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Monitoring Reclamation of Plant Biodiversity and Soil Parameters in an Area of Bauxite Mine Spoils (A Case Study of Greece)

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Abstract: In order to assess plant biodiversity in bauxite mine spoils, a fully randomized experiment was carried out with five treatments to find the ones that would best restore the soil and plant biodiversity in the spring seasons of 2020 and 2021. In the studied area, 68 plant species belonging to 54 genera with high ecological value that comprise the flora and represent 19 families were identified. Concerning the herbaceous plant species richness in several treatments, the highest value was calculated in the treatment of sludge (52 plant species), followed by the treatment of soil in the area (39), whereas the lowest plant species richness was recorded in the treatment of fertilization (27), the incorporation of soil with soils (26), and control (27), so our findings indicate that the area where sludge was applied showed the highest nutrient enrichment as well as the highest plant biodiversity, plant cover, and biomass. Apart from sludge, the mineral soil around the area was also composed of some materials that provided good results with regard to plant parameters. The main problems with the properties of the mine spoil material were the low organic matter content and the low clay percentage. The use of sludge, probably in combination with the soil around the area, might alleviate these problems. The plant parameters (Shannon diversity index, plant cover, and biomass) correlated positively and significantly with most of the macronutrients and micronutrients in soils.

Keywords: bauxite mine; soil; plants; reclamation; treatments

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1. Introduction

Opencast bauxite mining produces large deposits of waste materials. The properties of these materials are usually unfavorable or even prohibitive in some cases for the establishment of vegetation [1]. The adverse conditions are associated with the low concentrations of plant available nutrients, organic matter deficiency, low content of fine material (<2 mm), lack of structure, accelerated soil erosion, excessive leaching, compaction, reduced cation exchange capacity, decreased microbial activity, and finally, to a reduction in soil fertility [2,3]. To improve these conditions, several methods and soil amendments have been proposed by many researchers. Among them, the use of soil to cover the spoil materials or to incorporate it into them [4,5], the use of fertilizers [6] as well as sewage sludge and other organic amendment applications [7] include the most common interventions, in order to facilitate the establishment and evolution of vegetation.

Vascular plants are the dominant primary producers of terrestrial ecosystems and they are quite accurate indicators of the abiotic environment in which they grow. Plant species richness and diversity play an important role in evaluating the success of a restoration project [8]. In ecology, diversity is assessed by determining the number of species and their relative abundance in a community. Apart from diversity, two other parameters are usually assessed (i.e., plant cover and biomass) [9,10]. The rehabilitation of mining areas requires a pre-evaluation of its potential success. In other words, it is essential to conduct studies to determine the plant biodiversity and soil factors of the mining areas as natural habitats in industrialized post-mining landscapes. The latter have been subjected to less examination and are less understood [11].

Revegetation through forest plants is an efficient means to restore soil fertility through an increase in the soil organic matter content, concentrations of available nutrients, cation exchange capacity, and increased biological activities. Chauhan and Silori [12] carried out a successful reclamation of bauxite residue through afforestation activities in South India. Mensah [13] reviewed the effects of reclamation measures in mine soils in Ghana. He argued that forest plant establishment was one of the best methods to restore past ecosystems. However, he warned that this method would require long periods to restore the soil fertility as closely as possible to the original level [13]. Greece holds the position of being the twelfth largest global producer of bauxite mines, while simultaneously maintaining its status as the top producer within the European Union. In 2017, Greece achieved an annual production of 1800 thousand metric dry tons. Currently, the Greek bauxite reserves, which possess economic viability for production, are estimated to be over 250,000 thousand metric dry tons in the United States.

The aim of this work was to assess the reclamation of plant biodiversity and soils in a bauxite mine, in the spring seasons of 2020 and 2021, in Greece. In order to do so, five treatments were applied to find out their effects on the soil properties and various plant parameters. The null hypothesis, as usual, was that there was not any significant effect brought about by the treatments applied.

2. Materials and Methods

2.1. Study Area

The experiment was established at the "Rodia" site at a 580 m altitude on the SW slopes of Mount Parnassos, in the Eleonas municipality (38°34′25.72′′ N, 22°22′24.98′′ E). The area belongs to the Parnassos-Giona geological zone and consists of hard limestone. According to the bioclimatic maps of Greece [14], it belongs to the sub-humid bioclimatic zone with a mild winter (3 °C < m < 7 °C). The character of the bio climate is Meso-Mediterranean with 75–100 biologically dry days during the dry season. Annual precipitation, based on the rainfall map of Greece, ranges from 600 to 800 mm. The study area belongs to the evergreen broadleaf's zone, at the *Quercus coccifera* L. biotope, just above the biotope of *Pistacia lentiscus* L. The dominant species are *Q. coccifera* L., *Juniperus oxycedrus* L., *J. phoenicea* L., *Phillyrea media* L., *Olea oleaster* L., *Pistacia terebinthus* L., *Calicotome vilosa* (Poir.) Link, *Phlomis fruticosa* L., etc. The bauxite mining of the site started some decades ago (around the eighties).

2.2. Experimental Design

The design was carried out by the personnel of the Landscape Architecture and Environmental Rehabilitation Laboratory, Institute of Mediterranean Forest Ecosystem, ELGO DIMITRA. Site preparation including leveling and the removal of large stones and boulders was carried out by a bulldozer. The experiment consisted of five treatments with four replications for each. The replications were fully randomized. The area of each plot was 16 m^2. The five treatments were: (a) no intervention, bare spoils–control, (b) addition of 40 ton/ha of sludge, (c) fertilization with 20 kg/ha of NPK:11-15-15, (d) surface addition of a 15 cm layer of soil (fine earth) from the vicinity of the area (soil around the area), and (e) addition of the soil above-mentioned and incorporation into the spoils using a small rotary cultivator. The soil was excavated from the same area from a depth > 30 cm.

The sludge was spread homogeneously over the whole surface of each experimental plot and was incorporated into the soil at a depth of 30 cm by using the same rotary cultivator. The heavy metal concentrations were below the limits set by EEC regulation no. 86/278/EEC (OJ No L 181/4.7.86).

