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Gram-Negative Bacteria from Organic and Conventional Agriculture in the Hydrographic Basin of Loja: Quality or Pathogen Reservoir?

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Abstract: Organic and conventional agriculture are vital for the development of human society; however, the use of contaminated water and the inappropriate use of organic chemical fertilizers can lead to an increase in the microbial load (potentially pathogenic) of the normal microbiota of the agricultural soil. In this context, the aim of our study was to isolate Gram-negative bacteria from the superficial soil layer and irrigation water of agricultural areas (11 organic farms and nine conventional farms) and consider their potential ecological and health risk importance. Through culture isolation using three bacterial media (TSA) trypticase soy agar (general nutritive media); MacConkey Gram-negative bacteria and (EMB) eosin methylene blue agar (selective for Enterobacteriaceae) and classical biochemical tests, we recorded a total of 12 bacterial species, most belonging to the Enterobacteriaceae family, such as *Enterobacter*, *Escherichia*, *Klebsiella*, *Salmonella* and *Shigella*, which can be pathogenic for humans and animals. In contrast, bacteria such as *Pantoea agglomerans*, *Pseudomonas aeruginosa*, *P. fluorescens* and *Burkholderia mallei* could facultatively work as diazotrophic or plant growth-promoting rhizobacteria. Soil bacteria richness detected with the media applied was significantly higher than water bacteria, but we found no significant differences between organic and conventional agriculture. We conclude that the isolated bacteria in water and soil mostly belongs to enteropathogenic bacteria which could be pathogenic to animals and humans. While other bacteria like *Pseudomonas aeruginosa* could be viewed as useful by improving nutrient availability in agricultural soil.

Keywords: enteropathogens; organic manure; chemical fertilizer; biochemical tests; environmental contamination

1. Introduction

Agriculture is of vital importance for the development of human societies and has an enormous impact in the soil functional system [1], since almost 40% of the total land area is used for food production through agriculture [2]. Currently, organic agriculture has been revived and represents a growing proportion in the world economy [3], since the population seeks to obtain products which are "healthier" and free of chemicals harmful for humans and the environment [4]. Conventional agriculture is characterized by the usage of chemicals during its production [5]; on the contrary, organic agriculture is based on the effective natural management of plant-soil nutrition cycling [6] and makes use of natural organic compounds such as manure, compost and homemade fertilizers from animal waste such as chicken manure to compensate for nutrient deficiencies [5].

In Ecuador and especially Loja, the smallholder farmers lack scientific knowledge regarding the correct soil management in agricultural practices [7]. Jimenez et al. [7] indicate that the most common practice in the Loja province is manual tillage (57%), including the incorporation of crop residues and animal manure to improve the soil production. This production in Ecuador and Loja province mainly corresponds to crops like maize, oats, vetch, potatoes, onion and beans [7,8]. The correct applications of this traditional knowledge could help "conservation agriculture" as was experimented with in small-scale farming in Ecuador's highlands [8].

However, the inadequate application of these natural organic fertilizers or the use of water lacking proper treatment could result in an increase of microbial load both in the normal soil microbiota and in the products grown in these areas [9]. It is known that soil is one of the major reservoirs of microbial biodiversity (i.e., soil microbiota, which includes bacteria, archaea, fungi, protists and viruses), playing a crucial role in agricultural ecosystems [10,11]. Many of them fulfill roles as catalysts of nutrient cycling, carrying out stabilization and disposal of minerals or transforming organic compounds into amino acids or nitrates (e.g., nitrogen fixation, mineralization, solubilization), facilitating absorption in plants, boosting their growth and improving production in agricultural and natural soils around the world [12]. Within the soil bacterial diversity, species such as those belonging to the genera *Acinetobacter*, *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Brevibacterium*, *Caulobacter*, *Cellulomonas*, *Clostridium*, *Corynebacterium*, *Flavobacterium*, *Micrococcus*, *Micrococcus*, *Mycobacterium*, *Pseudomonas*, *Rhizobium*, *Staphylococcus*, *Streptococcus* and *Xanthomonas* [13] form communities with great influence on plant growth and vitality [14]. Other bacterial genera, such as *Shigella*, *Vibrio*, *Salmonella*, *Enterococcus* and *Escherichia* with *E. coli* species, can also be found in soil and are considered fecal indicators, being related to animal or human contamination [15,16]. The latter bacteria genera are potential pathogens for humans and animals [17].

Organic production is currently increasing and so is use of fruits and vegetables in their fresh form, which could lead to outbreaks of infectious diseases such as human gastroenteritis, due to the presence of bacterial pathogens and other microorganisms from water used for irrigation [18–20] or from soil previously treated with compost or animal manure [21].

Many studies indicate that bacterial pathogens for animals and humans survive easily in soil and water [22]. Likewise, they can persist in the plant spermosphere-rhizosphere or phyllosphere, due to the formation of bacterial biofilms facilitating their adherence [21]. Efforts to study and control the microbial load potentially harmful to humans in different types of artisanal organic fertilizers are scarce in Ecuador [23]. Most are limited to reporting colony-forming unit (CFU) counts of total coliforms (*Escherichia*, *Klebsiella*, *Enterobacter* and *Citrobacter*) and *Eschericha coli* in minimally processed food products [24,25]. *Escherichia coli* and *Salmonella* spp. are the main pathogens causing enteric infections and diarrhea in humans, being associated with the consumption of contaminated food, mainly from agriculture [26–29]. The present study seeks to determine for the first time the isolated Gram-negative bacteria through three media in the hydrographic basin of Loja. We examined the surface layer of agricultural soil and the irrigation water of areas used for agricultural production. Our objectives were related to: (i) the assessment of their biochemical characteristics and ecological roles (quality or pathogenic reservoir), (ii) the exploration of differences between bacteria shaped by organic and conventional agriculture. Based on these objectives we expected more Gram-negative species in organic farms than conventional farms.

2. Materials and Methods

2.1. SamplingArea

The studied sites were located around Loja city (canton) between 2000 to 2400 m a.s.l., with an environmental annual mean temperature of 20 °C, 80% relative humidity and 900 mm^3 of precipitation [30]. Loja basin is mainly covered by native vegetation, grasslands and urbanization [31] with a population of over 200,000 inhabitants (INEC = Instituto Nacional de Estadísticas y Censos). Twenty rural farms (11 organic farms and nine conventional farms) were evaluated, where vegetables such as lettuce, cabbage, carrots, potatoes and legumes are grown combined, mainly under mixed soils (Vertisol or Mollisols) [7]. In all cases, the land had been used as a vegetable farm for at least three years.

The selection criteria for soil and water samples for each type of farm were defined as follows:

I. Organic: farms that apply organic fertilizers such as poultry manure and/or hand-processed compost to the agricultural soil and avoid or restrict the use of chemical compounds for soil fertilization and plague control.
II. Conventional: farms that apply commercial chemical fertilizers (e.g., N10-P30-K10, urea 46%, ammonium nitrate 36%) and plague control (e.g., metaldehyde; chlorpyrifos) for agricultural management. All conventional farms also use animal manure as a base fertilizer before applying chemical treatments according to the particular needs of each farm.

2.2. Experimental Design and Sampling

A total of 40 samples were taken corresponding to 20 rural farms (replicates = 11 organic farms and nine conventional farms). For water analysis, 20 water samples (500 mL of piped, non-chlorinated water) were collected at the intakes at the entrance of the farms, which come directly from a stream, using sterile glass bottles. The water spring used for crop irrigation may be shared by more than one farm, as indicated in the map (Figure 1). The irrigation caption of water to each farm is protected by wood fencing against cattle.

