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Abstract: As rehabilitation efforts in Guyana are recent, there is little information on the effect of different ecological rehabilitation protocols for Guyana's mining sites on biogeochemical cycles and mercury mobility. This study was conducted to assess the impact of different ecological restoration protocols on soil quality with the use of soil microbial indicators and by estimating the mercury mobility. We sampled soil from six rehabilitated mining sites in French Guyana with different ecological restoration procedures. We carried out measurements of enzymatic activities and an analysis of mercury environmental speciation to assess its potential toxicity according to a mobility gradient. The results obtained in this study show that the rehabilitation of mining sites has been carried out in a heterogeneous manner and soil quality is very variable, even in nearby sites. Sites that have been rehabilitated with fabaceous species have positive soil quality indicators. In addition, the results highlight a change in mercury mobility that is 82.1% correlated after co-inertia analysis with soil texture properties, which also confirms a direct effect of rehabilitation on mercury mobility. The non-restored sites had a much higher potential of mercury mobility and toxicity than the sites where ecological restoration was successful. These results highlight the positive effect of controlled rehabilitation and ecological restoration on microbiological activities and the potential toxicity of mercury.

Keywords: ecological restoration; mercury mobility; microbial activities; biogeochemistry; gold mining activities; French Guiana

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1. Introduction

Deforestation is currently one of the sectors that emit the most greenhouse gases [1,2] and although forest areas are increasing globally, tropical forests are particularly affected by this crisis [3]. Among the causes of deforestation in the Amazon, agriculture, silviculture, cattle ranching, selective logging, coca farming, and artisanal scale gold mining (ASGM) are responsible for a large percentage of forest loss. In South America and in French Guiana in particular, the stakes of gold mining are serious for the development of the region, and are worrying for the preservation of the ecosystem. In Guyana, gold mining impacts land use and the functioning of the forest system, including the soil [4,5]. To exploit gold in alluvial terraces, it is necessary to remove all the vegetation and then to use powerful water jets (sluice) to eliminate the superficial layers of soil until reaching the gold-bearing layer [6]. In addition to the direct damage caused by deforestation, these practices lead to heavy soil losses through erosion, which will lead to increased turbidity in aquatic systems and the remobilization of toxic metallic trace elements such as mercury [7]. Due

to amalgamating chemical properties for gold mining, mercury was widely and legally used until 2006 and the problem of mercury in Guiana has already been described in many scientific studies [8,9]. It was shown that mercury of anthropogenic origin in Guyana was the most reactive, highly disorganized and often had suboxic physico-chemical conditions of the mining sites, which are favored its mobility and methylation [10,11].

The biogeochemistry of mercury is complex and depends on many physico-chemical parameters (i.e., temperature, redox potential, pH, metal oxides, organic matter, and mercury oxidation level) [12], but also biological parameters. Depending on these conditions, the forms of mercury in the soil will be affected and this will influence its mobility, bioavailability and toxicity to biological systems [13]. Free forms of mercury take different forms in soils such as alkyl mercury species such as methylmercury ($MeHg^+$) or ethylmercury(II) ($EtHg^+$) or inorganic soluble mercury species. These forms generally represent the most toxic component of mercury due to their high mobility. Metal mercury complexes, or certain amalgams, are considered to be less toxic because they are more difficult to mobilize without specific reactions, such as chemical or microbial reduction. The least toxic forms of mercury, such as mercuric sulphide or cinnabar (HgS) [14,15], are generally stable in soils, but may be weathered by simple dissolution in acidic environments [15] by significant root exudates such as those from fabaceae [16] or by microbial production of low molecular weight organic acid (LMMOAs) [17]. Determining mercury speciation at anthropized sites such as former mining sites is therefore very important for assessing the positive or negative health impact of ecological rehabilitation [15].

In order to limit the adverse effects associated with mercury mobility, ecological rehabilitation methods have been proposed with varying degrees of success in the Guyana basin. In the mining code, there is a difference between the principle of rehabilitation and restoration or revegetation [18]. Rehabilitation in the mining sense consists solely of reconstituting the original soil from the materials that have been extracted and moved, terracing the mixture, and if possible, covering it with so-called fertile horizons. Restoration will consist of applying a defined site revegetation protocol, if possible using local species in order to rebuild the original ecosystem [19,20]. While rehabilitation is mandatory, restoration is not explicitly requested in the mining code and too often remains at the discretion of mining companies. Among the methods used to evaluate the quality of ecological restoration protocols, enzymatic activity measurements and quantification of soil microbial biomass are among the most effective and widely used methods, particularly at mining sites [21,22].

While data on mercury speciation in natural soils and mining sites are fairly well documented [9,23], changes in carrier phases can alter mercury mobility and these mechanisms during ecological restoration processes are still not well known in Guyana and are essential to define a chemical quality status of the soil. In a context where the physico-chemical conditions (iron oxides, low pH and suboxic conditions) of rehabilitated sites could promote mercury mobility [11], environmental speciation measures could therefore be an interesting tool for assessing the health quality of these plots. One of the additional effects of good ecological rehabilitation is also to limit anoxic soil conditions through better regulation of the water cycle. In the context of tropical soils that are potentially rich in mercury, ecological restoration could therefore limit the conditions conducive to mercury mobility. While the effect of ecological restoration has already been shown to be positive for soil biological quality [22,24], it would also be relevant to assess the impact of different vegetation cover on mercury speciation by the use of environmental extraction procedures [25].

The main assumptions led to his work on the quality of rehabilitation, which was linked to an increase in plant cover and soil stability and can limit mercury mobility and promote soil microbial activities. We also hypothesize that the toxic fractions of mercury in the soil have a negative impact on microbial communities. In this context, the main physico-chemical properties of the soil, telluric enzymatic activities indicative of the functioning of biogeochemical cycles, as well as the environmental speciation of mercury were determined in different soil samples from former mining sites rehabilitated in French Guiana, restored

or not to see the effect of a vegetation gradient. The objectives of this study were: (1) assess the biological quality of mine sites in relation to the quality of rehabilitation and the level of ecological restoration; and (2) describe the influence of heterogeneous soil rehabilitation and different ecological restoration modalities on the fate and mobility of mercury in soils. To answer these questions, six sites undergoing ecological restoration have been selected in French Guiana, in the Yaoni and Belizon regions. These sites were chosen because of their similar gold mining liabilities, as well as for their ecological restoration gradients, both in terms of quality and age.

It is difficult to anticipate the different recovery trajectories of biogeochemical cycles on a wide range of rehabilitated mining sites; however, these results could contribute to improving the assessment of the sanitary quality of rehabilitated sites in French Guiana.

