

Article

Glutamic-N,N-Diacetic Acid as an Innovative Chelating Agent in Microfertilizer Development: Biodegradability, Lettuce Growth Promotion, and Impact on Endospheric Bacterial Communities

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Abstract: The search for new biodegradable fertilizers to increase the productivity of agricultural plants is an urgent task. In this study, a complex microfertilizer was developed based on a chelating agent—glutamic-N,N-diacetic acid (GLDA). The evaluation encompassed assessments of biodegradability and effectiveness in fostering lettuce plant growth in hydroponic and conventional soil settings. The impact on endospheric bacteria, a sensitive indicator, was also examined. Results indicated a 59.8% degradation rate of the GLDA complex on the 28th day. The most notable positive effects were observed in above-ground plant biomass, with a 4.6-fold increase for hydroponics and 1.5 to 1.8-fold increases for root and foliar treatments in soil. In hydroponics, GLDA-treated plants showed 24 and 45 operational taxonomic units (OTUs) for leaves and 272 and 258 for roots (GLDA-treated and control plants). In soil, the OTU counts were 270 and 101, 221 and 111, and 198 and 116 in the leaves and roots of GLDA-treated and control plants (under root and foliar treatments), respectively. Non-metric multidimensional scaling (NMDS) and Indicator Species Analysis (ISA) demonstrated significant distinctions in endospheric communities between substrates (hydroponics and soil) in the presence of GLDA. Importantly, GLDA use simplified the composition of endospheric bacterial communities.

Keywords: chelated fertilizer; glutamic-N,N-diacetic acid (GLDA); endophytic bacterial microbiome; soil and hydroponic cultivation

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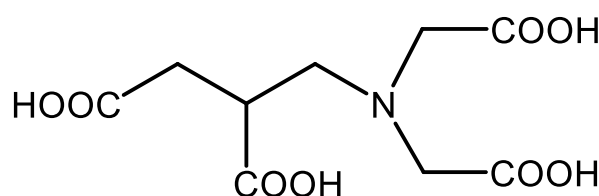
1. Introduction

Classical chelating agents such as ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA) exhibit up to 10 times higher microelement assimilation compared to traditional fertilizers in mineral forms. Despite their efficacy, chelates based on EDTA suffer from notable drawbacks, including low biodegradation rates, environmental accumulation, and mobilization of heavy metals [1]. EDTA contains six donor atoms and functions as a hexadentate ligand. The chemical structure of EDTA confers higher stability constants in metal binding compared to most chelators, as evidenced by scientific studies [2,3]. Investigations indicate that EDTA and EDTA-based chelates exhibit remarkable resistance to biodegradation [4]. In particular, using a CO₂ evolution test,

EDTA degradation rate was demonstrated to range between 6 and 10% within 28 days of incubation, and its complexes exhibited a degradation level of around 10–50% [5–7].

The search for and implementation of alternative chelating agents, characterized by high efficiency, biodegradability, and ecological compatibility, represent a contemporary challenge. This study explores the aminopolycarboxylate complex, specifically glutamic-N,N-diacetic acid (GLDA), as a potential alternative to traditional chelators. GLDA is a pentadentate chelating agent characterized by high water solubility and reduced tendency to crystallize in concentrated acid solutions [8]. The stability constants of chelate complexes based on GLDA are significantly lower than those of corresponding complexes with EDTA for the respective metals (iron and chromium) [9].

GLDA, hitherto unused in agriculture, finds applications in various industries, including oil well stimulation, cosmetics, hypoallergenic laundry detergents, high-performance dishwashing agents, and water treatment [10]. GLDA is known to form complexes with metal ions such as Ca^{2+} , Mg^{2+} , Sr^{2+} , Ba^{2+} , Hg^{2+} , Cu^{2+} , Cd^{2+} , Co^{2+} , Fe^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} , Zn^{2+} , Al^{3+} , and Fe^{3+} [11]. It exists in two isomeric states, with GLDA (Scheme 1) being considered biodegradable, displaying a degradation rate exceeding 60% over 28 days [2].



Scheme 1. Structural formula of a GLDA molecule.

However, information regarding the biodegradation of GLDA-based chelate complexes and their behavior in the environment remains limited compared to well-studied chelators such as EDTA.

The physiological intricacies of plantmicrobiome interactions underscore the importance of understanding the complex structure of microbial communities associated with plants [12]. Endophytic microbiomes, a pivotal component influencing plant health, engage in mutualistic relationships with plants, directly impacting the production of phytohormones, antibiotics, and mineral nutrient absorption, thereby fostering plant growth and development [13].

Both chemical and organic fertilizers can influence the structure of endophytic communities. Excessive use of chemical fertilizers during wheat cultivation has been reported to alter the root endophytic community structure, increasing wheat susceptibility to crown rot disease [14]. Researchers are also exploring potential risks to human health associated with endophytes, particularly concerning plants that are consumed raw. Wang et al. (2023) identified 20 potential human pathogens in the endophytic microbiome of lettuce, along with the presence of 137 antibiotic resistance genes and 31 mobile genetic elements [15,16]

In the context of this study, GLDA is investigated as an alternative to traditional chelators for micronutrient chelation in plants. A microfertilizer containing four GLDA-chelated elements (Fe, Cu, Mn, and Zn) and two unchelated elements (B and Mo) was formulated and evaluated for both biodegradability and efficacy in promoting lettuce growth under hydroponic and soil cultivation [17]. Given the pivotal role of endophytic bacterial communities in plant health and their potential as indicators of plant responses to external stimuli, the composition of endospheric bacterial communities was analyzed and compared with non-fertilized plants for discernible distinctions.

2. Materials and Methods

2.1. Characteristics of Microfertilizer

A GLDA solution (pH 6.66) was prepared by mixing individual solutions of the GLDA salts (GLDA*Fe, GLDA*Zn, GLDA*Cu, and GLDA*Mn) as well as H₃BO₃ and (NH₄)₆Mo₇O₂₄. Solutions of individual GLDA salts were prepared by reaction of Na₄GLDA solution with, respectively, FeSO₄*7H₂O, ZnSO₄*7H₂O, CuSO₄*5H₂O, and MnSO₄*H₂O, in the 1:1 molar ratio with respect to the metals [18]. Na₄GLDA was received from Nouryon as Dissolvine GL-47-S (47% water solution). Iron (II) sulfate heptahydrate, zinc (II) sulfate heptahydrate, copper (II) sulfate pentahydrate, manganese (II) sulfate monohydrate, orthoboric acid, and hexaammonium molybdate were purchased from Khimreaktiv, Russia (pure for analysis).

2.2. Degradability Analysis of GLDA-Based Chelates

The biodegradability of chelating agents was estimated according to the Test No. 301 OECD, with slight modifications [19]. For this purpose, chelated GLDA fertilizer was incubated in sealed containers with a volume of 0.03 l for 28 days at a constant temperature of 25 °C. Microorganisms were isolated from chicken manure and passaged 2 times. Before incubation, the concentration of microorganisms was 3 * 10⁵ CFU/mL. Vessels with microbial inoculums with glucose at a concentration of 30 mg/L were used as a control, with a theoretical value of carbon dioxide released (ThCO₂) of 3.3. To assess the degree of chemical degradation of GLDA, the chelated vials were incubated without microorganisms. The mixtures were incubated in the dark; on days 1, 4, 7, and 28, the CO₂ content in the gas–air mixture was determined by gas chromatography on a GC-2010 Plus device (Shimadzu, Japan). The biodegradation rate (Dm, %) was calculated as the difference between the mass of CO₂, released in the vessel, with the test compound and the average value of the mass of CO₂ released in the empty vessel related to ThCO₂.

2.3. Vegetation Experiment

2.3.1. Overall Experimental Design

The experiments were conducted in a greenhouse at the Kazan Federal University, Russia, between late spring and early summer 2021. The effect of GLDA on lettuce yield was analyzed in two different substrates – hydroponic and soil. In hydroponic lettuce cultivation, the efficiency of using GLDA-based micronutrients was compared with using micronutrients in the form of mineral salts in equivalent quantities. When growing lettuce in soil, the lettuce was fertilized with microelement fertilizers in chelated form in two ways: aqueous solutions were applied under the root and on the leaves (root and foliar treatment, respectively). Lettuce (*Lactuca sativa* var. *crispa* L. «Salad Bowl») seeds were sown in a tray filled with vermiculite and raised for 10 days, and lettuce seedlings were then planted in hydroponic (floating) systems. Plants were grown in a greenhouse with a light regime (light/night) – 16:8 h, at 22 °C for 28 days. On the 28th day of the experiment, the effect of the chelated microfertilizers on the length and biomass of lettuce plants as well as chlorophyll content in leaves were determined. In addition, plants were isolated and stored at –80 °C for further DNA extraction. Above-ground and below-ground parts of lettuce plants were analyzed separately. The sample variants are presented in Table 1.

Table 1. Plant samples analyzed in the present study.

