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Impact of 2-((1,2-dicarboxyethyl)amino)pentanedioic acid (IGSA) Chelated Fertilizer on Bacterial Communities in Hydroponic and Soil-Grown Lettuce

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Abstract

Ethylenediaminetetraacetic acid, commonly used in micronutrient fertilizers as a chelating agent, enhances the availability of micronutrients to plants. However, its accumulation in the environment has numerous negative effects. The search for alternative chelating agents without these drawbacks is highly relevant. This study evaluated the impact of a novel micronutrient fertilizer based on the chelating agent 2-((1,2-dicarboxyethyl)amino)pentanedioic acid on the endophytic microbial community of lettuce grown in soil and hydroponic systems. The OTU count in soil was 1595, compared to 449 in hydroponics. In hydroponic systems, the application of 2-((1,2-dicarboxyethyl)amino)pentanedioic acid altered the abundance of several dominant bacterial taxa. In soil experiments, the effect of 2-((1,2-dicarboxyethyl) amino)pentanedioic acid on bacterial taxa was less pronounced. For endophytic bacterial communities, 12 bacterial OTUs were identified as core for lettuce leaves and roots regardless of the substrate. The OTU count in control soil root samples was 296, compared to 178 in hydroponic roots. The application of 2-((1,2-dicarboxyethyl)amino)pentanedioic acid led to slight shifts in the abundance of certain endophytic bacteria, especially in hydroponic systems. Non-metric multidimensional scaling analysis demonstrated that substrate type has the most significant influence on the composition of endophytic and substrate bacterial communities. The application of 2-((1,2-dicarboxyethyl)amino)pentanedioic acid did not significantly alter the composition of the communities. The results suggest that while 2-((1,2-dicarboxyethyl)amino)pentanedioic acid chelated fertilizer can influence the abundance of specific microbial taxa, it does not drastically disrupt the endophytic bacterial communities' structure in lettuce grown in soil or hydroponics.

Keywords: 2-((1,2-dicarboxyethyl)amino)pentanedioic Acid, Micronutrient Fertilizers, Chelates, Ethylenediaminetetraacetic Acid, Plant Endophytic Microbiome, Bacterial Community, Soil, Hydroponics

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INTRODUCTION

Achieving high yields of agricultural crops necessitates comprehensive agronomic practices, including the use of various organic and mineral fertilizers. These fertilizers enhance the soil's overall condition and provide essential nutrients to plants.[1] Despite the evident benefits of fertilizers, there are significant drawbacks. Excessive and uncontrolled application of fertilizers can degrade soil structure, reduce fertility, contaminate groundwater and surface water, and increase greenhouse gas emissions from the soil.[2]

To enhance the efficiency of nutrient delivery to plants, synthetic micronutrient fertilizers based on chelating agents have been developed. Chelating fertilizers offer high protection against oxidation, precipitation, and immobilization. Chelation significantly improves nutrient uptake by plants, accelerates growth, strengthens plant immunity, and increases yields. Ethylenediaminetetraacetic acid (EDTA) is one of the most popular and effective chelating agents for producing micronutrient fertilizers.[3] However, EDTA's low biodegradability results in its accumulation in soils and groundwater.[4] In soil, EDTA converts heavy metals into a soluble form, leading to their accumulation in plants and contamination of drinking water. High concentrations of EDTA can reduce seed germination and plant biomass, slow growth, and delay the average emergence time of seedlings. In the presence of EDTA, plants may produce fewer photosynthetic pigments and generate more reactive oxygen species, resulting in electrolyte leakage. EDTA is also known to adversely affect soil microbial communities, altering both the number and diversity of microorganisms.[5]

Given the aforementioned disadvantages of EDTA-based fertilizers, the search for new biodegradable chelating agents that enhance environmental safety is imperative. Alternatives like iminodisuccinic acid (IDS), EDDHA, and N,N'-bis(2-hydroxyphenyl)ethylenediamine-N,N'-diacetic acid (HBED), which is an isomer of EDDHA, are commercially available and environmentally friendly.[6] In our previous work, the prospects of IGSA as such a chelate were demonstrated. The yield of lettuce treated with IGSA-based fertilizer was 1.4-1.6 times higher than with an EDTA-based fertilizer, while the biodegradability of IGSA-based fertilizer was 59% higher.[6]

To recommend IGSA as a full-fledged alternative to EDTA, information on the effect of IGSA chelates on plant-associated microorganisms is also needed. These microorganisms support plant function and protection and can directly influence human health, particularly when plants are consumed raw. The endophytic community of plants includes microorganisms residing in various plant parts, occupying intercellular or intracellular spaces.[6] The composition of a plant's microbial community is derived from seed microorganisms and those recruited from the environment. Plant seed endophytes are generally highly conserved, whereas the plant's endophytic microbial community can change during growth and development. It is also influenced by various abiotic and biotic factors, such as soil conditions, biogeography, plant genotype, and interactions among microorganisms and plants.[7]

