 included native expanding bushgrass (71%), whitetop (18%), cursed thistle (2%), Kentucky bluegrass (2%), and slit seeded red fescue (2%). Bushgrass is ideal succession dominant species (Prach and Pyšek 1999). It is a tall, wind-pollinated plant, often a geophyte capable of intensive lateral spread, requiring high nutrient supply and sufficient site moisture. Despite this conclusion, bushgrass proliferation is suppressed on untreated plots with slit seeded grass-legume mixture.

Soil characteristics. Soil organic matter carbon (C_{org}-MW) in the plots without biodegradable waste enrichment rose from 2.85% to 3.35% (Mine Slatinice); from 0.33% to 0.83% (Mine Most); and from 2.61% to 2.84% (Mine ČSA). Birdsfoot trefoil, meadow fescue, crownvetch, red fescue, and black medick are promising for plots without biodegradable waste enrichment. C_{org}-MW gradually increased regardless of biodegradable waste enrichment. Our conclusion is fully consistent with the results achieved by Anderson et al. (2008) on reclaimed soils at five surface coal mines located in semiarid regions of Wyoming. Across all mines, one half of reclaimed soils had, reflecting their ability to accumulate, soil organic C similar or greater than undisturbed soils. Use of wastewater sludge, together with paper mill waste (600 t/ha fresh mass), brought a gradual increase of C_{org}-MW from 0.33% to 4.22% (enrichment will be detectable over several decades through the extreme values of C_{org}-MW) but also a lack of physiologically available nitrogen for soil microbial communities (ratio NR/BR 1.5–1.4; Table 4). Nitrogen was observed in this study in two forms, as total Kjeldahl nitrogen (N_t; Table 3) and as nitrogen physiologically available for soil microbial communities within a respirometric test (ratio of NR/BR). A grass-legume mixture slit seeded each end of April from 2001–2003, in combination with mulching of all the mowed and crushed plant biomass produced (each first half of June and each first half of August from 2001 to 2008) brought a positive development, both in the case of C_{org}-MW and N_t (Table 3) and for the respiratory ratio NR/BR (Table 4). The negative development of the respiratory ratio NR/BR (from 1.3 to 2.0), brought only a gradual mineralization of the bark substrate Envima in the presence of slack admixture at Slatinice mine. The respiration ratio NR/BR revealed a deficiency in the nitrogen available to soil microbial communities. During the reclamation process, this ratio usually decreased to 1.3, and as low as 1.0 over the long-term in accordance with root development and exudation, especially of slit seeded plants (meadow fescue, birdsfoot trefoil, crownvetch, red fescue, black medick) and other spontaneously established plants. Surface strip mine enrichment by bark substrate, paper mill waste, and wastewater sludge after stabilization did not accelerate the decrease (Table 4), whereas slack admixture in surface materials tended to increase the ratio. Another ratio, GR/BR, reflected the respiration response to additional glucose-C. This ratio increased during the reclamation process up to a peak of 5.0 (Table 4), and in coming years could acquire even higher values (> 5.1).

The production of C-CO_2/h (BR) calculated on 1 g of MBC, called metabolic quotient (qCO_2), a

Table 4. Biological parameters of slit seeded coal mine reclamations

Locality	Years	BR[3] (mg C/kg DM/h)	NR/BR[4] ratio	GR/BR[5] ratio	MBC[6] (mg/kg DM)	(MBC/C_{org}) × 100 (%)	qCO_2[7] (BR/g MBC/h)
Mine Slatinice without enrichment	2001–2004	1.05[A]	1.5[A]	2.6[A]	458.5[A]	1.6[A]	2.3[A]
	2005–2008	3.87[A]	1.4[A]	4.2[A]	744.2[A]	2.2[A]	5.2[A]
Mine Slatinice[1]	2001–2004	1.15[A]	1.3[A]	2.2[A]	578.3[A]	1.8[A]	2.0[A]
	2005–2008	4.41[A]	2.0[A]	2.8[A]	772.2[A]	2.0[A]	5.7[A]
Mine Most without enrichment	2001–2004	0.86[A]	1.6[A]	3.8[A]	160.9[A]	4.9[A]	5.3[A]
	2005–2008	2.39[A]	1.3[A]	5.0[A]	302.3[A]	3.6[A]	7.9[A]
Mine Most[2]	2001–2004	2.05[A]	1.5[A]	4.6[A]	1479.1[B]	3.5[A]	1.4[A]
	2005–2008	8.82[B]	1.4[A]	5.0[A]	1410.0[B]	3.0[A]	6.3[A]
Mine ČSA without enrichment	2001–2004	1.66[A]	1.6[A]	3.2[A]	725.7[A]	2.8[A]	2.3[A]
	2005–2008	5.84[A]	1.4[A]	4.0[A]	1131.1[B]	3.9[A]	5.2[A]
Mine ČSA[1]	2001–2004	1.46[A]	1.6[A]	4.4[A]	908.3[A]	1.9[A]	1.6[A]
	2005–2008	5.92[A]	1.6[A]	4.6[A]	938.3[A]	1.9[A]	6.3[A]