2. Materials and Methods

2.1. Site Description and Soil Sampling

The work was undertaken in French Guiana, South America. The sampled soils (Figure 1) for this study were rehabilitated after gold mining extraction. After closing the alluvional mine pit, rehabilitation consisted of reconstituting the soil excavated during mine exploitation.

Figure 1. Illustration of some examples of different stages of ecological restoration on formers Guyanese gold mining sites and geographical location of the Yaoni and Belizon mines in French Guiana. Non-restored site (**top left**), ecological restoration with herbaceous species after 3 years ((**top right**), Bel1), ecological restoration with fabaceous species after 3 years ((**bottom left**), Bel3), ecological restoration with fabaceous species after 18 years ((**bottom right**), Yao3). The pictures were taken during a second sampling campaign in April 2016 on sites undergoing ecological restoration.

The first three sites were in the Belizon forest (N 04°22.200′/W 052°19.328′). The mining operation lasted five years and ended in January 2013. All sites in the Belizon have had the same mining history. In February 2013, an ecological restoration programme was initiated with macrocuts of *Clitoria racemosa*, a species of local fabacea. The recovery of *C. racemosa* macrocuts has not been effective on all sites in the Belizon forest. On the Bel1 site, vegetation covered between 5% and 10% of the soil surface with a majority of cyperaceae species (*Cyperus* sp. and *Carex* sp.). On the Bel2 site, vegetation covered more than 70% of the site and almost the entire site is dominated by *Lycopodiella* sp. with the presence of some cyperaceae species. On the Bel3 site, macrocuts of *C. racemosa* were recovered and the trees are nearly 5 m high.

The following three sites were taken from the former Yaoni mine site (N 04°30.930′/W 052°21.275′). Mining activity at the Yaoni sites lasted several years and ended in 1997. These three sites also have the advantage of having the same mining history. The first two Yaoni sites (Yao1 and Yao2) have not been restored by specific protocols and the low vegetation cover, mainly *Lycopodiella* sp., *Cyperus* sp. and *Carex* sp., is the result of

spontaneous vegetation development. The Yao3 site has been completely restored by associations of fabaceous species, notably *Acacia mangium* and *Clitoria racemosa*.

At each site, soil samples were collected from depths between 0–10 cm with an auger in April 2016. The sampling for the five sites consisted of sampling five plots (n = 5) per site, with three sub-samples per plot that were pooled at the laboratory. The sampling of five replicas per site was mainly conditioned by the topography of the terrain, the accessibility of the plots and the feasibility of sampling. Soil samples were immediately sealed in sterile hermetic polyethylene bags for transportation to the Cayenne IRD laboratory. Samples were then dried at ambient temperature (25 °C) until they were air dried (i.e., approximately three weeks). These 90 collected samples (6 sites × 5 plots × 3 samples per plot) were then sieved at 2 mm, homogenized, and hermetically sealed at 4 °C until used.

2.2. Soil Samples Characterization

Granulometry was determined in the fraction less than 2 mm, and five classes of particles have been distinguished according to the NF X 31-107 standard: clay (<2 µm), fine silts (2 to 20 µm), coarse silt (20 to 50 µm), fine sands (0.050 to 0.200 mm) and coarse sands (0.200 to 2 mm).

For the total Fe and Al analyses by inductively coupled plasma optical emission spectroscopy (ICP-OES, Spectroblue, Elancourt-France), the samples were first ground to 63 µm then an acid digestion was performed in cleaned Teflon digestion tubes (SCP Science, Villebon sur Yvette-France) using a hot block (Digiprep MS, SCP Science) with two digestion HNO_3 (VWR ref. 83872.330)-HCL (VWR ref. 83871.290) in a 1:3 ratio and HF (Sigma-Aldrich, Saint Louis, MO, USA, ref. 339261-100 ML).

Soil pH-H_2O and pH-KCl were measured in soil suspensions according to ISO 10390. Soil total carbon (Ctot) and total nitrogen (Ntot) were determined by the Dumas method (NF ISO 13878) by chromatography with a thermal conductivity detector (NA 1500 série 2 CARLO-ERBA). Total phosphorus (Ptot) was determined after the acid digestion of soil samples, by Murphy and Riley method [26]. Water extractions were performed for total organic carbon (TOC), and concentrations were measured with a Shimadzu TOC-500 apparatus (Shimadzu, Kyoto, Japan).

2.3. Soil Microbial Biomass and Enzyme Assays

The soil microbial biomass was estimated according to Anderson and Domsch's work [27]. Using the SIR method, soil microbial biomass C (SIR-biomass) was calculated using the following equation:

$$\text{SIR-biomass C } (\mu gC\ g^{-1}\ \text{soil}) = \text{SIR } (\mu L\ CO_2\ g^{-1}\ \text{soil}\ h^{-1}) \times 40.04 + 0.37 \quad (1)$$

The activity of acid (AcdP) and alkaline (AlkP) phosphatases, B-glucosidase (Glu) and arylsulfatase (Aryl) were assayed based on the amount of p-nitrophenol (pNP) released after cleavage of enzyme-specific substrates at the average pH of natural soil. These enzymes were measured, as described by Badiane et al. and Tabatabay and Bremner [28–30]. Dehydrogenase activity (DHA) was determined according to Klein et al. [31] using 2.3.5_tripenyltetrazolium chloride (TTC) as substrate. Urease activity (Ur) was determined according to Kandeler and Gerber [32] using urea as substrate. The total enzymatic activity (FDA) of soil microorganisms was determined by a spectrophotometric method using fluoresceine diacetate as substrate following the method developed by Green et al. [33].

2.4. Mercury Analysis

2.4.1. Total Mercury Content

Mercury concentrations (HgT) in soil samples were determined directly by thermal decomposition atomic absorption spectrometry after gold amalgamation using an automatic mercury analyzer (AMA 254) in Ultra High Sensitivity configuration (ref 002-001-001) with a detection limit of 0.001 ng and quantification limit of 0.003 ng.

2.4.2. Environmental Speciation of Mercury

- Extraction of "Highly mobile" mercury species

This extraction included three fractions (water soluble, exchangeable and bioavailable mercury fraction) of the most mobile and bioavailable forms of mercury. The sum of the three fractions (F1 + F2 + F3) in the next three paragraphs will be called E1.

Soluble mercury (F1) was estimated according to Rasmussen et al. [34] with ultrapure water. The exchangeable Hg fractions (F2) in soil samples were measured according to Silveira et al. [35] with 0.1 M $CaCl_2$ (Sigma-Aldrich, ref. 223506-500 G). The bioavailable mercury contents (F3) in soil samples were measured after a 1:10 (w/v) soil/0.01 M EDTA (Carl Roth, Karlsruhe-Germany, ref. 8040.3) + 1 M ammonium acetate (Merck, Darmstadt-Germany, ref. 1.01115.1000) following the standard AFNOR NF X31-120. Then, solutions were centrifuged, supernatants were filtered at 0.45 mm with Teflon filters (Minisart SRP 25, Sartorius, Dourdan-France), and then 200 µL of extracted supernatants were analyzed for exchangeable mercury content with AMA-254.