The literature documents various negative effects of different fertilizers on the endophytic microbial community. For instance, nitrogen fertilizer application altered the microbial community structure in seeds, roots, leaves, and stems, affecting the relative abundance of several beneficial microorganisms, as well as affected genes involved in the nitrogen cycle in the roots.[8,9] Alterations in the composition and structure of the plant endophytic microbiome can negatively impact plant immunity and resistance to infections, ultimately leading to crop losses.[10]

In this study, we investigated the potential of using a new chelating agent, IGSA, to develop a plant micronutrient fertilizer. The objective was to evaluate the effect of IGSA-based fertilizer on endophytic microorganisms of lettuce, cultivated on different substrates soil and hydroponics.

MATERIALS AND METHODS

Experimental design

The IGSA was synthesized at the Chemical Institute of Kazan Federal University (Russia). The structural formula and characteristics of IGSA, as well as the IGSA-based microfertilizer, are described in our previous work.[6] Lettuce plants (*Lactuca sativa* L.) were grown in two substrate systems-hydroponics and soil in a greenhouse. The

preparation of seeds, seedlings, and the design of soil and hydroponics experiments are detailed in.[6] In the hydroponics experiment, IGSA-based fertilizer was introduced into the water. In the soil experiment, it was applied in two ways: foliar (on leaf surfaces) and root application (at the plant roots). Both experiments (soil and hydroponics) included control variants without the application of IGSA-based fertilizer (Figure 1).

The characteristics of the soil, the content of micronutrients in the hydroponic solution, and the concentration of IGSA-based microfertilizer applied are described in Brusko et al.[6] Plants were cultivated in a greenhouse at 22 °C with a 16:8 hour light/dark cycle for 28 days. On the 28th day of vegetation, plants were harvested from the substrate. Filtrate, soil, leaves, and roots from each container were immediately used for bacterial DNA extraction.

DNA extraction and sequencing

DNA extraction from plant and soil samples was performed using the FastDNA Spin Kit for Soil (MP Bio., Irvine, CA, USA) following the manufacturer's instructions. DNA purification was conducted using the QIAquick PCR Purification Kit (Qiagen, Dusseldorf, Germany).

Sequencing was conducted according to the established protocol (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). Sequencing of the bacterial community was carried out on an Illumina platform (Illumina, San Diego, CA, USA). 16S rRNA sequencing data were analyzed using the Quantitative Insights Into Microbial Ecology (QIIME, East Lansing, Michigan, USA) platform, version 1.6.0.[11] Representative sequences were aligned using the PyNAST algorithm.[12] Within the high-throughput sequencing data analysis it was observed 1,777,897 qualified sequences from all samples for the bacterial microbial community. The total number of chimeras excluded from the analysis was 64,990 (Supplementary Table S1).

Statistical analysis

All measurements were conducted in at least three replicates. Each container for plant cultivation housed nine plants. Statistical analyses of the results were performed using the R Statistical Software package (version 3.6.1) and Microsoft Office Excel 2010 (Redmond, WA, USA) (R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, 2019). All graphical data are presented as mean values with standard deviations. The Mann–Whitney test was employed to determine the significance of differences at α = 0.05. The relative similarity of bacterial communities was assessed using non-metric multidimensional scaling (NMDS) based on the Bray-Curtis coefficient.[13] Shannon and Simpson indices were calculated to measure the alpha diversity of microbial communities.[14,15]

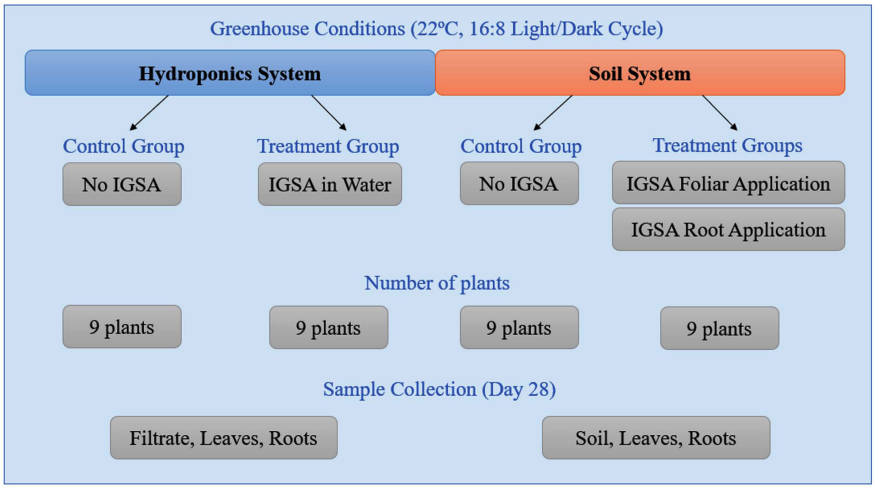

Figure 1. Scheme of the hydroponic and soil experiment

Venn diagrams illustrating shared and unique bacterial OTUs of microbial community samples were generated using an online tool (http://bioinformatics.psb.ugent.be/webtools/Venn/).

