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Effect of low-rank coal inoculated with coal solubilizing bacteria on edaphic materials used in post-coal-mining land reclamation: a greenhouse trial

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Abstract

Background: This study assessed, under greenhouse conditions, the use of low-rank coal (LRC) generated in the "El Cerrejón" (La Guajira Colombia) mine as a source of humified organic matter, which is released by the activity of coal solubilizing bacteria (CSB), in order to improve the properties of edaphic materials (EM) used in post-mining land reclamation processes.

Methods: In this trial, using pots with 10 kg of EM, the effect of LRC applications was tested, in contrast with applications of LRC inoculated with CSB at two different doses and EM without LRC. The responses of the cation exchange capacity (CEC), soil respiration, and fluorescein diacetate hydrolysis (FDA) were evaluated in each treatment. The contents of P and N and the dry weight were measured in maize seedlings. Soil microaggregates were observed with scanning electronic microscopy.

Results: After 12 months of treatment with LRC, an increase of 75 % was registered in the CEC, along with an increase of up to 59 % in the EM respiration (CO_2 production) and up to 50 % in the FDA activity. The LRC caused a significant increase in the dry weight and the total contents of N and P in the maize seedlings; the observations with the scanning electronic microscopy showed evidence of the formation of bigger aggregates, as compared to the control, and the presence of organic material on mineral particles in the treatments with LRC inoculated with CSB.

Conclusions: These results provided evidence that supports the use of LRC, alone or inoculated with CSB, as a humic amendment to improve the EM in soil reclamation processes (Technosols construction) after mining activities.

Keywords: Coal solubilization, Humic amendments, Humified organic matter, Technosols

Background

The "El Cerrejón" mine is the largest coal mining operation in Colombia and the second largest mine in the world, with an approximate influence area of 70,000 ha by the floodplain of the Rancheria river, in an area with warm, dry weather, and an aridity index of 0.4 (semiarid conditions). These conditions make it difficult to grow vegetation that would have a consequent contribution to the organic matter of an endogenous origin, which is important for post-mining land reclamation processes.

In open pit coal mining, the original soil is removed during the excavation phase, transported, and stored for several years in preservation banks, which drastically alter its properties, leading to a loss of the original characteristics, meaning the altered material is no longer considered soil and is instead called "edaphic material" (EM).

Once the coal extraction is carried out, backfilling of the excavated area is done using the rubble of the excavated rock material and the soil reconstruction phase starts; this type of constructed soil is called "Technosol" [1, 2]. This process entails land reconfiguration; the

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compacted surfaces are plowed and a new soil configuration (Technosol) is made with the superficial placement of the EM that was stored in preservation banks, forming uniform layers (similar to topsoil), on which a set of required management activities are carried out to ensure the growth of plant cover and the stabilization of a new Technosol. This process requires cementing agents and organic matter to group the edaphic materials and generate aggregation and structuring of the soil.

In this process, the presence of humified organic matter (HOM) is a key factor, which facilitates the physical reclamation of the soil [3], stimulates the activity of microbial communities [4], and promotes physiological processes in plants [5]. Under the semiarid conditions in the El Cerrejón mine area, in addition to the absence of vegetation that would provide organic matter, the lack of HOM is one of the most limiting factors in the initial stage of the land reclamation of this mine. Therefore, it is useful to adapt strategies to increase the content of HOM in the first stages of soil reclamation.

Low-rank coal (LRC) is a slightly evolved kind of coal that is generated as a by-product of open pit coal mining. This material has a high content of HOM [6] and has been recommended as a suitable raw material for the production of humic amendments for soils [7]. In a previous study, it was found that, in environments exposed to carbonaceous wastes that come from the extraction, transport, storage, and washing of coal in the El Cerrejón mine, there are bacterial populations that are able to biotransform the LRC and to release humic substances (HS) by solubilizing these materials [8]. Also, native strains of coal solubilizing bacteria (CSB) were selected and identified, which are able to grow in culture media with LRC as the sole carbon source and solubilize this material, generating HS [9].

Traditionally, the treatment of soils with organic amendments is done with direct applications of humic extracts obtained by alkaline treatments of raw materials or by applying easily released HOM-enriched materials such as compost, biosolids, or peat materials. These options are not technically or economically feasible for increasing the HOM content of EM used to rehab soil after coal extraction in the "El Cerrejón" mine, since in these semiarid conditions these organic raw materials are missing; however, LRC is an available by-product of mining, but no studies have demonstrated the effects of using this material as a humic amendment applied directly to the soil. Since there are native strains of CSB that transform LRC and release HOM, the following hypothesis was proposed: if LRC is inoculated with CSB and applied to EM, the CSB will help release HOM and, consequently, this HOM will improve the properties of the EM, resulting in similar changes to those commonly observed when soils are treated with HS that are obtained by the conventional method of alkaline extraction, which are related to the CEC, aggregate formation, stimulation of microbial activity, and changes in the root system of plants that result in better growth.