- Extraction of toxic, semi-mobile and non-mobile mercury

To assess the environmental speciation of mercury in soil, we followed Han et al. [14] procedure in order to determine the mobile and toxic, semi-mobile and non-mobile fractions of mercury in the restored soils and all fractions were analyzed by the AMA-254.

Briefly, the extraction of mobile and toxic mercury, called E2, involves the use of a solution of 2% HCl + 10% ethanol to extract the mobile mercury species from the soil. The target mercury species include toxic alkyl mercury species, such as $MeHg^+$ and $EtHg^+$ species, as well as inorganic mercury species that have great mobility, such as soluble Hg^{2+}. As this extraction targeted all forms of mobile mercury, including the three fractions detailed above, extraction E2 corresponded to the difference between the total mobile mercury extracted by this protocol and the sum of the fractions of extraction E1.

To extract the "semi-mobile" mercury, called E3, species including mainly Hg and mercury-metal amalgam, the sample remaining after the ethanol extraction was first sonicated with 5 mL of ultrapure water at 60 °C for 5 min. The sample was centrifuged, and the supernatant was discarded. Then, extraction was carried out with 5 mL 1:2 (v/v) HNO_3: Ultrapure water. The rest of the matric material could be directly analyzed by AMA-254 for "non-mobile" mercury species, called E4, including mainly Hg_2Cl_2 and HgS mercury species.

2.5. Statistical Analysis

The normality of the data distributions and the equal variance between treatments were tested using the Shapiro test and Bartlett's test, respectively. To study the effect of the restoration processes, we conducted different one-way ANOVA. Tukey's HSD multiple comparison method was used to test the difference between the different restoration processes. Pearson correlation was performed between mercury speciation and the dataset of enzyme activities and soil properties.

Co-inertia analyses, a two-table ordination method, were used to analyze the impact of soil properties (physics and chemistry) on enzymes activities and mercury speciation. This involves a simultaneous projection, at the same scale, of the PCA (data not showed) conducted on soil properties and the PCA conducted on enzyme datasets and a mercury speciation dataset. Permutation tests were conducted to assess the statistical significance of the co-variation between the different datasets. The R software was used for all statistical analyses (R version 3.3.2 (31 October 2016)).

2.6. Quality Assurance and Control

To avoid contamination, all materials used in this work were acid-washed twice with HNO_3 (5%) and then rinsed several times with Milli-Q water before used.

Concerning mercury assays in samples, the relative error was routinely ±5% and was always under ±10%. The detection limit (defined as three times the standard deviation

(SD) of the blank) was 0.03 µg kg^{-1}. Concentrations obtained for repeated analyses of certified reference materials never exceeded the published range of concentrations (i.e., 0.128 ± 0.017 µg g^{-1} and 0.091 ± 0.009 µg g^{-1} for BCR-277R and MESS-3, respectively).

3. Results

3.1. Soil Physical and Chemical Properties of the 6 Sites

Granulometry measurements (Table 1) showed heterogeneous textures between the rehabilitated sites. The Bel1, Bel3, Yao2 and Yao3 sites were predominantly sandy with significantly different fine sand/coarse sand ratios between the sites. The Bel2 site was mostly silty while the Yao1 site was mainly clayey. The soils rehabilitated after mining, with heterogeneous structures and textures, can be considered as anthroposols.

Table 1. TOC: Total Organic Carbon, C_{tot}, N_{tot}, and P_{tot}: Total C, N and P, Total Fe, Al: Total Iron, Aluminum content, (n = 5, mean ± SD). For each parameter, values followed by different letters differ significantly with $p < 0.05$ with Tukey HSD test.

Sites Characteristics	Bel1	Bel2	Bel3	Yao1	Yao2	Yao3
TOC (g·kg^{-1})	2.47 ± 0.22 **a**	5.84 ± 0.34 **b**	4.99 ± 0.37 **b**	3.88 ± 0.03 **ab**	3.51 ± 0.23 **ab**	15.58 ± 1.4 **c**
C total (g·kg^{-1})	3.52 ± 0.36 **a**	9.54 ± 0.73 **b**	7.01 ± 0.83 **b**	6.01 ± 0.32 **b**	4.29 ± 0.19 **a**	22.88 ± 1.51 **c**
N total (g·kg^{-1})	0.14 ± 0.02 **a**	0.45 ± 0.01 **b**	0.40 ± 0.04 **b**	0.40 ± 0.02 **b**	0.28 ± 0.03 **ab**	1.81 ± 0.04 **c**
C/N	25.14	21.20	17.52	15.02	15.32	12.64
P total (g·kg^{-1})	0.13 ± 0.01 **a**	0.27 ± 0.07 **b**	0.17 ± 0.02 **ab**	0.21 ± 0.01 **ab**	0.18 ±0.01 **ab**	0.75 ± 0.03 **c**
pH	5.3 ± 0.1 **a**	4.66 ± 0.08 **b**	5.00 ± 0.03 **ab**	5.26 ± 0.2 **a**	5.02 ± 0.03 **ab**	4.66 ± 0.2 **b**
Clay%	7.05 ± 2.7 **a**	22.24 ± 2.4 **b**	21.26 ± 1.5 **b**	46.44 ± 1.1 **c**	24.26 ± 0.5 **b**	19.78 ± 1.4 **b**
Fin silt%	18.62 ± 2.1 **b**	44.47 ± 2.6 **d**	17.68 ± 3.5 **b**	33.92 ± 1.2 **c**	10.10 ± 0.3 **a**	27.7 ± 0.8 **c**
Coarse silt%	2.39 ± 0.98 **a**	21.39 ± 0.92 **d**	7.10 ± 1.1 **b**	7.57 ± 0.45 **b**	10.40 ± 0.7 **c**	7.8 ± 0.5 **b**
Fin sand%	63.68 ± 1.4 **e**	2.67 ± 1.1 **a**	4.86 ± 0.6 **a**	8.59 ± 0.49 **b**	35.26 ± 0.7 **d**	14.17 ± 1.35 **c**
Coarse sand%	7.41 ± 0.33 **b**	9.14 ± 0.8 **b**	49 ± 5 **d**	3.30 ± 0.48 **a**	19.30 ± 0.5 **c**	22.22 ± 1.17 **c**
Fe (g·kg^{-1})	66.85 ± 6.2 **b**	44.4 ± 2.8 **b**	113.3 ± 15.2 **a**	65.04 ± 10 **ab**	75.7 ± 12.9 **b**	71.4 ± 4.9 **b**
Al (g·kg^{-1})	16.54 ± 2.12 **a**	41.4 ± 2.2 **a**	25.6 ± 2.15 **b**	53.6 ± 15.8 **b**	76.4 ± 4.8 **b**	43.3 ± 10.2 **b**

Among the six rehabilitated sites, the two sites restored Bel3 and Yao3 had significantly higher levels of total CNP and TOC content than the non-restored sites. The total carbon content, nitrogen and phosphorus varied, respectively, between 3.52 and 22.88 g·kg^{-1}; 0.14 and 1.81 g·kg^{-1} and 0.13 and 0.75 g·kg^{-1} between non-restored and restored sites. The C/N ratio of the Bel1 and Bel2 sites could indicate significant nitrogen deficiencies limiting ecological restoration processes. On the contrary, the old Yaoni sites have lower C/N ratios, indicative of a better cycle of organic matter degradation.