RESULTS AND DISCUSSION

The use of various substances in plant cultivation, targeting different purposes such as nutrition, disease protection, pest control, etc., is widespread in agriculture worldwide.[16,17] However, alongside the positive impact on the growth and development of crops, such substances can also have negative effects, particularly on the microbial communities in both soil and plant endophytes.[18,19] Given that IGSA, a chelated fertilizer, is a new product and there is insufficient data on its effects on microbial communities, this study was conducted.

Influence of IGSA on bacterial communities of substrates - hydroponics and soil

In the first stage, we assessed whether the application of IGSA affected the number of operational taxonomic units (OTUs) and their diversity in the substrate and the endosphere of lettuce. In hydroponic cultivation of lettuce, the control samples (without IGSA) had OTU counts in filtrate, leaves, and roots of 449, 51, and 178, respectively. The Simpson and Shannon indices, characterizing alpha diversity of microbial communities in the control filtrate samples, were 0.96 and 4.03, in leaves - 0.95 and 3.53, and in roots - 0.9 and 3.1. Figure 2 shows the OTU counts and the Simpson and Shannon indices expressed as percentages of the control. The application of IGSA did not significantly affect these parameters.

In the soil experiment control samples, the OTU count in soil was 1595, 3.5 times higher than in the control filtrate sample. In leaves, the

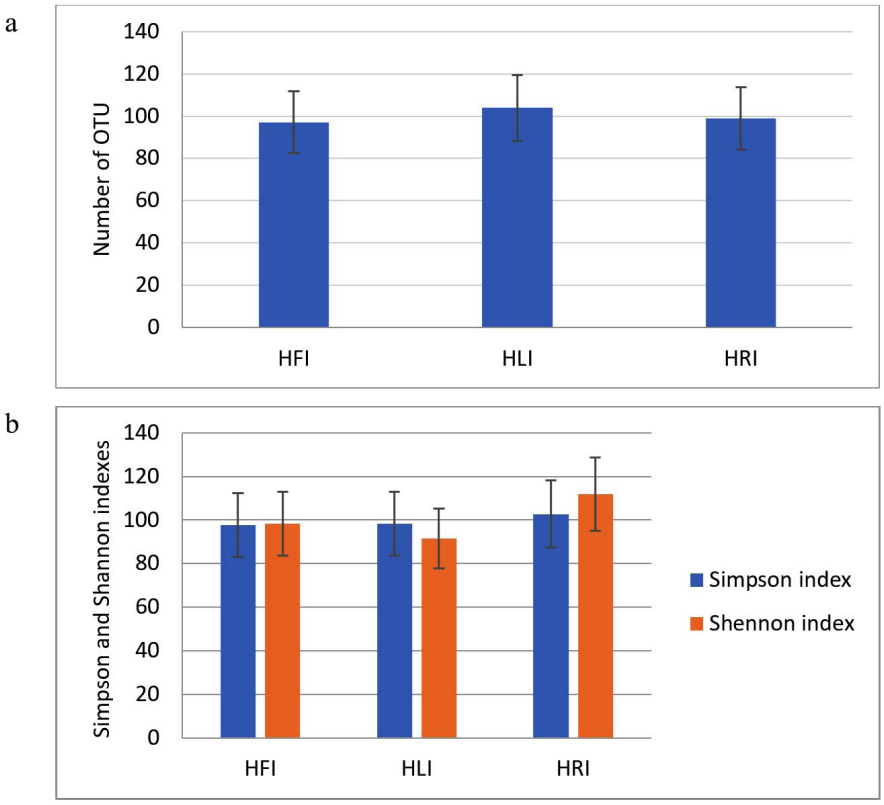

Figure 2. The number of OTUs (a), Simpson index, and Shannon index (b) in filtrate, leaves, and roots of hydroponically grown lettuce with IGSA chelated fertilizer addition

Table 1. Dominant (OTU abundance over 2% in at least one sample) bacterial taxa in the microbial community of hydroponic filtrate and soil of the experiment with IGSA chelated fertilizer

