minerals

MDPI

Article

Nitrogen Assessment in Amended Mining Soils Sown with *Coronilla juncea* and *Piptatherum miliaceum*

Juan Carlos Beltrá *, María Gabarrón, Ángel Faz, Raúl Zornoza, José A. Acosta and Silvia Martínez-Martínez

Sustainable Use, Management and Reclamation of Soil and Water Research Group, ETSIA, Universidad Politécnica de Cartagena, Paseo Alfonso XIII, 52, 30230 Cartagena, Spain; mariags86@gmail.com (M.G.); angel.fazcano@upct.es (Á.F.); raul.zornoza@upct.es (R.Z.); ja.acosta@upct.es (J.A.A.); silvia.martinez@upct.es (S.M.-M.)

* Correspondence: juan.beltra@upct.es; Tel.: +34-968-325-634

Abstract: Metalliferous mining activities generate a large amount of waste. This waste usually has high concentrations of pollutants such as metal(oid)s associated with the extractive processes, which, if not properly treated and reclaimed, put the ecosystem and the population at risk. One of the most used techniques for mine waste reclamation is aided phytostabilization, which is based on the use of plants that immobilize metals in the soil/roots aided by the use of amendments to improve the soil properties to favor plant growth. Although amendments increase nutrients and improve the soil properties, the concentration of these nutrients—especially N, the most limiting plant nutrient—decreases over time. Thus, this study focused on the evaluation of the relationship between different combinations of amendments (compost, biochar, zeolite and limestone) and plant growth (we introduced *Coronilla juncea* and *Piptatherum miliaceum*) on the evolution of soil N over time as well as the influence of *C. juncea* on soil N fixation. The results showed that the addition of amendments improved the soil characteristics in all plots favoring the growth of *C. juncea* and *P. miliaceum*. The compost provided higher concentrations of total N, nitrites, nitrates and ammonium due to the nature of this amendment and the biochar was less in measure. The limestone helped to elevate the pH and the zeolite controlled the exchangeable ions. Soils from *C. juncea* showed higher concentrations of N forms, suggesting that this legume contributes to the enrichment of soil N, likely due to biological fixation. Hence, the combinations limestone-zeolite-compost and limestone-zeolite-compost-biochar were the most suitable treatments for improving the soil fertility and favored plant growth. In addition, *C. juncea* seems to be a good candidate for reclaiming mining environments.

Keywords: reclamation; Technosol; tailings ponds; *Coronilla juncea*; *Piptatherum miliaceum*; inorganic N forms

Citation: Beltrá, J.C.; Gabarrón, M.; Faz, Á.; Zornoza, R.; Acosta, J.A.; Martínez-Martínez, S. Nitrogen Assessment in Amended Mining Soils Sown with *Coronilla juncea* and *Piptatherum miliaceum*. *Minerals* **2022**, *12*, 433. https://doi.org/10.3390/min12040433

Academic Editor: Maria Economou-Eliopoulos

Received: 23 February 2022
Accepted: 29 March 2022
Published: 31 March 2022

1. Introduction

Mine activity is considered to be one of the most environmentally unfriendly anthropogenic activities in the world. It results in negative impacts on public health and the environment because of heavy metals and metalloids that are accumulated in tailings ponds [1]. As a consequence, most of these tailings ponds remain without vegetation and show very low fertility and high toxicity [2]. Therefore, it is necessary to mitigate the environmental impacts such as the transfer of toxic trace elements by water and wind erosion as well as leaching or accumulation in edible plant species [3].

There are several remediation techniques available for tailings ponds. However, aided phytostabilization is considered to be the most promising phytoremediation technique for the site remediation of tailings ponds. It consists of the chemical stabilization of heavy metals and/or metalloids with the combined use of different inorganic or/and organic soil amendments to create a Technosol and appropriately selected plant species [4]. Adequate information on the different types of amendments is vital for the optimum restoration of degraded mining areas. Thus, the incorporation of organic amendments has been proposed as an economically feasible and environmentally sustainable practice because biowaste can improve soil properties and it contains the nutrients necessary for micro-organisms and plants [5,6]. The acidic nature of many tailings ponds makes the use of amendments that increase the pH necessary. Thus, alkaline materials such as carbonates are frequently used to improve the acidic conditions of the tailings as they are able to immobilize metals by precipitation such as oxides, sesquioxides, carbonates or phosphates, thus reducing toxicity [3,7]. In addition, soil amendments help to increase the capacity of the soil sorption complex [8]. The development of Technosols using these amendments may reduce the metal(oid) bioavailability and acid mine drainage (AMD). Moreover, these Technosols allow the establishment of new vegetation, improving the soil fertility and increasing the diversity of microbial communities as well as reducing wind and water erosion [9,10].

In recent years, the use of biochar as a soil amendment in aided phytostabilization for the reclamation of degraded soils and tailings has been widely studied, showing significant potential to immobilize metal(oid)s and increase soil fertility [6,7]. However, owing to the high recalcitrance of biochar, it is necessary to combine its application with a labile source of organic matter to activate the microbial activity that contributes to the formation of aggregates and the biogeochemical cycle [7]. The combined effect of biochar and compost has been demonstrated to be one of the best ways to improve soil quality and agronomic production [11,12]. This combination also helps to obtain a better aggregation of the particles and improves the soil structure to guarantee the correct development of micro-organisms and vegetation. In addition, the use of zeolite as a natural product has been proposed for soil reclamation based on their high cation exchange capacity and high surface adsorption [13]. Thus, zeolites have been proven to be a mobility reducer of metals such as Pb, Cu, Zn or Cd in contaminated soils, decreasing the absorption by plants [14,15]. The use of native species that are adapted to the soil and the climatic conditions of the area to be reclaimed are necessary to create a self-sustaining landscape by the phytoremediation technique and to avoid the introduction of invasive species that could reduce regional diversity [16,17].

Numerous studies have shown that there are many candidate plants to be used as phytostabilizers such as the grass *Piptatherum miliaceum*, which has good development in mining soils and tailings where amendments have been applied as well as a high ability to immobilize trace elements in the rhizosphere without the transport of metals to aerial parts [3].

The absence of N in tailings ponds is a recurrent issue in all mining environments worldwide [18]; therefore, it is essential to provide N externally with the use of amendments to enhance the creation of a Technosol and provide nutrients for the proliferation of organisms. Many legume and non-legume species have been widely used in phytoremediation; however, fewer studies have been carried out to evaluate the growth and physiological differences between legume and non-legume candidates in heavy metal-enriched soils. Legume plants commonly show a better performance and adaptation than non-legume species due to their wide-ranging capacities for N_2 fixation [19]. Nitrogen is one of the key nutrients in the terrestrial ecosystem and it is important in the maintenance of the plant community [20].

To stabilize nitrogen concentrations in tailings ponds after an amendment application, a legume species, *Coronilla juncea*, was introduced into the field plots of the present research, which allowed us to study if this N-fixer species was able to improve the soil characteristics. *C. juncea* was selected because it is a shrub native to the semi-arid regions of the Mediterranean basin. In addition, this species has previously been suggested to be included in programs for territorial planning and the rehabilitation of degraded semi-arid to arid ecosystems [21].

The lack of information in the scientific literature about the nitrogen dynamic in mining environments demonstrates the importance of carrying out this study. Therefore, the aim of this study was to assess the variations in soil N concentrations related to the different amendment combinations (limestone, biochar, zeolite and compost). Moreover, this investigation determined the availability of soil N and its relationship with the growing of *P. miliaceum* and *C. juncea* over time.

2. Materials and Methods

2.1. Study Area

The study area was located in the former Cartagena-La Unión mining district (southeast of Murcia Province, Spain) (Figure 1).