The fertilization, with 20 kg/ha, was applied twice on 4 November 2006 and 30 March 2007, respectively. The size of the basic unit (plot) was 4 X = 16 m^2. In total, there were 20 plots. The trial was established in the autumn (4 November) of 2006 and lasted 4 years.

2.3. Herbaceous Plant Sampling

The sampling of herbaceous plants and soils was carried out in the spring seasons of 2020 and 2021 in five selected plots of 0.25 m^2 (0.5 m $\times$ 0.5 m) each, for each treatment. The species richness, number of individuals of each species, and total percentage of plant cover given by all species were recorded in 0.25 m^2 sampling plots at each sampling site. The "*Flora Europaea*" [15,16], the "*Flora Hellenica*" [17], and the "*Vascular plants of Greece: An annotated checklist*" [18] were used in order to identify the plant species. Then, we took a surface cut of the vegetation from each sampling area and brought it back to the lab for analysis. The dry weight of the herbaceous plant biomass was determined by placing it in a drying oven at 60 °C for 48 h and then weighing it using a precision balance [19].

2.4. Soil Collection and Analysis

All soil samples, 20 in total (5 treatments $\times$ 4 replicates), after air drying, were passed through a 2-mm sieve and stored for analysis. Subsamples of the sieved soils were pulverized in a ball mill for the analysis of organic C, calcium carbonate, and Kjeldahl N. The texture of soils was determined by the hydrometer method, while the $CaCO_3$ content was measured by a calcimeter based on the reaction of $CaCO_3$ with HCl acid. The pH of soils (1:2.5 soil:water, ratio per weight) was measured by a glass electrode. The conductivity of the soil solution was determined with a conductivity meter in a soil water solution (1:5 soil:water, ratio per weight shaken for 1 h) and the result was multiplied by 6.4 [20]. Exchangeable cations (Ca, Mg, K, and Na) were extracted with 1 M NH_4-acetate solution at a pH of 7. Cation exchange capacity (CEC) of the samples was determined by the Na-acetate method [21]. The sodium saturation (ESP) was calculated as the percentage (%) of exchangeable sodium concentration over the CEC. Organic C was determined with the potassium dichromate method ($K_2Cr_2O_7$) [22]. Organic plus ammonium N was extracted with concentrated sulfuric acid (H_2SO_4) and its concentration was measured by the Kjeldahl distillation method. Ammonium and nitrate N, the so-called available N, was extracted after shaking the soil with a 2 M KCl solution [23]. The concentrations of ammonium N were measured with Kjeldahl distillation and that of nitrate N with a UV spectrophotometer at a wavelength of 220 nm [24]. Available P (Olsen) was extracted with a $NaHCO_3$ solution [25]. The available trace elements (Fe, Zn, Mn, and Cu) in soils were extracted with DTPA [26]. The concentrations of exchangeable cations and those of the available micronutrients were determined by flame atomic absorption spectroscopy.

2.5. Calculations and Statistical Analysis

The data were confirmed to be normally distributed using Levene's test. The average values, together with the coefficients of variations, were calculated for the soil properties found and the plant diversity parameters. The Shannon diversity index (H) takes into account the number of species present in the sample as well as the proportional number of individuals for each species and is utilized to quantify biodiversity. Less than 1.5 indicates a comparatively low level of species diversity, whereas greater than 2.5 indicates a high level. Plant diversity was assessed using the following biodiversity index [27,28], the formula of which is as follows:

$$H' = -\sum_{i=1}^{s} P_i \, \text{In} P_i$$

where H' is the species diversity index, s is the number of species, and P_i is the proportion of individuals of each species belonging to the *i*th species of the total number of individuals [29].

All soil parameters, together with the Shannon diversity index, the plant cover, and the plant biomass, were subjected to a one-way ANOVA analysis with 5 treatments and 4 replicates for each treatment, as mentioned in the experimental design section. The means were compared with the Tukey test.

Two correlation (Pearson) matrices were formed: one among the soil properties, and the other containing the soil properties and the plant parameters.

3. Results

3.1. Effects of Treatments on Soils

All of the results of the effects of then treatments, together with the coefficients of variations on the soil parameters, are shown in Table 1. The means were compared with the Tukey test (the same procedure took place for the plant parameters). Apart from pH and texture analysis, all of the other means had high correlation coefficients. This showed the heterogeneity of soils, even for the same replicate.

Although the pH did not change as a result of the treatments, the $CaCO_3$ content differed in the bauxite soil and the incorporated bauxite soil. It seems that the percentage of $CaCO_3$ is higher in the mining spoils than the excavated soils around the area. The organic C content was very low in all treatments with the exception of sludge. The C/N ratio was significantly higher in the control and lower in the bauxite soil. Another interesting result was that the bauxite soil had low C/N ratios. The organic N had the lowest value in the control and the highest value in the sludge treatment. The concentrations of the available NH_4+-N and NO_3^--N were significantly higher in the sludge treatment. It is worth noting that the NO_3^--N concentrations in this treatment was 10 times as high as its respective values in the other treatments. The fertilization treatments released some NO_3^--N (it ranked second in the NO_3^--N concentration after sludge). The clay content of the control and the fertilization treatment were significantly lower than those in the soil around the area and the incorporated soil. There were some nutrients, the concentration of which in all treatments was significantly lower than those in the sludge, that were also below the deficiency limits set in the literature. These were the available P, Cu, and Zn. Some other nutrients had significantly lower concentrations in the control and fertilization treatments than all the others and were below the deficiency levels. These nutrients were the exchangeable Mg and K and the CEC. The ratios of exchangeable Ca/Mg in the control and fertilization were very high due to the presence of $CaCO_3$. Nevertheless, the sludge treatment and the excavated soils had significantly lower ratios. The Pearson correlations among the nutrients (Table 2) will help explain the effects of the treatments on the soil properties.