Treatment Type	Growing Substrate					
	Hydroponics		Soil			
	Above-Ground Part of Plant (Leaves)	Below-Ground Part of Plant (Roots)	Above-Ground Part of Plant (Leaves) Root Treatment	Foliar Treatment	Below-Ground Part of Plant (Roots) Root Treatment	Foliar Treatment
Not fertilized (control)	H-Cont-A	H-Cont-B	S-Cont-A		S-Cont-B	
Fertilized (GLDA)	H-GLDA-A	H-GLDA-B	Sr-GLDA-A	Sf-GLDA-A	Sf-GLDA-B	Sf-GLDA-B

2.3.2. Hydroponic Cultivation

The hydroponic system was made up of three separate 12 L plastic tanks (water depth 19 cm) for each variant, with a polystyrene tray hosting 9 plants of lettuce (18 plants in total). The hydroponic water contained the following macronutrients: N-NO₃ (14.0 mM), N-NH₄⁺ (2.0 mM), P (2.0 mM), K (10.0 mM), Ca (4.5 mM), Mg (2.0 mM), and S-SO₄ (5.0 mM) [20]. The following salts were used to prepare the hydroponic solution: 5[Ca(NO₃)₂·2H₂O], NH₄NO₃, KH₂PO₄, MgSO₄·7H₂O, KNO₃, and K₂SO₄. Every 7 days, the hydroponic mixtures were replaced with fresh ones.

In the control sample, micronutrients were added to the hydroponic system in the form of mineral salts: iron (II) sulfate heptahydrate, zinc (II) sulfate heptahydrate, copper (II) sulfate pentahydrate, manganese (II) sulfate monohydrate, orthoboric acid, and hexaammonium molybdate. The micronutrients' content in the solution was the same as for the GLDA solution: B 53.6, Cu 4.6, Fe 27.9, Mn 22.1, Mo 1.2, and Zn 6.3 μM/l [21].

2.3.3. Soil Cultivation

The soil was taken from a backyard garden in the city (55.790934, 49.120356) at a depth of 20–40 cm. Physicochemical characteristics of the soil used in the study are presented in Table S1 (Supplementary Materials).

The experiment with model soil was organized as follows: 10 kg of soil was placed in black plastic containers (3 containers for each test sample). To supply plants with NPK elements, 0.133 g/kg soil of superphosphate and 0.35 g/kg soil of potassium nitrate were introduced into the soil once, at the beginning of the vegetation experiment.

Treatment with chelated and mineral fertilizers was carried out according to the FAO recommendations for specific plant species (EMSP for greenhouse vegetable crops in south-eastern Europe). Microelements in chelated form were added to the soil in the amounts B 129, Cu 11, Fe 67, Mn 53, Mo 3, and Zn 15 μM. Root fertilizers were applied on the 14th and 21st days of plant growth in the soil, according to recommendations for the use of commercial EDTA-based fertilizer with a similar microelement content (Aquadin, produced by the manufacturer «Buisy Chemical Plant»). For foliar feeding, the plants were sprayed 4 times during the vegetation experiment, in the evenings, with a portable sprayer, until the surfaces of the upper and lower leaves were completely wetted with a solution containing B 129, Cu 11, Fe 67, Mn 53, Mo 3, and Zn 15 μM. The volume of solution required for foliar treatment of plants in each box was around 100 mL. The first spray was given at the 4-leaf stage and the remaining 3 sprays were given at 1-week intervals.

2.4. Analytical Methods

Plants were harvested at the end of each experiment, and plant height, root length, and fresh biomass of 18 plants from each treatment were measured and recorded. The content of chlorophyll in lettuce *Lactuca sativa* was measured using Dualex ScientificTM (Force-A, France) [22].

After completing the soil and hydroponic growing experiments, the endophytic microbial community of lettuce leaves and roots was estimated according to [23]. Briefly, the surfaces of lettuce roots and leaves were washed under running water. Roots and leaves

were sterilized by sequentially soaking in 2.5% sodium hypochlorite for 2 min, rinsing in sterilized tap water, immersing in 70% ethanol for 3 min, rinsing 5 times in sterile tap water. The presence of bacteria in the final eluent was checked by monitoring the colony formation on an LB agar plate after incubation at 37 °C for 24 h. Then, sterilized leaves and roots, 0.3 g each, were ground in sterile mortars in liquid nitrogen. For DNA extraction, the MoBio Power Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) was used according to the manufacturer's instructions.

Analysis of the bacterial endophytic community association was performed using the Illumina MiSeq platform (Illumina, San Diego, CA, USA). The procedures for genomic library preparation, 16S rRNA gene sequencing, and data processing followed the same procedures previously described by Danilova N. [16]. The number of raw reads ranged between 13,375 and 53,479 for 16S rRNA amplicons. 16S pRNA sequencing data were analyzed using Quantitative Insights Into Microbial Ecology (QIIME) platform software and a table of sequence variants with taxonomy based on the Silva reference database version 132 was created [24]. Operational taxonomic units (OTUs) were grouped at a similarity threshold of 97%.

2.5. Statistical Analysis

All measurements were carried out at least in triplicate. The obtained data were processed using the Microsoft program Office Excel 2016 (Redmond, WA, USA). The data shown in Figures 1 and 2 are mean values, and the bars represent standard errors of the means. To assess the significance of differences, the nonparametric Mann–Whitney test at $p \leq 0.05$ was used. For bacterial community analysis, data on relative abundance of different OTUs were used. Venn diagrams presented in the study were generated using the Bioinformatics & Evolutionary Genomics tool [25]. Alpha diversity of the bacterial communities was assessed using the Shannon–Wiener and Simpson criteria [26,27]. To analyze the beta diversity of the communities, nonmetric multidimensional scaling (NMDS) analysis within the vegan package of R was performed [28]. It was based on indices of average statistical indicators, calculated using the Bray–Curtis coefficient [29]. Indicator species analysis (ISA) was conducted in R, using the Indicspecies package [30,31].

3. Results

3.1. Biodegradability of the Chelating Agents

The degradability of GLDA was evaluated by distinguishing between biotic and abiotic degradation through the involvement and exclusion of the microbial community, respectively. The temporal evolution of this process, assessed over a duration of 28 days, is depicted in Figure 1. On the initial day of observation, biological degradation accounted for 19%, whereas chemical degradation constituted only 0.2%. Subsequently, the degradation rate exhibited a progressive increase, reaching 59.8% on day 28. Notably, the relative contributions of biological and chemical processes underwent variation, with approximately one-quarter of the degradation attributed to chemical processes by the conclusion of the 28-day period.

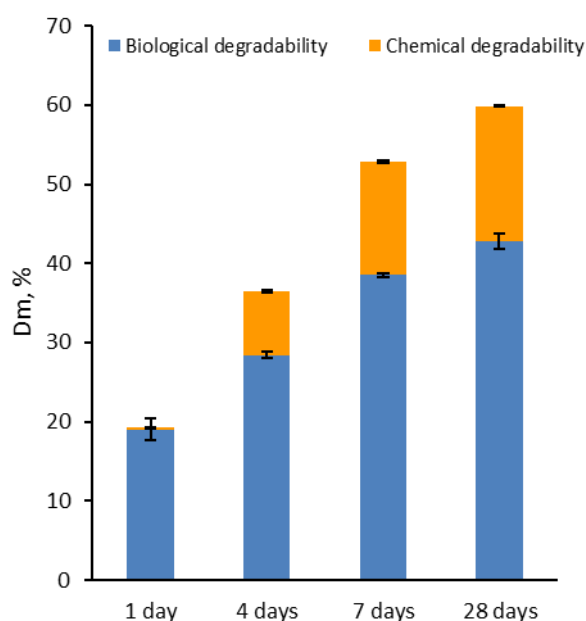


Figure 1. Biological and chemical degradability of GLDA as revealed in a 28-day-long experiment.

3.2. Influence of GLDA-Based Chelates on Lettuce Growth and Microelement Uptake

Subsequently, the impact of a GLDA-based fertilizer on the growth and development of lettuce plants cultivated on two distinct substrates was assessed. The first experiment employed soil as the substrate, while the second utilized a hydroponic mixture. Images of the plant samples are presented in Tables S2–S5.

In the control soil samples, with no GLDA in soil (S-Cont), the lengths and biomass of the above-ground part (A) and roots (B) of the lettuce plants were 22.3 ± 0.38 cm, 10.6 ± 0.31 cm, and 11.8 ± 0.97 g and 0.57 ± 0.058 g, respectively. In the hydroponic experiment (H-Cont), essential nutrients (N, P, K, B, Cu, Fe, Mn, Mo, and Zn) were introduced into the control solution in the form of mineral salts at appropriate concentrations. The corresponding values were 18.4 ± 1.21 cm, 12.2 ± 2.21 cm, and 10.9 ± 3.15 g and 1.2 ± 0.48 g. Chlorophyll content in the leaves of control variants was 11.86 ± 1.82 $\mu\text{g}/\text{cm}^2$ in soil and 7.83 ± 2.79 $\mu\text{g}/\text{cm}^2$ in hydroponics.

In hydroponics, GLDA variants (H-GLDA), Fe, Zn, Cu, and Mn mineral salts, were substituted with corresponding GLDA-based complexes. In the soil experiment, GLDA chelates were applied in two distinct manners—via root application and foliar application—resulting in two analyzed variants denoted as Sr-GLDA and Sf-GLDA, respectively. It was observed that the application of GLDA chelates led to an increase in the lengths and biomass of lettuce roots and leaves in both cultivation methods (H and S) and in both treatment variants in the soil experiment (Sr and Sf) (Figure 2a,b,d,e). The enhancement over the control amounted to 1.7, 1.8, 4.6, and 2.5 times for the H-GLDA variant for leaf and root lengths and leaf and root biomass, respectively. For the Sr-GLDA variant, such enhancement was 1.4, and 1.3 times, and for the Sf-GLDA variant, it was 1.5 and 1.8 times for leaf lengths and leaf biomass, respectively.