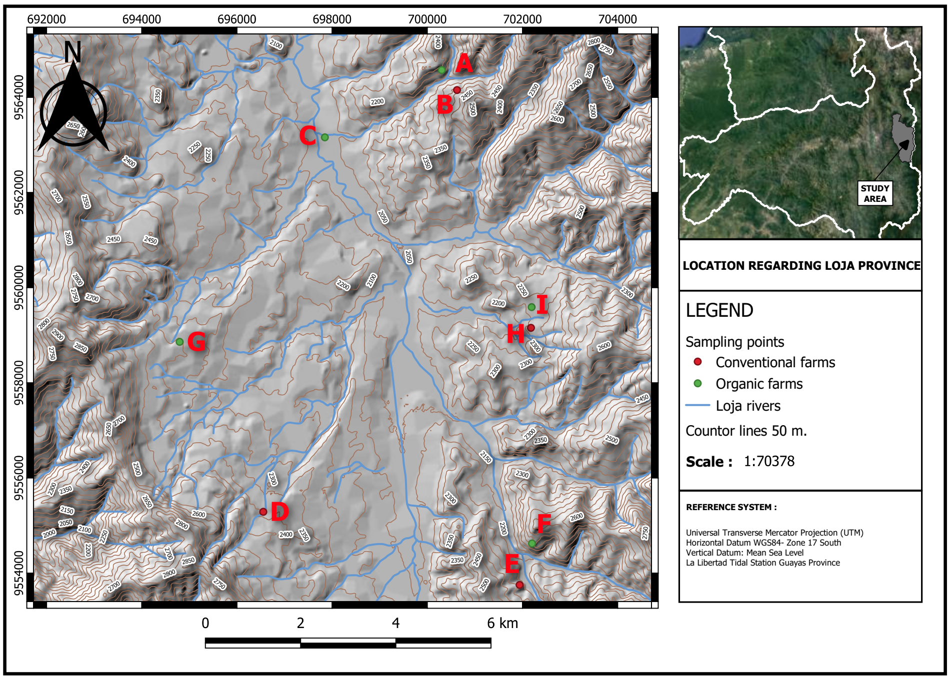

Figure 1. Location of the sampling zones in the basin of Loja city. (A, B) Shucos neighborhood, 3°56′18.4″, 79°11′45.3″ and 3°56′23.2″, 79°11′41.2″ respectively; (C) north zone, industrial park, 3°57′00.9″, 79°13′05.1″; (D) Colinas Lojanas zone, 4°01′17.3″, 79°13′46.5″; (E, F) Carmen neighborhood, 4°02′04.3″, 79°10′48.5″and 4°01′45.2″, 79°10′47.2″; (G) Carigan zone, 3°59′21.1″, 79°14′43.7″; (H, I) Yanacocha zone, 3°59′02.7″, 79°10′44.2″ and 3°58′56.8″, 79°10′44.0″. Type of farm: A, C, F, G, I (organic farm); B, D, E, H (conventional farm).

For soil microbiological analysis, 20 soil samples (1 kg) were obtained, one for each farm (11 organic farms and nine conventional farms), resulting from five randomly placed sub-samples. These sub-samples correspond to 200 g of sieved material (sieve 2 mm^2) from

500 g of soil taken at approximately 10 cm deep using a bore tool around the cultivated plants. All the samples were kept in a cooler (cooling boxes filled with ice blocks) and after sampling, these were analyzed immediately (1 h)

2.3. Isolation and Biochemical Identification of Bacterial Strains

For bacterial isolation, 1 g of each sample was suspended in 9 mL of 0.1% peptonized water and homogenized for 30 sec. Decimal dilutions of 10^{-1} to 10^{-2} were prepared from the water and soil samples, based on the probable microbial density (APHA, 1998), to obtain isolated colonies. The viable bacterial cells for both dilutions were counted as colony-forming units (CFUs). Duplicate aliquots of 100 µL of the dilutions were taken and spread on three types of solid media: trypticase soy agar (TSA) general nutritive media, MacConkey agar selective for Gram-negative bacteria and eosin methylene blue agar (EMB) selective for Enterobacteriaceae. Additional revision of colonies was done at 72 h to detect any color change or new growth of new colonies.

After the incubation period, 1 or 2 colonies per plate were selected for subculturing based on: differences in morphology, coloration or brightness and colony size. All pure cultures were analyzed via differential Gram staining. Then, another subculturing was performed on solid and semi-solid media to assure classical species identification with positive/negative biochemical reaction tests [32–34]. The latter test was carried out applying: Simmons citrate agar, iron-tripe sugar agar (TSI), urea agar, sulfide indole motility (SIM) and lysine iron agar (LIA). All subcultures and differential biochemical tests were incubated in aerobiosis for 24 h at 37 °C.

2.4. Data Analysis

The sampling effort of bacterial species in each sample type was estimated using species accumulation curves and a Chao 2 nonparametric richness estimator based on the incidence of species in a determined sample. The richness of microorganisms at the level of farm type (organic vs. conventional) and sample type (soil vs. water) was evaluated using an unpaired two-samples Wilcoxon test, because the data did not present a normal distribution ($p < 0.05$).

For community composition, a nonmetric multidimensional scaling analysis (NMDS) was performed to visualize the similarity between bacterial species composition according to farm type and sample type, using Euclidean distance and 999 Monte Carlo permutations. To analyze the effect of the farm type and sample type variables on the composition of the microorganism communities, a one-way analysis of similarities (ANOSIM) was performed. All analyses were done using the statistical "vegan" 2.5-7 package in the R environment [35].

3. Results

Biochemical Test

The previous colony-forming unit (CFU) count shows countable colonies between 30 and ≤ 300 for most farms (organic and conventional) for both dilutions (10^{-1} and 10^{-2}). Only two farms (one organic (C–Figure 1) and one conventional (D–Figure 1), presented uncountable ≥ 300 CFU in dilutions (10^{-1} and 10^{-2}).

From pure isolates (40 in total), 12 bacterial species were identified according to their biochemical reaction, out of which two were identified to genus level (Table 1). No new strains were obtained at 72 h.

Table 1. Bacterial species identified according to their biochemical reaction.

Bacterial Species	TSI	GAS	H_2S	CITRATE	UREA	MOTILITY	INDOL	LYSINE
Alkalescens dispar	ALC/A	−	−	−	−	−	+	+/−
Burkholderia mallei	A/A	−	−	+	−	−	−	−
Enterobacter cloacae	A/A	++	−	+	−	+	−	−
Enterobacter aerogenes	A/A	++	−	+	+/−	+	−	+
Escherichia coli	A/A	+	−	−	−	+/−	+	+

Table 1. *Cont.*

Bacterial Species	TSI	GAS	H_2S	CITRATE	UREA	MOTILITY	INDOL	LYSINE
Klebsiella oxytoca	A/A	++	−	+	+	−	+	+
Pantoea agglomerans	A/A	+/−	−	+	−	+	−	−
Proteus vulgaris	ALC/A	+/−	+	+/−	++	+	+	−
Pseudomonas aeruginosa	K/K	−	−	+	+	+	−	+/−
Pseudomonas fluorescens	K/K	−	−	+	−	+	−	+
Salmonella spp.	K/A	+	+	+	−	+	−	+
Shigella spp.	K/A	−	−	−	−	−	−	−

The references are show in methodology. Abbreviations and symbols: alkaline reaction (K), alkaline (ALC), acid (A), hydrogen sulfide (H_2S), iron-tripe sugar agar (TSI), positive (+), negative (−), variable reaction (+/−), GAS: Strong bubbles of gas that destroy the agar (++), Bubbles of gas without agar destruction (+). UREA: Slightly pinkish or fuchsia color (+), Strong fuchsia color (++).

Most bacteria can be correlated by urea, motility and lysine reactions and a few by strong generation of gas. On the other hand, *Shigella* spp., was not reactive for the biochemical test.

The highest isolated Gram-negative bacteria were found in soil samples taken from organic farms (Table 2). Five species present ubiquitous habitat, four species are frequently found in animals, humans or plants and two species are exclusively intracellular in human intestine. According to the ecology, eight bacteria species have a functional role related to plant-soil nutrition cycling or growth promotion. On the other hand, ecological information for three bacteria species was not found. Based on the bibliography, all bacteria species could present some pathogenic activity (Table 2).