Therefore, in the present study, a trial was designed under controlled greenhouse conditions in order to conduct a preliminary study on some effects of applying a lignite-type LRC inoculated with a microbial pool composed of three strains of native CSB on EM used for post-mining land reclamation in the "El Cerrejón" mine, using the LRC as a source of HOM and the CSB as an accelerating agent in the release of the coal HOM. This study aimed to determine some parameters for use in future field trials in the land rehabilitation area associated with the El Cerrejon mine.

Methods

Edaphic material (EM)

Preserved EM was used, taken from the bank for the "Tajo Patilla" pit, located at 11°05′46″ N and 72°40′46″ W in the "El Cerrejón" coal mine. The EM of this bank came from soils removed for mining intervention, corresponding to Fluventic haplustolls—Aridic lithic haplustolls and Aridic haplustalfs—Aridic Natrustalfs associations, which have moderate deep soils with moderate fine textures that are very well-drained; they show a moderate alkaline reaction, high to medium cationic exchange capacity, and moderate fertility. The EM was collected at the original storage site of the mine, transported to the greenhouse, and sieved with a 2-mm-diameter sieve in order to obtain a homogenous EM. Afterwards, samples were taken to determine the initial characteristics. The general characteristics of this EM were silt loam texture, 7.8 pH, low organic carbon content (0.36 %), low phosphorus content (2 ppm), low nitrogen content (0.035 %), high $CaCO_3$ content, and high base saturation (51.6; 5.2; 0.33; 0.5 cmol (+) kg^{-1} of Ca, Mg, K, and Na, respectively).

Low-rank coal (LRC)

A lignite-type LRC was used, generated in the "El Cerrejón" mine, which had a low heating value (3781 kcal kg^{-1}), 28.5 % moisture and contents of C (46.04 %), H (3.26 %), O (46.95 %), N (1.38 %), and S (0.13 %). This LRC was also previously used as a substrate for the bacterial activity and growth in the process of isolation and screening of CSB [8]. The diagnostic characteristics, HS content, and the extent of their biotransformation by activity of the microbial strains in this study were determined by Valero et al. [9].

The LRC had a NaOH-extractable HS (0.5 N) content of 45 %. The carbon contents in the total humic extract, humic acid carbon, and fulvic acid carbon were 32.91, 24.31, and 8.6 %, respectively. In vitro, the CSB used in this study had a LRC transformation capacity up to 37 % in 8 days, with the consequent HS release [9]. On the other hand, the main macromolecular characteristics of the LRC and the supramolecular characteristics of both the humic acids fraction (HA) obtained with the CSB activity and the HA obtained with the classic alkaline extraction method with NaOH were determined and compared [10]; Table 1 contains the results of the quantitative analysis ^{13}C-CPMAS-NMR, which determined the relative content of the major functional groups. For the different analyses, the LRC was sieved to obtain particles that were less than 5 mm in size in order to have uniform material sizes. The contents of As, Co, Pb, V, Cu, Zn, Ni, Cr, B, Mo, and Cd were determined following the standard methods of the American Section of the International Association for Testing Materials–ASTM (finding amounts of 0.71, 2.31, 1.73, 1.66, 0.55, 22.43, 3.35, 2.4, 15.11, 2.52, and 0.08 ppm, respectively) in order to estimate the toxicity risks from heavy metals in LRC applications on soils, which, considering these low values is negligible.

Coal solubilizing bacteria (CSB)

A mixed microbial inoculum, composed of biomass from the three bacterial strains previously isolated from the microenvironments influenced by LRC particles and carbonaceous wastes in the El Cerrejon mine, was developed. These microorganisms were selected because they exhibit greatest coal solubilizing activity in both solid and liquid media, in vitro, corresponding to strains of *Bacillus mycoides*, *Acinetobacter baumannii*, and *Microbacterium* sp. [9]. Beforehand, a lack of antagonism was confirmed in engagement trials using dual growth in Petri dishes. In addition, the survival of each CSB strain was observed in the EM mixed with LRC (data not shown).

The greenhouse trial

The experiment was conducted in a greenhouse with controlled climatic conditions, using an automated system and light (Licor PAR sensor—Lincoln Nebraska USA), temperature and moisture sensors; these sensors were connected to a SCADA system that periodically recorded the conditions. The temperature was between 28 and 32 °C; the relative humidity was between 65 and 80 %; and the photoperiod was 12/12 h (light-darkness). A completely randomized experiment was designed with 10 treatments (Table 2), each one with 10 repetitions. The experimental unit consisted of 10 kg of EM in plastic containers with a 15-cm diameter and 30-cm height. Two doses of LRC were assayed (0.1 and 1 %) and each one was added on the EM, alone or mixed with the CSB inoculum; for the latter, the inoculum of each bacterial strain was mixed with the LRC at 1×10^8 CFU g^{-1} LRC. Before the trial was definitively established, preliminary studies were conducted under the same conditions and using the same materials in order to determine the CSB dose for the evaluations, which included doses between 0.1 and 5 % of LRC and which resulted in the selection of the 0.1 and 1 % doses.