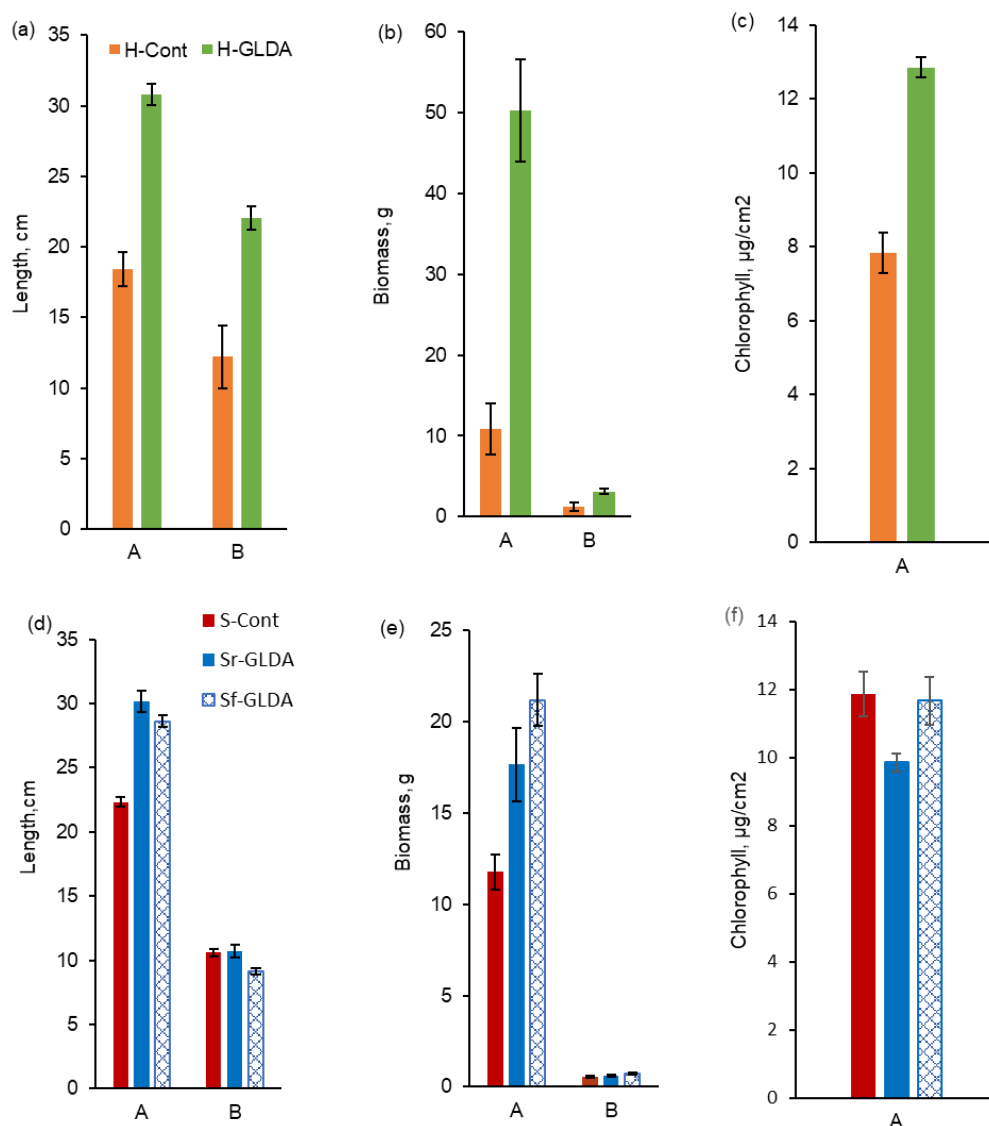


Figure 2. Length, biomass, and chlorophyll content of lettuce plants (*L. sativa*) cultivated in hydroponics (H) (a–c) and soil (S, variants Sf and Sr) or (d–f) treated with GLDA-based microfertilizer. Vertical bars indicate standard errors of the mean. A—above-ground parts of lettuce plants (leaves); B—below-ground parts of lettuce plants (roots).

The application of the chelated micronutrient fertilizer did not result in significant changes in chlorophyll content in the leaves of plants cultivated in soil, unlike those cultivated in hydroponics. In the latter case, the enhancement amounted to 1.6 times.

3.3. Influence of GLDA Chelate Fertilization on Leaf and Root Endophytic Bacterial Communities

The composition of endophytic bacterial communities was determined based on 16S rRNA amplicon sequencing using the Illumina MiSeq platform. The raw data are presented in Supplement Table S6. From the samples of leaves and roots of plants grown hydroponically or in soil (two types of treatments), 619 sequences were obtained. After chimera removal and quality control, the sequences were assigned to 603 OTUs.

In both leaf and root endophytic microbiomes, in both hydroponic and soil experiments, OTUs belonging to the phyla Proteobacteria (15–60%) and Bacteroidetes (14–24%) predominated. Additionally, Firmicutes was the dominant phylum in the leaf endophytic microbiome (35–46%) (Figure 3).

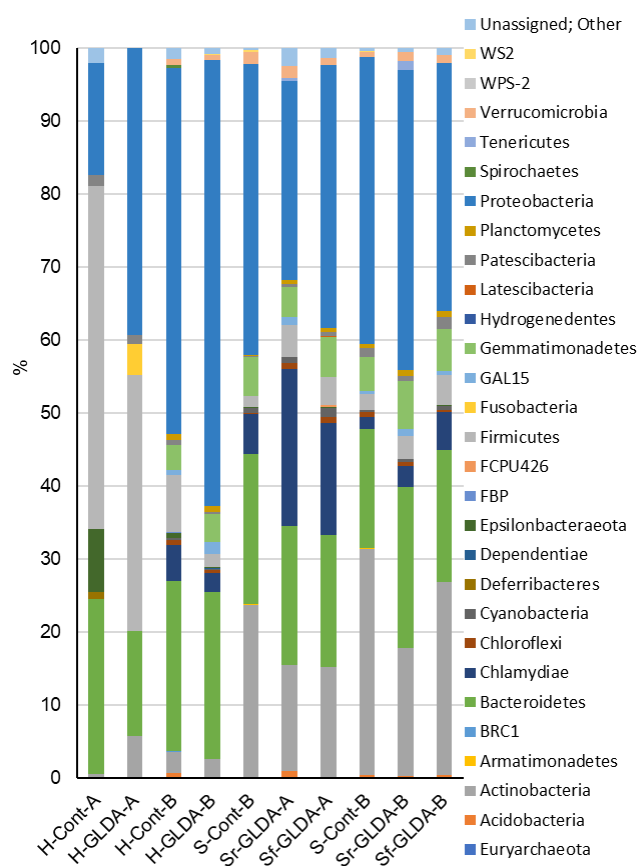


Figure 3. Bar chart of endophytic bacterial phyla composition in lettuce leaves and roots.

The evaluation of shared bacterial species within the endophytic community, identified in all leaf and root samples (Figure 4), presented an interesting perspective. A total of six common (core) bacterial species were identified in the endophytic microbiome of the leaves. These bacterial taxa are associated with the genera *Lactobacillus*, *Reyranella* (Proteobacteria), *Sphingomonas*, *Aquabacterium*, and bacteria of the order Sphingobacteriales. In contrast, the root endophytic microbiome exhibited 64 common species, with the highest prevalence observed among bacterial species such as *Pedobacter* (ranging from 9.4 to 17.8%), *Neochlamydia* (from 0.7 to 3.3%), *Sphingomonas* (from 5.7 to 14.7%), and *Pseudomonas* (from 1.4 to 13.9%).

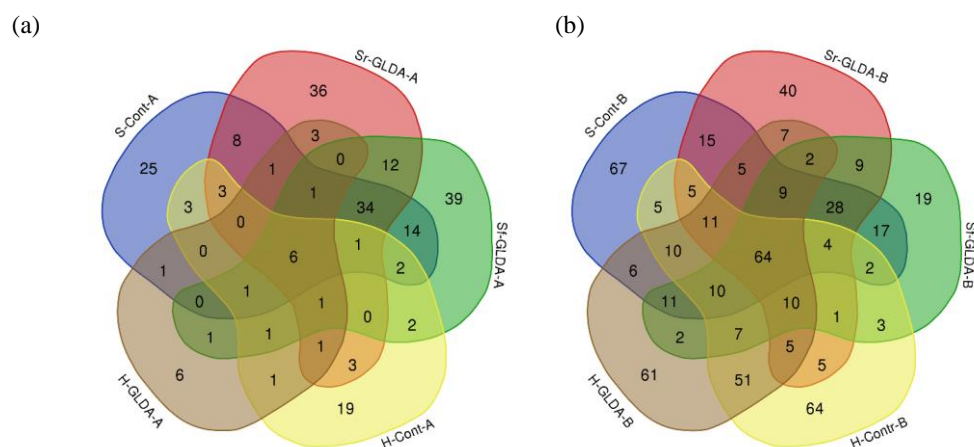


Figure 4. Venn diagrams of the number of unique and shared OTUs in endophytic bacterial in leaves (a) and roots (b) of lettuce plants grown in soil and hydroponic.

3.3.1. Influence of GLDA Chelate Fertilization on Endophytic Bacterial Communities of Plants Grown in Hydroponics

In the endophytic microbiome of lettuce leaves in the control variant grown in hydroponics, 44 OTUs were identified. The predominant bacteria in the control variant, based on relative abundance, were from the genera *Helicobacter* (*Proteobacteria*) (14%), *Lactobacillus* (*Firmicutes*) (10%), *Romboutsia* (*Firmicutes*) (10%), and an uncultured bacterium from the family *Peptostreptococcaceae* (*Firmicutes*) (7%) (Figure 5a).