Table 2. Gram-negative bacteria species isolated from water and soil samples for the two farm types and their ecology and pathogenicity based on literature.

Bacterial Species	Frequency–Number of Isolates by Species, Sample Type and Farm.				Habitat	Plant–Soil Nutrition Cycling or Growth Promotion	Pathogenicity	References
	O:W	C:W	O:S	C:S				
Burkholderia mallei			1		Exclusively: intracellular parasite.	Information not found.	Pathogen: equines, mammals including humans.	[36]
Enterobacter cloacae			5	1	Ubiquitous: terrestrial and aquatic environments including wastewater and plants. Human intestines.	Some strains alleviating salinity stress and promoting growth in plants due to high nitrogen fixation activity and produced iron carriers.	Nosocomial and opportunistic pathogens.	[37–40]
Enterobacter aerogenes	2		2	7	Ubiquitous: terrestrial and aquatic environments, including wastewater and plants. Intestine animals (including humans).	Some strains release high amounts of phosphorus and help their solubilization on soil.	Nosocomial and opportunistic pathogens.	[40–43]
Escherichia coli *Escherichia coli inactive (Alkalescens dispar)*	1		2	3	Frequently: intestines of animals (including humans).	Possible role in nitrogen cycle using ammonia and nitrate	Pathogen: animals (including humans).	[44,45]
Klebsiella oxytoca			2	1	Ubiquitous: terrestrial and aquatic environment and intestine of a wide range of animals.	Related to production of auxin (indole-3-acetic acid) and growth promotor for some plants.	Nosocomial and opportunistic pathogens.	[46–49]
Pantoea agglomerans			1		Frequently: Plants, soil and fecal matter of humans and animals.	Considered as diazotrophic endophyte in the stem of Japanese sweet potato	Pathogen: humans, plants and animals.	[50,51]
Proteus vulgaris			1		Frequently: environmental saprophyte, found on decaying animal matter and in contaminated water. Intestine of animals (including humans).	Volatile organic compounds on growth stimulation of Chinese cabbage	Nosocomial and opportunistic pathogens in animals (including humans).	[52–54]

Table 2. *Cont.*

Bacterial Species	Frequency–Number of Isolates by Species, Sample Type and Farm.				Habitat	Plant–Soil Nutrition Cycling or Growth Promotion	Pathogenicity	References
	O:W	C:W	O:S	C:S				
Pseudomonas aeruginosa		2			Ubiquitous: terrestrial and aquatic environments. Part of microbiota in animals (including humans).	Plant growth promoting through nitrogen accumulation, solubilization of phosphate, silicate and zinc. Additional positive for indole acetic acid.	Nosocomial and opportunistic pathogen in humans.	[55–57]
Pseudomonas fluorescens	1	1			Ubiquitous: terrestrial and aquatic environments. Non-pathogenic rhizobacteria.	Plant growth-promoting capacity through auxin-like phytohormones such as indole acetic acid.	Pathogen: many plants, and fish. Rarely pathogenic to humans.	[46,58–61]
Salmonella spp.	2	3	15	8	Frequently: intestine animals (including humans); survives in contaminated food and water.	Information not found.	Pathogen: animals (including humans).	[62,63]
Shigella spp.	6	1	4		Exclusively: human colon; survives in contaminated food and water.	Information not found.	Pathogen: humans.	[64,65]

Organic (O), conventional (C), water (W), soil (S).

The species accumulation curves indicated an adequate sampling for the two types of substrates (Figure 2). The Chao 2 estimator (https://search.r-project.org/CRAN/refmans/fossil/html/chao1.html, accessed on 4 May 2021) for the water samples indicated an adequate sampling, with six estimated and six observed species. As for the soil samples, the Chao 2 estimator indicated fifteen estimated species, compared to the 10 observed species, suggesting that the sampling is close to 70%.

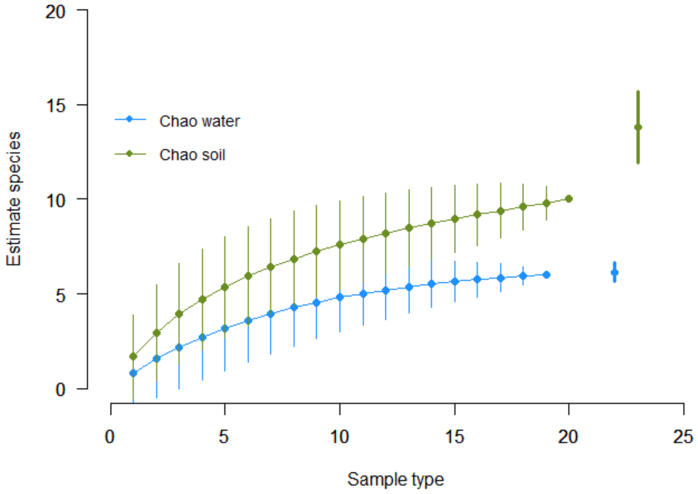

Figure 2. Rarefaction curves with 95% confidence intervals and Chao 2 estimator (points in the right of figure) according to substrate type.

Soil samples had a higher bacterial richness (Mann-Whitney test, $p < 0.0001$) compared to water samples (Figure 3). The type of farm did not have an effect on bacterial species richness ($p = 0.1433$).

The NMDS analysis indicated an ordering between bacterial communities related to farm and sample type (Figure 4). This was corroborated via ANOSIM analysis, which pointed out significant differences between the two sample types ($r = 0.1131$, $p = 0.0027$).

Conversely, type of farm (organic and conventional sampling points) does not show significant differences ($r = 0.0427$, $p = 0.1004$).

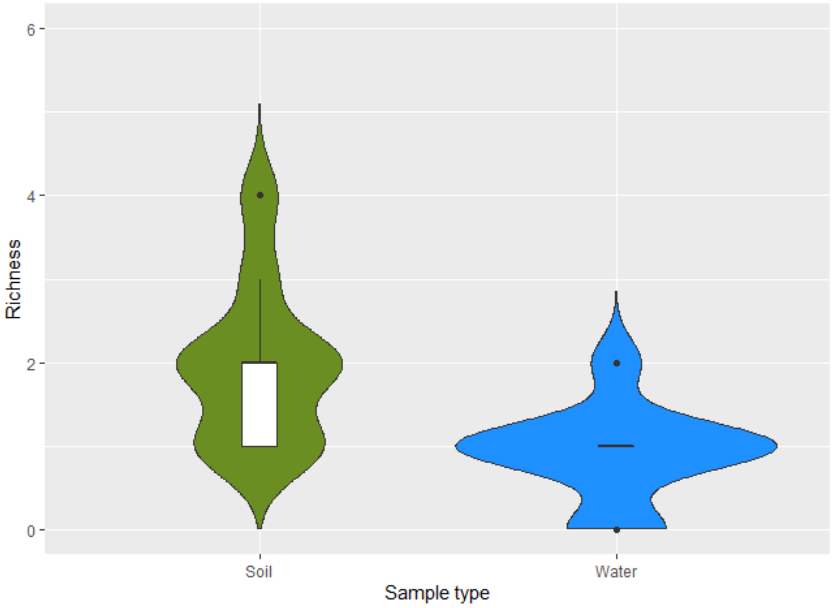

Figure 3. Bacterial richness according to sample type. Median richness (black horizontal bars); violin plots include the values within the min-max range; the width of the violin is proportional to kernel probability density of the data at different values.

