Effects of Organic Fertilization on the Evolution of Microbiological Properties of Chrome Mining Sterile

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ABSTRACT

In Camaguey, Cuba there is an surface chrome mine in need of restoration; the search for viable alternatives is an urgent need. One alternative used is initial micro organic stimulation of the soil with mineral fertilizers. However, due to the high costs of the procedure, organic sources were tried, as replacement for inorganic fertilizers. An experiment was set up at the Soil Laboratories, in Camaguey, Cuba, under semi-controlled conditions. A completely randomized design and six treatments were applied (mining sterile, ferromagnesial brownish-red fersialitic soil, originally found in the mine, from an unaltered area, mining sterile mixed with soils, manure (cattle or chicken), and a treatment using a mixture of mineral fertilizers: ammonium sulfate plus triple superphosphate, to determine the best microbiological variant. The results showed that the mining sterile was highly degraded, in comparison to the soils previously found in the site. The mixtures that contained organic fertilizers had higher microbial development, especially a treatment that included chicken manure.

KEY WORDS/: soil, mining, restoration, microbiology, organic fertilizers

INTRODUCTION

Soil is an important natural resource, considered a dynamic living system that makes up the interface between the atmosphere, lithosphere, biosphere, and hydrosphere, with a continuous exchange of matter and energy. Accordingly, it plays a key role in developing superficial biogeochemical cycles, with the capacity to develop a series of essential

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functions in the environment, ecology, economy, society and culture (Ortiz et al., 2007).

Soil is affected and altered in various ways, due to interaction with the environment, but human activity represents the most violent form to change and degrade soils.

Today, mining practices include rock extraction and processing into usable goods (Vilarruel and Márquez, 2014). They have played a key role in the economic development of human groups, making them a strategic sector for progress and economic growth.

Although mining has positive effects on the economy, it causes a series of negative impacts during extraction, considering surface mining is one of the most environmentally damaging activities, affecting the natural balance.

Surface mining uses techniques that lead to destruction and depletion of the earth´s ecosystems. The removal and destruction of the soil horizon, the contamination of surface and ground waters, the division of communities, and the violation of laws and rights, are commonly implemented practices in surface mining in many parts of the world (Villarruel and Márquez, 2014).

Cuba has also been affected by surface mining; since the 1990s, the country has linked economic to social development, based on the ethics of sustainable development to preserve and protect the environment.

To address this issue, the Cuban revolution is implementing actions to achieve soil restoration, according to a given local planning strategy. Such actions can be directed to agriculture, forestry, hydroeconomics, entertainment, hygiene, construction and protection of nature (NC, 1999).

In the municipality of Camaguey, there is an active mine of chrome adjacent to UBPC Victoria 2, with a test area for preservation of soils, water, and forests. It belongs to the Agricultural Enterprise of Camaguey. The negative effects caused by mining in the area can be observed in the ecosystem. The risks of turning the area into a lifeless land are high, and it is important to implement actions to reestablish productivity and economic relevance, to improve the environmental conditions.

To reduce the impact of mining and manage residuals, the best procedure is to return the previously extracted material to its original site, because it minimizes the environmental consequences; such as, accelerated erosion and visual impact, thus facilitating the site´s recovery (Enrique, 1995). However, the soil would be highly affected, with marked structural and compositional alterations, producing negative changes of microbiological properties.

Nowadays, several researchers agree that the level of recovery of affected soils must be assessed in terms of how similar are their microbiological properties to the features of the non-degraded natural soils in the area (Gil-Sotres *et al.*, 2002).

A common practice of sterile mine soil recovery uses inorganic fertilizers to achieve fast development of surface organic matter-rich surfaces, which tends to show similar properties to natural soils (Varela *et al.*, 1993). Because of the high costs of recovery for managements like that, the goal is to assess the effect of organic fertilizers as replacement for inorganic fertilization, to stimulate faster development of the soil´s microbiological properties of newly-formed soil on the mining sterile.

MATERIALS AND METHODS

Location and description of the area studied

The research was done at the Science and Technology Division of the Soil Laboratories, in Camaguey, from the Ministry of Agriculture. Sterile mining soil from the chrome mine adjacent to UBPC Victoria 2, from the Agricultural Enterpreise of Camaguey (20 m from the excavation site), were used. The test area for preservation of soils, water and forests, is located on site.