The lowest defined taxon	OTU	Hydroponics		Soil experiment		
		HFK	HFI	SSK	SSIr	SSIf
genus	*Glutamicibacter*	15.0	0.3	0.2	0.2	0.2
genus	*Pseudarthrobacter*	2.1	19.1	7.2	10.1	8.8
genus	*Flavobacterium*	5.5	0.1	0.2	0.3	0.4
family	*Simkaniaceae*	4.2	2.9	0.2	0.2	0.3
genus	*Bacillus*	0.0	0.0	3.0	3.3	3.6
family	*Saccharimonadaceae*	2.9	0.8	0.2	0.3	0.2
order	*Saccharimonadales*	1.9	14.6	0.7	0.9	0.9
genus	*Brevundimonas*	2.2	1.3	0.4	0.2	0.5
family	*Paracaedibacteraceae*	3.1	0.0	0.0	0.0	0.0
genus	*Reyranella*	3.0	0.9	0.2	0.2	0.2
genus	*Devosia*	5.4	2.0	1.0	1.0	1.4
genus	*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*	2.7	1.3	0.4	0.3	0.4
family	*Rhizobiaceae*	1.1	3.4	0.5	0.5	0.5
family	*Rickettsiaceae*	0.0	5.9	0.3	0.2	0.2
genus	*Novosphingobium*	2.2	0.2	0.2	0.3	0.4
genus	*Sphingobium*	3.7	1.1	0.3	0.4	0.2
genus	*Sphingomonas*	6.7	0.8	2.0	1.8	1.9
genus	*Limnobacter*	0.0	0.0	0.7	0.7	2.3
genus	*Methylophilus*	2.6	1.1	0.0	0.0	0.0

OTU count was 44, not significantly different from the hydroponic experiment. In roots, the OTU count was 296, 1.7 times higher than in the hydroponic control roots. The Simpson and Shannon indices were 0.99 and 5.65 for soil, 0.75 and 1.97 for leaves, and 0.92 and 3.66 for roots. In the soil experiment, two types of IGSA treatments were used: foliar and root application. As with the hydroponic experiment, IGSA did not affect the alpha diversity of the microbial communities in soil, leaves, and roots, nor the OTU counts in samples, except for the root sample treated with IGSA by root application (sample SRIf), where the OTU count was 73.6% of the control (Figure 3).

Thus, it was determined that in the substrates used for cultivating lettuce (soil, hydroponics), the number and alpha diversity of microorganisms were higher than in the roots and leaves of the plants. Soil contained significantly more microorganisms in both quantity and diversity compared to filtrate. Soil, unlike the aqueous medium, contains a wider range of soluble nutrients. Due to its structure and the presence of grains and aggregates of various sizes and textures, soil provides bacteria with a larger number of ecological niches. The higher number and diversity of microorganisms in the roots of soil-grown lettuce are likely due to some microorganisms being introduced from the soil environment. Bacterial penetration into root tissues can occur passively through root cracks or lateral root exit points, as well as through a number of active mechanisms.[20,21] Unlike roots, no differences in the number of OTUs and alpha diversity indices were observed in the leaves of lettuce grown on different substrates (soil, hydroponics).

Next, the influence of IGSA on the composition and abundance of dominant bacterial taxa (OTU abundance over 2% in at least one sample) in hydroponic filtrate and soil of the soil experiment was evaluated (Table 1). The same bacterial taxa were present among the dominants, but their abundance varied depending on the substrate type. In the control hydroponic filtrate, the dominant bacteria were from the family *Simkaniaceae* (abundance 4.2) and the genera *Glutamicibacter* (15.02), *Flavobacterium*

(5.5), *Devosia* (5.4), and *Sphingomonas* (6.7). In the control soil samples, the most frequently encountered bacteria were from the genera *Pseudarthrobacter* (abundance 7.2), *Bacillus* (2.9), and *Sphingomonas* (6.7).

Adding IGSA to the hydroponic filtrate altered the abundance of most dominant bacterial taxa. An increase in the abundance of bacteria belonging to the genus *Pseudarthrobacter*, order *Saccharimonadales*, families *Rhizobiaceae* and *Rickettsiaceae* was observed. The abundance of bacteria from the genera *Glutamicibacter*, *Flavobacterium*, *Reyranella*, *Devosia*, and *Sphingomonas*, and the families *Saccharimonadaceae* and *Paracaedibacteraceae* decreased.

In the soil experiment, the effect of IGSA on the abundance of bacterial taxa was less pronounced compared to the hydroponic system. This difference is likely due to the filtrate being a more homogeneous medium compared to soil, which increases IGSA bioavailability. An increase in the abundance of bacteria belonging to the genus Pseudarthrobacter was observed in the IGSA-treated variants, both foliar and root applications (samples SSIr and SSIf).

Thus, the application of IGSA leads to changes in the abundance of some dominant strains in the substrate, particularly in hydroponic solutions. However, no elimination of any OTUs or the appearance of new dominants compared to the control occurred. The method of IGSA application (root/foliar) did not affect the composition and abundance of dominant bacterial taxa in soil and filtrate.