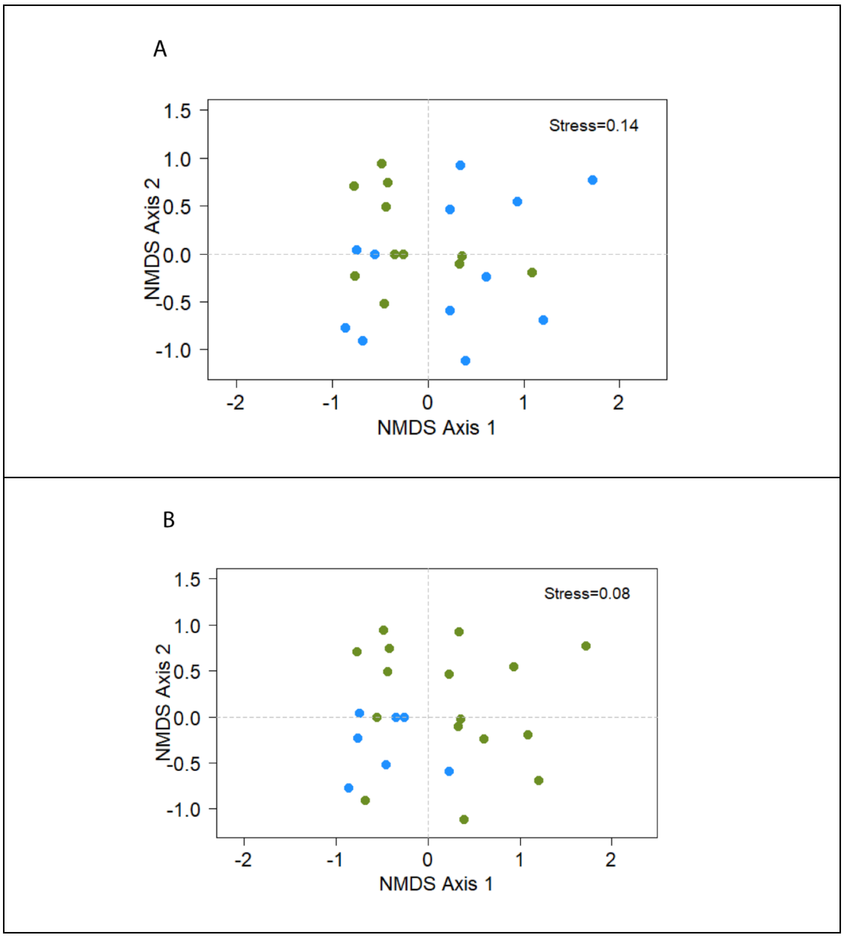

Figure 4. (**A**). Nonmetric multidimensional scaling (NMDS) plot of species composition and farms. Blue dots represent water samples and green dots represent soil samples. (**B**). Nonmetric multidimensional scaling plot of species composition and substrate. Blue dots represent water samples and green dots represent soil samples.

4. Discussion

Generally, microbiota are considered excellent catalysts, facilitating nitrogen fixation, mineralization and solubilization [10,11], transforming organic compounds, amino acids and proteins and improving their uptake for plants [66,67]. However, microbial diversity does not always have a positive impact on the functioning of ecosystems, including soil [68].

The results obtained in this study show a higher diversity of isolated Gram-negative bacteria in agricultural soil, compared to the water used for irrigation, which is consistent with previous research [21,69]. Although water can be a carrier of microorganisms, and thus a source of contamination, the fact that we have found a relatively low microbial load suggests that the water could be qualified as suitable for agricultural irrigation. However, it would be necessary to promote microbiological control of irrigation water as an additional element of food safety in the study area [70].

At the soil level, the dominant microbial pathogen group was *Salmonella* spp. This indicates a high contamination rate, with potential microbiological risk for humans, as demonstrated by Johannessen [71] and De Quadros et al. [72]. This bacterial load may be due to the high environmental survival rate of this type of bacteria; this can be for weeks in some types of cultivated plants such as lettuce or on substrates like compost [73]. Balkhair [74] indicates that several bacterial genera, amongst which are those found in our study, can have a high survival in soil, which can represent a pathogenic reservoir. This effect is frequent in soils considered organic, i.e., treated with animal manure (e.g., poultry manure) and compost [21,69,75,76], which generally change the composition of the agricultural soil microbiota [77]. On the other hand, the bacterium *Proteus vulgaris*, besides being an opportunist that causes urinary tract infections [78], possesses urease activity, which can increase the microbially-induced calcium carbonate precipitation (MIPC), thus affecting calcium availability and increasing soil hardness [79,80]. As mentioned before, all the farms sampled in the current study use animal manure as base fertilizer, applied 3–4 weeks before the planting of vegetables. This type of management may explain the overlap of bacterial species between organic and conventional farms, which are slightly different but non-statistically significant. High conservation of agrobiodiversity has been shown experimentally in small-scale organic farming in Ecuador's highlands [8]. Other fertilizers can be applied afterwards (e.g., N10-P30-K10; urea 46%; ammonium nitrate 36%) and plague control (e.g., metaldehyde; chlorpyrifos), usually between 10 to 15 days, or according to the particular needs of each of the conventional farms. This practice would explain the presence of pathogenic bacteria referenced as more resistant to extreme environmental conditions or chemicals [74,81].

Regarding bacteria isolated from water samples, the genus *Shigella* with the indicated species is the most common. Their presence is considered as an indicator of fecal pollution, exclusively from human excreta [82]. This genus easily accumulates at the interface formed between water and soil, in channels commonly called "MO deposits" [83]. Species of the genera (*Escherichia*, *Salmonella*, *Shigella*) are considered the main enteropathogens, causing diarrheal diseases in humans through food ingestion, many times coming directly from agriculture [26,29,84,85]. Similarly, *Burkholderia mallei* of the Burkholderiaceae family is considered pathogenic, causing "glanders" disease (contagious and re-emerging) in horses, donkeys and mules, but with low probability of transmission to humans, except for direct contacts with sick animals and other media [86]. Several genera of the Enterobacteriaceae family, such as *Enterobacter*, *Escherichia*, *Klebsiella*, *Salmonella* and *Shigella*, can be found in both soil and water; they include bacteria recognized as part of the microbiota of the digestive tract of animals such as cattle, horses and even humans, which can be natural dispersers to these ecosystems [87]. However, we cannot discard some human contamination due to the work of many farmers there who do not have knowledge about microbiological pollution.

On the other hand, our samples revealed the presence of the bacteria *Pseudomonas fluorescens*, a natural inhabitant of the phyllosphere, but which can survive and multiply in microhabitats associated with the rhizosphere and rhizoplane and be passively transported, forming biofilms in water flowing through saturated soils [88]. This species is a root-colonizing bacterium (rhizobacterium) [89], with plant growth-promoting capacity through auxin-like phytohormones such as indole acetic acid [61,90]. In addition, *P. fluorescens* is a pathogen controller via secondary metabolites, such as siderophores [91,92] or antibiotic-like substances (i.e., phenazine, pyrrolnitrin and pyocyanin) [93].

The present study indicates that sampled farmlands are a potential source of contamination and can affect human health, since the encountered type of bacteria has previously been reported by several authors as pathogenic [63,93–97]. The found frequency and the CFU measurements ($\leq$300) of isolation suggests that the risk of contamination of cultivated plants is a random process mostly associated to the specific manure in each farm, regardless of whether there are complementary organic or conventional management activities. Consequently, it is advisable to perform a microbiological quality analysis on these vegetables to establish acceptability for human consumption [72,98].

Probably some tests for solubilization and fixation of phosphorus, silicate, zinc and nitrogen or evaluation of the production of auxin (indole-3-acetic acid) or volatile organic compounds could be developed to evaluate our strains because some that are considered potential pathogens for humans are also important diazotrophic endophytes or PGPR, alleviating salinity stress and promoting growth in plants [39,41,45–47,54,59].

External environmental factors, chemicals and, most importantly, the introduction of foreign bacteria to the natural microbiota can upset the balance in the functionality of the natural microbiome by means of antagonistic reactions, parasitism, action of phytopathogens or natural competition for space [99]. Therefore, it is essential to maintain stability through the diversity ratio between "beneficial" and "harmful" microorganisms, especially in ecosystems such as those analyzed in this study.