To evaluate the effect of the plants (P), 5 pre-germinated yellow maize seeds from the V109 ICA variety were sown in pots from trials 2, 3, 4, 9, and 10 (Table 2). This maize variety was used because it is adapted to the warm conditions of the dry Colombian Caribbean. Moderate watering was applied three times a week, maintaining an approximate EM moisture of 50 % of the field capacity in order to avoid excessive water content; this condition was maintained until the end of the experiment. The maize plants were removed from the pots (by carefully cutting the shoots, avoiding disturbance of the EM) every 3 months and new pre-germinated seeds were planted in order to maintain the continuous growth of plants in the system.

After 3, 5, and 12 months, three experimental units of each treatment were randomly taken in order to

Table 1 Relative distribution (%) of area under the curve of the chemical shift in the ^{13}C-CPMAS-NMR spectra of LRC and three HA obtained through CSB activity in comparison to HA obtained through alkaline extraction with NaOH

HA-sample	C. carboxylic	C. phenolic	C. aromatic	C in carbohydrates and Di-*O* alkyl	C. *N*-alkyl methoxy	C. aliphatic	Aromaticity index
HA-CSB *A. baumannii*	17.37	5.72	35.51	11.10	7.55	22.74	0.41
HA-CSB *B. mycoides*	17.98	5.80	36.23	10.20	7.58	22.22	0.42
HA-CSB *Microbacterium sp*	17.37	6.86	41.67	10.71	5.90	17.49	0.49
AH-NaOH	18.27	10.64	48.71	6.75	3.93	11.69	0.59
LRC	11.83	8.14	48.06	8.55	3.05	20.37	0.56

Table 2 Description of the treatments used to evaluate the effect of LRC and CSB on an edaphic material used in post-mining land reclamation

Treatment	Name	Description
1	EM	Absolute control
2	EM + P	Relative control of the plants effect on the EM
3	EM + P + CSB	Relative control of the plants and CSB inoculum effect
4	EM + P + LRC 1 %	LRC (high doses) effect on EM in presence of plants
5	EM + LRC 1 %	LRC (high doses) effect on EM in absence of plants
6	EM + LRC 0.1 %	LRC (low doses) effect on EM in absence of plants
7	EM + CSB + LRC 1 %	Effect of LRC (high doses) mixed with CSB inoculum, in absence of plants
8	EM + CSB + LRC 0.1 %	Effect of LRC (low doses) mixed with CSB inoculum, in absence of plants
9	EM + P + CSB + LRC 1 %	Effect of LRC (high doses) mixed with CSB inoculum, in presence of plants
10	EM + P + CSB + LRC 0.1 %	Effect of LRC (low doses) mixed with CSB inoculum, in presence of plants

EM edaphic material, *P* plants (maize), *CSB* inoculum of coal solubilizing bacteria, *LRC* low-rank coal

determine the edaphic respiration, enzymatic activity, pH, and cationic exchange capacity (CEC). The EM respiration was determined using the respiration chamber method, as described by Alef and Nannipieri [11], with modifications; to do this, a glass container with 10 ml of NaOH 0.5 N was placed on the container surface and covered with a larger inverted bottle which acted as a bell respiration chamber, buried at a depth of 3 cm to minimize CO_2 diffusion over the edge; after 48 h, the bell was removed, the reaction was stopped with a $BaCl_2$ 0.5 M solution, and the remaining NaOH was titrated with HCl 0.5 N using phenolphthalein as the pH indicator; primary standards were used to standardize the HCl and NaOH. The results were expressed in terms of the EM surface covered by the respiration bell. The CEC was determined by saturation with the 1 N ammonium acetate method [12]. The EM microbiological activity was determined with the fluorescein diacetate enzymatic hydrolysis (FDA) method, using the optimized protocol for soil samples developed by Green et al. [13]; the microbiological activity was expressed in terms of the amount of fluorescein (mg/kg of EM/h) produced through FDA enzymatic hydrolysis.

In each of the samples (3, 5, and 12 months), the survival of the inoculum in the EM was verified. This was done by dilution of 10 g of EM in saline solution (0.85 % NaCl) and subsequent culture in petri dishes on minimum salt agar supplemented with LRC as the sole carbon source (selective condition for CSB growth) [8]. The presence of bacterial colonies was determined, which had the following morphological markers of each strain: (1) *Microbacterium* sp. (green-yellow pigment and typical microscopic morphology), (2) *Bacillus mycoides* (typical colony morphology and microscopic morphology), and (3) *Acinetobacter baumannii* (positive oxidase test, no fermenting colonies, paired cells in microscopic observations). It was determined that the inoculum had survived if colonies with the typical morphology of each strain were recovered that grew using LRC as the sole carbon source.

