# Coriander (*Coriandrum sativum*) Cultivation Combined with Arbuscular Mycorrhizal Fungi Inoculation and Steel Slag Application Influences Trace Elements-Polluted Soil Bacterial Functioning

Julien Langrand [1], Anissa Lounès-Hadj Sahraoui [1,*], Jérôme Duclercq [2], Robin Raveau [3], Frédéric Laruelle [1], Valérie Bert [4], Natacha Facon [1], Benoît Tisserant [1] and Joël Fontaine [1]

[1] Unité de Chimie Environnementale et Interactions sur le Vivant (UCEIV-UR 4492), Université Littoral Côte d'Opale, SFR Condorcet FR CNRS 3417, CS 80699, 62228 Calais, France
[2] Unité Écologie et Dynamique des Systèmes Anthropisés (EDYSAN UMR CNRS 7058 CNRS), Université de Picardie Jules Verne, UFR des Sciences, 80029 Amiens, France
[3] Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE), UMR Santé et Agroécologie du Vignoble (SAVE), Bordeaux Sciences Agro, ISVV, 33882 Villenave d'Ornon, France
[4] Unité Technologies Propres et Economie Circulaire, INERIS, Parc Technologique Alata, BP2, 60550 Verneuil en Halatte, France
* Correspondence: anissa.lounes@univ-littoral.fr; Tel.: +33–032-146–3658

**Abstract:** The cultivation of aromatic plants for the extraction of essential oils has been presented as an innovative and economically viable alternative for the remediation of areas polluted with trace elements (TE). Therefore, this study focuses on the contribution of the cultivation of coriander and the use of arbuscular mycorrhizal fungi (AMF) in combination with mineral amendments (steel slag) on the bacterial function of the rhizosphere, an aspect that is currently poorly understood and studied. The introduction of soil amendments, such as steel slag or mycorrhizal inoculum, had no significant effect on coriander growth. However, steel slag changed the structure of the bacterial community in the rhizosphere without affecting microbial function. In fact, *Actinobacteria* were significantly less abundant under slag-amended conditions, while the relative proportion of *Gemmatimonadota* increased. On the other hand, the planting of coriander affects the bacterial community structure and significantly increased the bacterial functional richness of the amended soil. Overall, these results show that planting coriander most affected the structure and functioning of bacterial communities in the TE-polluted soils and reversed the effects of mineral amendments on rhizosphere bacterial communities and their activities. This study highlights the potential of coriander, especially in combination with steel slag, for phytomanagement of TE-polluted soils, by improving soil quality and health.

**Keywords:** steel slag; phytomanagement; trace elements; coriander; arbuscular mycorrhizal fungi

## 1. Introduction

Soils provide fundamental ecosystem functions and play an important role in water and nutrient cycling, food production and the provision of renewable materials. Their ability to store carbon is essential for coping with climate change, and soil biodiversity is critical for soil fertility and biodiversity in general. Unfortunately, increasing pollution has made large areas of soil unsuitable for cultivation, a hazard to wildlife and humans, and uninhabitable for many living microorganisms. It is widely recognized that trace elements (TE), such as lead (Pb), cadmium (Cd), and zinc (Zn), can inhibit biological processes in ecosystems and affect the biodiversity, abundance, activity and function of soil microorganisms. These pollutants originate from human activities and, in particular, from smelters that have contributed to the production of significant amounts of dusts,

resulting in severe contamination of soils with TE [1]. They represent an increased toxicity for microorganisms living in the soil and are also responsible for soil acidification, small size of soil aggregates and poor soil structure due to the lack of organic matter (OM), humic acids and, by extension, nutrients [2–4]. Therefore, it is crucial to reduce TE-toxicity in polluted soils to restore soil ecosystem services. Phytomanagement technologies, such as phytostabilisation, are suitable options to manage agricultural soils polluted with TE ecologically and economically [5–7]. Therefore, a crucial point for successful phytomanagement is the selection of TE-tolerant plant species that grow rapidly and produce enough biomass to allow recovery [6,8]. Previous studies have shown that the cultivation of aromatic plants, such as coriander (*Coriandrum sativum*), should be a promising option for phytomanagement of TE-polluted soils [6,9]. Indeed, this aromatic belonging to the *Apiaceae* family is cultivated for the production of essential oils (EO), which are an important way of valorizing plant biomass. The EO produced on TE-polluted soils have antibacterial, antifungal and antioxidant activities, and can be used for various applications [10]. Although it has been demonstrated that coriander can grow in TE-polluted soils, this plant species accumulates Zn and Cd in its aboveground parts, which makes it impossible to recycle residues from the distillation of coriander (shoots or seeds), in the methanization process, or as compost [6,9,11].

For example, in the context of circular economy, the incorporation into the soil of mineral basic amendments could improve soil parameters, such as pH, nutrient mobilization or soil structure and microbial composition [12–14]. In addition, mineral basic amendments, such as slags, can contribute to mineral nutrition and thus improve crop yields, but also play an important role in stabilizing soil aggregates and resistance to plant diseases [12,13,15]. Some studies have shown the role of slag in TE immobilization, especially Cd, Zn and Pb [13,16,17]. Furthermore, steel slag has been described to increase microbial biomass, soil enzyme activity and the number of copiotrophic bacterial taxa, and is believed to provide beneficial ecosystem services to plants [14].

Therefore, an adequate supply of mineral, organic or biological amendments could be interesting, first to reduce TE-mobility and bioavailability, and also to improve the growth of coriander and soil function [11].

Biological amendments, such as arbuscular mycorrhizal fungi (AMF) inoculum, have also been shown to be useful for plant protection. Indeed, AMF enable host plants to grow vigorously under stressful conditions, especially in TE-polluted soils [18]. They contribute significantly to the uptake of soil nutrients, especially phosphorus [19], but also nitrogen, calcium, sulfur, potassium and zinc, thereby increasing plant biomass and improving plant tolerance to soil pollution [20,21]. In addition, AMF produce glomalin, an insoluble glycoprotein that has soil binding properties, but also melanin and chitin, which reduce metal bioavailability by metal immobilization into hyphae [18,22,23]. Soil microbial communities are indeed crucial for soil structure, OM degradation and C cycling [3,24]. They are often used to assess the degree of soil remediation [8,25].

Many recent studies have described a synergistic effect of AMF inoculation and biological and mineral amendments (olive residues, biochar, phosphate sludge from marble waste, compost, manure) on plant growth in TE-polluted soils [26–30]. So far, there are few scientific works dealing with the combined application of AMF and mineral amendments (steel slag), and their effects on microbial communities and their activities are generally poorly studied [16].

Therefore, under *in situ* conditions, this study focuses on the potential benefits of adding amendments (mineral amendment alone or in combination with AMF inoculum) to improve coriander growth and microbial function of historically TE-polluted soils. To this end, we investigated plant growth parameters, soil bacterial biomass (phospholipid fatty acid, PLFA), the functional activity (community-level physiological profile) and structure (16S rRNA metabarcoding) of rhizosphere bacterial communities.

## 2. Results

### 2.1. Coriander Growth Parameters

Although the plants had the same height after 11 weeks of cultivation regardless of the modality, we found that plants inoculated with AMF had a lighter aboveground dry weight than those non-inoculated (Table 1). In addition, the type and dose of the amendment did not alter the dry weight of coriander.

**Table 1.** Growth parameters and mycorrhizal rate of coriander. Data are presented as means ± standard error. The means were obtained from five replicates ($n = 5$) for height and dry weight of plants, and three replicates ($n = 3$) for mycorrhization rate. Means followed by the same letter are not significantly different, by one-way ANOVA comparison and post-hoc Fisher test ($\alpha = 0.05$).

| Amendment | Dose (t ha$^{-1}$) | Inoculation | Plant Aboveground Height (cm) | Dry Weight (g) | Mycorrhizal Rate (%) |
|---|---|---|---|---|---|
| Unamended | | NI | 69.6 ± 7.0 ab | 4.1 ± 1.1 bcd | 40.8 ± 10.7 abc |
| | | I | 71.6 ± 2.5 ab | 4.7 ± 0.6 cd | 35.6 ± 4.9 abc |
| Ladle slag | 1.5 | NI | 68.0 ± 4.0 ab | 7.0 ± 1.4 abc | 31.7 ± 0.8 c |
| | | I | 65.0 ± 2.5 ab | 3.2 ± 0.3 d | 35.8 ± 1.8 abc |
| | 45 | NI | 75.0 ± 9.8 a | 8.7 ± 2.1 a | 33.1 ± 3.9 bc |
| | | I | 72.7 ± 4.0 ab | 5.1 ± 1.1 abcd | 37.0 ± 1.3 abc |
| Oven slag | 1.5 | NI | 70.0 ± 5.1 ab | 6.1 ± 1.4 abcd | 37.5 ± 4.1 abc |
| | | I | 69.0 ± 4.3 ab | 4.9 ± 0.9 bcd | 51.0 ± 4.6 a |
| | 45 | NI | 73.8 ± 4.1 a | 8.0 ± 0.7 ab | 48.6 ± 7.5 ab |
| | | I | 62.0 ± 2.6 b | 6.0 ± 1.9 abcd | 47.9 ± 4.3 ab |
| **Dose** | | | NS | NS | NS |
| **Amendment** | | | NS | NS | ** |
| **Mycorrhizal inoculation** | | | NS | * | NS |
| **Dose * Amendment** | | | NS | NS | NS |
| **Dose * Mycorrhizal inoculation** | | | NS | NS | NS |
| **Amendment * Mycorrhizal inoculation** | | | NS | NS | NS |
| **Dose * Mycorrhizal inoculation * Amendment** | | | NS | NS | NS |

Significance: NS: not significant; *: $p < 0.05$; **: $p < 0.01$; according to multivariate ANOVA in bold. NI: not inoculated; I: inoculated.

### 2.2. Mycorrhizal Rate

The mycorrhizal rate of coriander roots ranged from 31.7 to 51%, and was significantly higher in plots amended with 1.5 t ha$^{-1}$ oven slag (51%) than in plots amended with 1.5 t ha$^{-1}$ ladle slag (31.7%). No difference was observed between NI and I conditions, indicating the presence of native AMF. The dose of amendment did not affect the mycorrhization rate.