5. Conclusions

The isolated Gram-negative bacteria determined in agricultural surface water and soil shows mostly the presence of enteropathogenic bacteria of animals and humans, along with other bacteria that, functionally, could be beneficial for improving nutrient availability in agricultural soil. The culture media as applied here does not allow isolate the whole Gram-negative bacteria diversity present in both types of samples, but allowed isolate the rare bacterium *Burkholderia mallei*.

Further studies are needed to improve the microbial culturability and understand the influence of organic and conventional management on the diversity of the natural microbiota of agricultural soil. Meanwhile, it is recommended that processes of maturation or previous fermentation of manure are implemented, which reduce the presence of pathogenic microorganisms.

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References

1. Uusitalo, V.; Kuokkanen, A.; Grönman, K.; Ko, N.; Mäkinen, H.; Koistinen, K. Environmental Sustainability Assessment from Planetary Boundaries Perspective—A Case Study of an Organic Sheep Farm in Finland. *Sci. Total Environ.* **2019**, *687*, 168–176. [CrossRef]
2. Foley, J.; Ramankutty, N.; Brauman, K.; Cassidy, E.; Gerber, J.; Johnston, M.; Mueller, N.; O'Connell, C.; Ray, D.; West, P.; et al. Solutions for a Cultivated Planet. *Nature* **2011**, *478*, 337–342. [CrossRef]
3. Nain, L.; Gaind, S.; Pandey, A.; Grover, M.; Sreekanthan, S.; Vasudevan, V. Practical Applications of Bioactive Compost in Organic Agriculture. In *Agriculturally Important Microorganisms*; Academic Word International: Bhopal, India, 2009; pp. 225–246.
4. Lans, C. Worldwide Opportunities on Organic Farms (WWOOF) as Part of the Existing Care Economy in Canada. *Geoforum* **2016**, *75*, 16–19. [CrossRef]
5. Fan, K.; Delgado-Baquerizo, M.; Guo, X.; Wang, D.; Zhu, Y.; Chu, H. Microbial Resistance Promotes Plant Production in a Four-Decade Nutrient Fertilization Experiment. *Soil Biol. Biochem.* **2020**, *141*, 107679. [CrossRef]
6. Hellequin, E.; Monard, C.; Quaiser, A.; Henriot, M.; Klarzynski, O.; Binet, F. Specific Recruitment of Soil Bacteria and Fungi Decomposers Following a Biostimulant Application Increased Crop Residues Mineralization. *PLoS ONE* **2018**, *13*, e0209089. [CrossRef]
7. Jiménez, L.; Jiménez, W.; Felicito, D.; Fierro, N.; Quichimbo, P.; Sánchez, D.; Capa-Mora, D. Rediscovering the edaphic knowledge of smallholder farmers in southern Ecuador. *Geoderma* **2021**, *406*, 115468. [CrossRef]
8. Barrowclough, M.; Stehouwer, R.; Alwang, J.; Gallagher, R.; Mosquera, V.H.B.; Dominguez, J.M. Conservation agriculture on steep slopes in the Andes: Promise and obstacles. *J. Soil Water Conserv.* **2016**, *71*, 91–102. [CrossRef]
9. Sanz-Cobena, A.; Lassaletta, L.; Estellés, F.; Del Prado, A.; Guardia, G.; Abalos, D.; Aguilera, E.; Pardo, G.; Vallejo, A.; Sutton, M.; et al. Yield-Scaled Mitigation of Ammonia Emission from N Fertilization: The Spanish Case. *Environ. Res. Lett.* **2014**, *9*, 125005. [CrossRef]
10. Yuan, H.; Ge, T.; Chen, C.; O'Donnell, A.; Wu, J. Significant Role for Microbial Autotrophy in the Sequestration of Soil Carbon. *Appl. Environ. Microbiol.* **2012**, *78*, 2328–2336. [CrossRef]
11. Zhao, Z.; He, J.; Quan, Z.; Wu, C.; Sheng, R.; Zhang, L.; Geisen, S. Fertilization Changes Soil Microbiome Functioning, Especially Phagotrophic Protists. *Soil Biol. Biochem.* **2020**, *148*, 107863. [CrossRef]
12. Delgado-Baquerizo, M.; Eldridge, D.; Ochoa, V.; Gozalo, B.; Singh, B.; Maestre, F. Soil Microbial Communities Drive the Resistance of Ecosystem Multifunctionality to Global Change in Drylands Across the Globe. *Ecol. Lett.* **2017**, *20*, 1295–1305. [CrossRef] [PubMed]
13. Konopka, A. Ecology microbial. In *Encyclopedia of Microbiology*, 4th ed.; Schaechter, M., Ed.; Elsevier: Amsterdam, The Netherlands, 2019; pp. 97–111.
14. Nihotimbere, V.; Ongena, M.; Smargiassi, M.; Thonart, P. Beneficial Effect of the Rhizosphere Microbial Community for Plant Growth and Health. *Biotechnol. Agron. Soc. Environ.* **2011**, *15*, 327–337.
15. Staley, Z.; He, D.; Edge, T. Persistence of Fecal Contamination and Pathogenic *Escherichia coli* O157:H7 In Snow and Snowmelt. *J. Great Lakes Res.* **2017**, *43*, 248–254. [CrossRef]
16. Uprety, S.; Dangol, B.; Nakarmi, P.; Dhakal, I.; Sherchan, S.; Shisler, J.; Jutla, A.; Amarasiri, M.; Sano, D.; Nguyen, T. Assessment of Microbial Risks by Characterization of *Escherichia coli* Presence to Analyze the Public Health Risks from Poor Water Quality in Nepal. *Int. J. Hyg. Environ. Health* **2020**, *226*, 113484. [CrossRef]
17. Heaton, J.; Jones, K. Microbial Contamination of Fruit and Vegetables and the Behavior of Enteropathogens in the Phyllosphere: A Review. *J. Appl. Microbiol.* **2008**, *104*, 613–626. [CrossRef]
18. Land & Water | Food and Agriculture Organization of the United Nations | FAO. Available online: http://www.fao.org/nr/water/topics_scarc_agri.html (accessed on 4 May 2021).
19. Matthews, K. Leafy vegetable. In *The Produce Contamination Problem. Causes and Solutions*, 2nd ed.; Academic Press: San Diego, CA, USA, 2014; pp. 187–206.
20. Slayton, R.; Turabelidze, G.; Bennett, S.; Schwensohn, C.; Yaffee, A.; Khan, F.; Butler, C.; Trees, E.; Ayers, T.; Davis, M.; et al. Outbreak of Shiga Toxin-Producing *Escherichia coli* (STEC) O157:H7 Associated with Romaine Lettuce Consumption, 2011. *PLoS ONE* **2013**, *8*, e55300. [CrossRef]
21. Szczech, M.; Kowalska, B.; Smolińska, U.; Maciorowski, R.; Oskiera, M.; Michalska, A. Microbial Quality of Organic and Conventional Vegetables from Polish Farms. *Int. J. Food Microbiol.* **2018**, *286*, 155–161. [CrossRef]
22. Yaron, S. Microbial Attachment and Persistence on Plants. In *The Produce Contamination Problem*, 2nd ed.; Academic Press: San Diego, CA, USA, 2014; pp. 21–57.
23. Ruiz-Pico, Á.; Pérez-Cuenca, Á.; Serrano-Agila, R.; Maza-Criollo, D.; Leiva-Piedra, J.; Salazar-Campos, J. Hydrochemical Characterization of Groundwater in the Loja Basin (Ecuador). *Appl. Geochem.* **2019**, *104*, 1–9. [CrossRef]
24. Hualpa, D.; Toledo, Z.; Meneses, M.; Feng, P. Microbiological Quality of Minimally Processed, Ready-to-Eat, Vegetables in Loja, Ecuador. *Rev. Politécnica* **2018**, *41*, 1–6.
25. Villa-Achupallas, M.; Rosado, D.; Aguilar, S.; Galindo-Riaño, M. Water Quality in The Tropical Andes Hotspot: The Yacuambi River (Southeastern Ecuador). *Sci. Total Environ.* **2018**, *633*, 50–58. [CrossRef]
26. Aijuka, M.; Buys, E. Persistence of Foodborne Diarrheagenic *Escherichia coli* in the Agricultural and Food Production Environment: Implications for Food Safety and Public Health. *Food Microbiol.* **2019**, *82*, 363–370. [CrossRef]