Site description

The chrome mine is located north of the city of Camaguey, 20km off city limits. The most important access route is Carretera de Nuevitas (Figure 1). The digging sites are located next to UBPC Victoria 2, in the small town of Altagracia, municipality of Camaguey. It is located on -77.72450890 north longitude and 21.48796890, east latitude, San Serapio Chart (4680-II-A), 1:25 000. The main limits are to the north: Military training field Lesca; to the south: Carretera de Nuevitas, Altagracia; to the east: La Lucha Chicken Farm road to Paso Lesca; and to the west: GENT Victoria 1.

The predominant soil in the mine and around it was ferromagnesial brown-red fersialitic soil on serpentine rocks, according to the Genetic Classification of Soils of Cuba (1999), correlated as Reddish-brown fersialitic soil, according to the New Classification of Soils in Cuba (Hernández *et al.*, 2015).

Experimental design

A laboratory experiment was made under semi-controlled conditions, to determine the evolution of microbiological properties of the different experimental variants in time (Table 1). A completely randomized experimental design was applied, with six treatments and three

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repetitions.

Polyethylene coated metal trays were used to prevent material contamination and tray deterioration. The mixtures were spread along the surface, and kept in semi-protected conditions at room temperature, with 70% maximum capacity of moisture retention.

The main material to elaborate the mixtures was the sterile soil from the crater interior of the mine. Unaltered soil samples were collected at the mining area, away from the digging sites, considering they were the same soil type as the one before the excavation. Both material were sieved (4 mm) to remove rocks and clods.

To enhance mining sterile soil, some fertilizing mixtures were used, like cattle and chicken composting manure, with 40% humidity; the mineral fertilizers used were triple superphosphate (TSP), and ammonium sulphate (AM).

The dose to use in every variant was determined, according to the amount of organic fertilizer used in soil improvement systems, under field conditions, suggested by Fuentes (2008). In terms of mineral fertilizers, the dose recommended by Varela *et al.* (1993) was applied.

Table 1. Treatments used in the study

Treat.	Variants
1	Mine sterile soils (2kg) (E).
2	Soil (2kg) (S).
3	Mine sterile soils (2kg) + soil (0.04kg) (E + S). (0,04kg) (E+S).
4	Mine sterile soils (2kg) + cattle manure (0.04 kg) + soil (0.04kg) (E + S + EV).
5	Mine sterile soils (2kg) + chicken manure (0.04kg) + soil (0.04kg) (E + S + G).
6	Mine sterile (2kg) + mineral fertilizer (TPS) 0.01809 kg + (SA) 0.00096 kg + soil (0.04) (E + S + FM).

Biological determinations were made three times (at beginning, 4, and 6 months). The chemical analysis were performed at the beginning, and 6 months, taking into account their variation time, regarding the biologicals (Anderson *et al.* (1997).

Indicators used. For chemical analysis: pH of Potassium chloride, using potentiometry NC ISO 10390 (1999); P2O5 and K2O, with Oniani NC 52 (1999); and for soil cation exchange capacity (CEC), the modified Mehlich method was used (Schactschabel) NC 65 (2000). The methods used for biological analyses were organic matter (OM) on the soil, determined by colorimetric Walkley-Black MINAG (1999); basal respiration (BR), moisturizing 25g of the soil at 70%

maximum capacity of retention, and CO_2 determination after 24 h of incubation, at 30°C (Calero *et al.*, 1999).

Real nitrifying capacity (RN) was performed through the phenol-disulfonic method suggested by Bolontina and Abramova (1968), and cellulose decomposition, using Erlenmeyers and cellulose paper strips, then quantification of 1% decomposed cellulose in time (Szegui, 1988).

Analysis and data processing

All the data were statistically processed through simple variance analysis (ANOVA) of means, to determine the significant differences between treatments. The Duncan multiple range test (P≤0.05) was used for mean comparison, with 95% reliability. SPSS for Windows, 11.5.1 (2002) was used for data processing.