The six sites were rich in iron with minor differences, which seems consistent with Guyanese soils [36–38]. Total iron content in soil varied between 44.4 and 85.04 g·kg^{-1} for Bel3 and Yao2, respectively. The aluminum contents were homogeneous for Bel3, Yao1, Yao2 and Yao3 sites and higher than Bel1 and Bel2 sites.

3.2. Soil Microbial Biomass and Enzyme Activities

The two sites restored with fabaceous species, Bel3 and Yao3 sites, had significantly higher soil microbial biomass levels than the non-restored sites (Table 2) with a maximum biomass of 1102 mg kg^{-1}.

Regardless of the soil enzyme assay (Table 2) and the rehabilitated sites, soil enzyme activities showed strong significant differences between restored and non-restored sites. The total enzymatic activities (FDA) varied between 0.49 and 3.22 µg·g^{-1}·h^{-1} of fluorescein for Yao2 and Yao3, respectively. For dehydrogenase activity, the maximum activity was for Yao3 site, while the minimum was for the Bel2 site with a value of 5.80 mg kg^{-1} and 0.34 µg TPF g^{-1} soil h^{-1}, respectively. For the β-glucosidase activity, the Yao3 site had significantly the highest activity with 18.6 µg pNP g^{-1} soil h^{-1}, while the other sites had a relatively homogeneous activity. For urease activity, the highest value was measured for Yao3, then for Bel3 with 4.74 and 1.52 µg NH_4^+-N g^{-1} soil h^{-1}, respectively. Acid

phosphatase activities were higher than for the other enzymes and significantly higher for the two restored sites and Yao2 than for the other non-restored sites. For alkaline phosphatase activity, the highest value was measured for Yao2, then for Bel2 with 12.07 and 4.17 µg pNP g^{-1} soil h^{-1}, respectively. For Arylsulfatase activity, the Yao3 and Bel3 sites reached values of 56.0 and 8.42 µg pNP g^{-1} soil h^{-1}, respectively, while the other sites had very little activity.

The results, in particular the microbial biomass, illustrated the positive impact of ecological restoration on the return of microbial communities in rehabilitated soils, in direct correlation with a return of enzymatic activities responsible for the turnover of nutrients in the soil and the functioning of ecosystems. The establishment of fabaceae species seemed to be a very interesting choice as a pioneer species to be used on rehabilitated sites.

Table 2. Soil Microbial Biomass and soil enzymes activities involved in soil biogeochemical cycles in the six rehabilitated sites (n = 5, mean ± SD). MBC: Soil microbial biomass carbon in mg·kg^{-1}, DH: Dehydrogenase activity in µg TPF (red-colored formazan) g^{-1} soil h^{-1}, B-Glu, AcdP, AlkP and Aryl: β-glucosidase, Acid phosphatase, Alkaline phosphatase and Arylsulfatase activities in µg pNP g^{-1} soil h^{-1}, Urease: Urease activity in µg NH_4^+-N g^{-1} soil h^{-1}, FDA: Global microbial enzymes activities in µg fluorescein g^{-1} soil h^{-1}. For each parameter, values followed by different letters differ significantly with $p < 0.05$ with Tukey HSD test.

Microbial Activities	Bel1	Bel2	Bel3	Yao1	Yao2	Yao3
MBC	312.6 ± 10.0 **c**	385.9 ± 21.5 **c**	844.4 ± 14.1 **b**	223.1 ± 10.8 **d**	248.1 ± 13.6 **d**	1102 ± 41 **a**
DH	0.43 ± 0.08 **d**	0.34 ± 0.02 **d**	2.10 ± 0.19 **b**	0.46 ± 0.07 **d**	1.03 ± 0.12 **c**	5.80 ± 0.44 **a**
B-Glu	1.60 ± 0.25 **a**	1.46 ± 0.78 **a**	5.54 ± 0.38 **a**	1.38 ± 0.10 **a**	3.48 ± 0.97 **a**	18.6 ± 3.4 **b**
Urease	0.46 ± 0.04 **c**	0.70 ± 0.02 **bc**	1.52 ± 0.06 **b**	0.14 ± 0.01 **d**	0.29 ± 0.02 **cd**	4.74 ± 0.28 **a**
AcdP	5.76 ± 0.90 **bc**	3.41 ± 0.80 **c**	11.78 ± 1.70 **b**	2.87 ± 0.98 **c**	10.98 ± 0.94 **b**	18.09 ± 0.7 **a**
AlkP	0.36 ± 0.09 **c**	4.17 ± 0.70 **b**	2.79 ± 0.47 **c**	1.02 ± 0.57 **c**	12.07 ± 3.01 **a**	4.02 ± 0.52 **b**
Aryl	1.57 ± 0.27 **bc**	nd	8.42 ± 1.22 **b**	1.04 ± 0.36 **c**	1.12 ± 0.50 **bc**	56.0 ± 3.5 **a**
FDA	1.22 ± 0.12 **b**	0.89 ± 0.10 **b**	1.28 ± 0.09 **b**	0.52 ± 0.04 **c**	0.49 ± 0.02 **c**	3.22 ± 0.21 **a**

3.3. Mercury Environmental Speciation

- Total mercury

Mercury levels (Table 3) were homogeneous between the rehabilitated sites with an average of 265 ng Hg g^{-1}. The Yao3 site had significantly higher mercury content with 338.8 ng Hg g^{-1}. These concentrations were still low compared to the gold mined soil in French Guiana [23]; however, it remained within the normal range of mercury measurements in French Guiana [23]. The total mercury is significantly and positively correlated with microbial biomass, total organic carbon and total iron, and negatively correlated with clay, total aluminum, and pH (Table 4).