27. Msolo, L.; Iweriebor, B.; Okoh, A. Pervasiveness of Diarrheagenic *E. coli* Pathotypes and *Salmonella* Species Among Gastroenteritis Patients in Some Selected Pastoral Hinterlands of the Amathole District Municipality, Eastern Cape, South Africa. *Hum. Microbiome J.* **2020**, *17*, 100074. [CrossRef]
28. Shekar, A.; Babu, L.; Ramlal, S.; Sripathy, M.; Batra, H. Selective and Concurrent Detection of Viable *Salmonella* spp., *E. coli*, *Staphylococcus aureus*, *E. coli* O157:H7, and *Shigella* spp. in Low Moisture Food Products By PMA-Mpcr Assay with Internal Amplification Control. *LWT* **2017**, *86*, 586–593. [CrossRef]
29. Zhi, S.; Stothard, P.; Banting, G.; Scott, C.; Huntley, K.; Ryu, K.; Otto, S.; Ashbolt, N.; Checkley, S.; Dong, T.; et al. Characterization of Water Treatment-Resistant and Multidrug-Resistant Urinary Pathogenic *Escherichia coli* in Treated Wastewater. *Water Res.* **2020**, *182*, 115827. [CrossRef]
30. Ochoa-Cueva, P.; Fries, A.; Montesinos, P.; Rodríguez-Díaz, J.A.; Boll, J. Spatial estimation of soil erosion risk by land-cover change in the Andes of southern Ecuador. *Land Degrad. Dev.* **2015**, *26*, 565–573. [CrossRef]
31. Chuquimarca, L.; Gaona, F.P.; Iñiguez-Armijos, C.; Benítez, Á. Lichen Responses to Disturbance: Clues for Biomonitoring Land-use Effects on Riparian Andean Ecosystems. *Diversity* **2019**, *11*, 73. [CrossRef]
32. MacFaddin, J. *Pruebas Bioquímicas para la Identificación de Bacterias de Importancia Clínica*, 3rd ed.; Médica Panamericana: Madrid, Spain, 2003.
33. Mota, R.; da Silva, L.; da Silva, K.; da Silva Neto, J.; da Cunha, A.; do Nascimento Sobrinho, E. Caracterización Bioquímica y Perfil de Sensibilidad Antimicrobiana in Vitro de Muestras de *Burkholderia mallei* Aisladas de Équidos de la Región Nordeste de Brasil. *Arq. Inst. Biol* **2005**, *72*, 7–11.
34. Mayz, J.; Manzi, L. Bacterias Hidrocarburoclásticas del Género *Pseudomonas* en la Rizosfera de Samanea Saman (Jacq.) Merr. *Rev. Colomb. De Biotecnol.* **2017**, *19*, 29–37. [CrossRef]
35. Oksanen, J.; Blanchet, F.; Friendly, M.; Kindt, R.; Legendre, P.; McGlinn, D.; Minchin, P.; O'Hara, R.; Simpson, G.; Solymos, P.; et al. Package "Vegan". Community Ecology. 2021. Available online: https://github.com/vegandevs/vegan (accessed on 22 September 2021).
36. Inglis, T.; Merritt, A. Burkholderia pseudomallei and Burkholderia mallei. *Mol. Med. Microbiol.* **2015**, *2*, 769–791.
37. Bhar, S.; Edelmann, M.; Jones, M. Characterization and Proteomic Analysis of Outer Membrane Vesicles from a Commensal Microbe, *Enterobacter cloacae*. *J. Proteom.* **2021**, *231*, 103994. [CrossRef] [PubMed]
38. Bhise, K.; Bhagwat, P.; Dandge, P. Plant Growth-Promoting Characteristics of Salt Tolerant Enterobacter Cloacae Strain KBPD and its Efficacy in Amelioration of Salt Stress in Vigna Radiata L. *J. Plant Growth Regul.* **2016**, *36*, 215–226. [CrossRef]
39. Ji, C.; Liu, Z.; Hao, L.; Song, X.; Wang, C.; Liu, Y.; Li, H.; Li, C.; Gao, Q.; Liu, X. Effects of *Enterobacter cloacae* HG-1 on the Nitrogen-Fixing Community Structure of Wheat Rhizosphere Soil and on Salt Tolerance. *Front. Plant Sci.* **2020**, *11*, 1094. [CrossRef]
40. Cooney, S.; O'Brien, S.; Iversen, C.; Fanning, S. Bacteria: Other Pathogenic Enterobacteriaceae—Enterobacter and other Genera. *Encycl. Food Saf.* **2014**, *1*, 433–441.
41. Collavino, M.; Sansberro, P.; Mroginski, L.; Aguilar, O. Comparison of in Vitro Solubilization Activity of Diverse Phosphate-Solubilizing Bacteria Native to Acid Soil and their Ability to Promote *Phaseolus vulgaris* Growth. *Biol. Fertil. Soils* **2010**, *46*, 727–738. [CrossRef]
42. Sindhu, S.; Phour, M.; Choudhary, S.; Chaudhary, D. Phosphorus Cycling: Prospects of Using Rhizosphere Microorganisms for Improving Phosphorus Nutrition of Plants. *Geomicrobiol. Biogeochem.* **2013**, *39*, 199–237.
43. Thapa, L.; Lee, S.; Park, C.; Kim, S. Metabolic Engineering of *Enterobacter aerogenes* to Improve the Production of 2,3-Butanediol. *Biochem. Eng. J.* **2019**, *143*, 169–178. [CrossRef]
44. Samanta, I.; Bandyopadhyay, S.; Bhatia, R. *Antimicrobial Resistance in Agriculture*; Academic Press: London, UK; San Diego, CA, USA, 2020; pp. 171–193.
45. Taabodi, M.; Hashem, F.; Oscar, T.; Parveen, S.; May, E. The Possible Roles of *Escherichia coli* in the Nitrogen Cycle. *Int. J. Environ. Res.* **2019**, *13*, 597–602. [CrossRef]
46. Pavlova, A.; Leontieva, M.; Smirnova, T.; Kolomeitseva, G.; Netrusov, A.; Tsavkelova, E. Colonization Strategy of the Endophytic Plant Growth-Promoting Strains of *Pseudomonas fluorescens* and *Klebsiella oxytoca* on the Seeds, Seedlings and Roots of the Epiphytic Orchid, *Dendrobium nobile* Lindl. *J. Appl. Microbiol.* **2017**, *123*, 217–232. [CrossRef] [PubMed]
47. Celloto, V.; Oliveira, A.; Gonçalves, J.; Watanabe, C.; Matioli, G.; Gonçalves, R. Biosynthesis of Indole-3-Acetic Acid by New *Klebsiella oxytoca* free and Immobilized Cells on Inorganic Matrices. *Sci. World J.* **2012**, *2012*, 1–7. [CrossRef]
48. Pongsapipatana, N.; Damrongteerapap, P.; Chantorn, S.; Sintuprapa, W.; Keawsompong, S.; Nitisinprasert, S. Molecular Cloning of Kman Coding for Mannanase from *Klebsiella oxytoca* KUB-CW2-3 And Its Hybrid Mannanase Characters. *Enzym. Microb. Technol.* **2016**, *89*, 39–51. [CrossRef]
49. Tominaga, T. Rapid Detection of *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Raoultella ornithinolytica* and Other Related Bacteria in Food by Lateral-Flow Test Strip Immunoassays. *J. Microbiol. Methods* **2018**, *147*, 43–49. [CrossRef] [PubMed]
50. Asis, C.; Adachi, K. Isolation of Endophytic Diazotroph *Pantoea agglomerans* and Nondiazotroph *Enterobacter asburiae* from Sweetpotato Stem in Japan. *Lett. Appl. Microbiol.* **2004**, *38*, 19–23. [CrossRef]
51. Völksch, B.; Thon, S.; Jacobsen, I.; Gube, M. Polyphasic Study of Plant- and Clinic-Associated *Pantoea agglomerans* Strains Reveals Indistinguishable Virulence Potential. *Infect. Genet. Evol.* **2009**, *9*, 1381–1391. [CrossRef]