RESULTS AND DISCUSSION

Chemical characterization of the material

The results of chemical characterization (Table 2) showed that the pH (KCl) ranged from neutral to slightly alkaline, except for FsRB soil (T2), where it was slightly acidic, on average (Hernández *et al.*, 2015). The highest value was observed in T5, which may have been caused by the kind of material used (chicken manure), coinciding with Tortosa *et al.* (2012), with high pH values (KCl) for the fertilizer, due to the inclusion of fallen eggs, feed residues and feathers that are mixed with the manure, on poultry farms.

Table 2. Initial characterization of chemical indicators

Treat.	pH	P_2O_5	K_2O	MO	Ca^{2+}	Na^{+}	K^{+}	Mg^{2+}	CIC
	(KCl)	mg.100g^{-1}		%	cmol$^{(+)}$.kg^{-1}				
1	7.65b	2.10c	1.35c	0.37c	0.63cd	0.19b	0.04c	16.11c	18.16bc
2	6.15cd	0.60d	15.21b	6.35a	5.44a	0.08c	0.39b	30.23a	38.54a
3	7.44a	1.35cd	2.02c	0.65b	0.61cd	0.19b	0.04c	16.9bc	18.81b
4	7.6ab	4.68b	12.25bc	0.70b	1.25b	0.21b	0.27bc	17.20b	18.55b
5	7.96d	10.52a	57.64a	0.78b	0.77c	0.52a	1.26a	16.44bc	16.82d
6	7.74bc	1.28cd	2.09c	0.55bc	0.46d	0.20b	0.05c	16.31bc	17.38cd
ESx	0.056*	0.435*	3.882*	0.078*	0.093*	0.016*	0.082*	0.310*	0.283*

a, b, c ... Means with the same letters do not differ from p≤0.05, according to the Duncan multiple range test

Elevated pH values (KCl) of the mixtures in relation to the soil were produced because all the mixtures included an important portion of mining sterile in

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their composition, making pH (KCl) increase in each of them. Similar results were achieved during analyses performed to mining wastes, by Aduvire *et al.* (2006). The results also concluded that mine soils may undergo extreme situations of their main chemical parameters, which may have an influence on restoration measures implemented.

The pH (KCl) values registered coincided with the values obtained by Huerta (2010) when assessing different soils contaminated with quicksilver from mining, in San Joaquín, Querétano, Mexico. Besides, the variations of this indicator on mining soils were achieved by Aridio (2012), by observing chemical deficiencies and extreme pH.

The P_2O_5 and K_2O contents generally show that the highest values were observed in the organic fertilizer treatments to form mixtures (T4 and T5). Elevated values of those nutrients were achieved in chemical analyses made to the organic fertilizers by Estrada (2005), for chicken manure; and by Crespo and Fraga (2006), for cattle manure. T5 showed the highest values for potassium (K2O and K+), because they had chicken manure, an organic fertilizer with elevated values of the nutrient. These results coincided with analyses made to different types of chicken manure, by Tortosa *et al.* (2012).

The content of organic matter (%) was significantly lower in all the treatments, in comparison to treatment 2, that contains soil. But it does not contain mining sterile, an almost inert material with low organic matter contents. Similar results were achieved for indicators Mg2+ and Ca2+, where the ratio Ca2+Mg2+ is below 1, commonly observed in this kind of soil Hernández *et al.*, 2015).

Generally, the mining sterile (T1) had the lowest organic matter and nutrient percentages, coinciding with studies made by CITME (2007), in terms of negative chemical effects caused by mining.

A chemical analysis six months after the mixtures (Table 3) showed decreased pH(KCI) values in all the treatments, in comparison to the initial sampling (Table 3), a recovery of the indicator. Similar results were achieved by Gómez *et al.* (2011), who claimed that organic fertilizers enhanced soil pH, facilitating nutrient release to the plants.

However, the values closest to neutral corresponded to mixtures 3, 4, 6. Moreover, though the pH of T5 decreased, it continues to be the most alkaline, due to the chemical composition of chicken manure contained in the mixture, also reported by Estrada (2005) and Tortosa *et al.* (2012), who assured that the inclusion of this kind of fertilizer may increase soil pH.