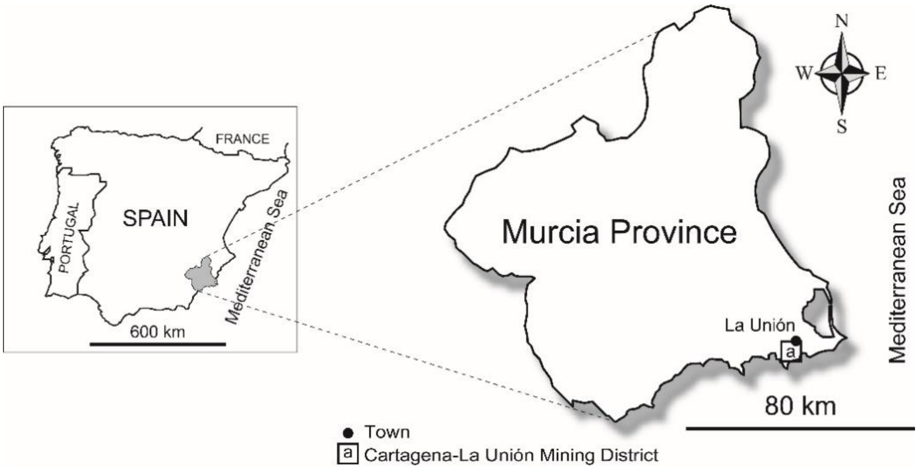

Figure 1. Location of the study area.

This area has a long mining history from ~200 B.C. until the middle of the 20th century, favoring the construction of tailings ponds that have originated severe negative impacts on the environment such as the formation of acidic mine drainage. The climate of the region is Mediterranean semi-arid with an average annual temperature of 18.36 °C and average annual rainfall of 315 mm in the last 20 years (SIAM, the agricultural information system of Murcia). The Cabezo Rajao tailings pond (La Unión, 37°37′ N, 0°54′ W) was selected in this study to set up the experimental plots. This tailings pond has an area of 16,000 m^2 and an altitude of 92 m. Its mining residues are characterized by a high acidity (pH ~3) and a high total metal(oid)s content: 2873 $mg \cdot kg^{-1}$ of Zn; 137 $mg \cdot kg^{-1}$ of As; 9 $mg \cdot kg^{-1}$ of Cd and 769 $mg \cdot kg^{-1}$ of Pb. It also has an absence of organic matter and nutrients as well as no plant cover.

2.2. Field Experiment

The field experiment consisted of the implementation of 21 amended plots of 3 m × 3 m ((6 treatments + 1 control) × 3 replicates). The following combination of amendments was used (Figure 2):

1. Control (CT);
2. Limestone + biochar (L + BC);
3. Limestone + compost (L + COM);
4. Limestone + biochar + compost (L + BC + COM);
5. Limestone + biochar + zeolite (L + BC + Z);
6. Limestone + compost + zeolite (L + COM + Z);
7. Limestone + biochar + compost + zeolite (L + BC + COM + Z).

Figure 2. Detail of the experimental plots.

For each plot, the total amount of amendment that could be added was calculated considering the density and volume of the tailings to treat as well as the surface and depth of the plot where the materials would be incorporated; the doses were then calculated. Thus, using these items, these doses could be extrapolated to any tailings pond in the world by making a few adjustments.

The specific doses of biochar (25 g·kg^{-1} of tailings) and compost (60 g·kg^{-1} of tailings) were calculated from the initial total carbon content to achieve the same rate of carbon at the plot to the natural soils of the surroundings (~2% of total carbon) [22]. In the case of treatments 4 and 7, which combined compost and biochar, the dose established for both maintained a carbon ratio of 1:1 with a dose of 12 g·kg^{-1} of biochar tailings and 30 g·kg^{-1} for compost tailings.

The dose of limestone (200 g·kg^{-1} of tailings) was calculated by a laboratory test consisting of a curve of neutralization as the amount of lime needed to neutralize all the potential acidity was predicted to reach a pH of 7.03 with 20% limestone. The dose of zeolites (20 g·kg^{-1} of tailings) was the optimum concentration found in the literature to retain the metals and increase the soil cation exchange capacity [23–26].

The implementation of the amendments was carried out for four days in February 2019 in the following order: limestone, compost, biochar and zeolite; one each day. After the application of the amendments, all materials were mixed at a depth of 0–40 cm to incorporate the amendments into the tailings and create a Technosol [27]. Once the different plots were created and conditioned, irrigation with 1050 L of water was carried out to favor the chemical reactions. After that, 17 individuals of *C. juncea* L. and 4 individuals of *P. miliaceum* were planted in each plot. *P. miliaceum* was also sown inside the plot in March 2019. Once the planting/sowing was completed, a single initial irrigation with 2100 L of water was applied to favor the adaptation and growth of the plants.

2.3. Sampling and Analytical Methods

The soil samples were collected at different sampling periods (S0, S1, S2 and S3). Sampling 0 (S0) was carried out before the amendment application in February 2019. Sampling 1 (S1) was performed after the amendment application and the sowing/planting of the plant species at each plot in March 2019. The rhizosphere soil (RS), non-rhizosphere soil (NRS) and plant samples were collected 4 and 8 months after the plot building in June 2019 (S2) and in November 2019 (S3). Three NRS samples within each plot were randomly collected (0–30 cm) with the S0, S1, S2 and S3 samplings. Three RS and plant samples (*C. juncea* and *P. miliaceum*) within each plot were collected only with the S2 and S3 samplings.

The soil and amendment samples were air-dried, passed through a 2 mm sieve and stored in plastic bags at room temperature prior to the laboratory analysis. A portion of the sample was ground for use in the elemental analysis of C and N.

The pH and electrical conductivity (EC) of the soil and amendments were measured in deionized water (ratio 1:2.5 and 1:5 w/v, respectively) [28,29]. The total nitrogen (TN) and total carbon (TOC) in the soil and amendments were measured with a CNHS-O 628 LECO elemental analyzer (LECO, St. Joseph, MI, USA). The anions and cations (NO_2^-, NO_3^-, NH_4^+) were extracted with deionized water (ratio 1:5 w/v) and measured by METROHM ion chromatography (model 850, Metrohm AG, Herisau, Switzerland).

Every month, the vegetation diversity, structure and plant cover were assessed by taking digital photographs of the marked surface. These images were processed with ImageJ software version 1.8.0 [30,31] and were used to obtain the survival rate, which was calculated as: (final number of individuals $\times$ 100)/initial number of individuals.

2.4. Statistical Analysis

The Kolmogorov-Smirnov normality test at $p < 0.05$ was used to ensure a normality fitting of the data. Data were transformed using logarithms to assure a normal distribution when needed. A one-way repeated measure ANOVA was carried out to assess the evolution of the N content through time and the treatments. The nitrogen-related soil properties at different sampling dates were used as a within-subject factor whereas the different treatments and the control were used as a between-subject factor. The residuals from the ANOVA were studied to confirm the normality fitting. When the null hypothesis was rejected, the separation of the means among the levels was made according to Tukey at $p < 0.05$. Two-way ANOVAs were carried out to assess the differences in the N content in the rhizosphere and non-rhizosphere soils separately for the S2 and S3 sampling dates with the type of soil and amendment as factors. A two-way ANOVA for S2 and S3 was independently carried out to check the effect of plant species (*P. miliaceum* and *C. juncea*) and amendments. Histograms of the residuals from the ANOVA were plotted for each variable to confirm that the normality assumption was plausible. The statistical analysis was performed with IBM SPSS statistics v.23 software.