Table 1. Average values of the properties of soils and the statistical effects of treatments. Values in parentheses depict the coefficients of variation.

	Control	Fertilization	Sludge	Soil from the Area	Incorporated Soil
pH	8.38 a (2.6)	8.53 a (2.1)	8.03 a (2.0)	8.39 a (1)	8.50 a (0.7)
$CaCO_3$ (%)	43.8 a (24)	47.0 a (37)	43.7 a (22)	16.9 c (60)	30.2 b (49)
Org. C (%)	0.39 c (40)	0.59 b (27)	3.37 a (30)	0.34 c (40)	0.44 b (31)
Org. N (mg kg^{-1})	252 d (80)	336 c (54)	2947 a (18)	478 b (24)	498 b (14)
NH_4-N (mg kg^{-1})	10.3 b (47)	13.6 b (24)	18.8 a (34)	10.0 b (15)	17.9 a (30)
NO_3-N (mg kg^{-1})	3.42 c (64)	7.72 b (67)	30.3 a (28)	2.06 c (22)	3.77 c (58)
Org. C/Org. N	26.3 a (90)	19.9 b (44)	11.1 d (24)	7.42 c (40)	8.69 c (22)
Sand (%)	66.8 a (14)	70.3 a (9.4)	71.3 a (4.2)	49.4 b (13)	55.9 b (17)
Clay (%)	17.2 b (36)	17.8 b (29)	14.6 b (7.9)	31.6 a (19)	26.3 a (27)
Silt (%)	16.0 a (23)	11.9 b (29)	14.0 a (21)	19.0 a (9)	17.8 a (12)
Conductivity (μS/cm)	608 b (10)	690 b (22)	854 a (37)	495 c (16)	505 c (7.5)
Exch. Ca (meq/100 g)	13.2 c (13)	12.4 c (7)	15.2 b (7)	16.7 a (7.9)	15.8 b (10)
Exch. Mg (meq/100 g)	0.167 c (34)	0.165 c (35)	0.348 c (39)	0.325 a (28)	0.258 b (22)
Ca/Mg	84 a (21)	82 a (21)	47 c (26)	54 b (23)	65 b (13)
Exch. K (meq/100 g)	0.116 b (46)	0.141 b (44)	0.224 a (60)	0.377 a (26)	0.305 a (30)
Exch. Na (meq/100 g)	0.005 a (75)	0.003 b (25)	0.007 a (7.2)	0.008 a (27)	0.008 a (19)
CEC (meq/100 g)	5.82 c (45)	4.55 c (50)	11.3 b (12)	15.1 a (26)	13.1 a (35)
Avail. P (mg kg^{-1})	2.83 b (29)	3.48 b (24)	47.6 a (11)	2.99 b (12)	3.46 b (72)
Avail. Mn (mg kg^{-1})	5.88 b (61)	4.57 b (40)	5.78 b (28)	11.9 a (3.1)	10.2 a (43)
Avail. Fe (mg kg^{-1})	5.84 c (23)	3.25 c (41)	21.3 a (32)	13.3 b (22)	11.2 b (51)
Avail. Cu (mg kg^{-1})	0.069 c (127)	0.113 c (69)	4.29 a (25)	0.494 b (26)	0.381 b (56)
Avail. Zn (mg kg^{-1})	0.188 b (73)	0.338 b (49)	12.9 a (32)	0.481 b (14)	0.763 b (64)

Different letters in the same row denote significance level for at least a 0.05 probability level.

Table 2. Pearson correlation coefficients of soil properties.

	pH	$CaCO_3$	Org. C	Kjeldahl N	Org. N	NH_4-N	NO_3-N	Clay	Exch. Ca	Exch. Mg	Exch. K	CEC	Avail. P	Avail Mn	Avail. Fe	Avail. Cu	Avail. Zn
pH	1	0.066	−0.792 **	−0.792 **	−0.793 **	−0.169	−0.773 **	0.060	−0.366	−0.599 **	−0.189	0.320	−0.739 **	−0.059	−0.592 **	−0.752 **	−0.733 **
$CaCO_3$	0.066	1	0.263	0.182	0.182	0.209	0.347	0.856 **	−0.805 **	−0.560 *	−0.777 **	−0.804 **	0.237	−0.738 **	−0.203	0.192	0.267
Org. C	−0.792 **	0.263	1	0.960 **	0.960 **	0.470 *	0.968 **	0.420	0.084	0.438	−0.020	0.077	0.939 **	0.295	0.660 **	0.943 **	0.953 **
Kjeldahl N	−0.792 **	0.182	0.960 **	1	1.000 **	0.490 *	0.958 **	0.329	0.218	0.515 *	0.076	0.221	0.980 **	−0.187	0.781 **	0.991 **	0.987 **
Org. N	−0.793 **	0.182	0.960 **	1.000 **	1	0.487 *	0.958 **	0.328	0.219	0.516 *	0.077	0.221	0.980 **	−0.185	0.780 **	0.991 **	0.987 **
NH_4-N	−0.169	0.209	0.470 *	0.490 *	0.487 *	1	0.491 *	0.296	−0.026	−0.052	−0.172	0.018	0.456 *	−0.344	0.491 *	0.516 *	0.510 *
NO_3-N	−0.773 **	0.347	0.968 **	0.958 **	0.958 **	0.491 *	1	−0.451 *	0.018	0.400	−0.051	0.024	0.932 **	−0.331	0.619 **	0.936 **	0.955 **
Clay	0.060	−0.856 **	−0.420	−0.329	−0.328	−0.296	−0.451 *	1	0.739 **	0.438	0.793 **	0.791 **	−0.430	0.855 **	0.113	−0.331	−0.412
Exch. Ca	−0.366	−0.805 **	0.084	0.218	0.219	−0.026	0.018	0.739 **	1	0.810 **	0.878 **	0.957 **	0.152	0.793 **	0.540 *	0.198	0.125
Exch. Mg	−0.599 **	−0.560 *	0.438	0.515 *	0.516 *	−0.052	0.400	0.438	0.810 **	1	0.839 **	0.783 **	0.465 *	0.527 *	0.570 **	0.470 *	0.445 *
Exch. K	−0.189	−0.777 **	−0.020	0.076	0.077	−0.172	−0.051	0.793 **	0.878 **	0.839 **	1	0.898 **	−0.008	0.791 **	0.339	0.044	0.004
CEC	−0.320	−0.804 **	0.077	0.221	0.221	0.018	0.024	0.791 **	0.957 **	0.783 **	0.898 **	1	0.139	0.787 **	0.580 **	0.219	0.139
Avail P	−0.739 **	0.237	0.939 **	0.980 **	0.980 **	0.456 *	0.932 **	0.430	0.152	0.465 *	−0.008	0.139	1	−0.265	0.733 **	0.972 **	0.972 **
Avail. Mn	−0.059	−0.738 **	−0.295	−0.187	−0.185	−0.344	−0.331	0.855 **	0.793 **	0.527 *	0.791 **	0.787 **	−0.265	1	0.184	−0.199	−0.270
Avail. Fe	−0.592 **	−0.203	0.660 **	0.781 **	0.780 **	0.491 *	0.619 **	0.113	0.540 *	0.570 **	0.339	0.580 **	0.733 **	0.184	1	0.822 **	0.766 **
Avail. Cu	−0.752 **	0.192	0.943 **	0.991 **	0.991 **	0.516 *	0.936 **	0.331	0.198	0.470 *	0.044	0.219	0.972 **	−0.199	0.822 **	1	0.989 **
Avail. Zn	−0.733 **	0.267	0.953 **	0.987 **	0.987 **	0.510 *	0.955 **	0.412	0.125	0.445 *	0.004	0.139	0.972 **	−0.270	0.766 **	0.989 **	1