Soil serves as a reservoir for a large number and diversity of microorganisms compared to the aquatic environment. The number of bacteria in soil varies from less than 500,000 to 3,863,000 cells/mL, while in water, these numbers are significantly lower. Soil has a high buffering capacity, consistent pH, salinity, and other physicochemical properties. Furthermore, soil is not subject to rapid temperature changes and

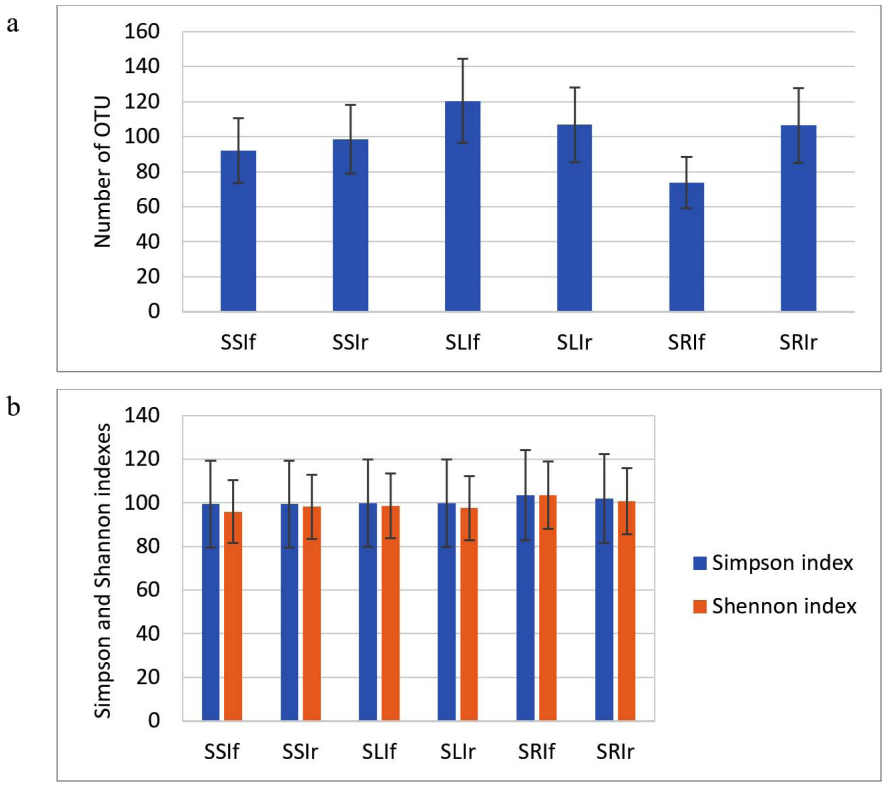

Figure 3. The number of OTUs (a), Simpson index, and Shannon index (b) in soil, leaves, and roots of lettuce grown in soil with IGSA chelated fertilizer addition

nutrient concentration fluctuations.[22,23] In water, conditions for the microbial community are more variable. Fluctuations in salinity, thermal and oxygen stratification, and concentrations of ammonium, nitrates, total chlorine, and monochloramine can significantly affect the number of aquatic microorganisms.[24,25] Nutrient deficiency limits microbial colonization of hydroponic systems. The number of microorganisms in it before planting is low, and the microbial community consists of a small number of species. Colonization of hydroponic systems by microorganisms mainly occurs through airborne droplets. For example, when cultivating plants using hydroponic systems, the filtrate can also be colonized by plant endophytes.[26,27]

Influence of IGSA on bacterial communities of Lettuce leaves and root endosphere

Next, the effect of IGSA chelated fertilizer on the endophytic microbial community of lettuce grown in soil and hydroponic systems

Table 2. Dominant (OTU abundance over 2% in at least one sample) bacterial taxa in the endophytic microbial community of lettuce grown in soil and hydroponic systems with IGSA chelated fertilizer