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Table 3: Final characterization of several chemical indicators

Treat	pH	P_2O_5	K_2O	MO	Ca^{2+}	Na^{+}	K^{+}	Mg^{2+}	CIC
	(KCl)	mg/100g		%	cmol$^{(+)}$.kg-1				
1	7.65b	1.15d	2.64c	0.37d	0.63b	0.19c	0.04e	16.11d	18.16d
2	6.15b	1.15d	19.00b	6.35a	5.44a	0.08d	0.39c	30.23a	38.54a
3	7.26a	1.38cd	3.06c	1.1c	0.68b	0.42b	0.80b	18.03bc	19.79c
4	7.33a	7.92b	12.29b	1.28c	0.82b	0.43b	0.24d	18.71b	21.01b
5	7.77b	13.92a	56.66a	1.48b	0.65b	0.63a	1.26a	17.53c	19.01cd
6	7.20a	1.81c	3.47c	1.1c	0.68b	0.42b	0.08e	17.53c	18.87cd
ESx	0.087*	0.164*	3.289*	0.060*	0.095*	0.019*	0.020*	0.347*	0.393*

a, b, c … Means with the same letters do not differ from $p \leq 0.05$, according to the Duncan multiple range test

The best results achieved in the rest of the indicators studied were observed in the treatments using organic fertilizers (T4 and T5), coinciding with results reported by Gómez *et al.* (2008) on beans (*Phaseolus vulgaris L.*) and radish (*Raphanus sativus L.*), in bio intensive gardens in the humid tropic of Tabasco, which confirmed that the application of organic fertilizers on the soil significantly increased the values of CEC, OM, Ca_{2+}, Mg_{2+}, P_2O_5 and K_2O. They also added that the favorable effects of the application of compost to soils is produced by its chemical composition, with high contents of OM, nitrogen, P_2O_5, K+, Ca_{2+} and Mg_2.

High P_2O_5 and K_2O contents were observed in the final analysis of organic fertilizer mixtures, in comparison to all the other treatments, especially T5, the same way as the initial analysis (Table 2). These results confirmed the effectiveness of such fertilizers to recover some of the chemical properties of the mine soils studied.

Evolution of microbiological indicators

Basal respiration (BR)

In terms of microbiological indicators, BR was null or close to zero for all the variants studied at the beginning, except for unaltered soil (T2) (Figure 2). This result is caused by the very low or null microbial activity observed in mining sterile, similar to inert minerals.

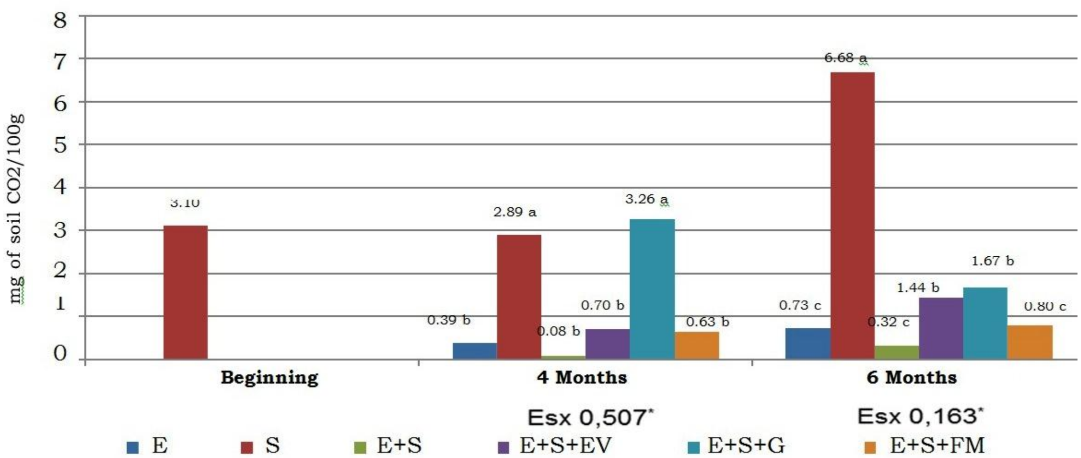

Figure 2: Behavior of basal respiration in different treatments studied

The results for BR showed that after four months of mixture, active microbial activity was observed in all the treatments, though T2 had the highest values. However, six months later, the chicken manure (T5) underwent increased activity compared to all the other treatments, without significant differences, regarding soil (T2). It may be caused by the high nutrient contents of T5 (P_2O_5 and K_2O), and organic matter contents. Similar results were achieved by Mogollón *et al*. (2004), using vermicomposting to achieve increased basal respiration of salt-degraded soils. Hence, cattle and chicken manure can help recover the initial microbial population of the soil.