Table 3. Mercury environmental speciation for the six sites. E1: Extraction of highly mobile mercury species, F1: H_2O soluble mercury fraction, F2: $CaCl_2$ exchangeable mercury fraction, F3: EDTA bioavailable mercury fraction, E2: Extraction of mobile and toxic mercury species, E3: Extraction of semi mobile mercury species, E4: Non-mobile mercury species. All values are expressed in ng g^{-1} soil. Extraction yield represents the quality of mercury extraction in the samples, the value is obtained with the following formula: (E1 + E2 + E3 + E4)/Total Hg × 100. For each parameter, values followed by different letters differ significantly with $p < 0.05$ with Tukey HSD test.

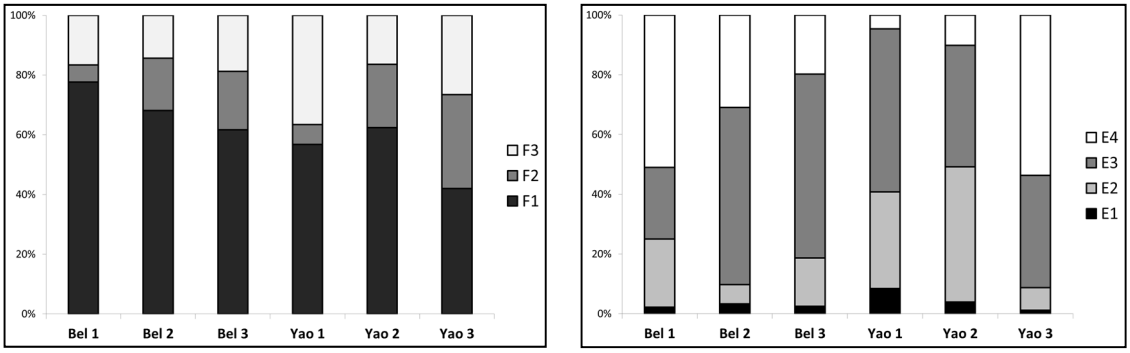
Extraction	Bel1	Bel2	Bel3	Yao1	Yao2	Yao3
E1	6.28 ± 2.16 **a**	7.30 ± 0.48 **a**	6.82 ± 0.49 **a**	18.32 ± 1.29 **b**	8.90 ± 0.88 **a**	4.07 ± 0.52 **a**
F1	4.87 ± 0.09 **b**	4.97 ± 0.12 **b**	4.21 ± 0.08 **b**	10.39 ± 0.82 **a**	5.55 ± 0.45 **b**	1.71 ± 0.21 **b**
F2	0.36 ± 2.06 **a**	1.27 ± 0.47 **b**	1.33 ± 0.43 **b**	1.23 ± 0.83 **b**	1.89 ± 0.54 **b**	1.28 ± 0.28 **b**
F3	1.04 ± 0.09 **a**	1.04 ± 0.19 **a**	1.27 ± 0.29 **a**	6.69 ± 0.19 **b**	1.46 ± 0.27 **a**	1.08 ± 0.53 **a**
E2	65.8 ± 14.9 **ab**	14.4 ± 1.6 **bc**	44.1 ± 8.3 **b**	70.4 ± 37.7 **ab**	103.1 ± 5.3 **a**	25.9 ± 4.0 **b**
E3	69.2 ± 5.1 **c**	132.5 ± 5.9 **b**	167.8 ± 9.8 **a**	118.6 ± 7.1 **bc**	92.8 ± 3.6 **bc**	128.6 ± 14.9 **b**
E4	147.1 ± 12.2 **a**	69.2 ± 4.0 **ab**	53.9 ± 15.2 **ab**	10.1 ± 3.7 **b**	23.1 ± 1.6 **b**	184.0 ± 4.8 **a**
Total Hg	292.8 ± 16.5 **ab**	224.1 ± 7.2 **b**	273.9 ± 4.6 **ab**	228.5 ± 11.5 **b**	232.7 ± 3.9 **b**	338.8 ± 11.6 **a**
Extraction yield (%)	98.5	99.7	99.5	103.1	97.9	101.1

Table 4. Pearson correlation coefficients (n = 30) between enzymes activities, soil parameters and mercury mobility; bold values are significant at the $p < 0.05$.

	E1	E2	E3	E4	F1	F2	F3	HgT
MBC	**−0.90**	**−0.84**	−0.26	**0.84**	**−0.93**	**−0.60**	**−0.83**	**0.92**
DH	**−0.89**	**−0.78**	−0.37	**0.85**	**−0.93**	**−0.59**	**−0.81**	**0.93**
Bglu	**−0.89**	**−0.79**	−0.39	**0.86**	**−0.93**	**−0.59**	**−0.81**	**0.93**
Urease	**−0.90**	**−0.84**	−0.35	**0.88**	**−0.93**	**−0.62**	**−0.82**	**0.93**
P5	**−0.91**	**−0.64**	**−0.42**	**0.79**	**−0.94**	**−0.51**	**−0.87**	**0.91**
P9	0.09	**0.45**	0.14	−0.37	0.09	**0.72**	−0.05	−0.31
Aryl	**−0.88**	**−0.81**	**−0.40**	**0.88**	**−0.91**	**−0.63**	**−0.79**	**0.94**
FDA	**−0.92**	**−0.84**	**−0.45**	**0.93**	**−0.94**	**−0.72**	**−0.83**	**0.97**
Clay	**0.94**	**0.59**	**0.69**	**−0.90**	**0.92**	**0.75**	**0.94**	**−0.86**
TOC	**−0.90**	**−0.86**	−0.30	**0.87**	**−0.93**	**−0.60**	**−0.83**	**0.92**
Al	**0.68**	**0.70**	**0.43**	**−0.79**	**0.66**	**0.92**	**0.60**	**−0.75**
Fe	**−0.46**	−0.12	−0.10	0.22	**−0.48**	−0.22	**−0.44**	**0.47**
pH	**0.70**	**0.83**	−0.08	**−0.62**	**0.73**	0.20	**0.70**	**−0.58**

- Total highly mobile and mobile mercury species

The quantities of highly mobile mercury E1 (Table 3), and their percentage in relation to total mercury (Figure 2b), were significantly higher for site Yao1 with a percentage of 8% for E1. Yao1 was also the richest in water soluble mercury (F1) and bioavailable mercury (F3) with 4.56 and 2.93%, respectively. For the other rehabilitated sites, the percentages of fractions F1, F2 and F3 (Figure 2a) did not show significant differences. Concerning mobile and toxic (E2) mercury, the Bel2 and Yao3 sites had the lowest grades with percentages of 6.46% and 7.68%, while Yaoni's two non-restored sites had average grades of 31.1% and 44.3% respectively. E1 and E2 are correlated negatively with microbial parameters and TOC and positively with total clay and aluminum (Table 4).