The lowest defined taxon	OTU	Hydroponics				Soil experiment					
		leaf		root		leaf			root		
		HLK	HLI	HRK	HRI	SLK	SLIr	SLIf	SRK	SRIr	SRIf
species	*Rhodococcus qingshengii*	0.0	0.7	0.0	0.0	31.8	33.8	34.6	19.8	11.5	12.4
genus	*Pseudarthrobacter*	6.7	2.2	0.0	2.5	0.3	0.0	0.1	3.0	0.4	0.2
genus	*Streptomyces*	0.0	0.0	0.2	5.8	0.0	0.0	0.1	2.7	0.7	2.5
family	*Muribaculaceae*	0.7	11.4	0.3	0.0	0.0	0.0	0.1	0.0	0.7	1.2
genus	*Myroides*	0.0	0.0	0.0	0.0	0.9	0.3	2.4	0.7	0.2	0.6
family	*NS11-12 marine group*	2.7	0.9	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.1
genus	*Pedobacter*	2.2	0.7	0.4	0.5	0.0	0.0	0.0	0.2	0.0	0.2
family	*env.OPS 17*	1.7	2.7	3.6	2.6	2.1	1.5	1.8	0.8	1.1	1.7
family	*Simkaniaceae*	5.3	1.8	0.5	0.1	0.0	0.0	0.0	2.9	6.5	5.5
genus	*Lactobacillus*	1.1	23.0	2.6	0.8	0.6	0.4	1.8	0.2	5.0	6.5
genus	*Candidatus Arthromitus*	0.0	2.4	0.0	0.0	0.0	0.0	0.0	0.0	0.6	1.0
genus	*Romboutsia*	0.0	0.2	3.6	0.1	0.0	0.0	0.0	0.0	0.4	0.5
family	*Peptostreptococcaceae*	0.0	0.1	3.2	0.2	0.0	0.1	0.1	0.0	0.6	0.3
order	*Saccharimonadales*	8.8	2.9	0.0	0.5	0.0	0.0	0.0	1.4	0.9	1.3
genus	*Reyranella*	4.1	2.4	0.8	1.8	0.8	0.9	0.0	0.4	0.3	0.3
genus	*Bosea*	1.7	0.6	0.0	2.3	0.0	0.0	0.0	0.2	0.0	0.1
genus	*Devosia*	2.1	0.7	0.1	1.2	0.0	0.1	0.0	2.5	0.5	0.7
genus	*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*	1.1	0.4	6.7	2.5	0.0	0.0	0.0	1.0	4.2	0.8
genus	*Sphingobium*	1.9	0.6	0.6	2.6	0.0	0.0	0.0	0.4	0.2	0.0
genus	*Sphingomonas*	10.2	9.5	13.0	10.9	5.1	8.6	8.9	6.8	6.1	7.9
genus	*Achromobacter*	0.0	0.0	0.1	3.2	0.0	0.0	0.0	0.1	0.4	0.0
genus	*Aquabacterium*	1.4	1.4	0.6	1.4	4.1	3.6	4.9	2.1	2.1	2.8
genus	*Massilia*	0.8	0.3	9.9	2.4	0.0	0.0	0.0	0.6	0.0	0.5
genus	*Methylophilus*	1.0	0.3	18.6	19.7	0.0	0.0	0.0	0.0	0.2	0.2
family	*Methylophilaceae*	0.0	0.0	0.4	0.4	0.0	0.0	0.0	1.1	2.5	1.4
family	*Enterobacteriaceae*	0.0	2.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
genus	*Pseudomonas*	0.0	0.4	5.1	1.7	37.5	35.0	30.8	13.5	12.5	6.9
genus	*Ureaplasma*	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.0	2.6	2.5

was evaluated (Table 2). Differences in dominant bacterial taxa in the lettuce endophytic microbiome were observed depending on the substrate used (soil/hydroponics). When using soil for lettuce cultivation, bacteria *Rhodococcus qingshengii* sp. with an abundance of 11.5-34.6 were found in leaves and roots, absent in the endophytic microbiome of plants grown in hydroponics. Conversely, bacteria of the genus *Methylophilus* were present only in hydroponic experiment samples.

In the control hydroponic lettuce leaf sample (HLK), the most abundant bacteria were from the genera *Sphingomonas* (abundance 10.2), *Pseudarthrobacter* (6.7), and *Reyranella* (4.1), family *Simkaniaceae* (5.3), and order *Saccharimonadales* (8.8). The endophytic microbial community of the root (HRK sample) was characterized by the following dominant genera: *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* (abundance 6.7), *Sphingomonas* (13), *Massilia* (9.9), *Methylophilus* (18.6), and *Pseudomonas* (5.1).

Using IGSA in the hydroponic experiment increased the abundance of bacteria from the families *Muribaculaceae* and *Enterobacteriaceae* and the genus *Lactobacillus* in leaves. At the same time, the abundance of bacteria from the genus *Pseudarthrobacter*, family *Simkaniaceae*, and order *Saccharimonadales* decreased. In the root microbial community, an increase in the abundance of bacteria from the genera *Pseudarthrobacter* and *Streptomyces* and a decrease in the abundance of bacteria from the families *Peptostreptococcaceae* and genera *Romboutsia*, *Massilia*, *Pseudomonas*, and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* occurred.