Likewise, Chaveli *et al*. (2009) had similar results in terms of microbial activity (BR), in a stability study of mineral and organic fertilizers, achieving better microorganism respiration 60 days after mixture application.

Real nitrifying capacity (RN)

The real nitrifying capacity (RN) refers to the presence and activity of microbial groups able to transform complex or poorly assimilated nitrogen forms in the simplest forms, and therefore, with the capacity to be assimilated by the plants. Figure 3 shows the results achieved for that indicator during the study, with a similar RB behavior. After four months, activity response reached the highest values in treatment 2 and 5, then decreasing in T5 (fowl manure), six months later. This behavior was similar to T4 (cattle manure), keeping t2 (unaltered soil) with the highest value.

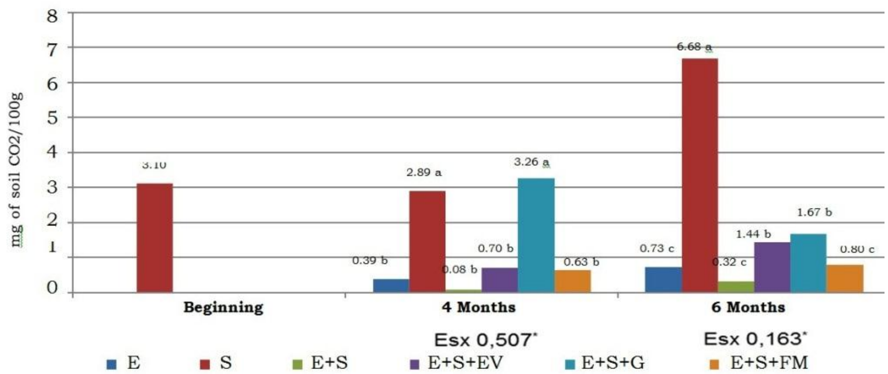

Figure 3: Behavior of real nitrifying in the different treatments studied

These results corroborate the ones achieved for BR (Figure 2), where the treatments that improved the mining sterile by using organic fertilizers had higher microbial activity. These results coincided with reports by Gil-Sotreset *et al.* (2002), who determined that Purina organic fertilizers contributed to faster development of properties that define soil quality in sterile soils, even better than inorganic fertilizers.

In T5 an explosion of microbial activity was observed in four months, then it decreased two months later. It may be caused by the presence of ammonium in that type of material, that tends to stabilize due to mineralization, and phosphorus and calcium contents that favor nitrification (Fernández and Novo, 1988).

Cellulose decomposition

Cellulose decomposition (CD), demonstrated microflora capacity to degrade complex carbonated compounds, such as cellulose commonly found in nature. Figure 4 shows that at the beginning of the study, little cellulolytic activity was observed, which is a feature of this type of soil (Hernández *et al.*, 2015), only T4 and T5 had some activity. As time went by, and under ideal temperature and humidity conditions, microbial activity was observed in all the treatments, an evidence of initial material recovery, now sterile.

However, the best behavior was always observed in the organic fertilizer mixtures used for recovery (T4 and T5). In that sense, the chicken manure treatment had a more active response to the extent of not producing significant differences regarding the soil (T2), six months later.