At the end of the trial, the maize plants were extracted from each treatment and the dry weight and P and N contents were determined for each plant according to the protocols of the Colombian soil laboratory of the Agustin Codazzi Geographic Institute, which are based on Soil Survey Laboratory Methods (US Department of Agriculture) [12]. EM samples of 100 g, without roots segments, were taken from each pot, handled carefully so as to not cause compaction or any other disturbance that would alter the aggregates; these samples were air dried for 48 h and then sieved to obtain aggregates that were less than 2 mm in diameter and a new sample of 1 g was taken that was sieved through a 400 sieve (Tyler). With these samples, observations were made with scanning electronic microscopy (SEM) to describe the changes in the microaggregate formation in order to note the bacterial presence and fragments of organic matter that act as aggregating agents of mineral particles. The analysis was performed with a JOEL JSM 5910 LV model; the samples were pre-plated with 2 nm colloidal gold particles; the equipment was operated in the high vacuum mode at 10 and 15 kV and a reading distance of 10 mm.

The data obtained for each of the evaluated quantitative variables were subjected to an ANOVA analysis and a mean comparison test after an analysis of normality of the data. SPS statistical software, version 18, was used.

Results

pH and cation exchange capacity (CEC)

During the 12 months of the experiment, the EM pH values in all of the treatments ranged between 7.7 and 8; there were no significant changes in any treatment from the initial EM pH (7.8).

Table 3 shows that the CEC had significantly higher values (DMS, $\alpha = 0.05$) in all of the measurements for the treatment group with the addition of the high-dose LRC (1 %). The relative control treatments (EM + P and EM + P + CSB) did not have significant differences that were greater than the absolute control (EM) in any of the measurements. The treatment group with the addition of the low-dose LRC (0.1 %) did not show differences in comparison with the control treatments (EM, EM + P, and EM + P + CSB) at 3 and 5 months; but, at 12 months, the differences were more noticeable, especially with the EM + P + CSB + LRC 0.1 % and LRC 0.1 % treatments, whereas in the high-dose LRC treatments, the increase in the CEC was high starting at 3 months.

Edaphic respiration

After 3 months of the experiment, some significant differences were observed, as compared to the absolute control treatment (EM), in the three treatments with the addition of 0.1 % LRC and the treatment with the addition of 1 % LRC for the presence of plant and microbial inoculum (EM + P + CSB + LRC 1 %) (Table 4).

At 5 months, a high edaphic respiration was noticed in all of the treatments except the absolute control treatment (EM) and treatment with 0.1 % LRC + CSB in the absence of plants; an increasing respiratory activity was found in the three treatments with 1 % LRC without CSB and 0.1 % LRC without microbial inoculum or plants. There were no significant differences between the four treatments that had the greater effect, but there were differences between these treatments and the others ($P < 0.05$, Tukey test), except for the EM + P + CSB + LRC 0.1 % treatment.

Table 3 CEC [cmol (+)/kg soil] at 3, 5, and 12 months in EM treated with LRC and CSB inoculum

Treatment	Cation exchange capacity [cmol (+)/kg soil]		
	3 months	5 months	12 months
EM	11.6 ± 0.7 a	11.6 ± 0.1 a	11.5 ± 0.3 a
EM + P	11.9 ± 0.6 a	11.9 ± 0.3 a	12.1 ± 0.4 ab
EM + P + CSB	11.9 ± 0.3 a	11.6 ± 0.2 a	12.2 ± 0.5 ab
EM + P + LRC 1 %	17.8 ± 0.3 c	16.7 ± 1 c	19.3 ± 0.7 c
EM + LRC 1 %	16.7 ± 1.3 c	14.6 ± 0.5 bc	18.9 ± 0.6 bc
EM + LRC 0.1 %	11.8 ± 0.2 a	11.9 ± 0.2 a	13.2 ± 0.5 ab
EM + CSB + LRC 1 %	19.4 ± 1.9 c	16.9 ± 1.8 c	17.1 ± 1.2 c
EM + CSB + LRC 0.1 %	12.6 ± 0.0 ab	11.4 ± 0.1 a	12.4 ± 0.9 ab
EM + P + CSB + LRC 1 %	19.9 ± 0.2 c	15.3 ± 0.4 c	20.1 ± 1.1 c
EM + P + CSB + LRC 0.1 %	12.2 ± 1 ab	12.0 ± 0.3 ab	14.2 ± 0.7 b

$n = 3$, ±SD = standard deviation

Treatments with different letters show significant difference (LSD, $\alpha = 0.05$)

Table 4 Edaphic material respiration (mg CO_2 m^2 day) at 3, 5, and 12 months after treatment with LRC and CSB inoculum