* and ** denote significance level at 0.05 and 0.01 probability levels.

3.2. Effects of Treatments on Plant Biodiversity Parameters

In the studied area, 68 plant species belonging to 54 genera with high ecological value that comprise the flora and represent 19 families were identified. Concerning the herbaceous plant species richness in several treatments, the highest value was calculated in the treatment of sludge (52 plant species), followed by the treatment of the soil in the area (39), whereas the lowest plant species richness was recorded in the treatment of fertilization (27), the incorporation of soil with soils (26), and the control (27) (Appendix A). The most numerous families were Poaceae (19.23%, 18.51%, 22.22%, 15.38%, and 14.81%) and Asteraceae (15.38%, 14.81%, 22.22%, 17.98%, and 22.22%) in the treatment of sludge, soil around the area, fertilization, and incorporation of soil with soils and the control, respectively. Also, the status of plant species were as follows: Alien/Established (1 plant species); Native/Non Range-Restricted (48) and Native/Range-Restricted (3) in the treatment of sludge; Native/Non Range-Restricted (37) and Native/Range-Restricted (2) in the treatment of soil around the area; Native/Non Range-Restricted (26) and Native/Range-Restricted (1) in the treatment of fertilization; Native/Non Range-Restricted (24) and Native/Range-Restricted (2) in the incorporation of soil with soils; Native/Non Range-Restricted (25) and Native/Range-Restricted (2) in the control.

Regarding the life forms, the plant species in each treatment were detected as follows: Chamaephyte (2 plant species), Hemicryptophyte (13), Hemicryptophyte, Chamaephyte (1), Phanerophyte, Chamaephyte (1), Therophyte (31), Therophyte, Hemicryptophyte (4) in the treatment of sludge; Circumtemperate (1), Cosmopolitan (3), European-SW Asian (6), Greek endemic (2), Mediterranean (14), Mediterranean-European (5), Mediterranean-SW Asian (7) in the treatment of soil around the area; Hemicryptophyte (7), Therophyte (18) and Hemicryptophyte (2) in the treatment of fertilization; Chamaephyte (1), Geophyte (1), Hemicryptophyte (2), Hemicryptophyte, Chamaephyte (1), Therophyte (19) and Therophyte, Hemicryptophyte (2) in the incorporation of soil with soils; Hemicryptophyte (7), Hemicryptophyte, Chamaephyte (1) and Therophyte (19) in the control.

Figures 1–3 show the effects of the treatments on the Shannon diversity index, plant cover, and plant biomass. It can be seen that the variation in the bars was not as high as the variability in the soils. In all treatments, the sludge gave the highest values. The excavated soil from the area ranked second in terms of nutrient magnitudes, as shown in Figures 2 and 3 (biomass and plant cover, respectively). The correlation of the plant parameters (Table 3) with those of the soil will be discussed below.