3. Results and Discussion

3.1. Effect of the Amendment Application on Soil Nitrogen Content

An initial characterization of the amendments was carried out in order to discover their main properties. The amendment pH values ranged from neutral (7.0) in the zeolites to alkaline (11.1) in the biochar (Table 1). The electrical conductivity (EC) of all the amendments was very low except for compost, which reached 12 mS·cm^{-1}; that supposed a contribution of soluble/insoluble salts to the soils where it was added. This high salinity was related to the nature and composition of the starting material, mainly by its salt concentration and to a lesser degree by the presence of ammonium or nitrate ions formed during the process [32]. In this study, the compost was made from manure, which contains a large amount of salt. Manures from concentrated animal feeding operations are usually high in salt content [33,34]. The other amendments (such as zeolite and limestone) had a low EC. Biochar is obtained from the pyrolysis of plant material and, therefore, had a low EC (1.5 mS·cm^{-1}) because plant material generally does not have a high concentration of salt [35]. For the organic amendments, the highest organic carbon content was found in the biochar (71.4%) whereas the compost reported a four-fold lower organic carbon content than the biochar. Conversely, for the total nitrogen content, the compost showed the highest input of N with 1910 mg·kg^{-1} followed by biochar with 760 mg·kg^{-1} although the predominant fraction of inorganic N in both amendments was N-NO_3 and whose concentration should be monitored to avoid negative impacts in nearby water bodies. With the limestone, acid mine drainage was drastically reduced because it completely neutralized the acidity, reducing the availability of dissolved heavy metals. If we also factored in the action of the zeolites, which retain these metals in their structure, the impact was considerably reduced.

Table 1. Geochemical parameters analyzed in the amendments used in the different treatments.

	pH	EC ($mS \cdot cm^{-1}$)	TOC (%)	TN ($mg \cdot kg^{-1}$)	$N\text{-}NO_2^-$ ($mg \cdot kg^{-1}$)	$N\text{-}NO_3^-$ ($mg \cdot kg^{-1}$)	$N\text{-}NH_4^+$ ($mg \cdot kg^{-1}$)
Biochar	11.1	1.5	71.4	760	0.89	12.5	0.7
Compost	9.5	12.1	17.7	1910	10.3	107.3	26.6
Zeolite	7.0	0.14	*	*	*	*	*
Limestone	8.7	0.04	*	*	*	*	*

EC: electrical conductivity; TOC: total organic carbon; TN: total nitrogen; $N\text{-}NO_2^-$: nitrogen-nitrite; $N\text{-}NO_3^-$: nitrogen-nitrate; $N\text{-}NH_4^+$: nitrogen-ammonium; *: non-significant (ns).

The addition of the amendments, especially the limestone, contributed to a significant increase of the pH in the soil from acidic values in S0 (3.7) to basic pH values reported in S3 (8.2), which was the pH target of the experiment. This pH increment implied better soil environment conditions for the development and proliferation of the plant species used and contributed to the creation of an effective soil structure. It is important to highlight that native soils from the area had a pH of 8 owing to high contents of carbonates and salt; it is essential to achieve this value to promote spontaneous vegetal colonization from the surroundings.

The soil EC was reduced with the addition of the amendments. The values decreased from slightly saline in S0 (3.40 $mS \cdot cm^{-1}$) to non-saline in S3 (1.95 $mS \cdot cm^{-1}$) [36,37]. This variation in the salinity was due to the coprecipitation of metallic salts with carbonates from the amendments [38] influenced by geochemical processes and the cation exchange capacity of the zeolite and biochar [39,40]. The TOC content was increased in S3 with an average value of 1.52% in plots where the compost and biochar were applied. The highest TOC value was 4.8% in treatment 7, which was similar to the values of the native forest soils of the area (3.4–4.3%) [41]. This increase in the TOC is vital to achieve the supply of nutrients that enhance the development of plant species. In this way, compost offered a faster mineralization of the organic compounds than the biochar and, therefore, a higher supply of nutrients. In contrast, the lower mineralization of the biochar could avoid a rapid depletion of nutrients by a slow release in the medium and long term.

The TN average content of all the treatments (including the control) increased from S0 (283 $mg \cdot kg^{-1}$) to S3 (1141 $mg \cdot kg^{-1}$); no increase was observed in the control treatment. Significant differences in the samplings and treatments were observed with the highest TN values where the compost was applied (treatments 3, 4, 6 and 7). The treatments containing the biochar as the sole carbon source showed a lower TN content than when applying the compost, probably because of the nature of the biochar, which contained 2.5 times less TN than the compost. In addition, it should be noted that the plots with the highest TN content were those with the compost as the only carbon source (treatments 3 and 6) (Figure 3A).

$N\text{-}NO_3^-$ and $N\text{-}NO_2^-$ showed a similar trend to TN. Thus, the $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ average contents of all treatments (including the control) increased from S0 (0.14 $mg \cdot kg^{-1}$ for $N\text{-}NO_2^-$; 3.35 $mg \cdot kg^{-1}$ for $N\text{-}NO_3^-$) to S3 (1.51 $mg \cdot kg^{-1}$ for $N\text{-}NO_2^-$; 5.73 $mg \cdot kg^{-1}$ for $N\text{-}NO_3^-$). Significant differences were observed in the samplings and treatments. The highest $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ contents were observed with the compost (treatments 3, 4, 6 and 7) whereas the treatments with the biochar as the sole carbon source had the lowest $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ contents (Figure 3B,C). The increase of $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ contents in S2 seemed to be related to the nitrification processes associated with the probable proliferation of different bacteria that metabolize or produce nitrites and nitrates; the decrease in content of both anions in S3 could be associated with the plant adsorption and N_2 release from the denitrification processes [42,43]. Although the $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ contents in the last sampling were lower than in the previous ones, the existence of these variations over time in the amended soils implied the release of nutrients that facilitated the growth of the introduced species and the possibility of the natural colonization of the vegetation.