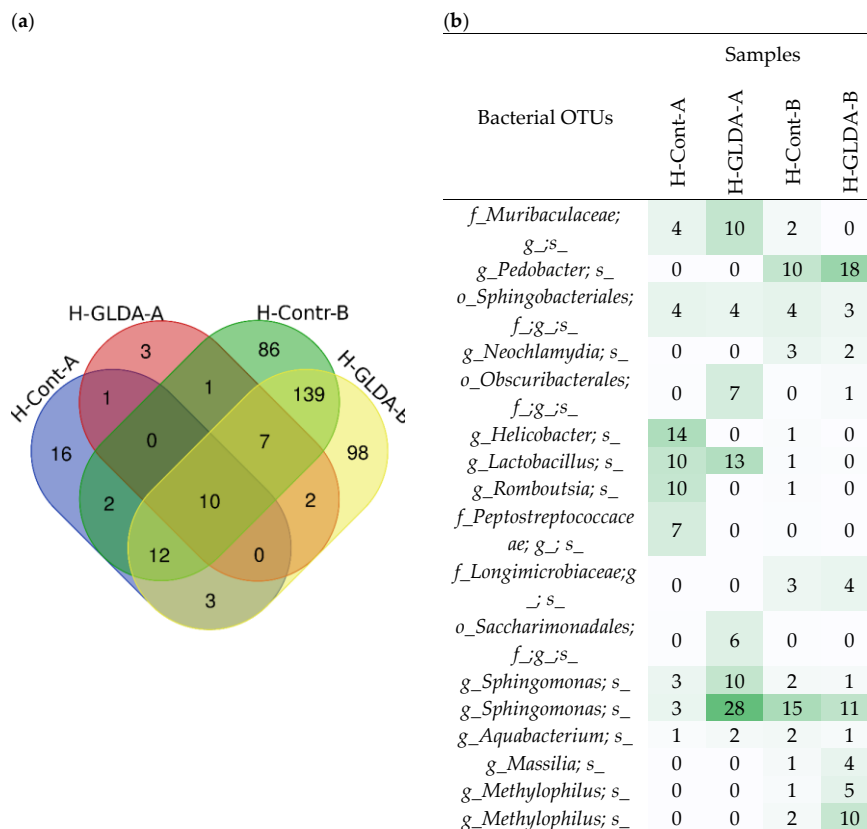


Figure 5. Venn diagram of the number of unique and shared OTUs (a) and heatmap of dominant bacterial genera (% relative sequence abundance ≥ 1) (b) in endophytic bacterial in leaves and roots of lettuce plants grown in hydroponics.

The use of the chelate fertilizer led to a reduction in the number of OTUs to 24, with 11 of them being common with the control variant. The proportion of shared OTUs in leaf endophytes between samples H-Cont-A and H-GLDA-A was 24% and 46%, respectively. The dominant species in the H-GLDA-A sample included two bacterial genera, *Sphingomonas* (Proteobacteria) (28% and 10%), *Lactobacillus* (13%), bacteria from the family *Muribaculaceae* (Bacteroidetes) (10%), and the order *Obscuribacterales* (Cyanobacteria) (10%) (Figure 5b).

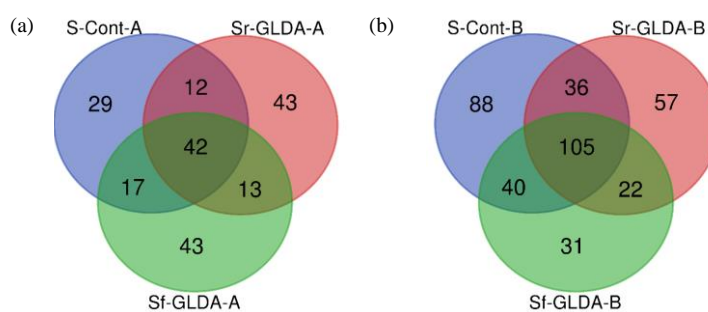
In the roots of lettuce plants, 6 to 11 times more bacterial OTUs were detected compared to the leaves (257 in sample H-Cont-B and 271 in sample H-GLDA-B). There were 168 common OTUs for both samples, constituting 65% and 62% for samples H-Cont-B and H-GLDA-B, respectively. In the root endophytic microbiome of control plants, OTUs from the genera *Sphingomonas* (Proteobacteria) (15%) and *Pedobacter* (Bacteroidetes) (10%) dominated. Additionally, OTUs from the order *Sphingobacteriales* (Bacteroidetes) (4%) and the genus *Neochlamydia* (Chlamydiae) (3%) were subdominant. In the GLDA-treated sample, alongside the dominant *Pedobacter* (Bacteroidetes) (18%) and *Sphingomonas* (Proteobacteria) (11%), subdominant OTUs included two different genera, *Methylophilus* (Proteobacteria) (5%) and *Massilia* (Proteobacteria) (4%) (Figure 5b).

3.3.2. Influence of GLDA Chelate Fertilization on Endophytic Bacterial Communities of Plants Grown in Soil

The composition of the endophytic bacterial community is strongly influenced by agrotechnology and the presence of various nutrient elements [32]. In addition to these factors, the diverse soil microbial community can exert a direct impact on the endophytic community. In contrast to plants grown hydroponically, the endophytic microbiome of soil-grown lettuce is characterized by a dominance of Actinobacteria bacteria in addition to Proteobacteria (27–41%) and Bacteroidetes (16–22%) (Figure 3).

The number of OTUs in the leaf endophytic microbiome of soil-grown lettuce exceeded that of hydroponically grown plants. In samples S-Cont-A, Sf-GLDA-A, Sr-GLDA-A, the number of OTUs was 100, 115, and 110, respectively. A total of 42 OTUs were shared among all three samples (Figure 6a). The percentage of shared OTUs in leaf endophytes was 42%, 37%, and 32% for S-Cont-A, Sf-GLDA-A, Sr-GLDA-A, respectively. Fertilizer use and application method did not significantly influence the dominant species. Dominant species in the leaves for samples S-Cont-A, Sf-GLDA-A, Sr-GLDA-A (Figure 6c) were identified as *Pseudomonas* (Proteobacteria) (23, 7, 12%), *Pedobacter* (14, 7, 6%), *Neochlamydia* (5, 21, 16%), *Longimicrobiaceae* (Gemmatimonadetes) (4, 2, 3%), and *Sphingomonas* (3, 2, 3%).

The observed OTUs in the root endophytic community of soil-grown lettuce were comparable to those of hydroponically grown plants. In samples S-Cont-B, Sf-GLDA-B, Sr-GLDA-B, the number of OTUs was 270, 198, 220, respectively, of which 105 OTUs were in common (Figure 6b). The percentage of shared OTUs in root endophytes was 39%, 53%, and 48% for S-Cont-B, Sf-GLDA-B, Sr-GLDA-B, respectively. Fertilizer use and application method did not significantly influence the dominant genera of the root endophytic microbiome, as this was true for leaves as well. Dominant genera in the roots for samples S-Cont-B, Sf-GLDA-B, Sr-GLDA-B (Figure 6c) were identified as *Rhodococcus* (Actinobacteria) (17, 13, 17%), *Pseudomonas* (11, 14, 12%), *Pedobacter* (9, 15, 10%), *Sphingomonas* (6, 8, 9%), and *Aquabacterium* (Proteobacteria) (2, 2, 2%).



(c)

Bacterial OTUs	Samples					
	S-Cont-A	Sf-GLDA-A	Sf-GLDA-A	S-Cont-B	Sf-GLDA-B	Sf-GLDA-B
<i>s_Rhodococcus qingshengii</i>	21	13	13	17	13	17
<i>g_Pseudarthrobacter; s_</i>	0	0	0	3	0	0
<i>g_Prauserella; s_</i>	1	0	1	1	0	1
<i>g_Streptomyces; s_</i>	0	0	0	2	1	2
<i>g_Rubrobacter; s_</i>	1	0	0	0	1	1
<i>g_Flaviaesturariibacter; s_</i>	0	2	1	0	0	0
<i>g_Lacibacter; s_</i>	1	2	3	0	1	1
<i>g_Parasegetibacter; s_</i>	0	3	2	1	0	0
<i>f_Microscillaceae; g; s_</i>	1	1	1	0	0	0
<i>g_Fluviicola; s_</i>	0	1	1	0	1	1
<i>g_Myroides; s_</i>	1	0	3	1	0	0
<i>g_Pedobacter; s_</i>	14	7	6	9	15	10
<i>o_Sphingobacteriales; f; g; s_</i>	1	0	1	1	1	1
<i>g_Neochlamydia; s_</i>	5	21	16	1	2	3
<i>f_Simkaniaceae; g; s_</i>	1	1	1	1	1	2
<i>g_Lactobacillus; s_</i>	0	2	2	0	1	0
<i>f_Longimicrobiaceae; g; s_</i>	4	2	3	4	6	5
<i>c_S0134 terrestrial group; o; f; g; s_</i>	1	1	1	0	0	0
<i>o_Rhizobiaceae; f; g; s_</i>	0	0	0	1	3	1
<i>g_Sphingomonas; s_</i>	1	0	1	1	1	1
<i>g_Sphingomonas; s_</i>	3	2	3	6	8	9
<i>g_Aquabacterium; s_</i>	2	0	1	2	2	2
<i>f_Methylophilaceae; g_</i>	2	2	2	1	1	1

Figure 6. Venn diagrams of the number of unique and shared OTUs in endophytic bacterial in leaves (a) and roots (b) of lettuce plants grown in soil and heatmap of dominant bacterial genera (c) (%)

relative sequence abundance ≥ 1) in endophytic bacterial in leaves and roots of lettuce plants grown in soil.