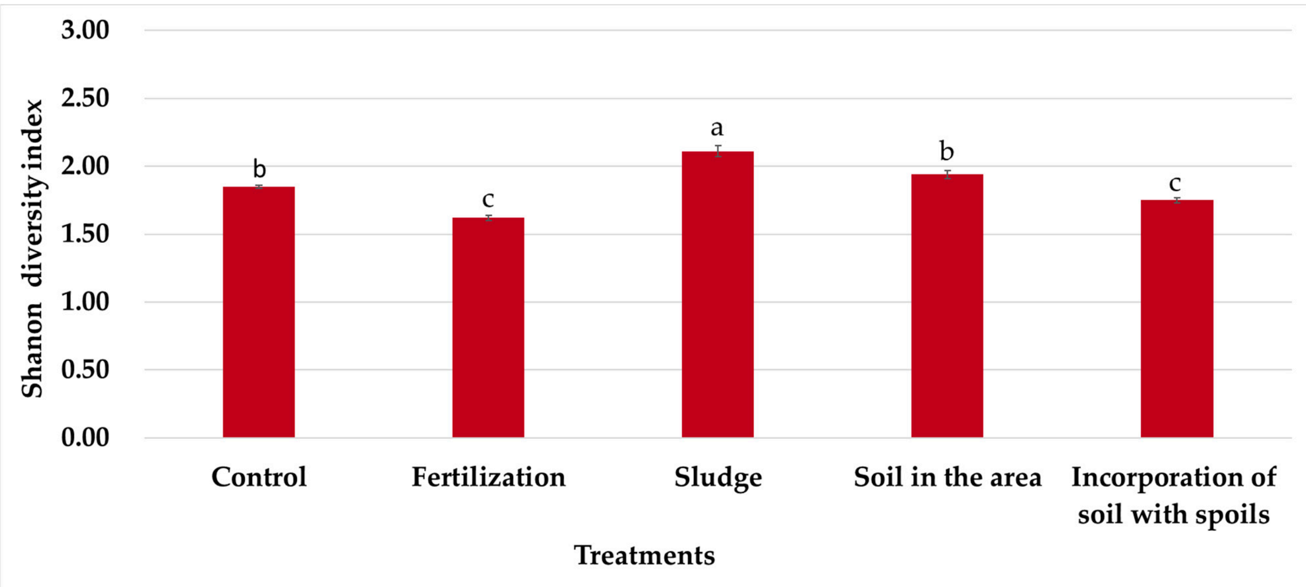

Figure 1. Plant Shannon diversity index in several treatments. For all treatments with the different letter, the difference between the means is statistically significant.

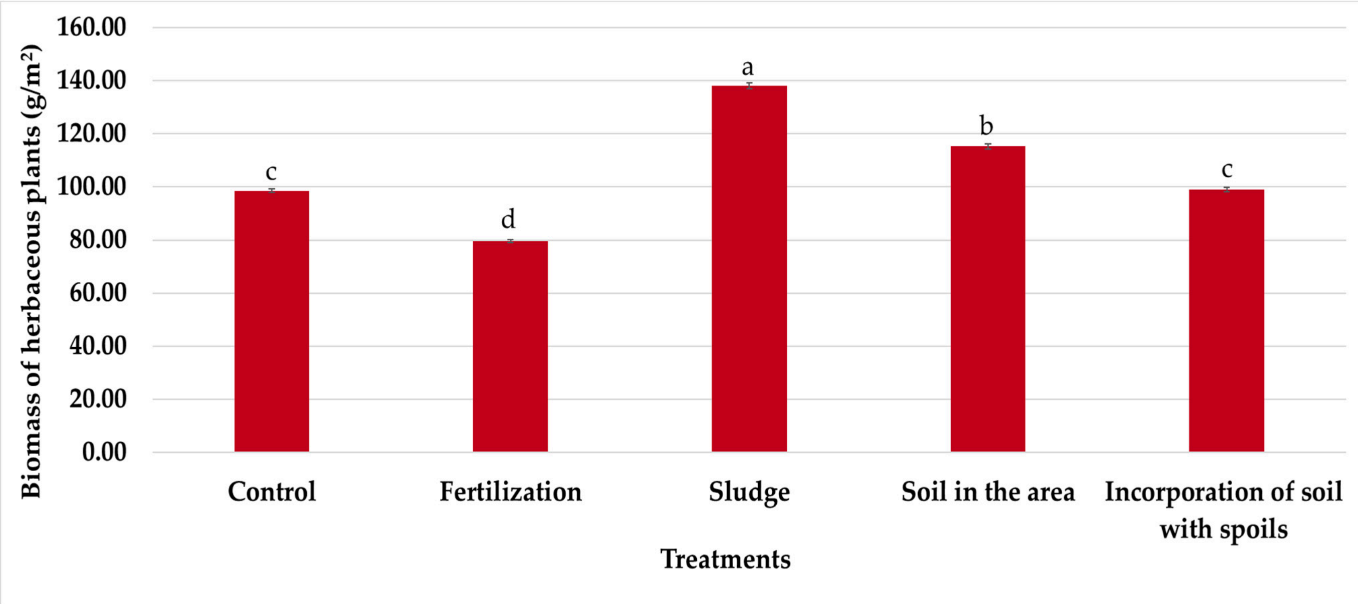

Figure 2. Mean biomass of herbaceous plants produced (g/m^2) in the several treatments. For all treatments with the different letter, the difference between the means is statistically significant.