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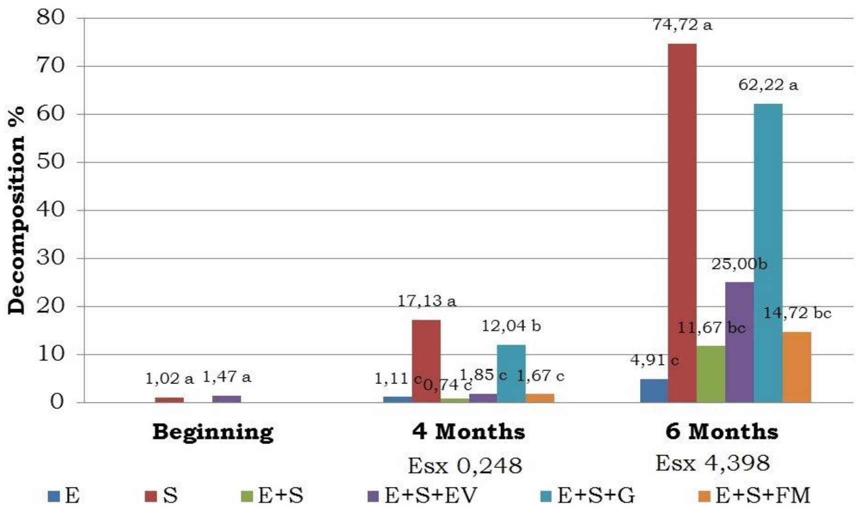

Figure 4: Behavior of cellulose decomposition four weeks after incubation of the treatments studied.

This result may be influenced by cellulolytic activity, which is stimulated in media with enough calcium, rich in nitrogen and assimilating phosphorus. It coincided with Burgos (2000). Chicken manure is characterized by high values of the previous elements. These parameters coincided with analysis made by Tortosa *et al.* (2012) to different types of chicken manure. They also noted that the content of calcium in chicken manure is higher in intensive caged raising, because often eggs fall down, enriching the fertilizer and becoming part of it.

Table 4 shows the CD dynamics during the first four weeks, six months after incubating the mixtures. Progressive development of antimicrobial activity can be observed. The organic fertilizers had the best values, with T5 behaving closest to T2 at the end of assessment.

Table 4: Dynamics of cellulose decomposition at six months of mixture (%)

Treatments	1	2	3	4
S	0.96	6.11	29.72	74.72

E+S	1.11	3.33	2.96	11.67
E+S+EV	1.11	2.78	6.94	25.00
E+S+G	5.83	13.33	35.28	62.22
E+S+FM	1.11	2.22	7.59	14.72
E	0.56	1.39	3.33	4.91
E $\overline{sx}$	0.632	1.815	3.727	4.398

In the first weeks T5 had a significant activity, even better than T2. This behavior lasted until week 3, when the soil cellulolytic activity was better than T5. It may have been caused by the high content of nitrogen in the fertilizer, coinciding with studies made by Estrada (2005); and Tortosa *et al.* (2012). The microbial activity of the mixture was stimulated until a reaching a point of balance.

Relation among the indicators assessed

The results achieved for CD are similar to others achieved for BR and RN, indicating the viability of organic fertilizers as soil enhancers, also demonstrated by Hernández *et al.* (2010), who admitted that apart from improving the soil microbiological properties, its physical and chemical properties were also improved, with a direct influence on plant growth.

These microbiological indicators have been used by different authors to diagnose degradation and erosion (Font *et al.*, 2003; Chaveli *et al.*, 2003), soil fertility (Calero *et al.*, 1999; Chaveli *et al.*, 2006; Font *et al.*, 2011), fertilization dosage for particular crops (Burgos, 2000; Font *et al.*, 2004), effects of heavy metals on the quality of organic substrates (Rodríguez *et al.*, 2012; Monaga *et al.*, 2015), and estimation of soil quality (Font 2007; Font *et al.,* 2012; Ginebra, 2014), with positive results.

Overall, all the mixtures improved chemical and biological characteristics in comparison to the mine sterile soil. However, the results of this study showed that the inclusion of soil in the sterile material with the dosage for treatment 3 was not effective to achieve proper recovery of the soil, even with the application of mineral fertilizers, where improvements in soil properties were not significant, because the quality of the mining sterile was poor, and the fertilizers used in the doses recommended were ineffective to recover soil quality in the area.

Additionally, the mixtures of organic fertilizers used (T4 and T5) showed similar indicators to T2, sometimes higher, demonstrating their feasibility to restore highly degraded soils. The chicken manure (T5) had the best indicators in most assessments.

CONCLUSIONS

The mining sterile was highly degraded, with low nutrient and organic matter contents, causing poor microbial activity. Moreover, the organic fertilizers improved the chemical properties of the mining sterile, and in six months it acquired a positive microbiological state, close to unaltered soils. It also favored the use of organic fertilizers rather than chemical fertilizers, with higher viability during recovery.

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