52. Delgado-Bermúdez, A.; Bonet, S.; Yeste, M.; Pinart, E. Long-Term Storage of Boar Seminal Doses Contaminated with *Proteus vulgaris*: A Dose-Dependent Effect on Sperm Motility and Sperm-Bacteria Interaction. *Anim. Reprod. Sci.* **2020**, *216*, 106349. [CrossRef] [PubMed]
53. Sun, L.; Sun, Y.; Jiang, M.; Luo, L.; Yu, X.; Yao, W.; Wu, Z. Isolation, Identification and Pathogenicity of *Proteus vulgaris* from Moribund Common Carp (*Cyprinus carpio*) Farmed in China. *Aquaculture* **2020**, *525*, 735294. [CrossRef]
54. Yu, S.; Lee, Y. Plant Growth Promoting Rhizobacterium *Proteus vulgaris* JBLS202 Stimulates the Seedling Growth of Chinese Cabbage through Indole Emission. *Plant Soil* **2013**, *370*, 485–495. [CrossRef]
55. Radhapriya, P.; Ramachandran, A.; Anandham, R.; Mahalingam, S. *Pseudomonas aeruginosa* RRALC3 Enhances the Biomass, Nutrient and Carbon Contents of *Pongamia pinnata* Seedlings in Degraded Forest Soil. *PLoS ONE* **2015**, *10*, e139881. [CrossRef] [PubMed]
56. Schinner, S.; Engelhardt, F.; Preusse, M.; Thöming, J.; Tomasch, J.; Häussler, S. Genetic Determinants of *Pseudomonas aeruginosa* Fitness During Biofilm Growth. *Biofilm* **2020**, *2*, 100023. [CrossRef]
57. Tripathy, S.; Kumar, N.; Mohanty, S.; Samanta, M.; Mandal, R.; Maiti, N. Characterisation of *Pseudomonas aeruginosa* Isolated from Freshwater Culture Systems. *Microbiol. Res.* **2007**, *162*, 391–396. [CrossRef]
58. Andreani, N.; Carraro, L.; Zhang, L.; Vos, M.; Cardazzo, B. Transposon Mutagenesis in *Pseudomonas fluorescens* Reveals Genes Involved in Blue Pigment Production and Antioxidant Protection. *Food Microbiol.* **2019**, *82*, 497–503. [CrossRef]
59. Ortiz-Castro, R.; Campos-García, J.; López-Bucio, J. *Pseudomonas putida* and *Pseudomonas fluorescens* Influence *Arabidopsis* Root System Architecture through an Auxin Response Mediated by Bioactive Cyclodipeptides. *J. Plant Growth Regul.* **2019**, *39*, 254–265. [CrossRef]
60. Nkoh, J.; Yan, J.; Xu, R.; Shi, R.; Hong, Z. The Mechanism for Inhibiting Acidification of Variable Charge Soils by Adhered *Pseudomonas fluorescens*. *Environ. Pollut.* **2020**, *260*, 114049. [CrossRef] [PubMed]
61. Siddiqui, I.; Shahid Shaukat, S. Suppression of Root-Knot Disease by *Pseudomonas fluorescens* CHA0 in Tomato: Importance of Bacterial Secondary Metabolite, 2,4-Diacetylpholoroglucinol. *Soil Biol. Biochem.* **2003**, *35*, 1615–1623. [CrossRef]
62. Patel, A.; Jeyasekaran, G.; Jeyashakila, R.; Anand, T.; Wilwet, L.; Pathak, N.; Malini, A.; Neethiselvan, N. Prevalence of Antibiotic Resistant *Salmonella* spp. Strains in Shrimp Farm Source Waters of Nagapattinam Region in South India. *Mar. Pollut. Bull.* **2020**, *155*, 111171. [CrossRef]
63. Krzyzanowski, F.; de Souza Lauretto, M.; Nardocci, A.; Sato, M.; Razzolini, M. Assessing the Probability of Infection by *Salmonella* due to Sewage Sludge Use in Agriculture Under Several Exposure Scenarios for Crops and Soil Ingestion. *Sci. Total Environ.* **2016**, *568*, 66–74. [CrossRef] [PubMed]
64. Han, S.; Kim, D.; Kim, B.; Chi, Y.; Kang, S.; Park, H.; Jung, S.; Lee, J.; Oh, T. Complete Genome Sequencing of *Shigella* sp. PAMC 28760: Identification of Cazyme Genes and Analysis of their Potential Role in Glycogen Metabolism for Cold Survival Adaptation. *Microb. Pathog.* **2019**, *137*, 103759. [CrossRef] [PubMed]
65. Lu, H.; Yan, P.; Xiong, W.; Wang, J.; Liu, X. Genomic Characterization of a Novel Virulent Phage Infecting *Shigella fiexneri* and Isolated from Sewage. *Virus Res.* **2020**, *283*, 197983. [CrossRef]
66. Jansson, J.; Hofmockel, K. The Soil Microbiome—From Metagenomics to Metaphenomics. *Curr. Opin. Microbiol.* **2018**, *43*, 162–168. [CrossRef]
67. Jacoby, R.; Peukert, M.; Succurro, A.; Koprivova, A.; Kopriva, S. The Role of Soil Microorganisms in Plant Mineral Nutrition—Current Knowledge and Future Directions. *Front. Plant Sci.* **2017**, *8*. [CrossRef]
68. Shade, A. Diversity is the Question, not the Answer. *ISME J.* **2016**, *11*, 1–6. [CrossRef]
69. Rusiñol, M.; Hundesa, A.; Cárdenas-Youngs, Y.; Fernández-Bravo, A.; Pérez-Cataluña, A.; Moreno-Mesonero, L.; Moreno, Y.; Calvo, M.; Alonso, J.; Figueras, M.; et al. Microbiological Contamination of Conventional and Reclaimed Irrigation Water: Evaluation and Management Measures. *Sci. Total Environ.* **2020**, *710*, 136298. [CrossRef]
70. Soonvald, L.; Loit, K.; Runno-Paurson, E.; Astover, A.; Tedersoo, L. The Role of Long-Term Mineral and Organic Fertilization Treatment in Changing Pathogen and Symbiont Community Composition in Soil. *Appl. Soil Ecol.* **2019**, *141*, 45–53. [CrossRef]
71. Johannessen, G. *Use of Manure in Production of Organic Lettuce*; Norwegian School of Veterinary Science, National Veterinary Institute: Oslo, Norway, 2005.
72. De Quadros, R.; Loiko, M.; Minéia Daniel de Paula, C.; Hessel, C.; Jacxsens, L.; Uyttendaele, M.; Bender, R.; Tondo, E. Microbiological Contamination Linked to Implementation of Good Agricultural Practices in The Production of Organic Lettuce in Southern Brazil. *Food Control* **2014**, *42*, 152–164. [CrossRef]
73. Jechalke, S.; Schierstaedt, J.; Becker, M.; Flemer, B.; Grosch, R.; Smalla, K.; Schikora, A. *Salmonella* Establishment in Agricultural Soil and Colonization of Crop Plants Depend on Soil Type and Plant Species. *Front. Microb.* **2019**, *10*. [CrossRef] [PubMed]
74. Balkhair, K. Modeling Fecal Bacteria Transport and Retention in Agricultural and Urban Soils Under Saturated and Unsaturated Flow Conditions. *Water Res.* **2017**, *110*, 313–320. [CrossRef] [PubMed]
75. Gerba, C. The Role of Water and Water Testing in Produce Safety. In *Microbial Safety of Fresh Produce*; Wiley-Blackwell: Hoboken, NJ, USA, 2009; pp. 129–142. [CrossRef]
76. Jiang, X.; Shepherd, M. The Role of Manure and Compost in Produce Safety. In *Microbial Safety of Fresh Produce*; Wiley-Blackwell: Hoboken, NJ, USA, 2009; pp. 143–166.
77. De Corato, U.; Viola, E.; Arcieri, G.; Valerio, V.; Zimbardi, F. Use of Composted Agro-Energy Co-Products and Agricultural Residues Against Soil-Borne Pathogens in Horticultural Soil-Less Systems. *Sci. Hortic.* **2016**, *210*, 166–179. [CrossRef]