3.3.3. Alpha and Beta Diversity of the Lettuce Endophytic Bacterial Communities

Based on the data obtained from 16S rRNA amplicon sequencing, biodiversity indices were computed for bacterial communities within the leaf and root endospheres of lettuce plants cultivated in soil and hydroponics. The results pertaining to two alpha diversity indices, Shannon and Simpson, are presented in Table 2.

Table 2. Alpha diversity among endophytic bacterial communities in the tissues of roots and leaves of plants grown in soil and hydroponics.

	Substrate	Sample	Index	
			Shannon	Simpson
Leaves	Hydroponic	H-Cont-A	2.83	0.91
		H-GLDA-A	2.00	0.82
	Soil	S-Cont-A	2.77	0.87
		Sr-GLDA-A	3.17	0.91
Roots	Hydroponic	Sf-GLDA-A	3.24	0.92
		H-Cont-B	4.03	0.96
	Soil	H-GLDA-B	3.45	0.92
		S-Cont-B	3.71	0.93
	Soil	Sr-GLDA-B	3.39	0.92
		Sf-GLDA-B	3.46	0.93

In control samples, the Shannon index was calculated as 2.83, 2.77, 4.03, and 3.71 for H-Cont-A, S-Cont-A, H-Cont-B, and S-Cont-B samples, respectively. These results fall within the range reported in the literature for plant bacterial endospheres, utilizing the same sequencing methods and bioinformatic protocols [33,34]. Notably, both Simpson and Shannon indices were lower in plants treated with GLDA compared to control plants, except for the leaf sample in soil (S-Cont-A). This trend persisted across both above- and underground plant parts, in both hydroponic and soil experiments, and irrespective of the type of treatment (foliar or root).

Specifically, the Shannon index in the leaf in the hydroponic control (H-Cont-A) was 1.4 times higher than in the sample with GLDA (H-GLDA-A). Meanwhile, the Shannon index in the leaf samples with GLDA via root (Sr-GLDA-A) and foliar treatment (Sf-GLDA-A) was 1.01 and 1.17 times higher than control, respectively. However, Shannon indices in root endophytic microbial communities did not exhibit significant differences between hydroponic and soil cultivation.

The beta diversity of the samples was estimated using NMDS analysis, as shown in Figure 7. Dots on the NMDS plot represent different bacterial communities, and the proximity of dots indicates higher similarity between communities. Dots representing S-Cont and S-GLDA samples are located close to each other, forming separate groups from the other dots. Within this group, two subgroups can be distinguished: one unifying root communities and the other unifying leaf communities. Dots representing H-Cont-A, H-GLDA-A, H-Cont-B, and H-GLDA-B samples are located far away from each other. Additionally, in the case of the leaf endophytic community of plants grown hydroponically, the points representing H-Cont-A and H-GLDA-A samples are located far from each other.

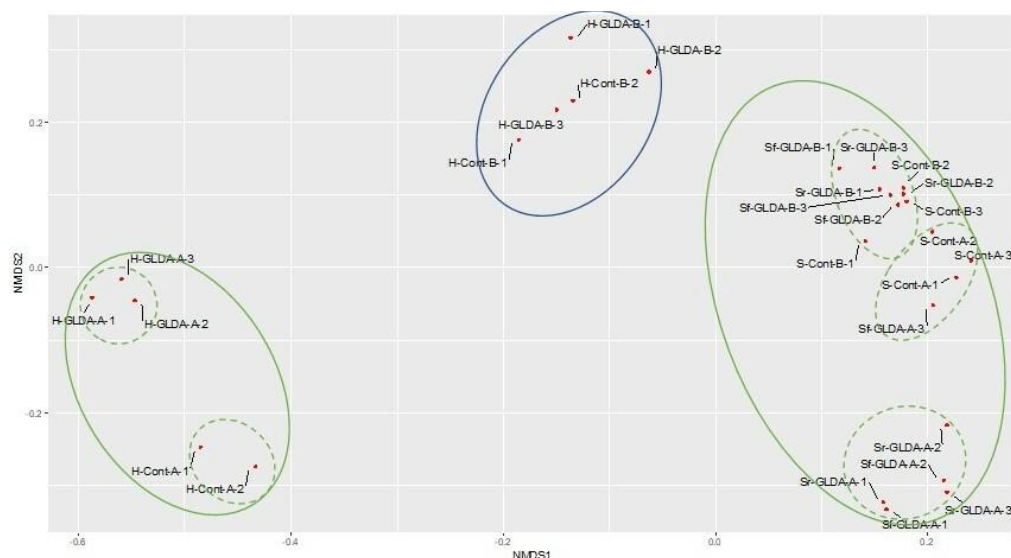


Figure 7. Betadiversity of the endophytic bacterial communities of the leaves and roots (letters represent the names of the variants and numbers the number of repetitions).

In addition, Indicator Species Analysis (ISA) was carried out using the relative abundance of species in the samples to determine the species that are most characteristic in the analyzed groups (Table 3).

Table 3. Indicator OTUs found for different samples' sets (where *, **, *** are *p*. value < 0.05, <0.01, and 0.001, respectively).

Bacterial OTUs	Samples					
	Cont	GLDA	S Cont	S GLDA	H Cont	H GLDA
<i>g_Acinetobacter; s_</i>	0.869 ***	0	0.783 **	0	0.974 **	0
<i>g_Ralstonia; s_</i>	0.788 *	0	0	0	0.952 *	0
<i>g_Staphylococcus; s_</i>	0.717 *	0	0	0	0	0
<i>g_Helicobacter; s_</i>	0.698 *	0	0	0	0.996 *	0
<i>g_Romboutsia; s_</i>	0.694 *	0	0	0	0.995 *	0
<i>g_Staphylococcus; s_</i>	0.666 *	0	0	0	0	0
<i>g_Flavisolibacter; s_</i>	0.548 *	0	0	0	0	0
<i>f_Burkholderiaceae; g; s_</i>	0.548 *	0	0	0	0	0
<i>f_Chitinophagaceae; g; s_</i>	0.542 *	0	0.707 *	0	0	0
<i>f_Sandaracinaceae; g; s_</i>	0.536 *	0	0.693 *	0	0	0
<i>g_Rhodococcus; s_</i>	0	0	0.814 *	0	0	0
<i>c_Acidimicrobiia; o; f; g; s_</i>	0	0	0.799 *	0	0	0
<i>g_Pseudarthrobacter; s_</i>	0	0	0.798 *	0	0	0
<i>g_Massilia; s_</i>	0	0	0.680 *	0	0	0
<i>p_WS2; c; o; f; g; s_</i>	0	0	0.672 *	0	0	0
<i>f_JG30-KF-CM45; g; s_</i>	0	0	0	0.814 *	0	0
<i>f_Lachnospiraceae; g; s_</i>	0	0	0	0	0.864 *	0
<i>o_Obscuribacterales; f; g; s_</i>	0	0	0	0	0	0.959 *

According to Table 3, the count of indicator species in both H and S control samples is represented by 10, with 8 in S-Cont samples and 5 in H-Cont samples. The combined group of GLDA samples showed no presence of indicator species, whereas S-GLDA and H-GLDA included one and one species, respectively.

4. Discussion

4.1. Biodegradability

The chemical stability and biodegradability of fertilizers represent critical characteristics in the evaluation of their efficacy. Frequently, the properties of novel chelators are compared with those of well-studied chelating agents, for which transformation and mineralization processes are well documented. However, even for the extensively studied chelating agent EDTA, available data often pertain to individual complexes (with iron, copper, chromium, etc.) rather than their mixtures. In our previous work [5], it was demonstrated that the degradation extent of a compositionally similar mixture of EDTA-based chelates (with Zn, Cu, Mn, and Fe) was approximately 14% over 28 days. Thus, the degradation extent of GLDA-based complexes determined in the current study was 4.3-fold higher.

The contributions of chemical and biological components to chelate degradation varied between EDTA and GLDA. For EDTA, the chemical contribution was negligible, whereas for GLDA, it was substantial.

The high degradation rate of GLDA established in this study, coupled with its favorable environmental indicators and demonstrated non-toxicity as shown by D. Kołodyńska et al. in 2013, suggests the viability of employing GLDA as a more environmentally friendly chelating agent [35].

4.2. Influence of GLDA-Based Chelates on Lettuce Growth and Microelements Uptake

The data obtained on the biomass of lettuce plants in our study are consistent with findings from other researchers. For instance, in the study by Ekinji M. et al. (2020), comparing different fertilizers for lettuce growth, the weight of plants in the control variant was 16.46 g [36]. In the work of Liu et al. (2020), cultivating lettuce in hydroponics under various LED lighting intensities, the lettuce fresh mass ranged from 41.5 to 109.9 g [37]. In the experiment by Cardarelli M. et al. (2023), on the 28th day of lettuce growth, the lettuce mass in the control variant was 132 g [38]. In addition, the literature indicates that the length of lettuce plants grown on different substrates can vary. A comparison of growth characteristics in soil and hydroponic lettuce cultivation in the experiment by Chunli Lei et al. (2021) showed a 10 cm increase in root length for hydroponically grown lettuce, while plant biomass in soil-grown lettuce was 1.2 times higher [39]. In our study, no significant differences ($p < 0.05$) in root length and plant biomass were found between hydroponic and soil-grown plants (control variants), whereas in above-ground length, there was a 1.2-fold difference (Figure 1).