### 2.3. Soil Microbial Biomass

Total bacterial biomass was not significantly different between the two unamended conditions. Analysis of variance (ANOVA) of the Gram-positive bacteria biomass showed an increase with the lower dose of amendment (1.5 t ha$^{-1}$) and with a combined effect of dose (1.5 t ha$^{-1}$) and vegetation (Table 2). Moreover, the biomass of Gram-positive bacteria was significantly higher (8.14 µg g$^{-1}$ of dry weight (DW) of soil) in soil amended with 1.5 t ha$^{-1}$ oven slag and under vegetated conditions than under the same non-vegetated conditions (2.54 µg g$^{-1}$ of DW of soil) (Table 2).

A significant effect of vegetation was observed on Gram-negative bacterial biomass. In fact, this increase was significantly higher in vegetated soils and NI condition supplemented with 1.5 t ha$^{-1}$ ladle slag (5.30 µg g$^{-1}$ soil) than under unvegetated modality (2.31 µg g$^{-1}$ soil, Table 2).

**Table 2.** Influence of amendments (ladle slag and oven slag used at 1.5 and 45 t ha$^{-1}$), inoculation and vegetation on Gram-positive, Gram-negative and total bacterial biomass ($\mu$g g$^{-1}$ of dry weight (DW) soil), and Gram-positive/Gram-negative ratio in trace element-polluted soil. Data are presented as means $\pm$ standard error. The means were obtained from three replicates (*n* = 3). Means followed by the same letter are not significantly different, by one-way ANOVA comparison and post-hoc Fisher test ($\alpha$ = 0.05).

| State | Amendment | Dose (t ha$^{-1}$) | Inoculation | Gram-Positive Biomass $\mu$g g$^{-1}$ of DW Soil | Gram-Negative Biomass $\mu$g g$^{-1}$ of DW Soil | Total Bacteria $\mu$g g$^{-1}$ of DW Soil | Gram-Positive/Gram-Negative Bacteria |
|---|---|---|---|---|---|---|---|
| **Unvegetated** | Unamended | | NI | 3.64 $\pm$ 3.64 abcd | 2.96 $\pm$ 1.58 abc | 6.60 $\pm$ 5.20 abc | 1.02 $\pm$ 0.60 bcd |
| | | | I | 5.50 $\pm$ 4.26 abcd | 2.85 $\pm$ 3.21 bc | 8.35 $\pm$ 7.34 abc | 2.93 $\pm$ 2.39 a |
| | Ladle slag | 1.5 | NI | 2.43 $\pm$ 0.57 d | 2.31 $\pm$ 0.94 c | 4.74 $\pm$ 0.40 c | 1.30 $\pm$ 0.92 bcd |
| | | | I | 4.30 $\pm$ 0.95 abcd | 4.11 $\pm$ 0.95 abc | 8.41 $\pm$ 1.88 abc | 1.05 $\pm$ 0.09 bcd |
| | | 45 | NI | 4.08 $\pm$ 1.76 abcd | 3.58 $\pm$ 0.85 abc | 7.65 $\pm$ 1.76 abc | 1.22 $\pm$ 0.64 bcd |
| | | | I | 2.05 $\pm$ 1.44 d | 2.82 $\pm$ 0.86 bc | 4.88 $\pm$ 1.85 c | 0.76 $\pm$ 0.47 cd |
| | Oven slag | 1.5 | NI | 6.97 $\pm$ 1.35 abc | 2.86 $\pm$ 0.77 bc | 9.83 $\pm$ 5.86 abc | 2.42 $\pm$ 0.65 ab |
| | | | I | 2.54 $\pm$ 1.13 bcd | 2.51 $\pm$ 1.41 bc | 5.05 $\pm$ 2.45 c | 1.17 $\pm$ 0.49 bcd |
| | | 45 | NI | 4.70 $\pm$ 0.86 abcd | 2.82 $\pm$ 0.91 bc | 7.52 $\pm$ 0.08 abc | 1.87 $\pm$ 0.98 abcd |
| | | | I | 3.26 $\pm$ 1.81 bcd | 2.82 $\pm$ 0.77 bc | 6.08 $\pm$ 2.57 abc | 1.09 $\pm$ 0.42 bcd |
| **Vegetated** | Unamended | | NI | 3.21 $\pm$ 2.40 bcd | 2.20 $\pm$ 1.15 c | 5.41 $\pm$ 3.54 c | 1.35 $\pm$ 0.40 abcd |
| | | | I | 2.42 $\pm$ 0.71 cd | 4.06 $\pm$ 1.29 abc | 6.47 $\pm$ 1.94 abc | 0.60 $\pm$ 0.09 d |
| | Ladle slag | 1.5 | NI | 3.65 $\pm$ 1.96 abcd | 5.30 $\pm$ 0.36 a | 8.96 $\pm$ 1.63 abc | 0.71 $\pm$ 0.43 cd |
| | | | I | 7.30 $\pm$ 3.49 ab | 4.82 $\pm$ 1.98 ab | 12.12 $\pm$ 5.45 a | 1.47 $\pm$ 0.20 abcd |
| | | 45 | NI | 1.77 $\pm$ 0.35 d | 2.31 $\pm$ 0.77 c | 4.08 $\pm$ 1.12 c | 0.78 $\pm$ 0.09 cd |
| | | | I | 3.97 $\pm$ 3.09 abcd | 4.37 $\pm$ 2.89 abc | 8.34 $\pm$ 5.95 abc | 0.88 $\pm$ 0.18 bcd |
| | Oven slag | 1.5 | NI | 7.34 $\pm$ 6.21 ab | 4.83 $\pm$ 1.05 ab | 12.16 $\pm$ 5.49 a | 1.72 $\pm$ 1.75 abcd |
| | | | I | 8.14 $\pm$ 4.66 a | 3.78 $\pm$ 0.34 abc | 11.92 $\pm$ 4.47 ab | 2.21 $\pm$ 1.29 abc |
| | | 45 | NI | 3.21 $\pm$ 1.92 bcd | 4.15 $\pm$ 2.18 abc | 7.36 $\pm$ 3.15 abc | 0.91 $\pm$ 0.80 bcd |
| | | | I | 2.83 $\pm$ 0.59 bcd | 2.86 $\pm$ 0.78 bc | 5.70 $\pm$ 0.81 bc | 1.05 $\pm$ 0.35 bcd |
| **Vegetation** | | | | NS | * | NS | NS |
| **Amendments** | | | | NS | NS | NS | NS |
| **Dose** | | | | * | NS | * | NS |
| **Mycorrhizal inoculation** | | | | NS | NS | NS | NS |
| **Vegetation * Amendment** | | | | NS | NS | NS | NS |
| **Vegetation * Dose** | | | | NS | NS | * | NS |
| **Vegetation * Mycorrhizal inoculation** | | | | NS | NS | NS | NS |
| **Mycorrhizal inoculation * Amendments** | | | | NS | NS | NS | NS |
| **Mycorrhizal inoculation * Dose** | | | | NS | NS | NS | NS |
| **Vegetation * Mycorrhizal inoculation * Amendments** | | | | NS | NS | NS | NS |

Significance: NS: not significant; *: *p* < 0.05; according to multivariate ANOVA in bold. NI: non-inoculated; I: inoculated.

The total bacterial biomass followed the same trend as the Gram-positive bacterial biomass. Indeed, a significant increase in total bacterial biomass was observed in the amended soil with 1.5 t ha$^{-1}$ oven slag and under vegetated conditions (11.92 $\mu$g g$^{-1}$ of DW soil) than in the same non-vegetated plots (5.05 $\mu$g g$^{-1}$ of DW soil). Examination of the ratio of the biomass of Gram-positive to Gram-negative bacteria showed no significant difference between diverse modalities (Table 2).

*2.4. Bacterial α-Diversity of Soil Biotopes*

To further investigate the effects of slags, vegetation and AMF inoculation on rhizospheric bacterial communities, the bacterial OTUs (Operational Taxonomic Units) were taxonomically assigned. The bacterial OTUs in the soil were assigned to 993 genera belonging to 33 phyla. *Actinobacteria* was the most represented phylum in each modality and accounted for between 41 and 67% of the total bacterial abundance (Figure 1).
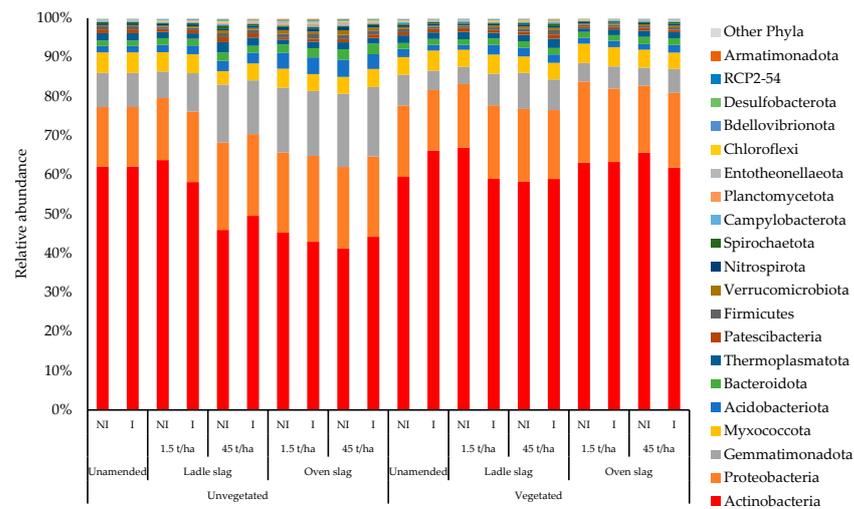
**Figure 1.** Relative abundance of bacterial operational taxonomic units (OTUs) grouped at phylum taxonomic rank in soil. NI, non-inoculated; I, inoculated.

Bacterial $\alpha$-diversity was calculated independently for each modality. According to the Chao1 index, soil bacterial species richness remains stable across modalities (average 325.3). Similarly, both bacterial richness and diversity (Shannon index) were not affected by the addition of slag, mycorrhizal inoculation or vegetation (Table 3).