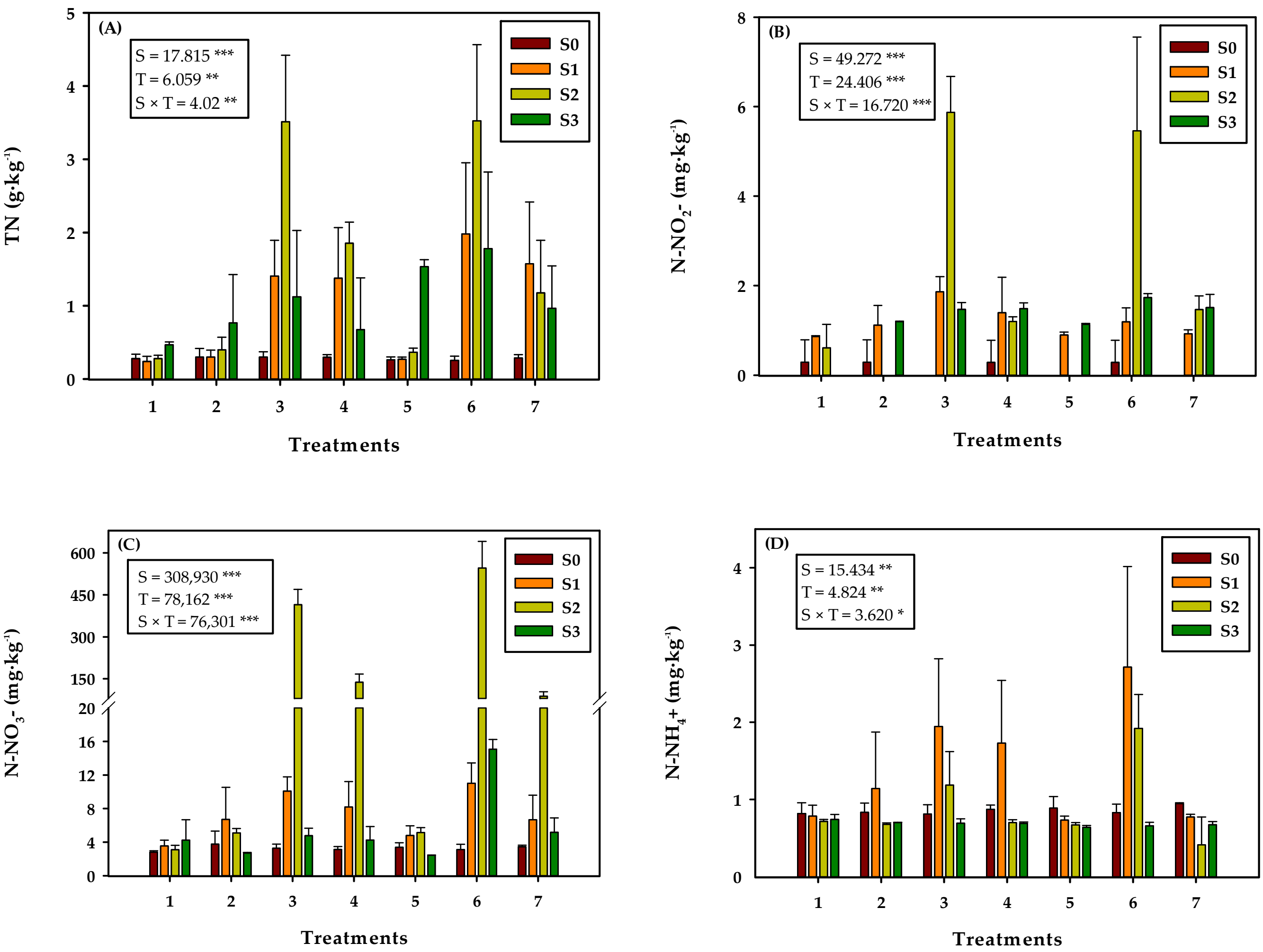

Figure 3. Mean concentration of TN (**A**), N-NO_2^- (**B**), N-NO_3^- (**C**) and N-NH_4^+ (**D**) in the different treatments (1–7). 1: control; 2: limestone and biochar; 3: limestone and compost; 4: limestone, biochar and compost; 5: biochar, limestone and zeolite; 6: limestone, compost and zeolite; and 7: limestone, biochar, compost and zeolite. S0: sampling 0, S1 sampling 1, S2: sampling 2; and S3: sampling 3. Error bars denote standard deviation (n = 3). F values and significance of the two-way repeated measures ANOVA are shown in each graph. S: sampling; T: treatment. Significant at * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$; ns: not significant ($p > 0.05$). (**A**) Mean concentration of TN in the different treatments (1–7). (**B**) Mean concentration of N-NO_2^- in the different treatments (1–7). (**C**) Mean concentration of N-NO_3^- in the different treatments (1–7). (**D**) Mean concentration of N-NH_4^+ in the different treatments (1–7).

Significant differences were observed for N-NH_4^+ among the samplings and treatments. The N-NH_4^+ average content of all treatments (including the control) showed an inverse trend to TN, N-NO_2^- and N-NO_3^- during the samplings, decreasing from S0 (0.86 mg·kg^{-1}) to S3 (0.68 mg·kg^{-1}). However, the N-NH_4^+ content showed a trend similar to the N-NO_2^- and N-NO_3^- contents and TN for all the treatments, registering the highest and lowest concentration values in the same treatments. These differences may have been due to the highest content of N-NH_4^+ in the compost (Table 1) whereas the treatments with the biochar as the sole carbon source had the lowest N-NH_4^+ content due to their composition (Figure 3D). The differences observed through the samplings may have been due, firstly, to the addition of amendments in S1, which supposed an increase in the N-NH_4^+ concentration that later decreased in S2 and S3 with the presence of nitrifying bacteria capable of transforming NH_4^+ into NO_2^- [44] or absorption by the plants [45]. NH_4^+ may also have been a source of N losses because of its transformation into NH_3^- that is quickly degraded to N_2.

3.2. Effect of the Soil and Plant Type on Soil Nitrogen Content

To obtain sustainable plant colonization in mining soils, several key factors should be considered such as a non-acid and stable pH, unavailability of heavy metals with toxic concentrations, a good nutrient content (especially N) and a low concentration of salts [46–48]. This condition can be achieved by the addition of organic and inorganic amendments. The pH was increased and stabilized with the limestone whereas nutrients were provided by the compost and biochar [49,50] as well as the retention of salts and metals by the zeolite [51]. The amendment application allowed the introduction of plant species with a higher survival rate although a few differences were observed during the colonization process of the plant species because their adaptive capacities and needs were different [52]. Several differences were observed in this study in the development of the planted species. *P. miliaceum* managed to grow and remained alive over time whereas *C. juncea* had greater difficulty in surviving (Table 2). As a consequence, the absence of this species in treatments 4 and 6 in the S2 and S3 samplings and the CT was observed. In the other treatments, *C. juncea* also had problems surviving although the survival rate was higher than in the above-mentioned treatments. The growing conditions were probably inadequate because of one or more of these factors: (a) a short period of development of the soil after the application of the amendments; (b) a slow release of nutrients and/or (c) an elevated content of available heavy metal(oid)s and salts. In plants, phytotoxicity particularly generates a reduction in root growth, biomass, transpiration, chlorosis and leaf necrosis as well as symptoms of senescence and abscission [53,54]. This high mortality rate may also be indicative of inadequate growth conditions for these species and of competitiveness among them (Table 2) [55].

Table 2. The % *C. juncea* survival in all the treatments (1–6 and control).

Treatment	T-Control	T-1	T-2	T-3	T-4	T-5	T-6
% survival	0.0	5.9	5.9	0.0	15.7	0.0	11.8

On the other hand, *P. miliaceum* managed to proliferate with a very good performance, reaching a survival rate of almost 100%. Similar results were observed by researchers in the phytoremediation of mining waste with amendment additions such as compost, biochar and limestone [2,7,56].

The TN content was significantly higher in the NRS than in the RS in S2 for both species—1014 mg·kg^{-1} and 800 mg·kg^{-1}, respectively—as well in S3, with 971 mg·kg^{-1} for NRS and 500 mg·kg^{-1} for RS. There was a significant effect of the treatment factor (T) in the S2 and S3 samplings whereas the interaction of the soil type × T was only significant in S2 (Figure 4A,E). The treatments 3, 5 and 7 showed the highest TN values, likely due to the compost application. It is important to note that the plant species were not significant for the TN variation in either S2 or S3.