The use of GLDA chelates positively influenced above-ground length and plant biomass in both cases (hydroponics and soil), with the greatest positive effect observed for the biomass of hydroponically grown plants. The biomass of H-GLDA-A samples exceeded that of soil-cultivated samples with root (Sr-GLDA-A) and foliar treatment (Sf-GLDA-A) by 2.8 and 2.3 times, respectively. The greater effect in the hydroponic experiment is likely associated with the following: soil organic matter can form complexes with metals, reducing their availability to plants, a blocking effect absent in hydroponic systems [39–41].

It is interesting to compare root length, above-ground length, and plant biomass in soil experiments with different treatment options—foliar and root. In some sources, it is claimed that foliar fertilization makes it possible to quickly supply plants with deficient nutrients, in case of both their deficiency in the soil and hindered uptake [42]. In our case, a comparable result was identified—with the use of a smaller volume of chelated fertilizer solution (80% less), root length and plant biomass did not significantly differ in Sr-GLDA and Sf-GLDA variants. Moreover, with foliar fertilization, above-ground length was 1.2 times higher. Plants can absorb nutrients, including through the above-ground part, through a complex process involving several layers of plant tissue. Nutrient absorption

through the leaf surface occurs through stomata, cuticular cracks, lenticels, ectodesmata, and pores [43].

The chlorophyll content in plant leaves is used to assess the impact of various factors (fertilization and the effect of pollutants) [22]. In our experiment, it is shown that the chlorophyll content in the leaves of control lettuce samples in soil (S-Cont-A) was 1.5 times higher than in hydroponics (H-Cont-A), and the use of GLDA chelates in hydroponics contributed to an increase in chlorophyll content to the levels determined in soil-grown plants.

4.3. Influence of GLDA-Chelate Fertilization on Leaf and Root Endophytic Bacterial Communities

According to modern understanding, microorganisms residing within plants play an equally significant role as the plant's own tissues and organs. Indeed, the endophytic microbiome plays a crucial role in promoting plant growth (PGP) and combating phytopathogens [44,45]. Endophytes can directly influence plant growth by producing phytohormones (e.g., jasmonic and salicylic acids) and inhibit the production of plant cells' ethylene. Additionally, they can impact pathogens by producing various hydrolytic enzymes (e.g., chitinases) that degrade structural elements of pathogen cell walls, synthesizing antibiotics, and limiting nutrients available to pathogens [32,46]. Therefore, when investigating new types of fertilizers, it is advisable to assess their impact not only on plant yield, morphometrics, and other plant characteristics, but also on its microbiome, particularly its variability under the influence of such fertilizers.

Recent research emphasizes the importance of studying the composition and changes in the bacterial endophytic community of plants consumed without thermal processing. The conservative part of the endophytic bacterial community in plants is often not affected by environmental factors, while satellite bacteria may lead to a so-called dysbiosis in the bacterial community. Investigating these changes is crucial, as alterations in the bacterial community can affect the health and productivity of plants, impacting not only the plants themselves but also humans [47].

Alterations in the endophytic plant microbiome can render its destabilization, resulting in decreased crop yield and fruit quality, influenced by various factors such as fertilizer application, a detrimental factor [48]. In many instances, investigations into plant endophytic microbial communities unveil considerable taxonomic overlaps at the phylum level. Yan-Zi Wang et al. (2023) illustrated that *Proteobacteria* and *Firmicutes* are frequently predominant phyla in the endophytic microbial communities of lettuce leaves and roots [15]. In our study, taxonomic overlaps at the phylum level were identified for all samples involving *Proteobacteria* and *Bacteroidetes*. Furthermore, *Firmicutes* bacteria prevailed in hydroponically grown lettuce leaves, whereas *Actinobacteria* bacteria were more abundant in the microbiomes of leaves and roots of soil-grown lettuce. Plant treatment with micronutrients did not exert an influence on the microbial community at the phylum level. However, analyses at lower taxonomic units revealed more discernible differences. The most pronounced distinctions in microbial communities were observed in the leaf endosphere for hydroponics and soil, in comparison to the roots (Figures 5 and 6).

The impact of chelating agents on environmental components, particularly microbial communities, remains a pertinent subject [21]. The majority of studies examining the influence of chelates on plants and soil microbial communities have focused on the remediation of heavy metal-contaminated soils [49,50].

In a 2014 study conducted by Junghun Lee et al., which assessed the impact of various chelates on phytoremediation and soil properties, it was revealed that EDTA emerged as the most effective chelating agent for phytoremediation in soils contaminated with heavy metals. However, adverse effects of EDTA on plants and soil enzymatic activity were also identified [51].

Data concerning the impact of chelates on endophytic bacterial communities in plants are limited. Nonetheless, there are studies detailing the effects of chelates,

predominantly EDTA, on bacterial communities in other environmental components [52]. The antimicrobial properties of EDTA, particularly its tetrasodium salt, against various clinical microorganisms, encompassing Gram-negative and Gram-positive bacteria, fungi, and yeast, have been elucidated [53].

Rong Huang et al.'s 2021 findings demonstrated that the introduction of EDTA into soil significantly diminished the richness and diversity of soil bacteria, resulting in a noteworthy alteration in the Actinobacteria to Proteobacteria ratio [54].

It is noteworthy that the lower values of alpha diversity indices in GLDA-treated plant samples revealed in our study coincide with increased biomass and length in these plants (Figure 2). This is likely due to the development of K-strategists and the competitive displacement of bacteria categorized as R-strategists (reference). In a study by Jonathan W. Leff et al. in 2015, the relative abundance of groups of soil bacteria generally considered more copiotrophic increased with nutrient addition, while the relative abundance of the predominantly oligotrophic phylum *Acidobacteria* decreased [55]. Thus, an increase in nutrients alters the life cycle strategies of microbiomes. Moreover, the results of Y. Wang et al. in 2023 indicated that excess fertilization reduced the richness and diversity of endophytic bacteria in wheat roots [14].

On the basis of NMDS analysis of relative ability data, it can be hypothesized that the substrate (soil vs. hydroponics, which can be considered as a complex substrate with a mineral–organic matter plus macro- and microelement solution vs. a simplified substrate with macro- and microelement solution only) is the primary factor shaping the endophytic microbial communities of lettuce plants. The second significant factor for the community composition is the part of the plant (above- or below-ground). Finally, the addition of chelated micronutrients that lead to the acceleration of plant growth is a third factor influencing microbial community composition in lettuce plants. From the results shown in Table 3, we assume that the way GLDA influences the community is the simplification (rarefaction) of its composition.

To confirm this assumption, Indicator Species Analysis was additionally conducted. ISA employs indices reflecting an organism's relative abundance and occurrence to assess the degree of its associations with predefined groups of interest (reference). Indeed, the number of species specific to control samples was higher than that of GLDA samples—10 and 0, respectively (Table 3). This holds true for the entire set of samples, both with chelate treatment (GLDA) and without treatment.

5. Conclusions

The research into the application of glutamic-N,N-diacetic acid (GLDA) as an innovative chelating agent in microfertilizer development has yielded significant insights into its biodegradability, effects on lettuce growth, and impact on endospheric bacterial communities. The study's findings underscore the potential of GLDA as a promising chelating agent, demonstrating both environmental benefits and agronomic efficacy. The biodegradability of GLDA was thoroughly evaluated by distinguishing between biotic and abiotic degradation over a 28-day period. Initial observations revealed that biological degradation accounted for 19% on the first day, while chemical degradation was a mere 0.2%. Over time, the degradation rate increased progressively, reaching 59.8% by day 28. Notably, chemical processes contributed approximately one-quarter of the degradation by the end of the study period. This high degradation rate, particularly the significant biological component, highlights GLDA's potential as an environmentally friendly alternative to traditional chelating agents like EDTA. The application of GLDA-based fertilizers was found to positively influence the growth and development of lettuce plants. In both soil and hydroponic systems, plants treated with GLDAchelates exhibited increased lengths and biomass of both roots and leaves compared to control groups. In the hydroponic experiments, the lengths and biomass of the lettuce leaves and roots increased by factors of 1.7, 1.8, 4.6, and 2.5 times, respectively. Similarly, soil experiments showed significant growth improvements with root and foliar applications of GLDAchelates. These enhancements

were observed across various growth metrics, including leaf length and biomass, which increased by 1.4 to 1.8 times in treated plants.

The chlorophyll content in leaves also increased significantly in hydroponic systems, suggesting improved photosynthetic efficiency and overall plant health.

The study also explored the impact of GLDA-based fertilization on the endophytic bacterial communities within the lettuce plants. Using 16S rRNA amplicon sequencing, the research identified shifts in the composition and diversity of these communities. In both hydroponic and soil-grown plants, dominant bacterial phyla included Proteobacteria and Bacteroidetes, with Firmicutes also being prevalent in leaf endophytes of hydroponically grown plants. The introduction of GLDAchelates resulted in a reduction in the number of operational taxonomic units (OTUs) in the bacterial communities, indicating a potential simplification or rarefaction of the microbiome. This was particularly evident in hydroponic systems, where the number of OTUs in leaves decreased significantly with GLDA treatment. However, alterations caused by GLDAchelates are less significant compared to other factors influencing endophytic community assemblies. Based on NMDS analysis of relative ability data, the primary factor shaping the endophytic microbial communities of lettuce plants is the substrate (soil versus hydroponics). The second significant factor is the part of the plant (above-ground or below-ground). The addition of chelated micronutrients, which accelerates plant growth, is only the third factor influencing microbial community composition.