(**a**) (**b**)

Figure 2. Mercury environmental speciation for the six sites as a percentage of each fraction relative to total mercury. (**a**) Focus on the most mobile fractions of mercury. F1: H_2O soluble mercury, F2: $CaCl_2$ exchangeable mercury, F3: EDTA bioavailable mercury. (**b**) Focus on the main extractions of mercury. E1: Extraction of highly mobile mercury, E2: Extraction of mobile and toxic mer cury, E3: Extraction of semi mobile mercury, and E4: Non-mobile mercury.

- Total semi-mobile mercury species

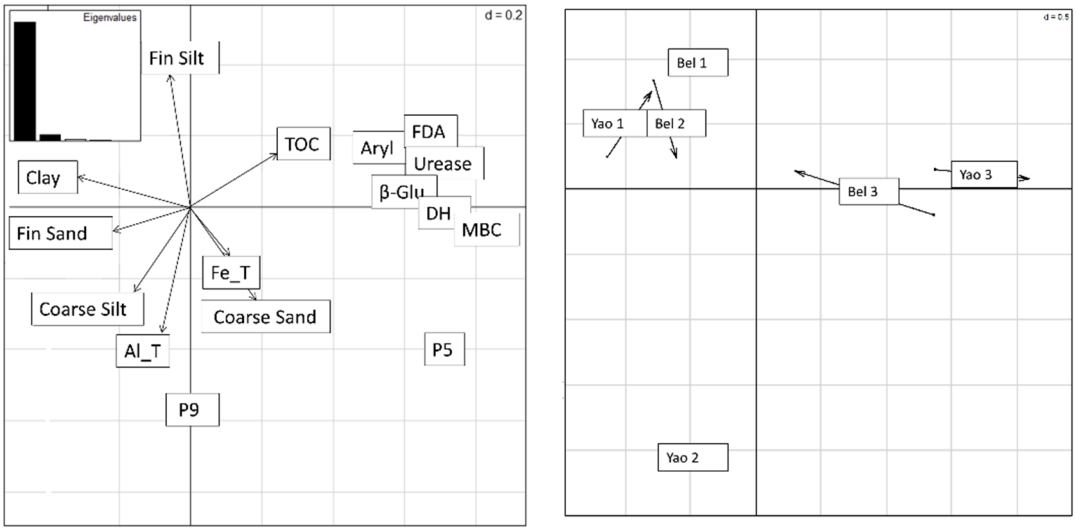
The highest percentages were detected for sites Yao1, Bel2 and Bel3 with values of 51.9%, 59.2% and 61.3%, respectively. The Bel1 site had the lowest quantity and percentage of semi-mobile mercury with 23.7%. The semi-mobile mercury fraction did not have a significant effect on microbial parameters and was significantly correlated with clay and aluminum content.

- Total non-mobile mercury species

The restored Yao3 site showed that mercury was most immobilized with 54% non-mobile mercury. The two non-restored Yaoni sites Yao1 and Yao2 had significantly lower levels of non-mobile mercury than the other sites with 4.4% and 9.9%, respectively. The two non-restored sites Bel1 and Bel2 had more mercury, 50.3% and 30.9%, respectively, than the restored site Bel3 with 19.7%. The correlation between non-mobile mercury and the dataset followed exactly the same pattern as the total mercury content.

3.4. Co-Inertia between Soil Parameters and Microbial Activities Dataset

The co-inertia analysis (Figure 3a) showed that soil parameters (six variables) had a highly significant correlation ($p < 0.001$) with the microbiological dataset (eight variables) with 41.86% of total variance explained. Total organic carbon was positively correlated to enzyme activities with the exception of acid and alkaline phosphatase. Soil pH, fine sand and clay content were negatively related to the microbiological dataset.



(**a**) (**b**)

Figure 3. (**a**) Co-inertia analysis between soil physico-chemical parameters and microbiological variables. The variables linked by lines refer to the physico-chemical variables, the other variables refer to the different enzymes activities and microbial biomass in the soil sample; (**b**) discriminant analysis on co-inertia variable of soil samples. Boxes represent the projected co-ordinates of physico-chemical dataset and microbial dataset of each rehabilitated site, respectively. The length of the arrow is proportional to the divergence between the data sets. Eigen values 86.74% and 9.08% for axis 1 to 2, respectively. Randtest: simulated *p*-value: 0.0019. Explained variance: 41.81%.

Discriminant analysis of co-inertia (Figure 3b) showed that soil samples were significantly distinguished ($p < 0.001$) according to their spatial distribution in terms of the quality of ecological restoration. In the axis 1 direction, soil samples collected from restored sites (Bel3 and Yao3) were predominated on the positive axis, and were clearly distinguished from non-restored sites, all on the negative side.

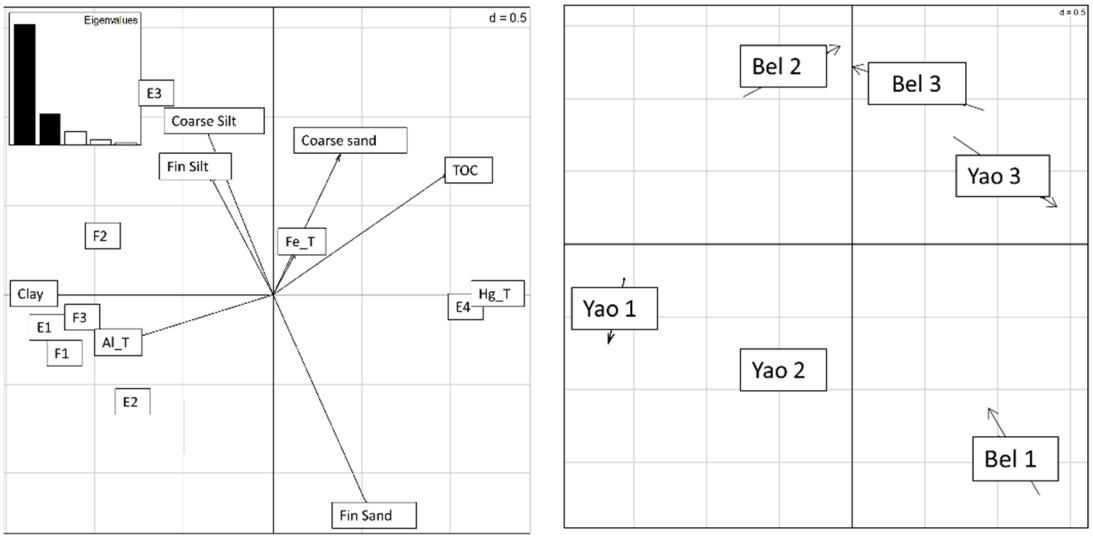
According to these data, the microbial biomass and all enzyme activities tested except for phosphatases would be correlated exclusively to the organic carbon concentration. However, we observed a negative correlation of the microbiological data with the concentration of clays and fine sands. Texture and structure are among the most important factors influencing the activity of telluric enzymes [39]. Enzyme activity is generally greater in fine textured soils than in coarse textured soils [40,41] and negative correlations between enzyme activity and clays appear to show opposite results. However, while the nature of clays also impacts enzymatic activities [42,43], it has been shown that the structural stability of soil aggregates, linked to the organic matter content, could play a more important role

than soil texture alone [40,44,45]. Organic matter stabilizes aggregate structure, increases soil retention capacity and improves nutrient bioavailability [46,47], thus stimulating soil microbial flora [48].