**Table 3.** Influence of amendments (ladle slag and oven slag used at 1.5 and 45 t ha$^{-1}$), inoculation and vegetation on bacterial richness and diversity indexes. Data are mean $\pm$ SD (*n* = 3) for each condition. Means followed by the same lowercase letter are not significantly different, by one-way ANOVA comparison and post-hoc Fisher test ($\alpha$ = 0.05).

| State | Amendment | Dose (t ha$^{-1}$) | Inoculation | Chao1 | Shannon |
|---|---|---|---|---|---|
| **Unvegetated** | Unamended | | NI | 298.7 $\pm$ 28.5 cd | 4.3 $\pm$ 0.1 a |
| | | | I | 305.1 $\pm$ 48.8 cd | 4.4 $\pm$ 0.1 a |
| | Ladle slag | 1.5 | NI | 333.3 $\pm$ 53.7 abc | 4.5 $\pm$ 0.1 a |
| | | | I | 318.3 $\pm$ 13.5 bcd | 4.5 $\pm$ 0.1 a |
| | | 45 | NI | 288.1 $\pm$ 11.1 a | 4.5 $\pm$ 0.1 a |
| | | | I | 343.1 $\pm$ 17.7 abc | 4.5 $\pm$ 0.0 a |
| | Oven slag | 1.5 | NI | 335.5 $\pm$ 31.8 abc | 4.4 $\pm$ 0.0 a |
| | | | I | 329.9 $\pm$ 16.7 abcd | 4.4 $\pm$ 0.0 a |
| | | 45 | NI | 312.5 $\pm$ 19.3 bcd | 4.3 $\pm$ 0.2 a |
| | | | I | 334.1 $\pm$ 28.6 abc | 4.4 $\pm$ 0.3 a |
| **Vegetated** | Unamended | | NI | 321.7 $\pm$ 25.7 abcd | 4.4 $\pm$ 0.2 a |
| | | | I | 324.0 $\pm$ 23.4 abcd | 4.4 $\pm$ 0.0 a |
| | Ladle slag | 1.5 | NI | 320.2 $\pm$ 1.6 bcd | 4.5 $\pm$ 0.1 a |
| | | | I | 365.4 $\pm$ 27.9 a | 4.4 $\pm$ 0.0 a |
| | | 45 | NI | 335.4 $\pm$ 28.2 abc | 4.5 $\pm$ 0.1 a |
| | | | I | 302.8 $\pm$ 13.3 cd | 4.5 $\pm$ 0.0 a |
| | Oven slag | 1.5 | NI | 350.7 $\pm$ 26.9 ab | 4.4 $\pm$ 0.1 a |
| | | | I | 310.2 $\pm$ 5.0 bcd | 4.5 $\pm$ 0.1 a |
| | | 45 | NI | 335.7 $\pm$ 33.5 abc | 4.4 $\pm$ 0.1 a |
| | | | I | 340.9 $\pm$ 24.3 abc | 4.5 $\pm$ 0.1 a |
| | **Vegetation** | | | NS | NS |
| | **Amendments** | | | NS | NS |
| | **Dose** | | | NS | NS |
| | **Mycorrhizal inoculation** | | | NS | NS |
| | **Vegetation * Amendment** | | | NS | NS |
| | **Vegetation * Dose** | | | NS | NS |
| | **Vegetation * Mycorrhizal inoculation** | | | NS | NS |
| | **Mycorrhizal inoculation * Amendments** | | | NS | NS |
| | **Mycorrhizal inoculation * Dose** | | | NS | NS |
| | **Vegetation * Mycorrhizal inoculation * Amendments** | | | NS | NS |

Significance: NS: not significant; *: *p* < 0.05; according to multivariate ANOVA. NI: non-inoculated; I: inoculated.

*Actinobacteria* associated with *Proteobacteria*, *Gemmatimonadota*, *Myxococcota* and *Acidobacteriota* accounted for over 85% of bacterial OTUs. In general, we found that the abundance of the different bacterial phyla, and thus the structure of the bacterial community, was affected by the conditions tested. One of the most important changes concerned the high abundance of *Actinobacteria*, which was even more pronounced under the vegetated conditions, than under the unvegetated conditions, except for the not inoculated and unamended condition (Table S2). In contrast, a decrease of the *Gemmatimonadota* and *Acidobacteriota* abundances was observed under all vegetated conditions (Figure 1).

In addition to these changes in the abundance of the bacterial phyla, we also noted changes within phyla (Figure 2). For example, within the phylum *Actinobacteria*, we found that the genera *Pseudarthrobacter*, *Arthrobacter* and *Streptomyces* were enriched under vegetated conditions, whereas the genus *Gemmatimonadota* was favored under non-vegetated conditions.
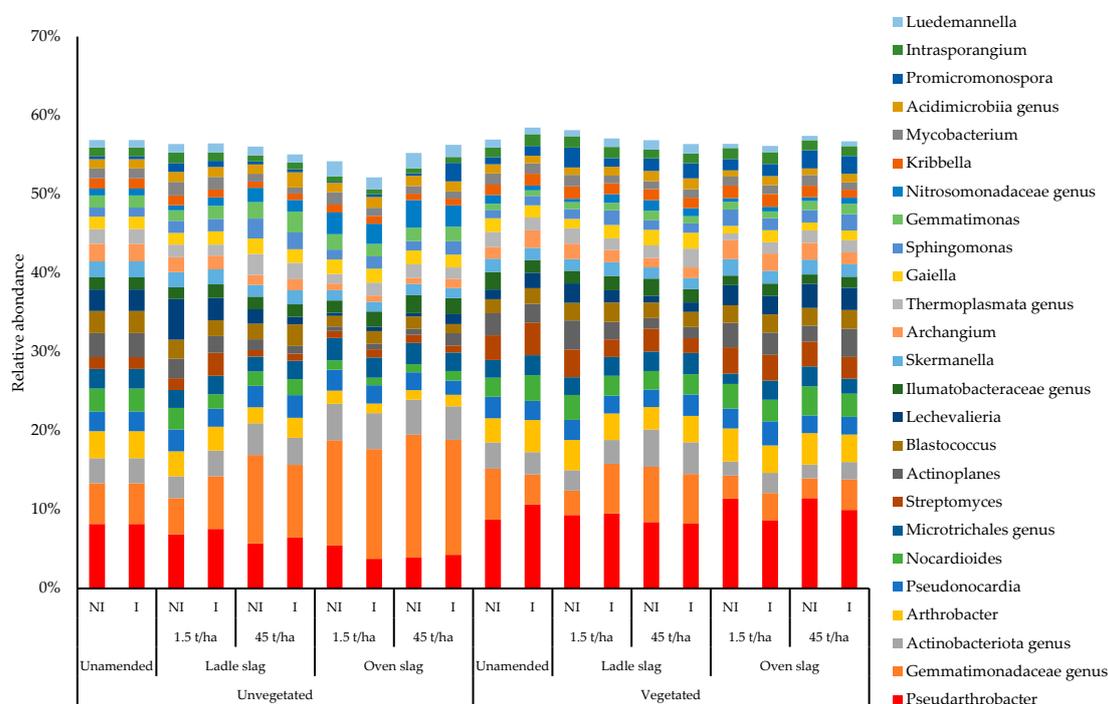


**Figure 2.** Abundances of the 25 most represented bacterial genus in soil. NI, non-inoculated; I, inoculated.

### 2.5. Metabolic Potential of Soil Microbial Communities

To understand the impact of taxonomic changes on the functionality of these microbial communities, a study was conducted on their ability to degrade different carbon sources. Overall, the metabolic potential of the soil microbial communities of each sample, expressed as average well color development (AWCD), was significantly higher under vegetated conditions than under the unvegetated conditions. The lowest value was found under the not inoculated, unvegetated and oven slag amended condition at 45 t ha$^{-1}$. In addition, there was no significant effect of inoculation, type of amendment or dose on AWCD level (Table 4).

A multivariate ANOVA showed that functional richness was significantly higher in vegetated conditions than in the unvegetated conditions. Neither the inoculation, nor the type or dosage of amendment had a significant influence on the values of functional richness (Table 4).

**Table 4.** Richness and diversity indexes of the 20 soil modalities. Data are mean $\pm$ SD (*n* = 3) for each condition. Means followed by the same lowercase letter are not significantly different, by one-way ANOVA comparison and post-hoc Fisher test ($\alpha$ = 0.05).