In summary, the study demonstrates that GLDA is an effective and environmentally friendly chelating agent that enhances lettuce growth and impacts endophytic bacterial communities. Its high biodegradability and positive agronomic effects make it a promising candidate for sustainable agriculture. Future research should continue to explore its applications and long-term effects on different crops and ecosystems.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Physicochemical characteristics of the soil used in the study; Table S2: Images of lettuce plant samples from above (grown hydroponically); Table S3: Images of lettuce plant samples from the side (grown hydroponically); Table S4: Images of lettuce plant samples from above (grown on soil); Table S5: Images of lettuce plant samples from the side (grown on soil); Table S6: OTUs of endophytic bacteria in leaves and roots.

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References

1. Kolodynska, D. Polyacrylate anion exchangers in sorption of heavy metal ions with the biodegradable complexing agent. *Chem. Eng. J.* **2009**, *150*, 280–288. <https://doi.org/10.1016/j.cej.2008.12.027>.
2. Bucheli-Witschel, M.; Egli, T. Environmental fate and microbial degradation of aminopolycarboxylic acids. *FEMS Microbiol. Rev.* **2001**, *25*, 69–106. <https://doi.org/10.1111/j.1574-6976.2001.tb00572.x>.
3. Jones, P.W.; Williams, D.R. Chemical speciation used to assess [S,S']-ethylenediaminedisuccinic acid (EDDS) as a readily-biodegradable replacement for EDTA in radiochemical decontamination formulations. *Appl. Radiat. Isot.* **2001**, *54*, 587–593. [https://doi.org/10.1016/S0969-8043\(00\)00297-9](https://doi.org/10.1016/S0969-8043(00)00297-9).

4. Henneken, L.; Nörtemann, B.; Hempel, D.C. Influence of physiological conditions on EDTA degradation. *Appl. Microbiol. Biotechnol.* **1995**, *44*, 190–197. <https://doi.org/10.1007/BF00164501/METRICS>.
5. Belyukova, M.; Kuryntseva, P.; Galitskaya, P.; Selivanovskaya, S.; Brusko, V.; Dimiev, A. Biodegradation rate of EDTA and IDS and their metal complexes. *Horticulturae* **2023**, *9*, 623. <https://doi.org/10.3390/HORTICULTURAE9060623>.
6. Metsärinne, S.; Ronkainen, E.; Tuhkanen, T.; Aksela, R.; Sillanpää, M. Biodegradation of novel amino acid derivatives suitable for complexing agents in pulp bleaching applications. *Sci. Total Environ.* **2007**, *377*, 45–51. <https://doi.org/10.1016/J.SCI-TOTENV.2007.01.097>.
7. Thomas, R.A.P.; Lawlor, K.; Bailey, M.; Macaskie, L.E. Biodegradation of metal-EDTA complexes by an enriched microbial population. *Appl. Environ. Microbiol.* **1998**, *64*, 1319. <https://doi.org/10.1128/AEM.64.4.1319-1322.1998>.
8. Almubarak, T.; Ng, J.H.; Ramanathan, R.; Nasr-El-Din, H.A. From initial treatment design to final disposal of chelating agents: A review of corrosion and degradation mechanisms. *RSC Adv.* **2022**, *12*, 1813. <https://doi.org/10.1039/D1RA07272B>.
9. Begum, Z.A.; Rahman, I.M.M.; Sawai, H.; Tate, Y.; Maki, T.; Hasegawa, H. Stability constants of Fe(III) and Cr(III) complexes with dl-2-(2-carboxymethyl)nitriolotriacetic acid (GLDA) and 3-hydroxy-2,2'-iminodisuccinic acid (HIDS) in aqueous solution. *J. Chem. Eng. Data* **2012**, *57*, 2723–2732. <https://doi.org/10.1021/JE3005936>.
10. Discover Our Dissolvine® Product Range. Available online: <https://www.nouryon.com/products/chelates/dissolvine/> (accessed on 9 January 2024).
11. Li, N.; Hu, Y.; Xiong, G.; Liu, P.; Xiong, Y.; Luo, Z.; Zhang, Q.; Li, Y.; Zhu, S.; Feng, W.; et al. Research and application of eco-friendly chelating agents in plugging removal systems: A review. *Geoenergy Sci. Eng.* **2023**, *229*, 212135. <https://doi.org/10.1016/J.GEOEN.2023.212135>.
12. Morelli, M.; Bahar, O.; Papadopoulou, K.K.; Hopkins, D.L.; Obradović, A. Editorial: Role of endophytes in plant health and defense against pathogens. *Front. Plant Sci.* **2020**, *11*, 577603. <https://doi.org/10.3389/FPLS.2020.01312/BIBTEX>.
13. Tiwari, P.; Bae, H. Trends in harnessing plant endophytic microbiome for heavy metal mitigation in plants: A perspective. *Plants* **2023**, *12*, 1515. <https://doi.org/10.3390/PLANTS12071515>.
14. Wang, Y.; Wu, Y.; Cao, C.; Han, S.; Zhao, W.; Li, Q.; Liu, X.; Kong, L. Effects of fertilizer reduction coupled with straw returning on soil fertility, wheat root endophytic bacteria, and the occurrence of wheat crown rot. *Front. Microbiol.* **2023**, *14*, 1143480. <https://doi.org/10.3389/FMICB.2023.1143480/BIBTEX>.
15. Wang, Y.Z.; Zhou, S.Y.D.; Zhou, X.Y.; An, X.L.; Su, J.Q. Manure and biochar have limited effect on lettuce leaf endophyte resistome. *Sci. Total Environ.* **2023**, *860*, 160515. <https://doi.org/10.1016/J.SCI-TOTENV.2022.160515>.
16. Danilova, N.; Galieva, G.; Kuryntseva, P.; Selivanovskaya, S.; Galitskaya, P. Influence of the antibiotic oxytetracycline on the morphometric characteristics and endophytic bacterial community of lettuce (*Lactuca sativa* L.). *Microorganisms* **2023**, *11*, 2828. <https://doi.org/10.3390/MICROORGANISMS11122828/S1>.
17. Smith, R.M.; Martell, A.; Motekaitis, R.; Smith, R.; Motekaitis, R. *NIST Critically Selected Stability Constants of Metal Complexes Database*; NIST: Gaithersburg, MD, USA, 2013; Volume 46.
18. Begum, Z.A.; Rahman, I.M.M.; Tate, Y.; Egawa, Y.; Maki, T.; Hasegawa, H. Formation and Stability of Binary Complexes of Divalent Ecotoxic Ions (Ni, Cu, Zn, Cd, Pb) with Biodegradable Aminopolycarboxylate Chelants (DL-2-(2-Carboxymethyl)Nitriolotriacetic Acid, GLDA, and 3-Hydroxy-2,2'-Iminodisuccinic Acid, HIDS) in Aqueous Solutions. *J. Solut. Chem.* **2012**, *41*, 1713–1728. <https://doi.org/10.1007/S10953-012-9901-9>.
19. *OECD Guideline for Testing of Chemicals Test No. 301: Ready Biodegradability*; OECD Guidelines for the Testing of Chemicals, Section 3; OECD: Paris, France, 1992. <https://doi.org/10.1787/9789264070349-EN>.
20. Puccinelli, M.; Landi, M.; Maggini, R.; Pardossi, A.; Incrocci, L. Iodine biofortification of sweet basil and lettuce grown in two hydroponic systems. *Sci. Hortic.* **2021**, *276*, 109783. <https://doi.org/10.1016/J.SCI-NTA.2020.109783>.
21. Brusko, V.; Garifullin, B.; Geniyatullina, G.; Kuryntseva, P.; Galieva, G.; Galitskaya, P.; Selivanovskaya, S.; Dimiev, A.M. Novel biodegradable chelating agents for micronutrient fertilization. *J. Agric. Food Chem.* **2023**, *71*, 14979–14988. <https://doi.org/10.1021/acs.jafc.3c03500>.
22. Cerovic, Z.G.; Masdoumier, G.; Ghozlen, N.B.; Latouche, G. A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. *Physiol. Plant.* **2012**, *146*, 251. <https://doi.org/10.1111/J.1399-3054.2012.01639.X>.
23. Zhang, Q.; Acuña, J.J.; Inostroza, N.G.; Mora, M.L.; Radic, S.; Sadowsky, M.J.; Jorquera, M.A. Endophytic bacterial communities associated with roots and leaves of plants growing in Chilean extreme environments. *Sci. Rep.* **2019**, *9*, 4950. <https://doi.org/10.1038/s41598-019-41160-x>.
24. Caporaso, J. G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F. D.; Costello, E. K.; Fierer, N.; Peña, A. G.; Goodrich, J. K.; Gordon, J. I.; Huttley, G. A.; Kelley, S. T.; Knights, D.; Koenig, J. E.; Ley, R. E.; Lozupone, C. A.; McDonald, D.; Muegge, B. D.; Pirrung, M.; Reeder, J.; Sevinsky, J. R.; Turnbaugh, P. J.; Walters, W. A.; Widmann, J.; Yatsunencko, T.; Zaneveld, J.; Knight, R. QIIME Allows Analysis of High-Throughput Community Sequencing Data. *Nature methods.* **2010**, *7*, 335–336. <https://doi.org/10.1038/NMETH.F.303>.
25. Bioinformatics & Evolutionary Genomics. Available online: <https://bioinformatics.psb.ugent.be/webtools/Venn/> (accessed on 9 January 2024).
26. Shannon, C.E. A mathematical theory of communication. *Bell Syst. Tech. J.* **1948**, *27*, 379–423. <https://doi.org/10.1002/J.1538-7305.1948.TB01338.X>.