In this study, the major factor of microbial activity was organic matter. In these rehabilitated soils, the contribution of organic matter is exclusively conditioned by the nature of the plant cover and therefore by the success of ecological restoration. The choice of the implantation of fabaceous species, fast-growing nitrogen-fixing species, seems judicious for their ability to bring a large amount of organic matter to the soil [49,50].

3.5. Co-Inertia between Soil Parameters and Environmental Mercury Speciation

The co-inertia analysis (Figure 4a) showed that soil parameters (eight variables) had a highly significant correlation ($p < 0.001$) with the environmental mercury speciation dataset (seven variables) with 82.06% of total variance explained, which could indicate relatively similar mercury retention and mobilization mechanisms between different rehabilitated sites.



(**a**) (**b**)

Figure 4. (**a**) Co-inertia analysis between soil physico-chemical parameters and mercury extraction. The variables linked by lines refer to the physico-chemical variables, the other variables refer to the different extractions and fractions of mercury in the samples; (**b**) discriminant analysis on co-inertia variable of soil samples. Circles and boxes represent the projected co-ordinates of physico-chemical dataset and mercury dataset of each rehabilitated site, respectively. The length of the arrow is proportional to the divergence between the data sets. Eigen values 67.96% and 20.34% for axis 1 to 2, respectively. Randtest: simulated *p*-value: 0.0001. Explained variance: 82.06%.

Clay was strongly correlated with highly mobile mercury species (E1, F1, and F3) and negatively correlated to total and non-mobile mercury, while silt was related to exchangeable mercury and semi-mobile mercury species. Mobile mercury species were correlated with coarse sand, but not with fine sand. Soil pH was significantly and positively correlated (Table 4) with mobile mercury species (E2) and negatively correlated with total Fe content.

Discriminant analysis of co-inertia (Figure 4b) showed that soil samples were significantly distinguished ($p < 0.001$) according to their spatial distribution in terms of the quality of ecological restoration. In axis 1 direction, soil samples collected from restored sites (Bel3 and Yao3) were predominated on the positive axis and were clearly distinguished from non-restored sites. The highly mobile clay and mercury variable appeared to strongly distinguish the Yaoni sites, while the percentages of silt, sand and semi-mobile mercury appeared to more differentiate the Belizon sites along axis 2.

4. Discussion

4.1. Positive Effect of Ecological Restoration on Mercury Mobility

In this study, the results showed very distinct mercury behaviors based on sites that were not solely related to vegetation density. Indeed, the three least covered sites (Bel1, Yao1 and Yao2) presented very different speciation results with nearly 50% non-mobile mercury for Bel1 and less than 10% non-mobile mercury for Yao1 and Yao2. In addition, the results showed a significant increase in mobile and toxic mercury for sites Yao1 and Yao2 with more than 40% compared to about 25% of mobile mercury for Bel1. The presence of tree cover did not positively impact the mobility of mercury on the Bel3 site compared to adjacent plot Bel2, which showed a higher proportion of semi-extractable and non-mobile mercury and less mobile mercury. However, mercury speciation over the oldest restored area of Yao3 showed a very significant decrease in mobility and potential toxicity of mercury with 90% of mercury present in non-extractable forms.

While HCL/ethanol extraction represents more of a mobility potential linked to leaching under acidic conditions, water extraction, $CaCl_2$ and EDTA represent more direct mobility close to natural conditions in soils. The F1, F2 and F3 extractions showed the lowest percentage of easily mobile mercury for the Yao3 site and the highest for Yao1 with 1 and 8%, respectively. While the low mobility of mercury species for the Yao3 site are close to those for the Asturias [51], Almaden [52] and Usagre [53] mines in Spain, the Cuyuni Basin [54] in Venezuela, old mining mines in Mexico [55], in the Yaoni region of French Guiana [16], and the mercury mobility and toxicity for Yao1 were significantly higher and comparable to mobility at some Wanshan mining sites in China [56]. Depending on rainfall, these sites can go into suboxic or even anoxic conditions and these results show that the pool of mercury available for rapid methylation diverges very strongly between the rehabilitated sites.

The results indicate the contribution of a dense cover of fabaceae on the Yao3 site to modify mercury speciation compared to the unrestored Yao1 and Yao2 sites. At this site, despite a higher total mercury content than at the other rehabilitated sites, most of the mercury corresponds to very stable chemical species with low mobility and high stability, such as amalgamated elemental mercury, strongly bound mercury (mineralized and metal-oxyhydroxides) and species in the form of sulphides and hydroxide oxides. For the restored site Bel3, the results are more contrasted, but are possibly due to the more recent restoration and more limited contributions in organic matter in only four years of vegetation recovery.

4.2. Effect of Soil and Microbial Properties on Environmental Speciation of Mercury

In view of the above conclusions, the nature of the vegetation cover is not only indicative of mercury mobility. The mercury speciation provide access to information on toxicity, but do not provide a clear picture of the influence of mercury-carrying phases in soils. In tropical soils, the mobility and retention of mercury are largely from the nature of carrier phases. More specifically, clay minerals, iron and aluminum (oxyhydr)oxides, and the quantity and nature of organic matter represent the most important carrier phases and vary according to the biochemical conditions of the environment [57–60].

Extractions E1, E2 as well as the highly soluble fractions F1, F2 and F3, are positively correlated with clay and total aluminum contents. In tropical soils, rich in kaolinite and aluminosilicates, the adsorption of mercury in clay minerals may be important, especially when the pH is above 5 [61]. This distribution in soil of soluble fractions of mercury in clay minerals is consistent with the literature and shows that mercury adsorbed on these soil phases appears to be readily mobilizable [61].