Treatment	Edaphic respiration(mg CO_2 m^2 day)		
	3 months	5 months	12 months
EM	4477 ± 918 a	4193 ± 1771 a	3309 ± 193 a
EM + P	4676 ± 365 a	6589 ± 1057 bc	3781 ± 640 ab
EM + P + CSB	5373 ± 262 abc	7388 ± 765 bc	4077 ± 447 ab
EM + P + LRC 1 %	5191 ± 553 abc	10.084 ± 1100 bcd	5140 ± 404 cd
EM + LRC 1 %	4511 ± 891 a	10.483 ± 998 cd	4963 ± 610 bcd
EM + LRC 0.1 %	5821 ± 608 bcd	9185 ± 326 c	4550 ± 524 bc
EM + CSB + LRC 1 %	4511 ± 753 abc	6390 ± 1756 abc	5081 ± 453 bcd
EM + CSB + LRC 0.1 %	6036 ± 140 cd	4393 ± 1031 ab	4491 ± 273 bc
EM + P + CSB + LRC 1 %	6209 ± 636 bcd	10.483 ± 1542 cd	5259 ± 226 d
EM + P + CSB + LRC 0.1 %	5622 ± 379 bcd	7688 ± 2337 bcd	4727 ± 386 bc

Treatments with different letters are significantly different (Tukey, $\alpha = 0.05$)

$n = 3$, ±SD = standard deviation

At 12 months, a general decline in respiration was noticed, with values close to those recorded at 3 months; this decline can be explained as a result of the normal decline of plants in an experiment confined to pots under greenhouse conditions. However, at 12 months, a trend in the stabilization in the response was observed, with all of the treatments with LRC at 1 and 0.1 % showing highly statistically significant differences ($P < 0.05$, Tukey test) in contrast to the three treatments used as controls (EM, EM + P, and EM + P + LRC); the high values corresponded to the four treatments with additions of LRC at 1 % (high dose).

Microbiological activity (FDA hydrolysis)

At 3 months, three of the four treatments with additions of LRC at 1 % (EM + LRC 1 %, EM + LRC 1 % + CSB and EM + LRC 1 % + P) had a higher microbiological activity with significant differences ($P < 0.05$, Tukey test), as contrasted with all of the other treatments; the other treatments did not show significant differences as compared to the controls. At 5 months, all of the treatments, except treatments (EM + LRC 0.1 % and EM + LRC 0.1 % + CSB), showed significant differences ($P < 0.05$), as contrasted with the absolute control (EM); the four treatments with 1 % LRC showed the highest activity, with no significant differences between them. At 12 months, the activity declined, but the trend for a greater activity was maintained in the treatments with 1 % LRC additions, without a significant difference between them, but with a significant difference from the controls (Table 5).

Table 5 Microbiological activity of EM (mg fluorescein. kg soil^{-1} h^{-1}) at 3, 5, and 12 months after application of LRC and CSB inoculum in the presence and absence of maize plants

Treatment	Microbiological activity of EM (mg fluorescein. kg soil^{-1} h^{-1})		
	3 months	5 months	12 months
EM	8.4 ± 1.9 a	8.1 ± 0.6 a	8.2 ± 1.2 ab
EM + P	11.4 ± 2.5 ab	10.8 ± 1.2 b	8.3 ± 0.5 ab
EM + P + CSB	13.5 ± 2.5 b	10.8 ± 1.0 b	9.0 ± 0.6 abc
EM + P + LRC 1 %	10.8 ± 1.8 ab	14.7 ± 1.6 c	10.7 ± 1.2 bcd
EM + LRC 1 %	25.6 ± 3.5 d	14.9 ± 4.6 c	12.3 ± 1.5 d
EM + LRC 0.1 %	10.7 ± 2.2 ab	10.3 ± 1.8 ab	8.0 ± 0.5 ab
EM + CSB + LRC 1 %	18.7 ± 2.2 c	12.7 ± 0.9 c	12.7 ± 1.3 d
EM + CSB + LRC 0.1 %	9.4 ± 1.4 a	10.3 ± 1.8 ab	7.8 ± 0.7 a
EM + P + CSB + LRC 1 %	18.8 ± 2.8 c	15.2 ± 3.2 c	11.5 ± 0.8 cd
EM + P + CSB + LRC 0.1 %	13.8 ± 3.3 b	10.7 ± 1.5 b	9.5 ± 0.3 abc

Treatments with different letters are significantly different (Tukey, $\alpha = 0.05$)

$n = 3$, ±SD = standard deviation

Observation of aggregates with scanning electronic microscopy (SEM)

Figure 1 has two detail levels of the aggregates in the control treatment and the treatments with the addition of high doses of LRC, with and without plants and CSB. In observations between the 85× and 100× magnification, evidence of larger aggregate formation in the LRC treatments in comparison with the absolute control treatment (EM) was observed.

In the pictures with 1000× and 2000× magnification, details of the aggregate surfaces can be observed in the control treatment (EM), with smooth surfaces of the larger particles and the presence of smaller particles and amorphous materials, with no significant organic matter fragments. In the EM + P + LRC1 % + CSB and EM + LRC1 % + CSB treatments, the mineral particles covered with organic materials and organic fractions that were joined to smaller particles were observed; these materials probably came from the LRC particle microbial biotransformation, from the spontaneous release of HS in the LRC particles and plant root exudates; also, the presence of bacterial cells on the surfaces was observed. In the EM + P + LRC1 % treatment, a large quantity of uncovered interstices and a lesser quantity of materials and organic fractions and bacteria were also observed.