| State | Amendment | Dose | Inoculation | AWCD | Functional Richness |
|---|---|---|---|---|---|
| **Unvegetated** | Unamended | | NI | 100.27 $\pm$ 18.70 ef | 23.00 $\pm$ 1.63 cde |
| | | | I | 109.00 $\pm$ 16.49 bcdef | 23.50 $\pm$ 2.65 bcde |
| | Ladle slag | 1.5 | NI | 101.51 $\pm$ 29.86 ef | 21.50 $\pm$ 4.43 de |
| | | | I | 131.28 $\pm$ 21.44 abcd | 25.25 $\pm$ 1.71 abc |
| | | 45 | NI | 105.58 $\pm$ 5.46 def | 24.50 $\pm$ 1.00 bcd |
| | | | I | 99.88 $\pm$ 17.08 ef | 21.50 $\pm$ 2.65 de |
| | Oven slag | 1.5 | NI | 107.53 $\pm$ 15.34 cdef | 23.75 $\pm$ 1.26 bcde |
| | | | I | 98.86 $\pm$ 18.16 ef | 21.00 $\pm$ 2.16 e |
| | | 45 | NI | 87.99 $\pm$ 23.07 f | 20.75 $\pm$ 2.87 e |
| | | | I | 96.76 $\pm$ 14.04 ef | 21.00 $\pm$ 2.83 e |
| **Vegetated** | Unamended | | NI | 134.96 $\pm$ 6.02 ab | 26.50 $\pm$ 1.91 ab |
| | | | I | 148.19 $\pm$ 20.41 a | 26.50 $\pm$ 1.29 ab |
| | Ladle slag | 1.5 | NI | 141.52 $\pm$ 15.20 a | 25.75 $\pm$ 0.50 ab |
| | | | I | 136.81 $\pm$ 25.96 a | 26.75 $\pm$ 1.50 ab |
| | | 45 | NI | 128.84 $\pm$ 25.97 abcd | 25.00 $\pm$ 3.65 abc |
| | | | I | 122.11 $\pm$ 19.30 abcde | 25.50 $\pm$ 2.52 abc |
| | Oven slag | 1.5 | NI | 135.67 $\pm$ 18.88 ab | 25.75 $\pm$ 1.71 abc |
| | | | I | 133.32 $\pm$ 19.25 abc | 25.25 $\pm$ 3.30 abc |
| | | 45 | NI | 147.66 $\pm$ 12.12 a | 28.00 $\pm$ 1.63 a |
| | | | I | 145.17 $\pm$ 24.64 a | 26.75 $\pm$ 1.89ab |
| **Vegetation** | | | | *** | *** |
| **Amendments** | | | | NS | NS |
| **Dose** | | | | NS | NS |
| **Mycorrhizal inoculation** | | | | NS | NS |
| **Vegetation * Amendments** | | | | * | NS |
| **Vegetation * Dose** | | | | NS | NS |
| **Vegetation * Mycorrhizal inoculation** | | | | NS | NS |
| **Mycorrhizal inoculation * Amendments** | | | | NS | NS |
| **Mycorrhizal inoculation * Dose** | | | | NS | NS |
| **Vegetation * Mycorrhizal inoculation * Amendments * Dose** | | | | NS | NS |

Significance: NS: not significant.*: $p < 0.05$; ***: $p < 0.001$ according to multivariate ANOVA. NI: non-inoculated; I: inoculated.

## 3. Discussion

The cultivation of aromatic plants for the EO extraction has been presented as an innovative and economically viable alternative for the phytoremediation of TE-polluted areas [6]. However, the effects of growing aromatic plants, such as coriander, in combination with mineral (steel slag) and microbial (AMF) amendments introduced into the soil on the structure and potential function of bacterial communities in the TE-polluted rhizosphere, have never been studied. Thus, our results showed that coriander was able to establish and develop well on heavily TE-polluted soil, confirming previous work [6]. The addition of steel slag had no overall effect on plant growth. This result could be explained by the low nutrient requirement of coriander, but also by the OM content of this agricultural soil [6,31]. Consequently, it is likely that the slag did not provide any supplemental nutrient gain to the plant. Indeed, the beneficial effect of slag is controversial. Some studies have shown that these mineral amendments could contribute to mineral supply and consequently improve crop yields, while others showed no change or diverse responses, depending on the experimental conditions [13,15,32–34].

We have shown that coriander was able to form a mycorrhizal symbiosis with the native AMF present in our TE-polluted soil. Therefore, no significant difference in root mycorrhizal colonization rates was found between NI and I conditions, while AMF inoculation had no significant effect on shoot growth. In contrast to our results, some studies have shown that the introduction of AMF inoculum into TE-polluted and unpolluted soils can lead to a significant improvement in overall mycorrhizal rates, indicating different responses in experiments depending on the plant species, but also on the type of mycorrhizal inoculum [6,35,36]. We cannot exclude that the natural presence of native AMF in the TE-polluted soils masked the contribution of the exogenous mycorrhizal inoculum on coriander growth. In contrast, an increase in mycorrhizal colonization rates was observed in the presence of oven slag. This increase could be due to a reduction in TE-bioavailability or changes in pH or soil texture, as suggested by Hu et al. [16].

Although no benefits were found for the growth of coriander after mineral amendment and inoculation with AMF, several studies reported the crucial role played by these mineral and microbial amendments, as well as vegetation, in restoring soil functions that might be disturbed by TE-pollution [16,26]. In our results, the high bacterial diversity and richness indexes were comparable to some indexes obtained on unpolluted soils [37,38]. Moreover, despite the soil TE pollution, the functionality of the AWCD-based microbial communities indicated that the studied soils had a high metabolic capacity, which is typical of functional soils [39–42]. However, numerous studies have also shown a reduction in the abundance, activity, and diversity of soil microbial communities following TE exposure [42–44]. For example, Fontaine et al. [45] demonstrated a relatively low ratio of fungi-to-bacteria in this agricultural polluted soil, indicating an extensively managed soil in combination with tillage, high fertilization and a low C:N ratio favored bacteria. In this study, more Gram-positive than Gram-negative bacteria were observed under all conditions. Adaptation to an environment rich in TE leads microorganisms to exhibit activities of biosorption, bioprecipitation, extracellular sequestration, transport mechanisms and/or chelation [46]. Gram-positive bacteria are known for their diverse physiological and metabolic properties that allow them to thrive in a wide range of environments. Compared to Gram-negative bacteria, they appear to accumulate higher TE-concentrations on their cell walls [47]. Therefore, these bacteria are able to adapt easier to adverse conditions, such as pollution, and should even be used for TE-removal, which could explain their frequent presence in this soil [44,48]. Specifically, *Actinobacteria*, *Proteobacteria* and *Gemmatimonadota*, were the most abundant bacterial phyla in our TE-polluted soils. The *Actinobacteria* phylum has been highlighted for its TE-tolerance and even investigated for potential applications in remediation [49]. It plays a role in the degradation of polysaccharides, e.g., cellulose, xylan and chitin, OM turnover and in nutrient cycling [50]. *Proteobacteria*, the second most abundant phylum, is known to contain a wide range of functional bacteria, including fast-growing bacteria and many plant promoters, as well as growth-promoting bacteria [51]. Furthermore, *Proteobacteria* taxa have been described to be more abundant in heavily TE-polluted soils [44,52]. The latter phylum, *Gemmatimonadota*, is widespread in ecosystems around the world, making it an important member of soil bacterial communities [53]. Therefore, the long-term response of the bacterial community to TE results from the ability of these microorganisms to develop TE-tolerance without affecting overall community structure, as proposed by Brandt et al. [54].

The addition of amendments, such as steel slags or mycorrhizal inoculum, altered the structure of the rhizosphere bacterial community without affecting its metabolic potential, which was assessed by the AWCD parameter. In fact, the low dose of the mineral amendment increased bacterial biomass and caused a shift in the bacterial community structure. The *Actinobacteria* phylum was significantly less abundant under the slag-amended conditions, while the relative proportion of *Gemmatimonadota* increased. These results are in agreement with those of Gremion et al. [40] on a TE-polluted soil, but also with the functional redundancy of these communities [55]. Mineral granulometry has been shown to act as a physical support for the fixation of microorganisms (i.e., bacteria, fungi, etc.)

and plants, as well as a nutrient reserve, and that the abundance of *Acidobacteria* decreases with increasing pH [56]. In this respect, and due to the alkaline nature of slags, the pH and mineralosphere can be important factors influencing microbial communities [56,57].

Cultivation of coriander also resulted in a change in the bacterial biomass and community structure. A significant increase in Gram-negative bacterial biomass under vegetated conditions and in the proportion of *Actinobacteria* phylum was detected. It is well known that plants secrete specific root exudates to shape the microbial community structures in the rhizosphere. These compounds, including sugars, amino acids and organic acids, have various effects on bacterial community composition and microbiome assembly [58]. In addition, aromatic plants are also known to be able to secrete secondary metabolites in their root exudates [59–61]. Due to their anti-microbial properties, some of these metabolites probably influence bacterial richness and diversity by targeting specific communities [62]. Some compounds may therefore affect microbial communities in the rhizosphere [60]. This is especially true for mono- and sesquiterpenes found in aromatic plants [60].

We noted an increase in the genus *Streptomyces* under vegetated conditions, a genus that has been described for its abilities as plant growth-promoting bacteria, but also for TE biosorption, making it particularly interesting in a phytostabilization context [63,64]. Moreover, the relative abundance of some genera (*Sphingomonas*, *Gemmatimonas*, *Kribbella*, . . . ) was very low, but they could play an important role in bio-geochemical cycles [65,66]. In fact, rare taxa are more modulated in abundance than dominant taxa [66], and therefore could be important factors in the resilience and adaptability of soil microorganisms [66]. Interestingly, some bacterial phyla have shown the ability to limit the accumulation of TE in plants. Indeed, some *Actinobacteria* and *Proteobacteria* can alter the bioavailability of heavy metals through redox, precipitation or sorption and desorption reactions [49]. These phyla, such as *Firmicutes* and *Bacteroidetes*, are known to possess TE-tolerance genes encoding proteins involved in efflux and sequestration of TE ions, such as AT-Pases, D-protein or B-protein (copper tolerance and transport, respectively) and oxidative stress attenuation proteins, such as superoxide dismutase, alkyl hyperoxide reductase or mycothiol reductase [67,68].

Therefore, coriander in TE-polluted soil activated the resilience of bacterial communities. This resilience of microbial communities and functions in TE-polluted soils is usually related to a shift from sensitive to tolerant species, genetic modifications leading to TE-resistance, a transfer of genes encoding resistance or tolerance to TE, or a decrease in TE-bioavailability [69]. The recruitment of bacterial species belonging to the genera *Pseudarthrobacter*, *Arthrobacter* and *Streptomyces* by root exudates of coriander to the detriment of *Gemmatimonadota* bacteria, was associated with an increase in soil functionality that corresponds to such a shift. In this case, species belonging to *Gemmatimonadota* could be considered as indicator species for resistance to TE. Similarly, species belonging to *Pseudarthrobacter*, *Arthrobacter* and *Streptomyces* could be more sensitive to TE, but can characterize the resistance of TE-polluted soils when suitable substrates are available, such as those provided by root exudates in phytomanagement.

## 4. Materials and Methods

### 4.1. In Situ Experimental Design

Study Area.