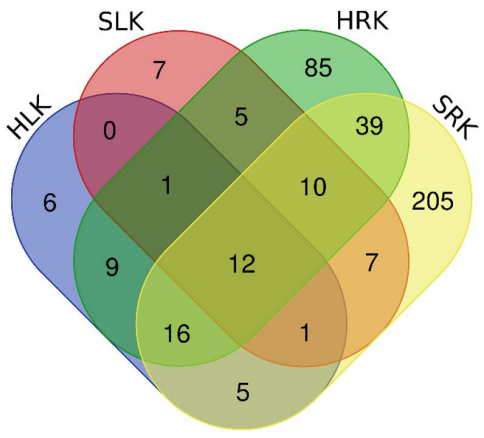

Figure 4. Overlapping bacterial OTUs in control samples of lettuce leaves and roots grown in soil and hydroponics

Table 3. Core bacterial OTUs in control samples of lettuce leaves and roots grown in soil and hydroponics

The lowest defined taxon	OTU
order	*Obscuribacterales*
genus	*Bradyrhizobium*
genus	*Reyranella*
family	*env.OPS 17*
genus	*Ralstonia*
family	*Caulobacteraceae*
genus	*Sphingomonas*
genus	*Pelomonas*
genus	*Methylobacterium*
genus	*Aquabacterium*
genus	*Sphingomonas*
genus	*Lactobacillus*

In the control soil-grown lettuce leaf sample, the dominant bacterial taxa were *Rhodococcus qingshengii* sp. (abundance 31.8) and genus *Pseudomonas* (37.5). In the root sample, the most frequent bacteria were *Rhodococcus qingshengii* sp. (abundance 19.8), genera *Sphingomonas* (6.8), and *Pseudomonas* (13.5). With the application of chelated fertilizer by both methods (root/foliar), an increase in the abundance of bacteria from the genus *Sphingomonas* and a slight decrease in the abundance of bacteria from the genus *Pseudomonas* were observed in the endophytic microbial community of lettuce leaves. Interestingly, using IGSA for lettuce grown in soil had a less pronounced effect on the number of dominant endophytic bacteria compared to its cultivation in hydroponics. The heterogeneous soil structure and sorption processes could have reduced the bioavailability of IGSA. Moreover, assembling the root microbiome in hydroponics fundamentally differs from that in soil, where plants can attract microbial partners from a more diverse pool. This assembly method leads to greater stability of the root microbiome in plants grown in soil compared to hydroponics.[26,28]

Overall, the results obtained for the endospheric communities of lettuce are similar to

those observed for substrates: the application of chelated fertilizer led to some changes in species composition and diversity, mainly when grown in hydroponics. However, no drastic changes in communities from the original (control) state occurred. The method of IGSA application did not lead to any additional changes in community composition.

Core endophytic microbiome of lettuce plants

Next, we identified bacterial taxa that constitute the core endophytic microbial community of lettuce leaves and roots, irrespective of the growing conditions (hydroponics/soil) (Figure 4). The core microbiome of plants consists of key microorganisms that contain genes selected through evolutionary mechanisms and necessary for plant function and adaptation.[29,30]

The core microorganisms for lettuce included 12 bacterial OTUs (Table 3). These microorganisms can inhabit various plants and many other ecological niches (soil, water, biofilms, aquifers, lake sediments).[31-34] Additionally, bacteria from some detected taxa (genus *Ralstonia* and genus *Sphingomonas*) are plant pathogens.[35-38]

Next, we identified unique OTUs present in control samples of lettuce leaves and roots depending on the substrate used. In lettuce grown in hydroponics, there were 6 and 85 unique OTUs in leaves and roots, respectively. When using soil, 7 and 205 unique bacterial OTUs were found in leaves and roots, respectively. Interestingly, using different substrates did not affect the number of unique OTUs in leaves, but significant differences were found in the number of unique OTUs in lettuce roots. Root-

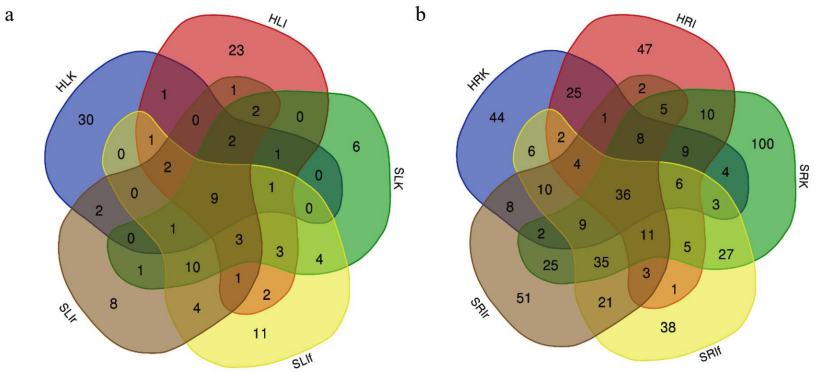

Figure 5. Overlapping bacterial OTUs in lettuce leaves (a) and root (b) samples grown in soil and hydroponics with IGSA chelated fertilizer