Concerning the relations between mercury and the organic component of the soil (organic matter and biomass), it would seem that organic matter is the main carrier phase of non-mobile and total mercury (r^2 = 0.87 and 0.92), which are results consistent with the literature [62], and are already observed on restored sites in French Guiana [16]. The increase in mercury content in restored soil in relation to organic matter content could be

related to the heterogeneity of mining soils, but could also be a key factor in the processes of mercury retention in the soil and in the limitation of losses through leaching and erosion of soil particles. The enrichment of organic matter levels through ecological restoration could therefore have an additional effect by potentially limiting mercury transfers. It would also appear that these non-mobile and total mercury concentrations do not have an impact on the development of soil microbial communities with positive correlations with microbial biomass and enzymatic activities, a result already shown in some mining areas with mercury loading in soil [63]. Nevertheless, the negative correlations between E2 (toxic mercury) and microbial biomass could indicate that a predominance of toxic forms of mercury, even at relatively low concentrations, could negatively affect the development of microbial communities and their enzymatic activities. Another major point is that these potentially toxic forms of mercury do not correlate with soil organic matter. In this context, microbial mineralization of organic matter would therefore be likely to release non-toxic forms of mercury, but would have a very limited impact on the transfer of toxic mercury into the soil.

This study also shows a significant correlation between total iron and total mercury. While iron oxide concentrations in soils were not measured in this study, these results confirm the importance of iron in the biogeochemistry of mercury and its retention in soil phases. This hypothesis also seems to be confirmed by the important correlations observed between semi-mobile mercury extraction and silty soil fractions, rich in metal-oxyhydroxides such as goethite or hematite. This study showed that the impact of non-homogeneous mine site remediation processes has altered mercury speciation by acting directly on soil texture. These results also showed that the level of vegetation cover, and the level of organic matter brought to the soil, has improved mercury retention and sanitary quality.

4.3. Positive Effect of Ecological Restoration on Microbial Activities

Soil biochemical parameters such as microbial biomass or soil enzymatic activities are sensitive and relevant indicators of stressed and disturbed soils and are used to describe soil quality status [15,64]. Generally, microbial biomass varies according to soil physico-chemical parameters, vegetation cover or potential contamination [15,65]. In this study, microbial biomass appeared to be very sensitive to the different modalities of ecological restoration and to the nature of the vegetation cover. The presence of a restored tree cover on the Bel3 and Yao3 plots had increased the microbial biomass by a factor of 2–4 compared to non-restored sites. The presence of *Lycopodiella* sp. on the Bel2 plot has also positively stimulated microbial biomass and shows that the increase in plant density, even herbaceous, has a significant impact on microbial biomass.

Soil enzymatic activities represent the overall degradation activity of organic soil components and are affected by many factors, such as pH, organic matter content or contamination level [66], and are mediated by microbial biomass. In our study, the restored Bel3 and Yao3 sites had the highest activities, except for alkaline phosphatase activities. However, the classification of soils according to the amount of microbial biomass is not the same as for enzymatic activities. Indeed, the Yao2 site, which has one of the lowest biomasses, has relatively important dehydrogenase, B-glucosidase, acid and alkaline phosphatase activities. This could show that these enzymatic activities are not only related to microbial biomass. These results indicated a significant and positive effect of controlled ecological restoration on certain components of biochemical activities in rehabilitated soils [67–70]. The very low level of activity on non-restored soils also highlights the stressed and anthropized nature of these former mining lands, which require adapted ecological rehabilitation protocols.

4.4. Footprint of Rehabilitation on Soil Functionning and Mercury Mobility

The various stages of rebuilding an ecosystem after human degradation, such as mining are complex, multidisciplinary and often involve ecological engineering. On mining

sites in French Guiana, these protocols have to be adapted according to the degree of site contamination, soil texture, water regime and of course the nature of the plant species used for restoration [14]. *Clitoria racemosa* is a fabacea native to South America and favors sandy-gravelly or even sandy-clay substrates. At the Belizon mine, difficult site rehabilitation and earthworks led to a high degree of textural heterogeneity, resulting in a mosaic of vegetation and partial success in ecological restoration. The recovery of the macrocuts was not effective on the overly draining and sandy substrate of the Bel1 plots, now colonized by *Cyperaceae* sp. On the Bel2 plots, the significant presence of silt led to a rapid covering of the site by *Lycopodiella* sp., preventing any recovery of *C. racemosa* plants. The rational use of other products, such as *Acacia mangium*, adapted to gravel surfaces, and *Erythrina fusca*, adapted to silty substrates on the Bel1 and Bel2 plots, respectively, could have allowed for a more widespread success of ecological restoration. These results showed the importance of adapting ecological restoration protocols according to the nature of the substrate, as already described by Loubry [14].

On the former Yaoni mine, the very significant differences in terms of organic constituents of the soil provide interesting elements for the restoration of mining sites. The very low CNP stocks on plots Yao1 and Yao2 showed that mining activity depletes organic constituents and soil fertility. Seventeen years after the end of the rehabilitation, these sites had still not found shrub or tree cover and ecological succession seemed to be stopped. The controlled recovery of vegetation on the Yao3 site has highlighted the positive effect of the establishment of a cover of fabaceae on the renewal of the stock of nutrients and organic matter. It would appear that the organic matter brought in during ecological restoration is also a driving force behind the retention of mercury and the limitations of potential transfers to aqueous media. A preliminary study [16] showed that ecological restoration could affect the nature of the mercury bearing phases. However, it would also seem that the potential toxicity of these plots under ecological restoration could be reduced by the establishment of a plant cover.

5. Conclusions

We evaluated the effect of the rehabilitation of Guyana's mining sites in order to link soil texture with the quality of ecological restoration to the biochemical quality of the soil and the potential toxicity of mercury. The results indicated very variable and heterogeneous soil textures, even on nearby sites. Some sites rehabilitated with too much silt or sand have not allowed for the ecological restoration of a tree canopy. The results also highlight a direct impact of the success of ecological restoration on the return of microbial activities related to biogeochemical cycles. The provision of bedding using fabaceous species seems to be one of the key factors for the success of ecological restoration. The results also showed an impact of soil texture on mercury mobility and toxicity. Environmental speciation measures showed a link between mercury mobility and the nature of rehabilitation. The least restored sites have potentially higher concentrations of mobile and semi-mobile mercury, related to the quantities of clays and silts. The homogeneous texture of the restored Yaoni site, as well as the establishment of a dense tree canopy, has contributed to limiting mercury mobility by modifying its speciation towards more insoluble forms, such as cinnabar.

Author Contributions: Conceptualization: N.B. and E.C., The sampling campaigns for the sites presented in this study are the result of a second sampling campaign on mining sites undergoing ecological restoration. The samples were taken by E.C. and N.B., with Christian Pernaut as field guide. The methodology: E.C. and N.B.; validation, N.B.; formal analysis, E.C., V.A., A.L. and S.G.-M.; investigation, E.C.; resources, E.C.; data curation, E.C.; writing—original draft preparation, E.C.; writing—review and editing, E.C.; visualization, E.C.; supervision, N.B.; project administration, N.B.; funding acquisition, N.B. All authors have read and agreed to the published version of the manuscript.

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