The study took place on an agricultural soil, historically polluted by TE ($50°25'55.5''$ N, $3°02'25.5''$ E; elevation 23 m) in the "Hauts de France" region (northern France). The experimental site was located 600 m away from a former Pb and Zn smelter, Metaleurop Nord, whose activity generated significant amounts of TE-rich dust, resulting in TE-pollution of the topsoil (0–30 cm) [70,71]. The topsoil is characterized as silt loam, with a slightly alkaline water pH (7.9). It contains high TE total concentrations of Cd, Pb and Zn (7, 394 and 443 ppm, respectively), of which the $NH_4NO_3$ extractable fractions account for 0.114, 0.078, and 0.930 ppm, respectively [6]. These concentrations were approximately 17, 11 and 6-fold higher, respectively, than the regional background values for agricultural soils [72].

### 4.2. Experimental Design

The *in situ* experimental design was divided into 20 equal plots of 9 m$^2$, to investigate the effects of one biological (AMF) and two mineral amendments on soil function, with or without planting an annual herbaceous aromatic plant species, coriander (*Coriandrum sativum* L.). The mineral amendments, ladle and oven slags, were by-products of steel production, often used in agriculture due to their buffering capacity [73]. Their main constituent was CaO and $Fe_2O_3$, respectively. The biological amendment was the commercial mycorrhizal inoculum SYMBIVIT$^®$ (INOCULUMplus, Bretenière, France), which contained six AMF species: *Rhizophagus irregularis*, *Funneliformis mosseae*, *Glomus microaggregatum*, *Glomus claroideum*, *Claroideoglomus etunicatum* and *Funneliformis geosporum*. The surface of each test area was 9 m$^2$ (3 m × 3 m), and each test area was randomly arranged and separated from each other by a 1 m wide corridor. A total of 20 conditions were designed (Table S1). Before sowing, the soil of the test plots was amended or not with 2 different concentrations of 1.5 and 45 t ha$^{-1}$ mineral amendment (ladle and oven slags) and/or the biological amendment at a rate of 10 kg ha$^{-1}$. These plots were then sown (7.5 kg ha$^{-1}$) or not (unvegetated condition) with coriander (seeds were provided by Iteipmai, France) in March 2019. The unvegetated plots were kept in this condition by regular manual weeding.

### 4.3. Sample Collection

At the beginning of the *in situ* experiment, 5 soil samples representing the original condition were taken with a soil auger in the 0–20 cm soil horizon, and stored at −20 °C until analysis. Three weeks after sowing, germination rate was assessed by counting the number of coriander plants in 3 random squares (1 m$^2$) for each vegetated plot. After 11 weeks of cultivation, 5 new soil samples were collected in each plot from the 0–20 cm soil horizon and stored at −20 °C until analysis; the aboveground biomass and roots of 5 plants were also harvested from the vegetated area. The height of the aerial parts was measured first, before the plants were dried and weighed, while the fresh roots were washed and stored at 4 °C until stained for AMF root colonization determination. The roots were cleared in 10% (*w/v*) KOH and stained with 0.05% (*w/v*) trypan blue (Alfa Aesar) [74] to determine root colonization [75]. Briefly, root fragments were placed on microscopic slides and mycorrhizal structures (arbuscules and vesicles) were counted for each fragment observed. Three counts per fragment were performed. The percentage of total colonization was equal to the number of intersections with mycorrhizal roots × 100/number of total intersections. This percentage of colonization was expressed as the percentage of arbuscules, vesicles and total colonization.

### 4.4. Fatty Acid Analysis

After removing plant debris from the soil samples, 3 g of freeze-dried soil (3 samples per test area) were used for the analysis of fatty acid content. Lipid extraction was performed according to Frostegård et al., with a mixture of chloroform:methanol:citrate buffer—0.15M, pH 4.0—(1:2:0.8 *v/v/v*), under agitation for 2 h [76]. The lipid material was fractionated on Solid Phase Extraction (SPE) columns containing silica (6 mL volume, 500 mg sorbent, Interchim, Montluçon, France) by successive elutions with chloroform, acetone and methanol (1:2:1, *v/v/v*) into neutral lipids (NLFA), glycolipids and polar lipids containing phospholipid (PLFA) [76]. The NLFA and PLFA were trans-esterified with 0.2 M KOH in methanol to obtain free fatty acid methyl esters. The resulting fatty acid methyl esters were analyzed using a gas phase chromatography-mass spectrometer (GC-MS) Shimadzu QP-2010 Ultra (Shimadzu, Japan) equipped with a single quadrupole mass detector and simultaneously coupled with a flame ionization detector (FID). Samples were analyzed in split mode (80:1 ratio) on a ZB-1MS fast capillary column (10 m length × 0.1 mm inner diameter × 0.1 μm phase thickness, 100% dimethylpolysiloxane, Zebron, Phenomenex, Torrance Calif, CA, USA) using helium as the carrier gas at a constant linear velocity (40 cm s$^{-1}$). The injector temperature was 280 °C, and the detector temperatures were 330 °C and 280 °C for FID and for the ion source, respectively. The temperature program

started with an initial temperature of 175 °C and increased by 25 °C every minute to reach a final temperature of 275 °C, which was maintained for 0.5 min. The ionization mode was electronic impact at 70 eV and the mass range between 50.0 and 400.0 u was scanned. The single impact monitoring (SIM) mode was used simultaneously. Quantification of fatty acids was performed using nonadecanoic acid methyl ester (C19:0, Sigma-Aldrich) as an internal standard. Fatty acids were identified by comparing their relative retention time with that of commercial fatty acid methyl ester standards (C4-C24:1, Sigma-Aldrich), and by comparing them with spectra either obtained from commercial standards and/or published in the literature (NIST Standard Reference Database). Phospholipid fatty acid (PLFA) i15:0, a15:0, i16:0, i17:0, a17:0 and PLFA cy17:0, C18:1ω7 and cy19:0 were used as indicators of Gram-positive and negative bacterial biomasses, respectively [77].

### 4.5. DNA Extraction

Genomic DNA was extracted in triplicate directly from 250 mg fresh soil (*n* = 60) using the NucleoSpin Soil® kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. The quality of the extracted DNA was checked using 1% (*w/v*) agarose gels. Quantification of the extracted DNA was performed using a SpectraMax® iD3 spectrophotometer (Molecular Devices LLC, Sunnyvale, CA, United States). The extracted DNA was stored at −20 °C until use.

Polymerase Chain Reaction and sequencing of bacterial 16S rRNA gene Hypervariable regions V3–V4 of the *16S rRNA* gene were amplified from 1 ng of genomic DNA using a PCR thermal cycler (Agilent Surecycler 8800) with the forward primer CS1_341_F (ACACTGACGACATGGTTCTACACCTACGGGNGGCWGCAG) and the reverse primer CS2_805_R (TACGGTAGCAGAGACTTGGTCTCTGACTACCAGGGTATCTAATC) [78]. Two independent PCR reactions were performed per DNA sample.

### 4.6. Illumina MiSeq Sequencing

All PCR products were pooled (*n* = 60) and sent to the Genome Quebec Innovation Centre (Montreal, QC, Canada) for sequencing, using Illumina MiSeq platform, producing paired-end 2 bp × 300 bp.

### 4.7. Data Analysis–Bioinformatic Processing

Sequences were loaded into the Galaxy instance (v.2.3.0) of the Genotool bioinformatics platform (http://sigenae-workbench.toulouse.inra.fr, accessed on 27 December 2022) to be processed in the pipeline FROGS (Find Rapidly OTU with Galaxy Solution, [79]). Sequences with ambiguous bases (N) or without the specific primers were removed. The Cutadapt software [80] was used to search and cut primer sequences with less than 10% difference. The number of sequences identified was 2,660,014 and sequence clustering was performed using the SWARM algorithm (v2.1.5; [81]). A first denoising step was performed to build very fine clusters with minimal differences (d = 1), and a second one with an aggregation distance of 3. The resulting representative sequences for each cluster, or OTUs (Operational Taxonomic Units), were subjected to chimera detection using the VSEARCH algorithm [82] and eliminated, as well as those with an abundance <0.001% of the total abundance. In the end, 48,251 OTUs were retained, corresponding to 2,264,807 sequences. The taxonomic classification of each OTU against the Silva database (v138.1) was done using RDPClassifier [83]. The abundance of the different samples ranged from 65,070 to 16,243. As a result, a rarefaction of OTUs at species level was performed with 16,243 as the rarefaction value ("rrarefy" function of the vegan package). Following this, diversity indices were determined. Comparisons of means were made (Figures Shannon, and Chao1). As the Shapiro–Wilk group tests for the different indices indicated significant *p*-values (samples not following a normal distribution), comparisons via Wilcoxon–Mann–Whitney and Kruskal–Wallis tests were performed.

*4.8. Nucleotide Sequence Accession Numbers*

The 16S rRNA gene sequences of the whole dataset have been deposited in NCBI Sequence Read Archive (SRA) database and can be found under accession number PRJNA918629.

*4.9. Soil Bacterial Metabolic Profiles*

The metabolic potential of soil communities was determined from community-level physiological profiles (CLPPs) using Biolog EcoPlates (Biolog Inc.; [55]). For each soil sample, an EcoPlate containing 31 different carbon sources (and one blank with no carbon source) in triplicate was inoculated, and incubated for 196 h at 25 °C in an OmniLog® system (Biolog Inc.). The rate of carbon source utilization was recorded by the reduction of tetrazolium, a redox color indicator that changes from colorless to purple and is detected at a wavelength of 590 nm. Data were recorded every 15 min of incubation and saved in OmniLog® units generated by Biolog Data Analysis software (v1.7). Values for each well were calculated by subtracting the blank values from each well of the plate. A single absorbance time point at 50 h was used for the comparisons, as recommended in [84]. The metabolic potential of the soil microbial communities in each sample, expressed as the average well color development (AWCD), was calculated at the determined time point by dividing the sum of the optical density data by 31 (number of substrates). The total number of wells in a replicate with an absorbance above 25 OmniLog® units was counted to determine the functional richness of the soil microbial community.