Dry weight, N, and P contents in the maize seedlings

A high dry weight was seen in the plants, corresponding to the treatment with high doses of LRC. On the other hand, the foliar values of P and N in the treatments were significantly lower in relation to the ones with an addition of LRC; however, the total amounts of P and N assimilated into the biomass were higher in these treatments (EM + P + LRC 1 % and EM + P + LRC 1 %) (Table 6). Furthermore, qualitative observations of the freshly harvested plants showed a higher development of the root system and larger plants in all of the treatments with additions of LRC and LRC + CSB.

In addition, at 3, 5, and 12 months, all of the EM samples that had the addition of CSB inoculum had bacterial colonies that showed typical morphological markers for the three strains that made up the CSB inoculum, and also had the ability to grow in the culture medium containing LRC as the sole carbon source; however, it was not possible to count each of the strains due to the complexity of the growth type on the Petri dishes.

Discussion

Evidence was found that there was an increasing CEC in the EM that depended on the concentration of the applied LRC, as observed in the results that demonstrated a higher CEC in the treatments with a high dose of LRC; this fact may be related to the release of HS in the edaphic environment, which acts as a polyelectrolyte, or to the LRC properties themselves because this material has numerous cation exchange sites due to the presence of high quantities of phenolic and carboxylic groups [6], as can also be seen in Table 1. The treatments without addition of LRC (EM + P and EM + P + CSB) had no difference in the CEC with respect to absolute control (EM), indicating that the presence of LRC can increase the CEC. The CEC is a property directly related to the amount of soil organic matter, especially the humic fraction, which is involved in the formation of the clay-humic complex that helps to improve a soil's ability to retain cations that are important for plant nutrition and, subsequently, facilitates assimilation by the roots, meaning an increase in the CEC is favorable for the establishment of vegetation in soil reclamation processes. In addition, the CEC is a property that naturally changes slowly in soils. They are incorporated as fractions of HOM that are important and beneficial for the establishment of vegetation under aridity conditions, high temperatures, and zero contribution of endogenous organic matter as found in the post-mining soil reclamation area of the El Cerrejon mine, so that the fact trigger an increase in the CEC after an addition of LRC, even if the increase is small is important in these boundary conditions.

Edaphic respiration is considered a strong parameter as an indicator of the total metabolic activity of soil microorganisms [14]. This parameter changes in response to the physical and chemical conditions that allow the development of microbial metabolic processes and its action on organic substrates. In this sense, the assessment result