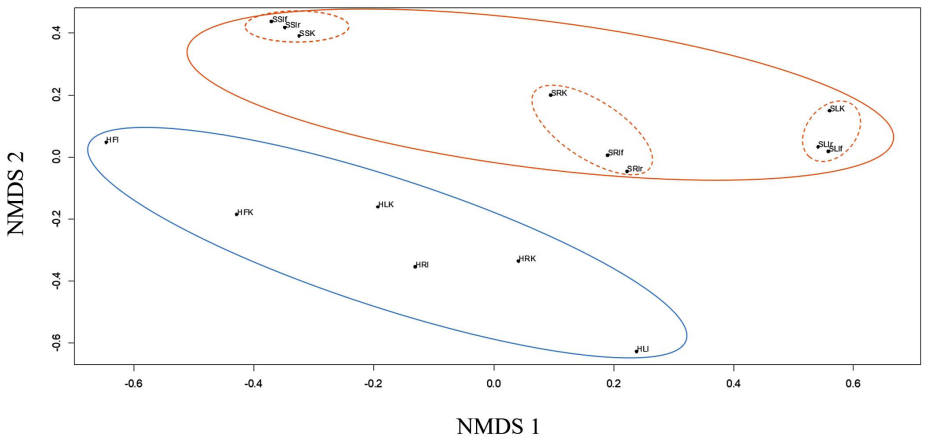

Figure 6. NMDS analysis of the bacterial communities of filtrate, soil and lettuce without and with IGSA chelated fertilizer

associated microorganisms mainly come from soil. The rhizosphere microbial community strongly influences the root microbiome composition. Soil microbial community structure and environmental parameters, including the substrate, are considered more critical factors for root bacterial community structure than plant genotype or species.[39,40]

Next, the microbial communities of lettuce leaves and roots grown on different substrates with and without IGSA chelated fertilizer were analyzed separately (Figure 5 a, b). Among the leaf microbial community, 9 core microorganisms were found regardless of the substrate and fertilizer treatment. More unique taxa (30 OTUs and 23 OTUs) were found in lettuce leaf samples grown in hydroponics, regardless of IGSA treatment (samples HLK and HLI). Overall, comparing microbial communities in root samples revealed more core and unique OTUs than in leaf samples. For example, 36 root core OTUs were found, three times the number of core OTUs in leaf samples. The analysis of core OTUs in microbial communities revealed 8 common endophytes for leaves and roots, present in lettuce plants regardless of growing conditions and IGSA treatment. Notably, the most significant differences in the number of unique OTUs were found between different root samples rather than leaf samples.

Beta-diversity of bacterial communities of soil, hydroponics, and Lettuce plants endosphere

Finally, non-metric multidimensional scaling (NMDS) was used to analyze the similarities and differences in microbial communities of lettuce leaves and roots, soil, and hydroponics. In Figure 6, each point represents an individual sample, and a greater distance between them indicates greater differences between the communities of the respective samples. The figure shows that points representing endophytic communities and substrate communities can be divided into two groups, H and S by substrate type. Within the S group, three subgroups of points corresponding to soil, leaf, and root communities can be distinguished.

Within each subgroup, distances between points representing communities in control and IGSA-treated variants do not differ. NMDS analysis confirms the data obtained using other sequencing analysis methods: the substrate type (soil, hydroponics) has the most significant influence on forming endophytic communities. Chelate application does not affect the composition of endophytic or substrate communities. Lettuce root and leaf communities differ significantly, especially when grown in soil, likely due to rhizosphere microorganisms migrating into plant tissues.

CONCLUSION

This study demonstrates that the new IGSA chelating micronutrient fertilizer does not adversely affect the endophytic microbial community of lettuce. The substrate type remains the most significant factor influencing microbial community composition and diversity. The findings support the potential use of IGSA as a safe and effective alternative to traditional EDTA-based fertilizers, contributing to more sustainable agricultural practices. The results suggest that the use of IGSA as a chelating agent in micronutrient fertilizers is a promising and environmentally safe alternative to EDTA. Given the minimal impact of IGSA on the endophytic microbial communities of lettuce, it could be recommended for broader agricultural use. The study highlights the importance of considering substrate types in agricultural practices, as they significantly influence microbial communities associated with plants. Future research should explore the long-term effects of IGSA on various crops and in different environmental conditions. Additionally, understanding the mechanisms behind the interaction between IGSA and plant-associated microorganisms will provide deeper insights into optimizing fertilizer use for sustainable agriculture.

SUPPLEMENTARY INFORMATION

Supplementary information accompanies this article at https://doi.org/10.22207/JPAM.20.2.43

Additional file: Table S1.

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None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

ND, GG and PG conceptualized the study and applied methodology. ND, LB, PK and GG performed Investigation, material preparation, data collection and formal analysis. ND and GG wrote the manuscript. SS performed supervision and project administration. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and/or in the supplementary files.

ETHICS STATEMENT

Not applicable.

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