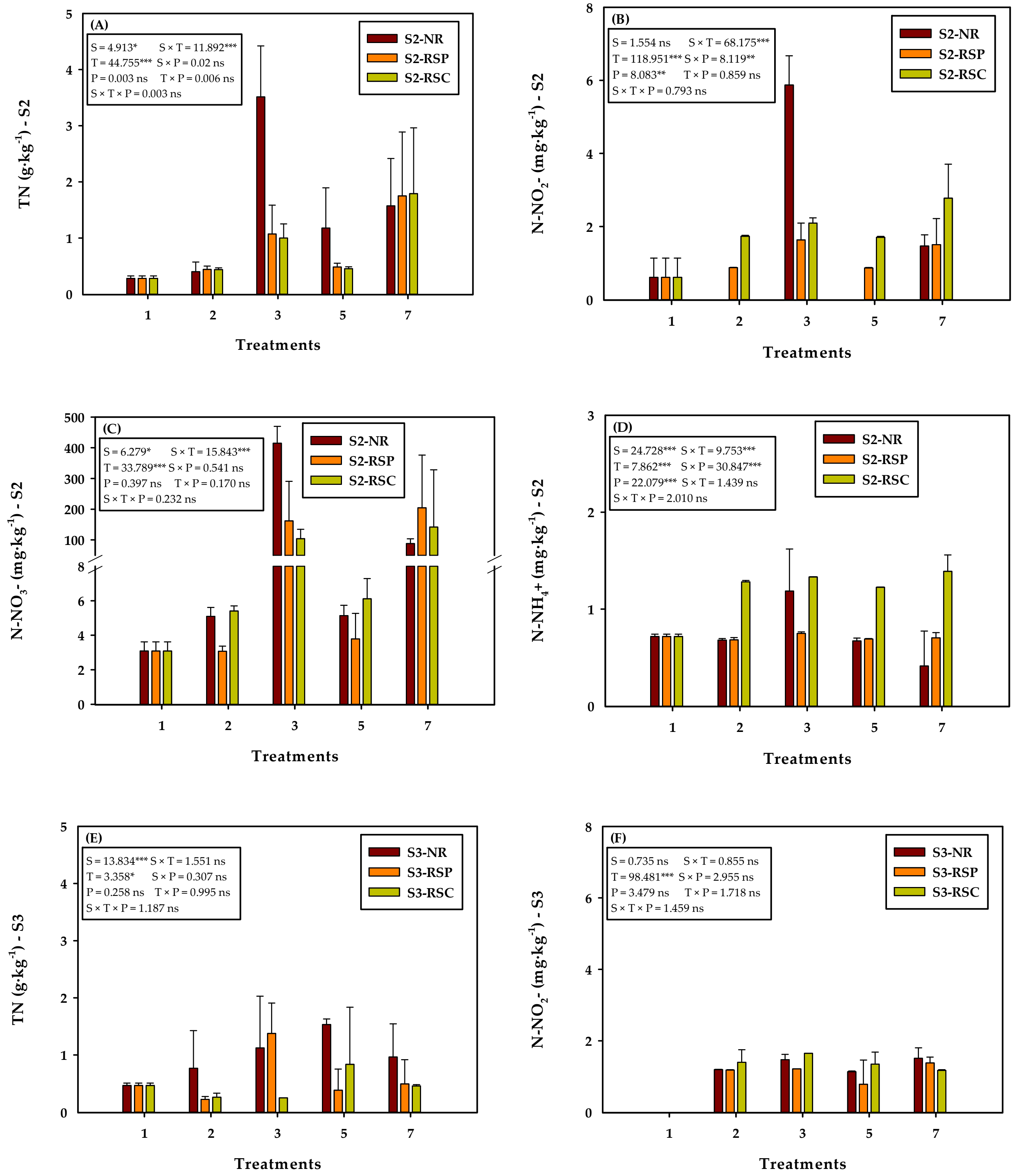

Figure 4. *Cont.*

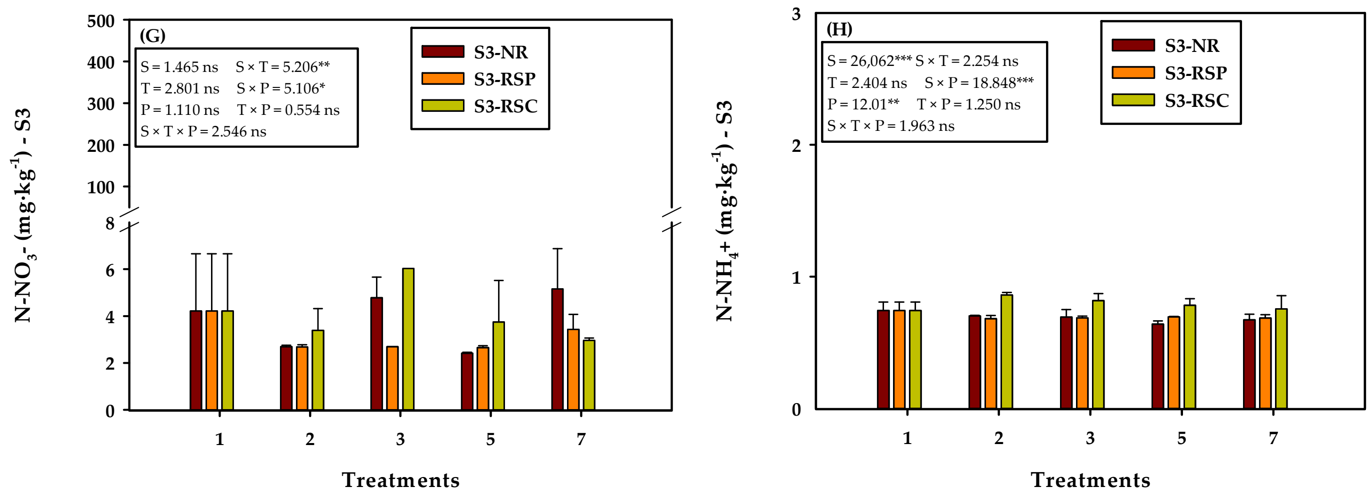

Figure 4. Mean concentration of total TN (**A**,**E**), N-NO_2^- (**B**,**F**), N-NO_3^- (**C**,**G**) and N-NH_4^+ (**D**,**H**) in the different treatments (1, 2, 3, 5 and 7): 1: control; 2: limestone and biochar; 3: limestone and compost; 5: biochar, limestone and zeolite and 7: limestone, biochar, compost and zeolite. S2: sampling 2; S3: sampling 3. NR: non-rhizosphere soil; RS: rhizosphere soil; P: *P. miliaceum*; C: *C. juncea*. Error bars denote standard deviation (n = 3). F values and significance of the two-way repeated measures ANOVA are shown in each graph. S: soil type; T: treatment; P: plant type. Significant at * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$; ns: not significant ($p > 0.05$). (**A**) Mean concentration of TN in the different treatments (1, 2, 3, 5 and 7). (**B**) Mean concentration of N-NO_2^- in the different treatments (1, 2, 3, 5 and 7). (**C**) Mean concentration of N-NO_3^- in the different treatments (1, 2, 3, 5 and 7). (**D**) Mean concentration of N-NH_4^+ in the different treatments (1, 2, 3, 5 and 7). (**E**) Mean concentration of TN in the different treatments (1, 2, 3, 5 and 7). (**F**) Mean concentration of N-NO_2^- in the different treatments (1, 2, 3, 5 and 7). (**G**) Mean concentration of N-NO_3^- in the different treatments (1, 2, 3, 5 and 7). (**H**) Mean concentration of N-NH_4^+ in the different treatments (1, 2, 3, 5 and 7).

Regarding the inorganic forms of N, higher concentrations of N-NO_2^- were observed in the rhizosphere soil for both species (1.4 mg·kg^{-1}) than in the non-rhizosphere soil (0.33 mg·kg^{-1}) in S2 (Figure 4B). This suggested higher nitrification rates in the rhizosphere were likely due to the symbiotic association between legumes and nitrogen-fixing bacteria [49] or the activation of microbial communities by the root exudates in both species. Moreover, the three factors of study (treatment, soil type and plant species) were significant in explaining the variation in N-NO_2^- in addition to the interactions between the soil type × treatment and the soil × plant species, suggesting that the different combinations of amendments and plants influenced the denitrification process. Thus, in both S2 and S3 samplings, the treatments 2, 3, 5 and 7 had significantly higher concentrations of N-NO_2^- than treatment 1 (control). This might have been be due to the contribution and generation of N-NO_2^- from the amendments, mainly the biochar and compost.

Regarding the type of plant, in S2, significant differences were observed in the concentrations of N-NO_2^- in the rhizosphere soil of *C. juncea*, which acted as a nitrogen-fixing species and reported a higher concentration in all treatments except in treatment 1 (control). However, in S3, significant differences were only observed between the treatments and not between the soil types or species (Figure 4F). This might have been due to the lower concentrations of N-NO_2^- presented by treatment 1 (control) compared with the other treatments (with amendments) that presented similar concentrations because N-NO_2^- originates mainly from organic amendments (in this case, the compost and biochar).