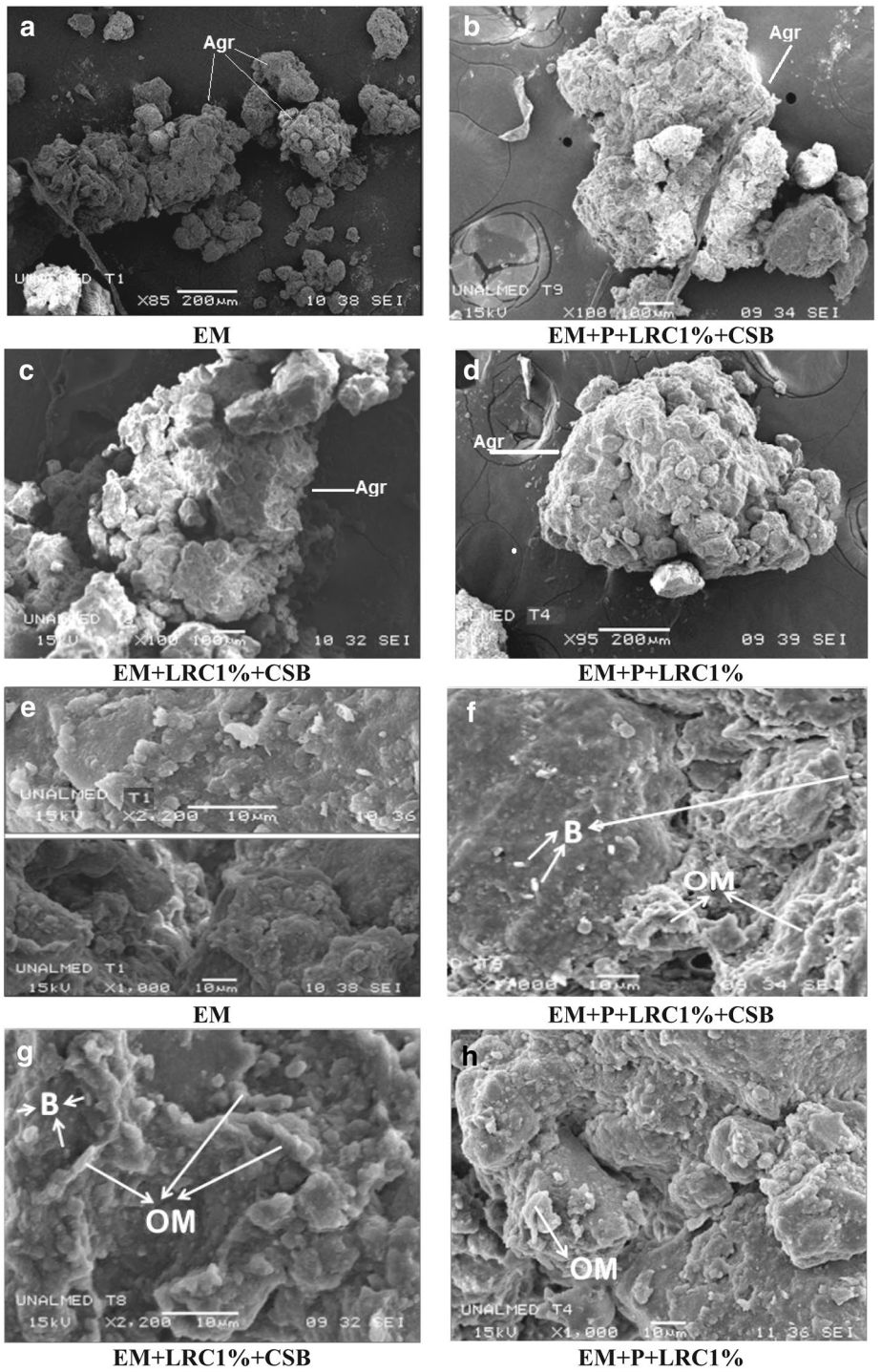

Fig. 1 Scanning electronic microscopy images of EM aggregates with an addition of LRC and CSB. **a**–**d** (85–100× magnification), **e**–**h** (surfaces of aggregates detail: 1000–2000× magnification). *Agr* soil aggregates, *B* bacteria, *OM* organic materials

of the respiration in this study suggests a stimulus of the microbial activity due to the LRC effect, even in the absence of organic inputs from the plants. Moreover, the short-term increase of soil respiration due to the addition of LRC was noticeable, but the effect was apparently independent of the presence of plants and the CSB

Table 6 Dry weight and contents of N and P in plants of Z. *mays*, grown in an EM with applications of LRC and CSB inoculum

Treatment	Dry weight (g)/plant	% foliar N	% foliar P	Total N (mg)/plant	Total P (mg)/plant
EM + P	3.53 ± 1.6 a	1.6 ± 0.3 b	0.14 ± 0.02 b	56.5	5.1
EM + P + CSB	2.71 ± 1.2 a	1.5 ± 0.1 b	0.14 ± 0.01 b	40.6	3.8
EM + P + LRC 1 %	6.79 ± 1.2 b	1.2 ± 0.1 a	0.12 ± 0.01 a	81.5	8.2
EM + P + CSB + LRC 1 %	5.15 ± 0.6 ab	1.2 ± 0.1 a	0.12 ± 0.006 a	61.8	6.0

Treatments with different letters show significant difference (LSD, $\alpha = 0.05$)

$n = 3$, ±SD = standard deviation

microbial inoculum. This result can be explained by the high surface area and porosity of LRC [15] because they are characteristics that help aeration and water retention, promoting a favorable habitat for the performance of the microbiota. At 12 months, a trend of increased respiratory activity in the treatments with an addition of LRC was observed, indicating a possible effect due to the HS that was slowly released by the action of the CSB, which contributed to the formation of aggregates in the soil [16] and stimulated the biological activity.

Aggregation is a soil property that changes slowly and the formation of new aggregates depends on binding agent that cement the mineral particles, these binding agents may be inorganic, organic or biological in origin. The HS, along with microbial waste, are the principal microaggregate forming agents, while the formation of these aggregates provide a surface for microbial biofilms, established on the surface of the newly formed aggregates, these new aggregates also allow the generation of micropores that favor water retention [17]. As a result and given the evidence of aggregate formation from the SEM images (Fig. 1), in which fractions of organic materials were observed that acted as binding agents in the treatments with the addition of LRC, it can be concluded that the release of the HOM contained in the LRC occurred, which allowed for the formation of aggregates and, consequently, the stimulation of the establishment of the microbiota on the surface of the new aggregates; however, this type of aggregation can be considered temporary, so it requires more time for the consolidation of stable aggregates.

The soil microbiological activity includes all cellular metabolic reactions, their interactions and the biochemical processes that are intervened on or made by soil microorganisms; in this sense, the FDA hydrolysis trial measured the enzymatic activity of the microbial populations, but it is considered non-specific because it is sensitive to the activity of several kinds of enzymes, including lipases, esterases, and proteases [18]. It is useful because it can provide an estimate of the total microbial activity in an environmental sample [13]; therefore, in this case, it is a valid indicator to infer changes in the microbiological activity caused by the treatment of EM with LRC as a humic amendment. Therefore, the results of the FDA hydrolysis trial suggested that 1 % LRC stimulated the microbial activity in the soils, but its effect could be independent of the presence of CSB and plants, which reinforces the idea that its action is due to a physical effect of LRC on the soil; therefore, this effect provides better conditions for the microbial activity, possibly due to the aggregate effect of the HS, as previously discussed.