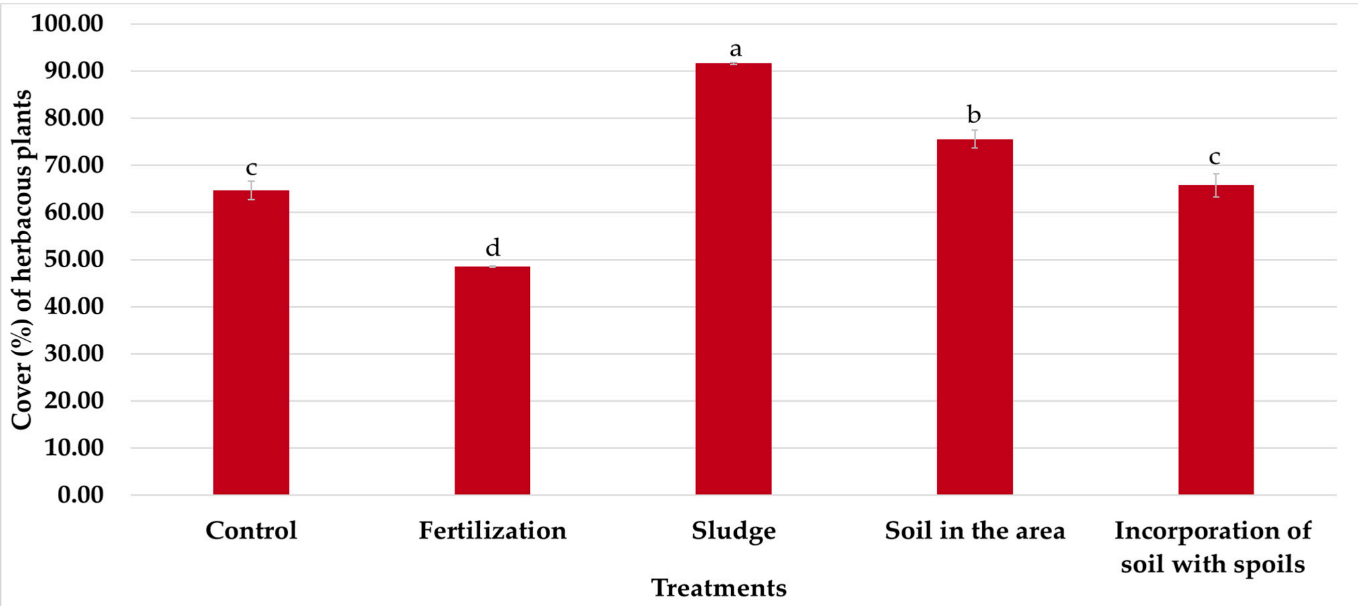

Figure 3. Plant cover (%) of herbaceous plants in several treatments. For all treatments with the different letter, the difference between the means is statistically significant.

Table 3. Correlation of the soil properties with the plant parameters.

	Shannon Diversity Index	Plant Cover (%)	Biomass
pH	0.639 **	−0.663 **	−0.749 **
$CaCO_3$	−0.119	−0.192	−0.064
Org. C	0.588 **	0.676 **	0.782 **
Org. N	0.706 **	0.762 **	0.867 **
NH_4-N	0.164	0.225	0.310
NO_3-N	0.549 *	0.589 **	0.721 **
Org. C/Org. N	−0.213	−0.230	−0.276
Clay	−0.093	0.018	−0.128
Exch. Ca	0.412	0.532 *	0.455 *
Exch. Mg	0.503 *	0.620 **	0.610 **
Ca/Mg	−0.505 *	−0.596 **	−0.606 **
Exch. K	0.194	0.340	0.234
CEC	0.696 **	0.536 *	0.436
Avail. Mn	0.083	0.216	0.059
Avail. Fe	0.735 **	0.813 **	0.836 **
Avail. Cu	0.723 **	0.778 **	0.874 **
Avail. P	0.728 **	0.751 **	0.864 **
Avail. Zn	0.682 **	0.728 **	0.839 **

* and ** denote significance level at 0.05 and 0.01 probability levels.

4. Discussion

4.1. Soils

The characteristics of the mining spoils (showed by the control values) are the high content of $CaCO_3$ and the low concentrations of organic C and clay. Only the last two treatments, which are covered by the mineral soils of the area, had different concentrations of $CaCO_3$ and clay. These characteristics affected the soil properties and nutrient concentrations to a great deal. For example, the magnitude of the CEC was low in the control and fertilization treatments. The significant and positive correlation of clay and CEC (Table 2) testifies to the effect of clay. The high ratios of Ca/Mg in the control and fertilization treatments can depress the Mg uptake by plants. The sludge treatment had by far the highest concentrations of organic C, total and available N, available P, and available trace elements. The concentrations of NO_3^--N in sludge was rather high with regard to the other treatments. This was due to the combination of the high total N content, the decomposability of the organic matter in sludge, and the alkaline environment in soils, which is favorable for nitrification. In general, the correlations of organic C and the total N, available N, available P, available Fe, and available Zn and Cu were significant and positive (Table 2). In contrast, the soil pH had a negative relationship with plenty of the soil parameters, even with NO_3^--N, which, according to theory, should be the other way. This was due to the negative significant correlation that pH had with the organic C. Organic C, as above-mentioned, had a positive relationship with plenty of nutrients. It is probable that the low pH brings about a delay in the organic matter decomposition. Many times in correlation matrices, a third variable affects the relationship of two variables.

Available Mn correlated significantly with the clay content. Despite the inorganic fertilization, its respective treatment did not have sufficient P and trace element concentrations. It is probable that the very low content of clay contributed to this result. The conductivity of soil solution and the exchangeable Na concentrations did not raise any alarm as their magnitudes were low.

4.2. Plant Biodiversity Parameters

Our study showed that the most numerous families were Asteraceae and Poaceae, which reflects the prevailing situation in the Greek area, as these are among the two most numerous families in Greece and the Mediterranean [30,31]. According to Gilliam [32], the understory is an important component of the ecosystems; it influences energy flow and nutrient cycling, biodiversity, and regeneration ability. Furthermore, the understory responds quickly to both natural and manmade disturbances [33] such as avoiding erosion and creating favorable microenvironments for the development of other species [34], microenvironments, and stand conditions [35]. The life form spectra of the vegetation in all treatments indicated that therophytes had the highest contribution in the study area of the total recorded species. A possible explanation is that therophyte life forms tend to occur in sites with warm and dry conditions and is linked to disturbances in Mediterranean ecosystems [36], which in the study area, have been disturbed by human activities (mining).

As the majority of the treatments (apart from the sludge one) were deficient in most of the soil nutrients, the correlations in Table 3 can be explained. The three plant parameters correlated significantly and positively with all of the micronutrients and macronutrients. There was a rather strange relationship with the soil pH. There was a positive significant correlation between the pH and the Shannon diversity index and negative with the biomass and plant cover. In the literature, there is an explanation for this biodiversity enrichment. Ewald [37] argued that the Pleistocene range conditions caused the extinction of more acidophilus species than calciphilus because acid soils were much rarer when refuge areas were at their minimum. Therefore, calciphilus species developed an ability to thrive in calcareous soils. The increase in plant cover and plant biomass with pH reduction was probably due to the better soil conditions in a lower pH environment. For example, the available P in soils requires lower pH values as it can be fixed by $CaCO_3$.

The NH_{4+}-N in soils did not significantly correlate with the plant parameters, probably due to the alkaline soil conditions. In contrast, the NO_3^--N had a positive and significant relationship with the plant parameters (Table 3). This form of N resulted from the nitrification of organic N compounds. There is a feedback between the availability of N and the Shannon diversity index. In general, the rates of N mineralization increase with plant species diversity [38,39]. On the other hand, the composition and diversity affect soil fertility through the differential species effects on the nutrient inputs.