*4.10. Statistical Analysis*

Statistical analyses were performed using XLSTAT software (v2021.1.1; Addinsoft, Paris, France). Normality of the data was first subjected to the Shapiro–Wilk test. Depending on the result of this test, parametric (ANOVA) or non-parametric (Kruskal–Wallis) tests were used, followed by Fisher's post-hoc test (Least Significant Difference, LSD). Data expressed as percentages (AMF colonization rate) were converted to arcsine values (ASIN function in Microsoft® Excel v16.50) before statistical analysis. Bacterial community analysis was performed using the R environment (v4.2.2; http://www.r-project.org/, accessed on 27 December 2022). Permutational multivariate analysis of variance (PERMANOVA), based on the Bray–Curtis dissimilarity, were performed with 1000 permutations using the "adonis" function of the vegan [85] R package. Bacterial richness (Chao1) and diversity (Shannon) indexes were calculated using the Phyloseq (v1.36.0; [86]) R package.

## 5. Conclusions

This study investigated the effect of coriander cultivation and introduction of AMF in combination with mineral amendments (steel slags) on the bacterial function of the rhizosphere, an aspect that is poorly understood and still poorly studied. The introduction of amendments, such as steel slags or mycorrhizal inoculum, into the soil had no significant effect on coriander growth. However, these amendments altered the structure of bacterial communities in the rhizosphere, without affecting their metabolic potential. In fact, *Actinobacteria* were significantly less abundant under the slag-amended conditions (55% on average), while the relative proportion of *Gemmatimonadota* increased (10% on average). In contrast, soil vegetation by coriander increased Gram-negative bacterial biomass (mean 2.96 to 3.87 $\mu g\,g^{-1}$ of DW soil) and the proportion of *Actinobacteria* phylum (62%). Thus, the vegetation improved soil quality and health by increasing the diversity of bacterial metabolic functions and diversity. Our results showed that coriander activated the resilience of bacterial communities in TE-polluted soils. Consequently, our results suggest that steel slag, which contributes to the circular economy through its recycling, in combination with soil vegetation that includes aromatic plants such as coriander, potentially provides long-term ecological benefits to TE-polluted soils and should be considered as a reliable tool for future TE-polluted soil remediation. Further studies are needed to confirm the contribution of steel slag to assisted TE-phytostabilization and its persistence in soil.

## References

1. Douay, F.; Roussel, H.; Pruvot, C.; Loriette, A.; Fourrier, H. Assessment of a Remediation Technique Using the Replacement of Contaminated Soils in Kitchen Gardens Nearby a Former Lead Smelter in Northern France. *Sci. Total Environ.* **2008**, *401*, 29–38. [CrossRef] [PubMed]
2. Gutiérrez, M.; Mickus, K.; Camacho, L.M. Abandoned Pb–Zn Mining Wastes and Their Mobility as Proxy to Toxicity: A Review. *Sci. Total Environ.* **2016**, *565*, 392–400. [CrossRef]
3. Gupta, V.V.S.R.; Germida, J.J. Distribution of Microbial Biomass and Its Activity in Different Soil Aggregate Size Classes as Affected by Cultivation. *Soil Biol. Biochem.* **1988**, *20*, 777–786. [CrossRef]
4. Epelde, L.; Burges, A.; Mijangos, I.; Garbisu, C. Microbial Properties and Attributes of Ecological Relevance for Soil Quality Monitoring during a Chemical Stabilization Field Study. *Appl. Soil Ecol.* **2014**, *75*, 1–12. [CrossRef]
5. Mench, M.; Vilela, J.; Pereira, S.; Moreira, H.; Castro, P.; Ávila, P.; Vega, A.; Maestri, E.; Szulc, W.; Rutkowska, B.; et al. *Guide of Best Practices for Phytomanaging Metal(Loid)-Contaminated Soils: GT1 Characterization and Risk Assessment of Contaminated/Degraded Sites and Implementation of Suitable Phytomanagement Options*; European Regional Development Fund: Maastricht, The Netherlands, 2019; 66p.
6. Raveau, R.; Fontaine, J.; Bert, V.; Perlein, A.; Tisserant, B.; Ferrant, P.; Lounès-Hadj Sahraoui, A. In Situ Cultivation of Aromatic Plant Species for the Phytomanagement of an Aged-Trace Element Polluted Soil: Plant Biomass Improvement Options and Techno-Economic Assessment of the Essential Oil Production Channel. *Sci. Total Environ.* **2021**, *789*, 147944. [CrossRef] [PubMed]
7. Kidd, P.; Mench, M.; Álvarez-López, V.; Bert, V.; Dimitriou, I.; Friesl-Hanl, W.; Herzig, R.; Olga Janssen, J.; Kolbas, A.; Müller, I.; et al. Agronomic Practices for Improving Gentle Remediation of Trace Element-Contaminated Soils. *Int. J. Phytoremediation* **2015**, *17*, 1005–1037. [CrossRef] [PubMed]
8. Deng, J.; Bai, X.; Zhou, Y.; Zhu, W.; Yin, Y. Variations of Soil Microbial Communities Accompanied by Different Vegetation Restoration in an Open-Cut Iron Mining Area. *Sci. Total Environ.* **2020**, *704*, 135243. [CrossRef]
9. Perlein, A.; Zdanevitch, I.; Gaucher, R.; Robinson, B.; Papin, A.; Lounès-Hadj Sahraoui, A.; Bert, V. Phytomanagement of a Metal(Loid)-Contaminated Agricultural Site Using Aromatic and Medicinal Plants to Produce Essential Oils: Analysis of the Metal(Loid) Fate in the Value Chain. *Environ. Sci. Pollut. Res.* **2021**, *28*, 62155–62173. [CrossRef]
10. Raveau, R.; Fontaine, J.; Soltani, A.; Jemâa, J.M.B.; Laruelle, F.; Lounès-Hadj Sahraoui, A. In Vitro Potential of Clary Sage and Coriander Essential Oils as Crop Protection and Post-Harvest Decay Control Products. *Foods* **2022**, *11*, 312. [CrossRef]
11. Zheljazkov, V.D.; Craker, L.E.; Xing, B.; Nielsen, N.E.; Wilcox, A. Aromatic Plant Production on Metal Contaminated Soils. *Sci. Total Environ.* **2008**, *395*, 51–62. [CrossRef]
12. Agnello, A.C.; Potysz, A.; Fourdrin, C.; Huguenot, D.; Chauhan, P.S. Impact of Pyrometallurgical Slags on Sunflower Growth, Metal Accumulation and Rhizosphere Microbial Communities. *Chemosphere* **2018**, *208*, 626–639. [CrossRef] [PubMed]

13. Branca, T.A.; Colla, V. Possible Uses of Steelmaking Slag in Agriculture: An Overview. In *Material Recycling—Trends and Perspectives*; InTech: London, UK, 2012.

14. Das, S.; Gwon, H.S.; Khan, M.I.; Jeong, S.T.; Kim, P.J. Steel Slag Amendment Impacts on Soil Microbial Communities and Activities of Rice (*Oryza sativa* L.). *Sci. Rep.* **2020**, *10*, 6746. [CrossRef]

15. Ning, D.; Liang, Y.; Song, A.; Duan, A.; Liu, Z. In Situ Stabilization of Heavy Metals in Multiple-Metal Contaminated Paddy Soil Using Different Steel Slag-Based Silicon Fertilizer. *Environ. Sci. Pollut. Res.* **2016**, *23*, 23638–23647. [CrossRef]

16. Hu, Z.H.; Zhuo, F.; Jing, S.H.; Li, X.; Yan, T.X.; Lei, L.L.; Lu, R.R.; Zhang, X.F.; Jing, Y.X. Combined Application of Arbuscular Mycorrhizal Fungi and Steel Slag Improves Plant Growth and Reduces Cd, Pb Accumulation in Zea Mays. *Int. J. Phytoremediat.* **2019**, *21*, 857–865. [CrossRef]

17. Kumpiene, J.; Lagerkvist, A.; Maurice, C. Stabilization of As, Cr, Cu, Pb and Zn in Soil Using Amendments—A Review. *Waste Manag.* **2008**, *28*, 215–225. [CrossRef] [PubMed]

18. Cicatelli, A.; Todeschini, V.; Lingua, G.; Biondi, S.; Torrigiani, P.; Castiglione, S. Epigenetic Control of Heavy Metal Stress Response in Mycorrhizal versus Non-Mycorrhizal Poplar Plants. *Environ. Sci. Pollut. Res.* **2014**, *21*, 1723–1737. [CrossRef] [PubMed]

19. Javot, H.; Pumplin, N.; Harrison, M.J. Phosphate in the Arbuscular Mycorrhizal Symbiosis: Transport Properties and Regulatory Roles. *Plant Cell Environ.* **2007**, *30*, 310–322. [CrossRef]

20. Meier, S.; Borie, F.; Bolan, N.; Cornejo, P. Phytoremediation of Metal-Polluted Soils by Arbuscular Mycorrhizal Fungi. *Crit. Rev. Environ. Sci. Technol.* **2012**, *42*, 741–775. [CrossRef]

21. Smith, S.; Read, D. *Mycorrhizal Symbiosis*; Elsevier: Amsterdam, The Netherlands, 2008; Volume 3, ISBN 9780123705266.