Other studies have shown that the hydrolysis of FDA is an effective indicator of the soil quality in degraded areas in restoration processes [19, 20] due to the fact that FDA assays and soil respiration are biochemical standards that change rapidly and show sensitivity to stress and soil reclamation [21].

HS not only act on the soil but also on the root system of plants, generally resulting in more branching and longer roots, and stimulate the metabolic activity of plants, inducing a better assimilation of nutrients [5] [22].The difference in the dry weight of the maize plants (Table 6) indicates that the addition of LRC to the EM stimulated plant growth. This result suggests that the HS contained in the LRC possibly had a physiological effect on the plants. According to this, the HS bioactivity effect (like the auxin effect) on the maize plants has been documented; this results in metabolic activity stimulation and the proliferation and elongation of roots, leading to general growth and increased primary and secondary metabolisms [5, 23–25].

However, in this experiment, despite the increase in the dry weight of the plants that grew in the EM treated with 1 % LRC and a greater amount of P and total N absorbed by the plants, the % of P and N in the tissues of these plants was lower when compared with the plants not treated with an LRC addition (Table 6). This can be explained by the low contents of P and N in the EM (2 ppm of P and 0.035 % of N), which means that the stimulation of plant growth in the treatments with 1 % LRC resulted in a more abundant root system and in greater nutrient uptake, but, due to very low

concentrations of P and N in the substrate, their quantities were not reflected in a higher concentration in the larger plant tissues. These results were consistent with the "microbial loop" model described by Puglisi et al. [4], which says that the HS in contact with the roots causes stimulation in plant physiology (like the auxin effect), which produces plant cell elongation and the development and increase of the photosynthetic rate; therefore, the plants probably increased the release of root exudates. A greater amount of exudates in the rhizosphere also stimulates the beneficial activity of plant-growth promoting microorganisms [5].

Given the fact that the microbiological activity in the EM increased as a result of treatment with LRC, we might think that, within the microbial population, plant-growth promoting microorganisms, such as nitrogen fixing or phosphate solubilizing, can be stimulated; however, to elucidate these aspects, it is necessary to design new experiments aimed at monitoring this activity after treatment of EM with LRC.

This experiment aims to explain whether changes in a set of EM variables can be indicators of the effect of HOM resulting from the addition of LRC inoculated with a consortium of CSB. The fact that bacterial colonies growing on minimal salt agar with LRC as the sole carbon source, with the typical morphological characteristics of the strains used in the CSB consortium may indicate that the EM inoculum survived until the end of the experiment should be taken into account; however, it was not possible to quantify the magnitude of the microbial population of each CSB at each sampling; therefore, there was evidence that the inoculum persisted, but it is uncertain whether the population increased or decreased. Therefore, considering the fact that the treatments with applications of the CSB consortium did not generate significant differences in the evaluated parameters for the treatments with the addition of uninoculated LRC, it was not possible to confirm that the observed changes in the EM properties were completely influenced by the CSB activity on the LRC. This results in the need for additional studies to better monitor the consortium and its activity in order to clarify these issues. However, it was found that the addition of LRC to the EM resulted in positive changes for the variables that commonly respond to treatments with humic amendments.

While some of the evaluated variables showed changes that can be considered relatively low for a year of observation, the present study showed trends that were significant when compared to assessments that have shown that remnant lignites can act like native soil organic matter after long periods of 14–37 years [26], after which the geogenic origin organic matter is incorporated into the edaphic subcycle of the carbon. Therefore, changes detected in the CEC, aggregate formation, microbial activity, and stimulation of plant growth in a relatively short time (1 year) can be considered evidence that an acceleration has occurred in the rate at which HOM containing in the LRC and EM interact, therefore, waste from open pit coal mining could be a potential resource to increase the humic content in post-mining land reclamation processes.

Conclusions

In the short-term, the application of LRC to EM under greenhouse conditions leads to effects that are widely known and described as a typical response of the effect of humic soil amendments, including an increase in the CEC, growth plant stimulation, and plant and microbiological activity stimulation, as evidenced by increased EM respiration and FDA enzymatic hydrolysis magnitudes. Therefore, evidence was found for a possible beneficial role played by the use of LRC mixed with EM in increasing the EM content and enhancing beneficial effects in the early stages of land reclamation for soils affected by coal mining by making Technosols.

Authors' contributions

NV: general design and management of the greenhouse trial, laboratory analysis, data analysis, and written manuscript. LMM: research consultation, trial design and execution, data analysis, and academic critical review of manuscript. RR: trial design, soil analysis, guidance in aggregate observation with scanning electronic microscopy, and manuscript review. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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