[1]enrichment by bark substrate Envima (400 t/ha FM) once in 2000; FM – fresh mass; DM – dry mass of soil (105°C; 24 h); [2]enrichment by paper mill waste (400 t/ha FM) together with anaerobically digested wastewater sludge (200 t/ha FM) once in 2000; [3]basal respiration (BR; mg C/kg DM/h): CO_2 is released by the soil sample after DW addition; [4]potential respiration (NR) with ammonium sulfate: CO_2 released after addition of 0.4 mg N-$(NH_4)_2SO_4$/g field-moist soil; [5]potential respiration (GR) with glucose: CO_2 released after addition of 4 mg C-glucose/g field-moist soil; [6]microbial biomass-C determined after soil sterilization at 64°C (24 h) in forced-air ventilation oven and microwave soil extract digestion (800 J/mL; 250 W, 77 s, 24 mL); [7]metabolic quotient (CO_2 carbon released (mg/h) per gram of MBC; (BR/MBC) × 1000; [AB]different characters indicate a significant difference (One-way ANOVA [Analysis of variance; Multiple range tests]; Scheffe's test; $P \leq 0.05$)

frequently used soil bio-ecological indicator (ISO 16072) that is linked with development changes in coal mine reclamation and with the intensity of root growth, was distinctively influenced by the increase in rhizosphere soil in comparison with loose soil. The result was an increase of qCO_2 to 7.9 mg C/g MBC/h (Table 4) over the standard values for North Bohemian Brown Coal Basin Anthrosols (3.1 ± 2.3 mg C/g MBC/h (Růžek et al. 2006). The standard applies to all Anthrosols analyzed during 1991–2005, most without biodegradable waste enrichment.

Humus quality (A 400/A 600) has a significant correlation with the content of fulvic acids (Swift 1996). Larger A 400/A 600 values are associated with lower molecular weight (Christl et al. 2000). The observed range (5.4–7.6; 6.7 ± 0.9) confirmed relatively young strip mine reclamation (starting after the year 2000) without the use of deposited topsoil.

North Bohemian strip mine Anthrosols were characterized by a medium level of C-K_2SO_4 (48 mg/kg DM). The extreme values of 143.7 and 140.1 mg/kg DM (Table 3) were associated with application (in 2000) and partial mineralization of waste organic matter. Similarly MBC ranged from 160.9 to extreme value 1479.1 mg/kg DM (Table 4). For comparison, Anderson et al. (2008) in their study, which was conducted to determine the long-term influence (>10 years) of a number of reclamation management practices in semiarid regions of Wyoming in all cases without enrichment of biodegradable waste/substrate, came to comparable results with Most mine (untreated plots) in this study. Soil organic C in their case moved at five surface coal mines in profile 0–30 cm from 0.24% to 1.06%, MBC ranged from 45 to 510 mg/kg, ratio C/N 9.0–16.4 and pH 6.1–8.1.

The ratio of biomass C to soil organic C (MBC/C_{org}) is a bio-indicator of environmental changes in surface strip mine materials and a well-known criterion for evaluation. It represents the portion of metabolic active carbon located in microbial cells. In the present study, surface strip mine materials more than five years after reclamation achieved a ratio of 1.9–3.9%; however, in the early stages of the reclamation process at sites low in soil organic mass it was found to be as high as 4.9% (Table 4).

Birdsfoot trefoil is the most frequently mentioned seed in grass-legume mixtures used for slit seeding in connection with land reclamation (Evanylo et al. 2005). It is a suitable legume for the use on reclaimed surface soils enriched by biodegradable wastes at 20% of the makeup of the mix for slit seeding, and up to 30% on non-enriched soils. Crownvetch at similar proportions to that of birdsfoot trefoil was found to be an equally suitable legume for reclamation. Růžková et al. (2011) indicate mulched fallow with *Lotus corniculatus* L. and *Festuca pratensis* L. as a form of herbaceous cover which the best preserved of soil biological properties. The selection of plant species is critical for the successful establishment and long-term maintenance of vegetation on reclaimed surface mined soils (Evanylo et al. 2005). They studied growth of 16 forage grass and legume species (in monocultures and mixes) on reclaimed Appalachian mine soils amended with biosolids, applied at a rate of 368 t/ha dry weight, agreeing with our results with respect to birdsfoot trefoil and crownvetch. Crownvetch was among the species with the greatest persistence and biomass production after ten or more years, due to physiological and reproductive characteristics, low fertility requirements, and drought and moisture tolerance (Evanylo et al. 2005).

General suggestion. A grass-legume mixture slit seeded each end of April from 2001 to 2003 in combination with mulching of the all produced plant biomass mowed and crushed (each first half of June and each first half of August from 2001 to 2008) brought beneficial changes in microbiological parameters (Tables 3–4). Available organic carbon (C-K_2SO_4) showed a notable tendency toward 10–66 mg C/kg DM values. MBC achieved 302–1131 mg/kg DM. The ratio MBC/C_{org} increased to 2.2–3.9%. The respiration ratio NR/BR decreased to 1.3. Metabolic quotient increased to 5.2–7.9 mg C/g MBC/h.

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