The negative relation of the Ca/Mg ratio with the plant parameters shows the suppressed uptake of Mg by plants. It seems that the Ca released by $CaCO_3$ intervenes in the Mg uptake.

The positive and significant correlation of the plant parameters with available P, Fe, Zn, and Cu mean that plants need these elements, but to what extent we do not know. Only fertilization trials can verify such conclusions.

5. Conclusions

The use of sludge was the treatment that most restored of the original soil environment. It was found that the area where sludge was applied showed the highest nutrient enrichment as well the highest plant biodiversity, cover, and biomass. The use of soil around the area is also a factor to be considered. Perhaps the combination of the two treatments could offer better results. The final proof of nutrient deficiency can be verified by fertilization trials. These can be conducted by foliar spray of the chosen fertilizer. Some of the plants recorded including *Melica ciliata*, *Scrophularia canina*, *Capparis spinosa*, *Centranthus ruber Melilotus albus*, *Medicago lupulina*, *Ononis pusilla*, *Vicia villosa*, and *Dorycnium hirsutum*, in addition to their importance for biodiversity, have a special interest in restoration. First of all, their presence in the deposits shows that they adapt to these difficult conditions, and as perennial plants, they can successfully be used in the restoration of vegetation, offering soil fixation and coverage, while legumes can create symbiosis and enrich the materials with N. These results will increase the environmental awareness of the reclamation of plant biodiversity in mines, and most importantly, they will induce and guide further work, especially field-orientated studies on this subject in Greece. No management strategy can be designed unless a thorough knowledge of the subject exists. Also, future research should focus on the evaluation of the environmental impacts on plant diversity, which could be utilized in decision making for conservation and the sustainable use of biodiversity and ecosystem services in the study area.

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Appendix A

Table A1. Plant species in the treatment of the incorporation of soil with soils.

Plant Species	Family
Aegilops neglecta Bertol.	Poaceae
Aegilops triuncialis L.	Poaceae
Avena sterilis L.	Poaceae
Biscutella didyma L.	Brassicaceae
Bromus tectorum L.	Poaceae
Bunias erucago L.	Brassicaceae
Clypeola jonthlaspi L.	Brassicaceae
Convolvulus althaeoides L.	Convolvulaceae
Crucianella angustifolia L.	Rubiaceae
Crucianella latifolia L.	Rubiaceae
Cynodon dactylon (L.) Pers.	Poaceae
Dianthus hispidus (Boiss. & Balansa)	Caryophyllaceae
Echium plantagineum L.	Boraginaceae
Matricaria recutita L.	Asteraceae
Medicago disciformis DC.	Fabaceae
Minuartia confusa (Boiss.) Maire & Petitm.	Caryophyllaceae
Picnomon acarna (L.) Cass.	Asteraceae
Polygonum aviculare L.	Polygonaceae
Reseda lutea L.	Resedaceae
Silene congesta Sm.	Caryophyllaceae
Silene vulgaris (Moench) Garcke	Caryophyllaceae
Silybum marianum (L.) Gaertn.	Asteraceae
Sonchus oleraceus L.	Asteraceae
Torilis nodosa (L.) Gaertn.	Apiaceae
Trifolium angustifolium L.	Fabaceae
Trifolium stellatum L.	Fabaceae

Table A2. Plant species in the fertilization treatment.

Plant Species	Family
Achnatherum bromoides (L.) P. Beauv.	Poaceae
Acinos suaveolens (Sm.) Loudon	Lamiaceae
Arenaria serpyllifolia L.	Caryophyllaceae
Aurinia saxatilis (L.) Desv.	Brassicaceae
Biscutella didyma L.	Brassicaceae
Bromus hordaceous L.	Poaceae
Bromus tectorum L.	Poaceae
Centranthus ruber (L.) DC.	Valerianaceae
Cerastium glomeratum Thuill.	Caryophyllaceae
Crepis incana Sm.	Asteraceae
Crucianella latifolia L.	Rubiaceae
Hordeum murinum L.	Poaceae
Knautia integrifolia (L.) Bertol.	Caprifoliaceae
Lactuca serriola L.	Asteraceae
Lactuca viminea (L.) J. Presl & C. Presl	Asteraceae
Lomelosia brachiata (Sm.) Greuter & Burdet	Dipsacaceae
Melica ciliata L.	Poaceae
Minuartia confusa (Boiss.) Maire & Petitm.	Caryophyllaceae
Misopates orontium (L.) Raf.	Plantaginaceae
Picnomon acarna (L.) Cass.	Asteraceae
Polygonum aviculare L.	Polygonaceae
Silene auriculata Sm.	Caryophyllaceae
Sonchus oleraceus L.	Asteraceae
Stellaria media (L.) Vill.	Caryophyllaceae
Tolpis barbata (L.) Gaertn.	Asteraceae
Velezia hispida Boiss. & Balansa	Caryophyllaceae
Vulpia myuros (L.) C.C. Gmel.	Poaceae

Table A3. Plant species in the sludge treatment.

Plant Species	Family
Achnatherum bromoides (L.) P. Beauv.	Poaceae
Acinos suaveolens (Sm.) Loudon	Lamiaceae
Aegilops triuncialis L.	Poaceae
Alyssum montanum L.	Brassicaceae
Anthoxanthum odoratum L.	Poaceae
Arenaria serpyllifolia L.	Caryophyllaceae
*Aurinia saxatilis (*L.) Desv.	Brassicaceae
Avena sterilis L.	Poaceae
Biscutella didyma L.	Brassicaceae
Brachypodium distachyon (L.) P. Beauv.	Poaceae
Bromus hordaceous L.	Poaceae
Bromus tectorum L.	Poaceae
Capparis spinosa L.	Capparaceae
Catapodium rigidum (L.) C.E. Hubb.	Poaceae
Cerastium glomeratum Thuill.	Caryophyllaceae
Chondrilla ramosissima Sm.	Asteraceae
Clypeola jonthlaspi L.	Brassicaceae
Crepis incana Sm.	Asteraceae
Crucianella latifolia L.	Rubiaceae
Dianthus hispidus (Boiss. & Balansa)	Caryophyllaceae