22. Ferrol, N.; Tamayo, E.; Vargas, P. The Heavy Metal Paradox in Arbuscular Mycorrhizas: From Mechanisms to Biotechnological Applications. *J. Exp. Bot.* **2016**, *67*, 6253–6565. [CrossRef]

23. González-Chávez, M.C.; Carrillo-González, R.; Wright, S.F.; Nichols, K.A. The Role of Glomalin, a Protein Produced by Arbuscular Mycorrhizal Fungi, in Sequestering Potentially Toxic Elements. *Environ. Pollut.* **2004**, *130*, 317–323. [CrossRef]

24. Liu, B.; Gumpertz, M.L.; Hu, S.; Ristaino, J.B. Long-Term Effects of Organic and Synthetic Soil Fertility Amendments on Soil Microbial Communities and the Development of Southern Blight. *Soil Biol. Biochem.* **2007**, *39*, 2302–2316. [CrossRef]

25. Zhang, B.; Kong, W.; Nan, W.; Zhang, Y. Bacterial Diversity and Community along the Succession of Biological Soil Crusts in the Gurbantunggut Desert, Northern China. *J. Basic Microbiol.* **2016**, *56*, 670–679. [CrossRef] [PubMed]

26. García-Sánchez, M.; Cajthaml, T.; Filipová, A.; Tlustoš, P.; Száková, J.; García-Romera, I. Implications of Mycoremediated Dry Olive Residue Application and Arbuscular Mycorrhizal Fungi Inoculation on the Microbial Community Composition and Functionality in a Metal-Polluted Soil. *J. Environ. Manag.* **2019**, *247*, 756–765. [CrossRef] [PubMed]

27. Xiao, Y.; Zhao, Z.; Chen, L.; Li, Y. Arbuscular Mycorrhizal Fungi and Organic Manure Have Synergistic Effects on Trifolium Repens in Cd-Contaminated Sterilized Soil but Not in Natural Soil. *Appl. Soil Ecol.* **2020**, *149*, 103485. [CrossRef]

28. Raklami, A.; Tahiri, A.I.; Bechtaoui, N.; Abdelhay, E.G.; Pajuelo, E.; Baslam, M.; Meddich, A.; Oufdou, K. Restoring the Plant Productivity of Heavy Metal-Contaminated Soil Using Phosphate Sludge, Marble Waste, and Beneficial Microorganisms. *J. Environ. Sci.* **2021**, *99*, 210–221. [CrossRef]

29. Liu, L.; Li, J.; Yue, F.; Yan, X.; Wang, F.; Bloszies, S.; Wang, Y. Effects of Arbuscular Mycorrhizal Inoculation and Biochar Amendment on Maize Growth, Cadmium Uptake and Soil Cadmium Speciation in Cd-Contaminated Soil. *Chemosphere* **2018**, *194*, 495–503. [CrossRef]

30. Curaqueo, G.; Schoebitz, M.; Borie, F.; Caravaca, F.; Roldán, A. Inoculation with Arbuscular Mycorrhizal Fungi and Addition of Composted Olive-Mill Waste Enhance Plant Establishment and Soil Properties in the Regeneration of a Heavy Metal-Polluted Environment. *Environ. Sci. Pollut. Res.* **2014**, *21*, 7403–7412. [CrossRef]

31. Laribi, B.; Kouki, K.; M'Hamdi, M.; Bettaieb, T. Coriander (*Coriandrum sativum* L.) and Its Bioactive Constituents. *Fitoterapia* **2015**, *103*, 9–26. [CrossRef]

32. Mench, M.J.; Dellise, M.; Bes, C.M.; Marchand, L.; Kolbas, A.; Le Coustumer, P.; Oustrière, N. Phytomanagement and Remediation of Cu-Contaminated Soils by High Yielding Crops at a Former Wood Preservation Site: Sunflower Biomass and Ionome. *Front. Ecol. Evol.* **2018**, *6*, 123. [CrossRef]

33. O'Connor, J.; Nguyen, T.B.T.; Honeyands, T.; Monaghan, B.; O'Dea, D.; Rinklebe, J.; Vinu, A.; Hoang, S.A.; Singh, G.; Kirkham, M.B.; et al. Production, Characterisation, Utilisation, and Beneficial Soil Application of Steel Slag: A Review. *J. Hazard. Mater.* **2021**, *419*, 126478. [CrossRef]

34. Mench, M.; Vangronsveld, J.; Didier, V.; Clijsters, H. Evaluation of Metal Mobility, Plant Availability and Immobilization by Chemical Agents in a Limed-Silty Soil. *Environ. Pollut.* **1994**, *86*, 279–286. [CrossRef]

35. Firmin, S.; Labidi, S.; Fontaine, J.; Laruelle, F.; Tisserant, B.; Nsanganwimana, F.; Pourrut, B.; Dalpé, Y.; Grandmougin, A.; Douay, F.; et al. Arbuscular Mycorrhizal Fungal Inoculation Protects Miscanthus×giganteus against Trace Element Toxicity in a Highly Metal-Contaminated Site. *Sci. Total Environ.* **2015**, *527–528*, 91–99. [CrossRef] [PubMed]

36. Kapoor, R.; Giri, B.; Mukerji, K.G. Mycorrhization of Coriander (*Coriandrum sativum* L.) to Enhance the Concentration and Quality of Essential Oil. *J. Sci. Food Agric.* **2002**, *82*, 339–342. [CrossRef]

37. Gui, H.; Purahong, W.; Hyde, K.D.; Xu, J.; Mortimer, P.E. The Arbuscular Mycorrhizal Fungus Funneliformis Mosseae Alters Bacterial Communities in Subtropical Forest Soils during Litter Decomposition. *Front. Microbiol.* **2017**, *8*, 1120. [CrossRef] [PubMed]

38. Morvan, S.; Meglouli, H.; Lounès-Hadj Sahraoui, A.; Hijri, M. Into the Wild Blueberry (*Vaccinium angustifolium*) Rhizosphere Microbiota. *Environ. Microbiol.* **2020**, *22*, 3803–3822. [CrossRef] [PubMed]
39. Feigl, V.; Ujaczki, É.; Vaszita, E.; Molnár, M. Influence of Red Mud on Soil Microbial Communities: Application and Comprehensive Evaluation of the Biolog EcoPlate Approach as a Tool in Soil Microbiological Studies. *Sci. Total Environ.* **2017**, *595*, 903–911. [CrossRef]
40. Gremion, F.; Chatzinotas, A.; Kaufmann, K.; Von Sigler, W.; Harms, H. Impacts of Heavy Metal Contamination and Phytoremediation on a Microbial Community during a Twelve-Month Microcosm Experiment. *FEMS Microbiol. Ecol.* **2004**, *48*, 273–283. [CrossRef]
41. Borymski, S.; Cycon, M.; Beckmann, M.; Mur, L.A.J.; Piotrowska-Seget, Z. Plant Species and Heavy Metals Affect Biodiversity of Microbial Communities Associated with Metal-Tolerant Plants in Metalliferous Soils. *Front. Microbiol.* **2018**, *9*, 1425. [CrossRef]
42. Xie, Y.; Fan, J.; Zhu, W.; Amombo, E.; Lou, Y.; Chen, L.; Fu, J. Effect of Heavy Metals Pollution on Soil Microbial Diversity and Bermudagrass Genetic Variation. *Front. Plant Sci.* **2016**, *7*, 755. [CrossRef]
43. Giller, K.E.; Witter, E.; McGrath, S.P. Heavy Metals and Soil Microbes. *Soil Biol. Biochem.* **2009**, *41*, 2031–2037. [CrossRef]
44. Schneider, A.R.; Gommeaux, M.; Duclercq, J.; Fanin, N.; Conreux, A.; Alahmad, A.; Lacoux, J.; Roger, D.; Spicher, F.; Ponthieu, M.; et al. Response of Bacterial Communities to Pb Smelter Pollution in Contrasting Soils. *Sci. Total Environ.* **2017**, *605–606*, 436–444. [CrossRef] [PubMed]
45. Fontaine, J.; Duclercq, J.; Facon, N.; Dewaele, D.; Laruelle, F.; Tisserant, B.; Lounès-Hadj Sahraoui, A. Coriander (*Coriandrum sativum* L.) in Combination with Organic Amendments and Arbuscular Mycorrhizal Inoculation: An Efficient Option for the Phytomanagement of Trace Elements-Polluted Soils. *Microorganisms* **2022**, *10*, 2287. [CrossRef] [PubMed]
46. Haferburg, G.; Kothe, E. Microbes and Metals: Interactions in the Environment. *J. Basic Microbiol.* **2007**, *47*, 453–467. [CrossRef] [PubMed]
47. Rani, A.; Goel, R. Strategies for Crop Improvement in Contaminated Soils Using Metal-Tolerant Bioinoculants. In *Microbial Strategies for Crop Improvement*; Springer: Berlin/Heidelberg, Germany, 2009; pp. 1–358. ISBN 9783642019791.
48. Biswas, R.; Halder, U.; Kabiraj, A.; Mondal, A.; Bandopadhyay, R. Overview on the Role of Heavy Metals Tolerance on Developing Antibiotic Resistance in Both Gram-Negative and Gram-Positive Bacteria. *Arch. Microbiol.* **2021**, *203*, 2761–2770. [CrossRef]
49. Cui, H.; Le Liu, L.; Dai, J.R.; Yu, X.N.; Guo, X.; Yi, S.J.; Zhou, D.Y.; Guo, W.H.; Du, N. Bacterial Community Shaped by Heavy Metals and Contributing to Health Risks in Cornfields. *Ecotoxicol. Environ. Saf.* **2018**, *166*, 259–269. [CrossRef] [PubMed]
50. Baldrian, P.; Kolaiřík, M.; Štursová, M.; Kopecký, J.; Valášková, V.; Větrovský, T.; Žifčáková, L.; Šnajdr, J.; Rídl, J.; Vlček, Č.; et al. Active and Total Microbial Communities in Forest Soil Are Largely Different and Highly Stratified during Decomposition. *ISME J.* **2012**, *6*, 248–258. [CrossRef] [PubMed]
51. Bauddh, K.; Ma, Y. *Advances in Microbe-Assisted Phytoremediation of Polluted Sites*; Elsevier: Amsterdam, The Netherlands, 2022; Volume 1, p. 522.
52. Gupta, R.S. The Phylogeny of Proteobacteria: Relationships to Other Eubacterial Phyla and Eukaryotes. *FEMS Microbiol. Rev.* **2000**, *24*, 367–402. [CrossRef]
53. DeBruyn, J.M.; Nixon, L.T.; Fawaz, M.N.; Johnson, A.M.; Radosevich, M. Global Biogeography and Quantitative Seasonal Dynamics of Gemmatimonadetes in Soil. *Appl. Environ. Microbiol.* **2011**, *77*, 6295–6300. [CrossRef]
54. Brandt, K.K.; Frandsen, R.J.N.; Holm, P.E.; Nybroe, O. Development of Pollution-Induced Community Tolerance Is Linked to Structural and Functional Resilience of a Soil Bacterial Community Following a Five-Year Field Exposure to Copper. *Soil Biol. Biochem.* **2010**, *42*, 748–757. [CrossRef]
55. Alahmad, A.; Decocq, G.; Spicher, F.; Kheirbeik, L.; Kobaissi, A.; Tetu, T.; Dubois, F.; Duclercq, J. Cover Crops in Arable Lands Increase Functional Complementarity and Redundancy of Bacterial Communities. *J. Appl. Ecol.* **2018**, *56*, 651–664. [CrossRef]
56. Wang, C.Y.; Zhou, X.; Guo, D.; Zhao, J.H.; Yan, L.; Feng, G.Z.; Gao, Q.; Yu, H.; Zhao, L.-P. Soil PH Is the Primary Factor Driving the Distribution and Function of Microorganisms in Farmland Soils in Northeastern China. *Ann. Microbiol.* **2019**, *69*, 1461–1473. [CrossRef]
57. Uroz, S.; Kelly, L.C.; Turpault, M.P.; Lepleux, C.; Frey-Klett, P. The Mineralosphere Concept: Mineralogical Control of the Distribution and Function of Mineral-Associated Bacterial Communities. *Trends Microbiol.* **2015**, *23*, 751–762. [CrossRef] [PubMed]
58. Hartmann, A.; Schmid, M.; van Tuinen, D.; Berg, G. Plant-Driven Selection of Microbes. *Plant Soil* **2009**, *321*, 235–257. [CrossRef]
59. Wu, L.; Wang, J.; Huang, W.; Wu, H.; Chen, J.; Yang, Y.; Zhang, Z.; Lin, W. Plant-Microbe Rhizosphere Interactions Mediated by Rehmannia Glutinosa Root Exudates under Consecutive Monoculture. *Sci. Rep.* **2015**, *5*, 15871. [CrossRef] [PubMed]
60. Misra, P.; Maji, D.; Awasthi, A.; Pandey, S.S.; Yadav, A.; Pandey, A.; Saikia, D.; Babu, C.S.V.; Kalra, A. Vulnerability of Soil Microbiome to Monocropping of Medicinal and Aromatic Plants and Its Restoration Through Intercropping and Organic Amendments. *Front. Microbiol.* **2019**, *10*, 2604. [CrossRef] [PubMed]
61. Adamovic, D.; Djalovic, I.; Mrkovacki, N. Microbial Abundance in Rhizosphere of Medicinal and Aromatic Plant Species in Conventional and Organic Growing Systems. *Ratar. Povrt.* **2015**, *52*, 1–6. [CrossRef]
62. Köberl, M.; Schmidt, R.; Ramadan, E.M.; Bauer, R.; Berg, G. The Microbiome of Medicinal Plants: Diversity and Importance for Plant Growth, Quality, and Health. *Front. Microbiol.* **2013**, *4*, 400. [CrossRef]
63. Adamovic, D.; Djalovic, I.; Mrkovacki, N.; Burdock, G.A.; Carabin, I.G.; Choudhary, S.; Mishra, B.K.; Singh, R.; Sharma, R.; Delgado-Baquerizo, M.; et al. Intercropping With Aromatic Plants Increased the Soil Organic Matter Content and Changed the Microbial Community in a Pear Orchard. *Trop. Ecol.* **2015**, *5*, 616932. [CrossRef]