The factors of soil type and treatment were significant in explaining the variation in N-NO_3^- in S2 as well as the interaction between the soil type × treatment. Significantly higher values of N-NO_3^- were found in the rhizosphere soil (65 mg·kg^{-1}) than in the non-rhizosphere soil (17 mg·kg^{-1}), which suggested that the ammonium adsorbed in soil or provided by the organic amendments (compost or biochar) was nitrified by microorganisms, accumulated and available as a nutrient for plant uptake [57]. Differences between the treatments in S2 seemed to be mainly related to the highest N-NO_3^- content in the compost composition (treatments 3 and 7) whereas the treatments with the biochar (treatments 2 and 5) had the lowest N-NO_3^- contents due to the low concentration. The high N-NO_3^- content observed in S2 (Figure 4C) may have been related to the nitrification processes, which, as mentioned above in the case of N-NO_2^-, was largely provided by the amendment (in this case, compost). Nitrifying bacteria may also have been acting, which transforms the ammonium in the soil into nitrites and nitrates, offering one of the main nutrients to the plant [58].

The factors of soil type and treatment were not significant in explaining the variation in N-NO_3^- in S3. Only the interactions of the soil type × treatment and the soil type x plant type were significant. We observed mean values (for all treatments and the two plant species including the control) of 4 mg·kg^{-1} of N-NO_3^- in all treatments in S3 (Figure 4G). The decrease of N-NO_3^- in S3 could be associated with the plant absorption and the release of N_2 by denitrification processes. The existence of these variations over time (S2–S3) in the amended soils implied the release of nutrients that facilitated the implantation of the sown species as well as the colonization of spontaneous species in the rainy season where opportunistic species of the first ecological succession were growing.

The mineralization of organic matter may lead to the formation of NH_4^+ in the soil from organic N. The factors of soil type and plant type were significant in explaining the variation in N-NH_4^+ in addition to the interactions between the soil type × plant type both in S2 and S3. We observed mean values (for all treatments and the two plant species including the control) of 0.85 mg·kg^{-1} of N-NH_4^+ for the rhizosphere soil and 0.65 mg·kg^{-1} for the non-rhizosphere soil in S2 and S3 (Figure 4D,H). In addition, it was observed that the rhizosphere soil of *C. juncea* showed significantly higher concentrations of N-NH_4^+ than the rhizosphere soil of *P. miliaceum* or the non-rhizosphere soil, which was noticeable in all treatments except in treatment 1 (control), as expected. This difference between the plant species may be due to the action of *C. juncea* as a nitrogen-fixing species (legume) that is able to supply nitrogen-based nutrients to the soil, which has a significant importance in the soil biological cycle [45]. This is even more important in Technosol created from tailings with very low fertility because the supply of nutrients depends on the type of materials used in the mixture, which may be exhausted with time. Thus, the presence of legumes should be highly encouraged to foster the maintenance of an adequate N level in the soil so the ecosystem can be self-sustaining [59,60].

The TN and the different inorganic fractions studied showed a slight decrease in their levels from S2 to S3, together with a homogenization of the S3 contents for all the treatments. It indicated that stabilization was achieved in the plots and, therefore, more stable concentrations of TN and inorganic fractions were obtained that could facilitate and guarantee the viability of the formation processes in these mining soils.

4. Conclusions

All the Technosols created increased the pH, TOC and TN contents and decreased the EC. NO_2^-, NO_3^- and NH_4^+ were mainly provided by the compost whereas in the biochar, these compounds demonstrated a greater retainment due to its high recalcitrance. For the organic amendments, the compost performed better than the biochar in terms of the total N, NO_2^-, NO_3^- and NH_4^+ whereas for the time evolution, higher values of NO_2^-, NO_3^- and NH_4^+ were observed in the soil sampling when *P. miliaceum* and *C. juncea* were planted or sown in the plots. These nutrients tended to decrease over time, likely due to the growth of the plant species. The best combinations of amendments were limestone + compost + zeolite and limestone + compost + biochar + zeolite, owing to the presence of compost and zeolite. Thus, compost is preferable to biochar to provide soil fertility and foster plant development in Technosols created with tailings. *P. miliaceum* showed better behavior and adaptation in all treatments than *C. juncea*. Nonetheless, *C. juncea* showed a high content of ammonium in the rhizosphere soil, suggesting biological nitrogen fixation, which may help the soil evolution and development of micro- and macrobiota. The results obtained during the studied period (9 months) suggested the need to provide a labile source of organic matter (in our study, compost) to promote the development of plant species. It is possible that *C. juncea* required better soil conditions or a longer time between the application of the amendments and sowing/planting to ensure its survival. *P. miliaceum* is a good, resistant and robust plant species to effectively reclaim tailings ponds.

The results of this study showed that a combination of organic amendments rich in N with the leguminous *C. juncea* could enhance the concentration and availability of nitrogen compounds for plant uptake, leading to a faster vegetal colonization of the reclaimed ponds in the future. The applied methodology could be implemented in any mining area with similar tailings characteristics and weather conditions.

Monitoring activities are necessary to evaluate the evolution of these parameters over time and to promote the integrated reclamation of mining areas.

Author Contributions: Data curation, J.C.B., R.Z. and J.A.A.; Formal analysis, Á.F.; Investigation, J.C.B., Á.F. and R.Z.; Methodology, J.C.B., M.G. and S.M.-M.; Software, J.C.B., M.G. and R.Z.; Supervision, J.A.A. and S.M.-M.; Writing—review & editing, S.M.-M.; Conceptualization, R.Z. and Á.F.; Resources, R.Z. and Á.F.; Writing—original draft preparation, J.C.B., M.G. and S.M.-M.; Visualization, J.C.B. and Á.F.; Project administration, Á.F. and R.Z.; Funding acquisition, R.Z. and Á.F. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Fundación Séneca (Agency of Science and Technology of the Region of Murcia, Spain) (grant number 20947/PI/18).

Data Availability Statement: Not applicable.