78. Drzewiecka, D. Significance and Roles of *Proteus* spp. Bacteria in Natural Environments. *Microb. Ecol.* **2016**, *72*, 741–758. [CrossRef] [PubMed]
79. Chaparro-Acuña, S.; Becerra-Jiménez, M.; Martínez-Zambrano, J.; Rojas-Sarmiento, H. Soil Bacteria that Precipitate Calcium Carbonate: Mechanism and Applications of the Process. *Acta Agronómica* **2018**, *67*. [CrossRef]
80. Ghobadi Nia, M.; Rahimi, H.; Sohrabi, T.; Naseri, A.; Tofighi, H. Potential Risk of Calcium Carbonate Precipitation in Agricultural Drain Envelopes in Arid and Semi-Arid Areas. *Agric. Water Manag.* **2010**, *97*, 1602–1608. [CrossRef]
81. Cui, H.; Sun, W.; Delgado-Baquerizo, M.; Song, W.; Ma, J.; Wang, K.; Ling, X. The Effects of Mowing and Multi-Level N Fertilization on Soil Bacterial and Fungal Communities in a Semiarid Grassland are Year-Dependent. *Soil Biol. Biochem.* **2020**, *151*, 108040. [CrossRef]
82. Bliven, K.; Lampel, K. *Shigella in Foodborne Diseases*, 3rd ed.; Dodd, C.E.R., Aldsworth, T., Stein, R.A., Cliver, D.O., Riemann, H., Eds.; Academic Press: Cambridge, MA, USA, 2017; pp. 171–188. [CrossRef]
83. Duffy, E.; Lucia, L.; Kells, J.; Castillo, A.; Pillai, S.; Acuff, G. Concentrations of *Escherichia coli* and Genetic Diversity and Antibiotic Resistance Profiling of *Salmonella* Isolated from Irrigation Water, Packing Shed Equipment, and Fresh Produce in Texas. *J. Food Prot.* **2005**, *68*, 70–79. [CrossRef]
84. Googoolee, A.; Takooree, S.; Goburdhun, D.; Neetoo, H. Characterizing the Cultivation Practices and Microbiological Quality of Watercress. *J. Agric. Food Res.* **2020**, *2*, 100057. [CrossRef]
85. Schroeder, G.; Hilbi, H. Molecular Pathogenesis of Shigella Spp.: Controlling Host Cell Signaling, Invasion, and Death by Type III Secretion. *Clin. Microbiol. Rev.* **2008**, *21*, 134–156. [CrossRef] [PubMed]
86. Saxena, A.; Pal, V.; Tripathi, N.; Goel, A. A Real-Time Loop Mediated Isothermal Amplification Assay for Molecular Detection of *Burkholderia mallei*, the Aetiological Agent of a Zoonotic and Re-Emerging Disease Glanders. *Acta Tropica* **2019**, *194*, 189–194. [CrossRef]
87. Kanso, S.; Dasri, K.; Tingthong, S.; Watanapokasin, R. Diversity of Cultivable Hydrogen-Producing Bacteria Isolated from Agricultural Soils, Waste Water Sludge and Cow Dung. *Int. J. Hydrog. Energy* **2011**, *36*, 8735–8742. [CrossRef]
88. Danhorn, T.; Fuqua, C. Biofilm Formation by Plant-Associated Bacteria. *Annu. Rev. Microbiol.* **2007**, *61*, 401–422. [CrossRef] [PubMed]
89. Gobbin, D.; Rezzonico, F.; Gessler, C. Quantification of the Biocontrol Agent *Pseudomonas fluorescens* Pf153 In Soil Using a Quantitative Competitive PCR Assay Unaffected by Variability in Cell Lysis- And DNA-Extraction Efficiency. *Soil Biol. Biochem.* **2007**, *39*, 1609–1619. [CrossRef]
90. Meza, A.; Rojas, P.; Cely-Veloza, W.; Guerrero-Perilla, C.; Coy-Barrera, E. Variation of Isoflavone Content And DPPH• Scavenging Capacity of Phytohormone-Treated Seedlings After In vitro Germination of Cape Broom (Genista Monspessulana). *S. Afr. J. Bot.* **2020**, *130*, 64–74. [CrossRef]
91. Jiang, J.; Wang, J.; Yang, P.; Xu, Z.; He, T.; Gao, Q.; Wang, L.; Li, Q. Interactive Effects Between Cadmium Stabilized by Palygorskite and Mobilized by Siderophores from *Pseudomonas fluorescens*. *Ecotoxicol. Environ. Saf.* **2019**, *181*, 265–273. [CrossRef]
92. Williamson, A.; Folens, K.; Matthijs, S.; Paz Cortes, Y.; Varia, J.; Du Laing, G.; Boon, N.; Hennebel, T. Selective Metal Extraction by Biologically Produced Siderophores During Bioleaching from Low-Grade Primary and Secondary Mineral Resources. *Miner. Eng.* **2021**, *163*, 106774. [CrossRef]
93. Suresh, P.; Vellasamy, S.; Almaary, K.; Dawoud, T.; Elbadawi, Y. Fluorescent pseudomonads (Fps) as a Potential Biocontrol and Plant Growth Promoting Agent Associated with Tomato Rhizosphere. *J. King Saud Univ. Sci.* **2021**, *33*, 101423. [CrossRef]
94. Gerbal, C.; Smith, J. Sources of Pathogenic Microorganisms and Their Fate During Land Application of Wastes. *J. Environ. Qual.* **2005**, *34*, 42–48.
95. Jiménez, B.; Austin, A.; Cloete, E.; Phasha, C.; Beltrán, N. Biological Risks to Food Crops Fertilized with Ecosan Sludge. *Water Sci. Technol.* **2007**, *55*, 21–29. [CrossRef] [PubMed]
96. Pepper, I.; Zerzghi, H.; Brooks, J.; Gerba, C. Sustainability of Land Application of Class B Biosolids. *J. Environ. Qual.* **2008**, *37*, S58–S67. [CrossRef]
97. Navarro, I.; Jiménez, B.; Lucario, S.; Cifuentes, E. Application of Helminth Ova Infection Dose Curve to Estimate the Risks Associated with Biosolid Application on Soil. *J. Water Health* **2008**, *7*, 31–44. [CrossRef] [PubMed]
98. Turner, G.; Green, R.; Alae-Carew, C.; Dangour, A. The Association of Dimensions of Fruit and Vegetable Access in the Retail Food Environment with Consumption; a Systematic Review. *Glob. Food Secur.* **2021**, *29*, 100528. [CrossRef] [PubMed]
99. Hibbing, M.; Fuqua, C.; Parsek, M.; Peterson, S. Bacterial Competition: Surviving and Thriving in the Microbial Jungle. *Nat. Rev. Microbiol.* **2009**, *8*, 15–25. [CrossRef]