64. Mosbah, R.; Sahmoune, M.N. Biosorption of Heavy Metals by Streptomyces Species—An Overview. *Cent. Eur. J. Chem.* **2013**, *11*, 1412–1422. [CrossRef]

65. Jousset, A.; Bienhold, C.; Chatzinotas, A.; Gallien, L.; Gobet, A.; Kurm, V.; Küsel, K.; Rillig, M.C.; Rivett, D.W.; Salles, J.F.; et al. Where Less May Be More: How the Rare Biosphere Pulls Ecosystems Strings. *ISME J.* **2017**, *11*, 853–862. [CrossRef]

66. Kurm, V.; Geisen, S.; Gera Hol, W.H. A Low Proportion of Rare Bacterial Taxa Responds to Abiotic Changes Compared with Dominant Taxa. *Environ. Microbiol.* **2019**, *21*, 750–758. [CrossRef] [PubMed]

67. Chen, Y.; Jiang, Y.; Huang, H.; Mou, L.; Ru, J.; Zhao, J.; Xiao, S. Long-Term and High-Concentration Heavy-Metal Contamination Strongly Influences the Microbiome and Functional Genes in Yellow River Sediments. *Sci. Total Environ.* **2018**, *637–638*, 1400–1412. [CrossRef] [PubMed]

68. Hemmat-Jou, M.H.; Safari-Sinegani, A.A.; Mirzaie-Asl, A.; Tahmourespour, A. Analysis of Microbial Communities in Heavy Metals-Contaminated Soils Using the Metagenomic Approach. *Ecotoxicology* **2018**, *27*, 1281–1291. [CrossRef] [PubMed]

69. Griffiths, B.S.; Philippot, L. Insights into the Resistance and Resilience of the Soil Microbial Community. *FEMS Microbiol. Rev.* **2013**, *37*, 112–129. [CrossRef] [PubMed]

70. Labidi, S.; Fontaine, J.; Laruelle, F.; Tisserant, B.; Dalpé, Y.; Grandmougin-Ferjani, A.; Douay, F.; Lounès-Hadj Sahraoui, A. Fly Ash-Aided Phytostabilisation of Highly Trace Element Polluted Topsoils Improves the Telluric Fungal Biomass: A Long-Term Field Experiment. *Appl. Soil Ecol.* **2015**, *85*, 69–75. [CrossRef]

71. Sterckeman, T.; Douay, F.; Proix, N.; Fourrier, H.; Perdrix, E. Assessment of the Contamination of Cultivated Soils by Eighteen Trace Elements around Smelters in the North of France. *Water Air Soil Pollut.* **2002**, *135*, 173–194. [CrossRef]

72. Sterckeman, T.; Douay, F.; Baize, D.; Fourrier, H.; Proix, N.; Schvartz, C. Référentiel Pédo-Géochimique Du Nord-Pas de Calais: Méthode et Principaux Résultats. *Etude Gest. Sols* **2007**, *14*, 153–168.

73. Mäkelä, M.; Watkins, G.; Pöykiö, R.; Nurmesniemi, H.; Dahl, O. Utilization of Steel, Pulp and Paper Industry Solid Residues in Forest Soil Amendment: Relevant Physicochemical Properties and Heavy Metal Availability. *J. Hazard. Mater.* **2012**, *207–208*, 21–27. [CrossRef] [PubMed]

74. Phillips, J.M.; Hayman, D.S. Improved Procedures for Clearing Roots and Staining Parasitic and Vesicular-Arbuscular Mycorrhizal Fungi for Rapid Assessment of Infection. *Trans. Br. Mycol. Soc.* **1970**, *55*, 158–161. [CrossRef]

75. McGonigle, T.P.; Miller, M.H.; Evans, D.G.; Fairchild, G.L.; Swan, J.A. A New Method Which Gives an Objective Measure of Colonization of Roots by Vesicular—Arbuscular Mycorrhizal Fungi. *N. Phytol.* **1990**, *115*, 495–501. [CrossRef]

76. Frostegård, Å.; Tunlid, A.; Bååth, E.; Frosteg, A.; Frostegård, Å.; Tunlid, A.; Bååth, E. Microbial Biomass Measured as Total Lipid Phosphate in Soils of Different Organic Content. *J. Microbiol. Methods* **1991**, *14*, 151–163. [CrossRef]

77. Frostegård, Å.; Tunlid, A.; Bååth, E. Changes in Microbial Community Structure during Long-Term Incubation in Two Soils Experimentally Contaminated with Metals. *Soil Biol. Biochem.* **1996**, *28*, 55–63. [CrossRef]

78. Herlemann, D.P.R.; Labrenz, M.; Jürgens, K.; Bertilsson, S.; Waniek, J.J.; Andersson, A.F. Transitions in Bacterial Communities along the 2000 Km Salinity Gradient of the Baltic Sea. *ISME J.* **2011**, *5*, 1571–1579. [CrossRef]

79. Escudié, F.; Auer, L.; Bernard, M.; Mariadassou, M.; Cauquil, L.; Vidal, K.; Maman, S.; Hernandez-Raquet, G.; Combes, S.; Pascal, G. FROGS: Find, Rapidly, OTUs with Galaxy Solution. *Bioinformatics* **2018**, *34*, 1287–1294. [CrossRef] [PubMed]

80. Martin, M. Cutadapt Removes Adapter Sequences from High-Throughput Sequencing Reads. *EMBnet. J.* **2011**, *17*, 10–12. [CrossRef]

81. Mahé, F.; Rognes, T.; Quince, C.; de Vargas, C.; Dunthorn, M. Swarm: Robust and Fast Clustering Method for Amplicon-Based Studies. *PeerJ* **2014**, *2*, e593. [CrossRef] [PubMed]

82. Rognes, T.; Flouri, T.; Nichols, B.; Quince, C.; Mahé, F. VSEARCH: A Versatile Open Source Tool for Metagenomics. *PeerJ* **2016**, *4*, e2584. [CrossRef]

83. Wang, Q.; Garrity, G.M.; Tiedje, J.M.; Cole, J.R. Naïve Bayesian Classifier for Rapid Assignment of RRNA Sequences into the New Bacterial Taxonomy. *Appl. Environ. Microbiol.* **2007**, *73*, 5261–5267. [CrossRef] [PubMed]

84. Weber, K.P.; Grove, J.A.; Gehder, M.; Anderson, W.A.; Legge, R.L. Data Transformations in the Analysis of Community-Level Substrate Utilization Data from Microplates. *J. Microbiol. Methods* **2007**, *69*, 461–469. [CrossRef]

85. Oksanen, A.J.; Blanchet, F.G.; Kindt, R.; Legen-, P.; Minchin, P.R.; Hara, R.B.O.; Simpson, G.L.; Solymos, P.; Stevens, M.H.H. Community Ecology Package. *Ecol. Packag.* **2013**, *2*, 321–326.

86. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-Resolution Sample Inference from Illumina Amplicon Data. *Nat. Methods* **2016**, *13*, 581–583. [CrossRef] [PubMed]