Acknowledgments: This work was supported by Fundación Séneca and the work of the research group, Sustainable Use, Management and Reclamation of Soil and Water.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Davila, R.B.; Fontes, M.P.F.; Pacheco, A.A.; Ferreira, M. Heavy metals in iron ore tailings and floodplain soils affected by the Samarco dam collapse in Brazil. *Sci. Total Environ.* **2020**, *709*, 136151. [CrossRef]
2. Parraga-Aguado, I.; González-Alcaraz, M.N.; Schulin, R.; Conesa, H.M. El uso potencial de *Piptatherum miliaceum* para la fitoterapia de relaves mineros en áreas semiáridas: Papel de la fertilidad del suelo y la competencia de las plantas. *Rev. Ambient. Ambient.* **2015**, *158*, 74–84.
3. Kabas, S.; Faz, A.; Acosta, J.A.; Zornoza, R.; Martínez-Martínez, S.; Carmona, D.M.; Bech, J. Efecto de los desechos de mármol y lodo de cerdo sobre el crecimiento de la vegetación nativa y la movilidad de metales pesados en un estanque de relaves de una mina. *Rev. De Explor. Geoquímica* **2012**, *123*, 69–76.
4. Carrillo-González, R. Fitorremediación asistida con enmiendas y fitoestabilización de elementos potencialmente tóxicos. *Agro Product.* **2017**, *10*, 4.
5. Asensio, V.; Covelo, E.F.; Kandeler, E. Soil management of copper mine tailing soils—Sludge amendment and tree vegetation could improve biological soil quality. *Sci. Total Environ.* **2013**, *456*, 82–90. [CrossRef] [PubMed]
6. Beesley, L.; Moreno-Jiménez, E.; Gomez-Eyles, J.L.; Harris, E.; Robinson, B.; Sizmur, T. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. *Environ. Pollut.* **2011**, *159*, 3269–3282. [CrossRef] [PubMed]
7. Moreno-Barriga, F.; Faz, Á.; Acosta, J.A.; Soriano-Disla, M.; Martínez-Martínez, S.; Zornoza, R. Uso de *Piptatherum miliaceum* para el fitomando de tecnosoles modificados con biocarbón derivados de relaves piríticos para mejorar la agregación del suelo y reducir la movilidad de los metales (loides). *Geoderma* **2017**, *307*, 159–171. [CrossRef]
8. Radziemska, M.; Bęś, A.; Gusiatin, Z.M.; Cerdà, A.; Jeznach, J.; Mazur, Z.; Brtnický, M. Assisted phytostabilization of soil from a former military area with mineral amendments. *Ecotoxicol. Environ. Saf.* **2020**, *188*, 109934. [CrossRef]
9. Martínez-Martínez, S.; Zornoza, R.; Gabarrón, M.; Gómez-Garrido, M.; Rosales, R.M.; Muñoz, M.A.; Gómez-López, M.D.; Soriano-Disla, J.M.; Faz, A.; Acosta, J.A. Is aided phytostabilization a suitable technique for the remediation of tailings? *Eur. J. Soil Sci.* **2019**, *70*, 862–875. [CrossRef]
10. Morugan-Coronado, A.; Soriano-Disla, M.; Moreno-Barriga, F.; Linares, C.; Faz, A.; García-Orenes, F.; Zornoza, R. Uso de *Piptatherum miliaceum* para permitir el establecimiento exitoso de Salvia rosmarinus en tecnosoles desarrollados a partir de relaves piríticos. *Quimiosfera* **2021**, *267*, 129281.
11. Agegnehu, G.; Srivastava, A.K.; Bird, M.I. El papel del biocarbón y el compost de biocarbón en la mejora de la calidad del suelo y el rendimiento de los cultivos: Una revisión. *Ecol. Suelo Apl.* **2017**, *119*, 156–170. [CrossRef]

12. Oldfield, T.L.; Sikirica, N.; Mondini, C.; López, G.; Kuikman, P.J.; Holden, N.M. Biochar, compost y ambient-compost blend como opciones para recuperar nutrientes y secuestrar carbono. *Rev. Ambient. Ambient.* **2018**, *218*, 465–476.
13. Misaelides, P. Aplicación de zeolitas naturales en la remediación ambiental: Una breve revisión. *Mater. Microporosos Mesoporosos* **2011**, *144*, 15–18. [CrossRef]
14. Castaldi, P.; Santoña, L.; Melis, P. Inmovilización de metales pesados por enmiendas químicas en un suelo contaminado e influencia en el crecimiento del lupino blanco. *Chemosphere* **2005**, *60*, 365–371. [CrossRef]
15. Shi, W.Y.; Shao, H.B.; Li, H.; Shao, M.A.; Du, S. Avances en la remediación de suelos peligrosos contaminados con metales pesados mediante zeolita natural. *D. Mater. Peligr.* **2009**, *170*, 1–6.
16. Méndez, M.O.; Maier, R.M. Fitorremediación de relaves mineros en ambientes templados y áridos. *Rev. Environ. Sci. Bio/Technol.* **2008**, *7*, 47–59.
17. Ghosh, D.; Maiti, S.K. Fitorremediación asistida por biocarbón y eliminación de biomasa en suelos mineros contaminados con metales pesados: Una revisión. *Rev. Int. Fitorremediación* **2021**, *23*, 559–576.
18. Hossner, L.R.; Hons, F.M. Reclamation of mine tailings. In *Soil Restoration*; Lal, R., Stewart, B.A., Eds.; Advances in Soil Science; Springer: New York, NY, USA, 1992; Volume 17, pp. 311–350.
19. Fagorzi, C.; Checcucci, A.; DiCenzo, G.C.; Debiec-Andrzejewska, K.; Dziewit, L.; Pini, F.; Mengoni, A. Aprovechamiento de los rizobios para mejorar la fitorremediación de metales pesados por las legumbres. *Genes* **2018**, *9*, 542. [CrossRef]
20. Chapin, F.S., III; Shaver, G.R.; Kedrowski, R.A. Controles ambientales sobre las fracciones de carbono, nitrógeno y fósforo en Eriophorum vaginatum en la tundra de matas de Alaska. *La Rev. Ecol.* **1986**, *74*, 167–195.
21. Robles, A.B.; Allegretti, L.I.; Passera, C.B. *Coronilla juncea* es un arbusto forrajero nutritivo y útil en la rehabilitación de tierras agrícolas marginales mediterráneas abandonadas. *Rev. Entornos Áridos* **2002**, *50*, 381–392.
22. Martínez-Martínez, S.; Acosta, J.A.; Cano, A.F.; Carmona, D.M.; Zornoza, R.; Cerda, C. Assessment of the lead and zinc contents in natural soils and tailing ponds from the Cartagena-La Unión mining district, SE Spain. *J. Geochem. Explor.* **2013**, *124*, 166–175. [CrossRef]
23. Toro, F.D.J.C.; Benítez, L.M.L.; Herrera, M.I.Á. La zeolita en la mitigación ambiental. *Rev. Lasallista Investig.* **2006**, *3*, 30–34.
24. Doula, M.K.; Kavvadias, V.A.; Elaiopoulos, K. Zeolitas en procesos de remediación de suelos. *Man. Zeolitas Nat.* **2012**, 519–568.
25. Querol, X.; Alastuey, A.; Moreno, N.; Alvarez-Ayuso, E.; García-Sánchez, A.; Cama, J.; Simón, M. Immobilization of heavy metals in polluted soils by the addition of zeolitic material synthesized from coal fly ash. *Chemosphere* **2006**, *62*, 171–180. [CrossRef] [PubMed]
26. Boros-Lajszner, E.; Wyszkowska, J.; Kucharski, J. Uso de zeolita para neutralizar el níquel en un ambiente de suelo. *Monit. Y Evaluación Ambient.* **2018**, *190*, 1–13.
27. Schad, P. Tecnosoles en la Base Mundial de Referencia para los Recursos del Suelo: Historia y definiciones. *Cienc. Suelo Nutr. Veg.* **2018**, *64*, 138–144.
28. Peech, M. Actividad de Iones de Hidrógeno. In *Métodos de Análisis de Suelos: Parte 2 Propiedades Químicas y Microbiológicas*; American Society of Agronomy: New York, NY, USA, 1965; Volume 9, pp. 914–926.
29. Cobertera, E. *Edafología Aplicada. Suelos, Producción Agraria, Planificación territorial e Impactos Ambientales*; Gráficas Rógar, S.A.: Madrid, Spain, 1993; 326p.
30. Ramírez-García, J.; Almendros, P.; Quemada, M. Ground cover and leaf area index relationship in a grass, legume and crucifer crop. *Plant Soil Environ.* **2012**, *58*, 385–390. [CrossRef]
31. Ramírez-García, J.; Gabriel, J.L.; Alonso-Ayuso, M.; Quemada, M. Quantitative characterization of five cover crop species. *Rev. Cienc. Agrícolas* **2015**, *153*, 1174–1185. [CrossRef]
32. Sánchez-Monedero, M.A.; Roig, A.; Paredes, C.; Bernal, M.P. Transformación de nitrógeno durante el compostaje de residuos orgánicos por el sistema de Rutgers y sus efectos sobre el pH, EC y madurez de las mezclas de compostaje. *Tecnol. Biorecursos* **2001**, *78*, 301–308.
33. Zubillaga, M.S.; Branzini, A.; Lavado, R.S. Problemas de fitotoxicidad en compost. *Pilquen-Sección Agron.* **2008**, *9*, 1.
34. Barbaro, L.; Karlanian, M.; Rizzo, P.; Riera, N. Caracterización de diferentes compost para su uso como componente de sustratos. *Chil. J. Agric. Anim. Sci.* **2019**, *35*, 126–136. [CrossRef]
35. Montero, R.G.; Medina, P.P.; Rastrero, M.R.; Herráiz, M.J.S.; Guirado, M.; Torres, R.M.G. Biochar y sus aplicaciones potenciales en el suelo. *Técnica Ind.* **2021**, *328*, 44–53.
36. Flores, A. *Suelos Salinos y Sódicos*; Instituto Superior de Ciencias Agropecuarias de la Habana, Facultad de Agronomía, Departamento de Suelos y Riego: La Habana, Cuba, 1991; 32p.
37. Ramirez, L.R.; José, G.L.D.; Rosales, J.G.; Edgar, R.C.; Hernández, J.D. Validación del modelo LEACHM para predecir la salinidad en un suelo del valle de Quibor, con cultivo de cebolla bajo riego localizado. *Rev. For. Latinoam.* **2005**, *38*, 97–119.
38. Zornoza, R.; Moreno-Barriga, F.; Acosta, J.A.; Muñoz, M.A.; Faz, A. Estabilidad, disponibilidad de nutrientes e hidrofobicidad de biochares derivados de estiércol, residuos de cultivos y desechos sólidos municipales para su uso como enmiendas del suelo. *Chemosphere* **2016**, *144*, 122–130. [CrossRef] [PubMed]
39. Escalante Rebolledo, A.; Pérez López, G.; Hidalgo Moreno, C.; López Collado, J.; Campo Alves, J.; Valtierra Pacheco, E.; Etchevers Barra, J.D. Biocarbón (biochar) I: Naturaleza, historia, fabricación y uso en el suelo. *Terra Latinoam.* **2016**, *34*, 367–382.
40. Espejel-Ayala, F.; Solís-López, M.; Schouwenaars, R.; Ramírez-Zamora, R.M. Síntesis de zeolita P utilizando jales de cobre. *Rev. Mex. Ing. Química* **2015**, *14*, 205–212.