27. Beisel, J.N.; Moreteau, J.C. A simple formula for calculating the lower limit of Shannon's diversity index. *Ecol. Model.* **1997**, *99*, 289–292. [https://doi.org/10.1016/S0304-3800\(97\)01954-6](https://doi.org/10.1016/S0304-3800(97)01954-6).
28. Ham, B.; Choi, B.Y.; Chae, G.T.; Kirk, M.F.; Kwon, M.J. Geochemical influence on microbial communities at CO₂-leakage analog sites. *Front. Microbiol.* **2017**, *8*, 264914. <https://doi.org/10.3389/FMICB.2017.02203/BIBTEX>.
29. Faith, D.P.; Minchin, P.R.; Belbin, L. Compositional dissimilarity as a robust measure of ecological distance. *Vegetatio* **1987**, *69*, 57–68. <https://doi.org/10.1007/BF00038687>.
30. De Cáceres, M.; Legendre, P. Associations between species and groups of sites: Indices and statistical inference. *Ecology* **2009**, *90*, 3566–3574. <https://doi.org/10.1890/08-1823.1>.
31. Rahman, M.S.; Hoque, M.N.; Puspo, J.A.; Islam, M.R.; Das, N.; Siddique, M.A.; Hossain, M.A.; Sultana, M. Microbiome signature and diversity regulates the level of energy production under anaerobic condition. *Sci. Rep.* **2021**, *11*, 19777. <https://doi.org/10.1038/s41598-021-99104-3>.
32. Ali, M.; Ali, Q.; Sohail, M.A.; Ashraf, M.F.; Saleem, M.H.; Hussain, S.; Zhou, L. Diversity and taxonomic distribution of endophytic bacterial community in the rice plant and its prospective. *Int. J. Mol. Sci.* **2021**, *22*, 10165. <https://doi.org/10.3390/IJMS221810165/S1>.
33. Lopez-Echartea, E.; Strejcek, M.; Mukherjee, S.; Uhlik, O.; Yrjälä, K. Bacterial succession in oil-contaminated soil under phytoremediation with poplars. *Chemosphere* **2020**, *243*, 125242. <https://doi.org/10.1016/J.CHEMOSPHERE.2019.125242>.
34. Ma, B.; Lv, X.; Warren, A.; Gong, J. Shifts in diversity and community structure of endophytic bacteria and archaea across root, stem and leaf tissues in the common reed, *Phragmites australis*, along a salinity gradient in a marine tidal wetland of northern China. *Antonie Van Leeuwenhoek Int. J. Gen. Mol. Microbiol.* **2013**, *104*, 759–768. <https://doi.org/10.1007/S10482-013-9984-3>.
35. Kolodynska, D. Application of a new generation of complexing agents in removal of heavy metal ions from different wastes. *Environ. Sci. Pollut. Res. Int.* **2013**, *20*, 5939. <https://doi.org/10.1007/S11356-013-1576-2>.
36. Ekinci, M.; Kul, R.; Turan, M.; Yildirim, E.; Bilgisi, Y. Effects of organic fertilizers on plant growth, yield and mineral content of lettuce (*Lactuca sativa* L.). *J. Erciyes Agric. Anim. Sci.* **2020**, *1*, 1319–1324.
37. Liu, W.; Zha, L.; Zhang, Y. Growth and nutrient element content of hydroponic lettuce are modified by LED continuous lighting of different intensities and spectral qualities. *Agronomy* **2020**, *10*, 1678. <https://doi.org/10.3390/AGRONOMY10111678>.
38. Cardarelli, M.; El Chami, A.; Iovieno, P.; Roupheal, Y.; Bonini, P.; Colla, G. Organic fertilizer sources distinctively modulate productivity, quality, mineral composition, and soil enzyme activity of greenhouse lettuce grown in degraded soil. *Agronomy* **2023**, *13*, 194. <https://doi.org/10.3390/AGRONOMY13010194>.
39. Liphadzi, M.S.; Kirkham, M.B. Availability and plant uptake of heavy metals in EDTA-assisted phytoremediation of soil and composted biosolids. *South Afr. J. Bot.* **2006**, *72*, 391–397. <https://doi.org/10.1016/J.SAJB.2005.10.010>.
40. Fonseca-García, C.; Coleman-Derr, D.; Garrido, E.; Visel, A.; Tringe, S.G.; Partida-Martínez, L.P. The Cacti Microbiome: Interplay between habitat-filtering and host-specificity. *Front. Microbiol.* **2016**, *7*, 150. <https://doi.org/10.3389/FMICB.2016.00150/BIBTEX>.
41. Arif, N.; Yadav, V.; Singh, S.; Singh, S.; Ahmad, P.; Mishra, R.K.; Sharma, S.; Tripathi, D.K.; Dubey, N.K.; Chauhan, D.K. Influence of high and low levels of plant-beneficial heavy metal ions on plant growth and development. *Front. Environ. Sci.* **2016**, *4*, 217521. <https://doi.org/10.3389/FENV.S.2016.00069/BIBTEX>.
42. Januszkiewicz, R.; Kulczycki, G.; Samoraj, M. Foliar fertilization of crop plants in Polish agriculture. *Agriculture* **2023**, *13*, 1715. <https://doi.org/10.3390/AGRICULTURE13091715>.
43. Shahid, M.; Dumat, C.; Khalid, S.; Schreck, E.; Xiong, T.; Niazi, N.K. Foliar heavy metal uptake, toxicity and detoxification in plants: A comparison of foliar and root metal uptake. *J. Hazard. Mater.* **2017**, *325*, 36–58. <https://doi.org/10.1016/J.JHAZMAT.2016.11.063>.
44. Bulgarelli, D.; Schlaeppi, K.; Spaepen, S.; Van Themaat, E.V.L.; Schulze-Lefert, P. Structure and functions of the bacterial microbiota of plants. *Annu. Rev. Plant Biol.* **2013**, *64*, 807–838. <https://doi.org/10.1146/ANNUREV-ARPLANT-050312-120106>.
45. Galieva, G.S.; Galitskaya, P.Y.; Selivanovskaya, S.Y. Plant microbiome: Origin, composition, and functions. *Uchenye Zap. Kazan. Universiteta. Seriya Estestv. Nauk.* **2023**, *165*, 231–262. <https://doi.org/10.26907/2542-064X.2023.2.231-262>.
46. Leach, J.E.; Triplett, L.R.; Argueso, C.T.; Trivedi, P. Communication in the phytobiome. *Cell* **2017**, *169*, 587–596. <https://doi.org/10.1016/J.CELL.2017.04.025>.
47. Nadarajah, K.; Abdul Rahman, N.S.N. The microbial connection to sustainable agriculture. *Plants* **2023**, *12*, 2307. <https://doi.org/10.3390/PLANTS12122307>.
48. Resendiz-Nava, C.N.; Alonso-Onofre, F.; Silva-Rojas, H.V.; Rebollar-Alviter, A.; Rivera-Pastrana, D.M.; Stasiewicz, M.J.; Nava, G.M.; Mercado-Silva, E.M. Tomato plant microbiota under conventional and organic fertilization regimes in a soilless culture system. *Microorganisms* **2023**, *11*, 1633. <https://doi.org/10.3390/MICROORGANISMS11071633>.
49. Epelde, L.; Hernández-Allica, J.; Becerril, J.M.; Blanco, F.; Garbisu, C. Effects of chelates on plants and soil microbial community: Comparison of EDTA and EDDS for lead phytoextraction. *Sci. Total Environ.* **2008**, *401*, 21–28. <https://doi.org/10.1016/J.SCI-TOTENV.2008.03.024>.
50. Gul, I.; Manzoor, M.; Hashim, N.; Shah, G.M.; Waani, S.P.T.; Shahid, M.; Antoniadis, V.; Rinklebe, J.; Arshad, M. Challenges in microbially and chelate-assisted phytoextraction of cadmium and lead—A review. *Environ. Pollut.* **2021**, *287*, 117667. <https://doi.org/10.1016/J.ENVPOL.2021.117667>.
51. Lee, J.; Sung, K. Effects of chelates on soil microbial properties, plant growth and heavy metal accumulation in plants. *Ecol. Eng.* **2014**, *73*, 386–394. <https://doi.org/10.1016/J.ECOLENG.2014.09.053>.

52. Kaurin, A.; Gluhar, S.; Tilikj, N.; Lestan, D. Soil washing with biodegradable chelating agents and EDTA: Effect on soil properties and plant growth. *Chemosphere* **2020**, *260*, 127673. <https://doi.org/10.1016/J.CHEMOSPHERE.2020.127673>.
53. Finnegan, S.; Percival, S.L. EDTA: An antimicrobial and antibiofilm agent for use in wound care. *Adv. Wound Care* **2015**, *4*, 415. <https://doi.org/10.1089/WOUND.2014.0577>.
54. Huang, R.; Cui, X.; Luo, X.; Mao, P.; Zhuang, P.; Li, Y.; Li, Y.; Li, Z. Effects of plant growth regulator and chelating agent on the phytoextraction of heavy metals by *Pfaffia glomerata* and on the soil microbial community. *Environ. Pollut.* **2021**, *283*, 117159. <https://doi.org/10.1016/J.ENVPOL.2021.117159>.
55. Leff, J.W.; Jones, S.E.; Prober, S.M.; Barberán, A.; Borer, E.T.; Firn, J.L.; Harpole, W.S.; Hobbie, S.E.; Hofmockel, K.S.; Knops, J.M.H.; et al. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 10967–10972. <https://doi.org/10.1073/pnas.1508382112>.

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