Table A3. *Cont.*

Plant Species	Family
Euphorbia rigida M. Bieb.	Euphorbiaceae
Galium divaricatum Lam.	Rubiaceae
Galium murale (L.) *All.*	Rubiaceae
Geranium robertianum L.	Geraniaceae
Knautia integrifolia (L.) *Bertol.*	Caprifoliaceae
Lactuca intricata Boiss.	Asteraceae
Lactuca serriola L.	Asteraceae
Lamium amplexicaule L.	Lamiaceae
Malabaila aurea (Sm.) Boiss.	Apiaceae
Malva sylvestris L.	Malvaceae
Matricaria recutita L.	Asteraceae
Medicago lupulina L.	Fabaceae
Melica ciliata L.	Poaceae
Melilotus albus Medik.	Fabaceae
Mentha longifolia (L.) Huds.	Lamiaceae
Minuartia confusa (Boiss.) Maire & Petitm.	Caryophyllaceae
Misopates orontium (L.) *Raf.*	Plantaginaceae
Ononis pusilla L.	Fabaceae
Phacelia tanacetifolia Benth.	Hydrophyllaceae
Picnomon acarna (L.) *Cass.*	Asteraceae
Polygonum aviculare L.	Polygonaceae
Psilurus incurvus (Gouan) Schinz & Thell.	Poaceae
Rumex pulcher L.	Polygonaceae
Scandix pecten-veneris L.	Apiaceae
Sedum hispanicum L.	Crassulaceae
Silene guicciardii Boiss. & Heldr.	Caryophyllaceae
Sonchus oleraceus L.	Asteraceae
Stellaria media (L.) *Vill.*	Caryophyllaceae
Tolpis barbata (L.) *Gaertn.*	Asteraceae
Torilis nodosa (L.) *Gaertn.*	Apiaceae
Trifolium campestre Schreb.	Fabaceae
Trifolium scabrum L.	Fabaceae

Table A4. Plant species in the treatment of soil around the area.

Plant Species	Family
Aegilops neglecta Bertol.	Poaceae
Aegilops triuncialis L.	Poaceae
Alyssum montanum L.	Brassicaceae
Arenaria serpyllifolia L.	Caryophyllaceae
Astragalus hamosus L.	Fabaceae
Avena sterilis L.	Poaceae
Biscutella didyma L.	Brassicaceae
Bromus hordaceous L.	Poaceae
Bromus tectorum L.	Poaceae
Bunias erucago L.	Brassicaceae
Centaurea solstitialis L.	Asteraceae
Cerastium glomeratum Thuill.	Caryophyllaceae
Chondrilla ramosissima Sm.	Asteraceae
Clypeola jonthlaspi L.	Brassicaceae
Crepis incana Sm.	Asteraceae
Crucianella latifolia L.	Rubiaceae
Cynodon dactylon (L.) Pers.	Poaceae
Echium plantagineum L.	Boraginaceae
Erodium cicutarium (L.) L'Hér.	Geraniaceae
Knautia integrifolia (L.) Bertol.	Caprifoliaceae

Table A4. *Cont.*

Plant Species	Family
Lolium rigidum Gaudin	Poaceae
Medicago disciformis DC.	Fabaceae
Medicago orbicularis (L.) Bartal.	Fabaceae
Minuartia confusa (Boiss.) Maire & Petitm.	Caryophyllaceae
Papaver rhoeas L.	Papaveraceae
Picnomon acarna (L.) Cass.	Asteraceae
Polygonum aviculare L.	Polygonaceae
Reseda lutea L.	Resedaceae
Scrophularia canina L.	Scrophulariaceae
Sherardia arvensis L.	Rubiaceae
Silene guicciardii Boiss. & Heldr.	Caryophyllaceae
Silene vulgaris (Moench) Garcke	Caryophyllaceae
Silybum marianum (L.) Gaertn.	Asteraceae
Sonchus oleraceus L.	Asteraceae
Tolpis barbata (L.) Gaertn.	Asteraceae
Tordylium maximum L.	Apiaceae
Trifolium angustifolium L.	Fabaceae
Trifolium scabrum L.	Fabaceae
Trifolium stellatum L.	Fabaceae

Table A5. Plant species in the control treatment.

Plant Species	Family
Achnatherum bromoides (L.) P. Beauv.	Poaceae
Arenaria serpyllifolia L.	Caryophyllaceae
Avena sterilis L.	Poaceae
Biscutella didyma L.	Brassicaceae
Bromus tectorum L.	Poaceae
Catapodium rigidum (L.) C.E. Hubb.	Poaceae
Cerastium glomeratum Thuill.	Caryophyllaceae
Chondrilla ramosissima Sm.	Asteraceae
Clypeola jonthlaspi L.	Brassicaceae
Convolvulus althaeoides L.	Convolvulaceae
Crepis incana Sm.	Asteraceae
Crucianella latifolia L.	Rubiaceae
Dorycnium hirsutum (L.) Ser.	Fabaceae
Knautia integrifolia (L.) Bertol.	Caprifoliaceae
Lomelosia brachiata (Sm.) Greuter & Burdet	Dipsacaceae
Medicago lupulina L.	Fabaceae
Minuartia confusa (Boiss.) Maire & Petitm.	Caryophyllaceae
Misopates orontium (L.) Raf.	Plantaginaceae
Ononis pusilla L.	Fabaceae
Picnomon acarna (L.) Cass.	Asteraceae
Ptilostemon afer (Jacq.) Greuter	Asteraceae
Reichardia picroides (L.) Roth	Asteraceae
Silene vulgaris (Moench) Garcke	Caryophyllaceae
Tolpis barbata (L.) Gaertn.	Asteraceae
Tordylium maximum L.	Apiaceae
Velezia hispida Boiss. & Balansa	Caryophyllaceae
Vicia villosa P.W. Ball	Fabaceae

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