41. Zornoza, R.; Faz, Á.; Carmona, D.M.; Acosta, J.A.; Martínez-Martínez, S.; de Vreng, A. Mineralización de carbono, actividad microbiana y dinámica de metales en estanques de relaves modificados con purín de cerdo y residuos de mármol. *Chemosphere* **2013**, *90*, 2606–2613. [CrossRef]
42. Valero, C.; Yazmin, T. *Desnitrificación del Suelo Bajo dos Tratamientos de Riego para Estimar el Rendimiento y Emisión de Metano en Arroz*; Repositorio institucional de la Universidad Nacional Agraria La Molina: Lima, Peru, 2020.
43. Cabrera, O.G.; Vermoesen, A.; Van Cleemput, O.; Cabriales, J.P. Efecto del tipo de suelo, humedad y fuente de nitrógeno en las emisiones de N_2 y N_2O. *Terra Latinoam.* **2000**, *18*, 1–9.
44. Hayatsu, M.; Tago, K.; Saito, M. Diversos actores en el ciclo del nitrógeno: Diversidad y funciones de los microorganismos implicados en la nitrificación y desnitrificación. *Cienc. Suelo Nutr. Veg.* **2008**, *54*, 33–45.
45. Salisbury, F.B.; Ross, C.W. *Fisiología Vegetal*; Grupo Editorial Iberoamérica, México, D.F.: Lima, Peru, 1994.
46. Lee, C.; Chon, H.; Jung, M. Heavy metal contamination in the vicinity of the Daduk Au–Ag–Pb–Zn mine in Korea. *Appl. Geochem.* **2001**, *16*, 1377–1386. [CrossRef]
47. Galán, A.; Romero, E. Contaminación de Suelos Por Metales Pesados. Macla Revista de la Sociedad Española de Mineralogía 10. 2008, pp. 48–60. Available online: http://www.ehu.eus/sem/macla_pdf/macla10/Macla10_48.pdf (accessed on 28 August 2021).
48. Jordanova, D.; Rao, S.; Kotsev, T.; Jordanova, N. Industrial contamination of alluvial soils near Fe–Pb mining site revealed by magnetic and geochemical studies. *Geoderma* **2013**, *192*, 237–248. [CrossRef]
49. Román, P.; Martínez, M.; Pantoja, A. Manual de Compostaje del Agricultor. Santiago de Chile (Chile). 2013. Available online: http://www.fao.org/docrep/019/i3388s/i3388s.pdf (accessed on 16 November 2021).
50. Díaz, L.; Laguna, H.; Gutiérrez, Y.; Melo, A.; Vega, A. Tratamiento de suelos mineros mediante compostaje con Biochar, estiércol ovino y residuos orgánicos domiciliarios. *Rev. Medio Ambiente Min.* **2020**, *5*, 11–18.
51. Rodríguez Valdivia, M. *Evaluacion de la Capacidad de Adsorción de Nh4+ y Metales Pesados Pb2+, Cd2+, Cu2+, Zn2+ y Mn2+ Empleando Zeolitas Naturales y Sintéticas*; UNAS: Arequipa, Peru, 2017.
52. Bochet, E.; García-Palacios, P.; Peco, B.; Tormo, J.; García-Fayos, P. Procesos Ecológicos y Restauración de la Cubierta Vegetal. In *Restauración Ecológica de Áreas Afectadas por Infraestructuras de Transporte: Bases Científicas para Soluciones Técnicas*; Fundación Biodiversidad: Madrid, Spain, 2011; pp. 101–141.
53. Vázquez, M.; Poschenrieder, C.; Barcelo, J. Pulvinus structure and leaf abscission in cadmium-treated bean plants (Phaseolus vulgaris). *Can. J. Bot.* **1989**, *67*, 2756–2764. [CrossRef]
54. Barceló, J.; Poschenrieder, C. Respuestas de las plantas a la contaminación por metales pesados. *Suelo Planta* **1992**, *2*, 345–361.
55. Vilagrosa, A.; Caturla, R.N.; Hernández, N.; Cortina, J.; Bellot, J.; Vallejo, V.R. Reforestación en ambiente semiárido del sureste peninsular. Resultados de las investigaciones desarrolladas para optimizar la supervivencia y el crecimiento de especies autóctonas. In Montes para la sociedad del nuevo milenio. III Congreso Forestal Español. *Junta Andal. Cons. Medio Ambiente* **2001**, *4*, 5.
56. García-Gil, J.C.; Plaza, C.; Senesi, N.; Brunetti, G.; Polo, A.A. Efectos de la enmienda de lodos de depuradora sobre los ácidos húmicos y las propiedades microbiológicas de un suelo mediterráneo semiárido. *Biol. Fertil. Suelos* **2004**, *39*, 320–328.
57. Rincón, L.E.C.; Gutiérrez, F.A.A. Dinámica del ciclo del nitrógeno y fósforo en suelos. *Rev. Colomb. de Biotecnol.* **2012**, *14*, 285–295.
58. Klotz, M.G.; Stain, L.Y. Nitrifier genomics and evolution of the nitrogen cycle. *FEMS Microbiol. Lett.* **2008**, *278*, 146–156. [CrossRef]
59. Noguez-Inesta, A.; López-Sánchez, A.S.; Carrillo-González, R.; González-Chávez, M.C.A. Uso de leguminosas (Fabaceae) en fitorremediación. *Agroproductividad* **2017**, *10*, 4.
60. Paredes Campos, P.D.S.; Rodríguez Rojas, J.J. Especies Vegetales en la Fitorremediación de Suelos Contaminados por Metales Pesados. Bachelor's Thesis, Universidad César Vallejo, Trujillo, Peru, 2020. Available online: Repositorio.ucv.edu.pe (accessed on 14 March 2021).