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

Dario Cruz 1,*, Rodrigo Cisneros 1, Ángel Benítez 1, Wilson Zúñiga-Sarango 1, Jhoan Peña 1, Heriberto Fernández 2 and Andrea Jaramillo 1

1 Microbial Systems Ecology and Evolution MS2E, Biodiversity of Tropical Ecosystems BIETROP, and ECOSLAB Research Groups, Biology School, Department of Biological and Agricultural Sciences, Technical University of Loja Particular, San Cayetano Alto s/n, Loja 1101608, Ecuador; rcisneros@utpl.edu.ec (R.C.); arbenitez@utpl.edu.ec (A.B.); wlzuniga@utpl.edu.ec (W.Z.-S.); jmpena@utpl.edu.ec (J.P.); acjaramillo4@utpl.edu.ec (A.J.)
2 Institute of Clinical Microbiology, Medicine School, Austral University of Chile, Valdivia 5090000, Chile; hfernand@uach.cl
* Correspondence: djcruz@utpl.edu.ec

Received: 22 September 2021
Accepted: 15 November 2021
Published: 22 November 2021

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 Escherichia 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. Sampling Area

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 mm³ of precipitation [30]. Loja basin is mainly covered by native vegetation, grass-
lands 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; chlorpyri- fós) 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.

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) from

![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).]
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$_2$S | 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 | H₂S | 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 | +   | +   | −       | −    | +        | −     | −      |

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. O:W C:W O:S C:S | Habitat                                                                 | Plant–Soil Nutrition Cycling or Growth Promotion | Pathogenicity | References          |
|----------------------------|----------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|-----------------------------------------------|--------------|---------------------|
| 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 inactive   | 1                                                                                | Frequently: intestines of animals (including humans).                                                                 | Possible role in nitrogen cycle using ammonia and nitrate | Pathogen: animals (including humans). | [44,45]            |
|                          | (Alkalescens dispar)                                                            |                                                                                                   |                                               |              |                     |
| 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 promotors for some plants. Considere as diazotrophic endophyte in the stem of Japanese sweet potato | Nosocomial and opportunistic pathogens. | [46–49]            |
| Pantoea agglomerans        | 1                                                                                | Frequently: Plants, soil and fecal matter of humans and animals.                                      | Related to production of auxin (indole-3-acetic acid) and growth promotors for some plants. Considere 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]            |

The references are show in methodology. Abbreviations and symbols: alkaline (K), alkaline (ALC), acid (A), hydrogen sulfide (H₂S), 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 (++).
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%.

Agronomy 2021, 11, x FOR PEER REVIEW 7 of 14

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 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 ($p = 0.1433$).

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.

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 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.

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.

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

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
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 (≤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.

**Author Contributions:** Conceptualization and methodology, D.C., R.C. and A.J.; sampling, R.C. and W.Z.-S.; bacteriological analysis, A.J., J.P. and D.C.; statistical analyses, Á.B.; contributed with their experience to improve the whole manuscript, H.F. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded indirectly by the Department of Biological and Agricultural Sciences of the Universidad Técnica Particular de Loja, using materials and reagents left over from different projects.

**Data Availability Statement:** All data are included into this document.

**Acknowledgments:** The authors would like to thank Diana Szekely for their critical language revision and some advice on writing. The authors are very grateful to the student Diego Piedra and many other students belonging to the Biological School and Biochemistry and Farmacy School who helped during sampling and laboratory work.

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

1. Uuisitalo, 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*, 337–343. [CrossRef]

3. Nain, L.; Gaid, S.; Pandey, A.; Grover, M.; Sreekantan, 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.* 2012, *78*, 2328–2336. [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.; Estellé, F.; Del Prado, A.; Guardia, G.; Abalos, D.; Aguilara, 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 Prostistics. *Soil Biol. Biochem.* 2020, *146*, 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. Upreti, S.; Dangol, B.; Nakarmi, P.; Dhakal, I.; Sherrahn, S.; Shisler, J.; Jutla, A.; Amarasi, 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. Hg. Environ. Health* 2020, *226*, 113484. [CrossRef]

17. Heaton, I.; 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, S.; Smolinska, 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, A.; 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]
78. Drzewiecka, D. Significance and Roles of Proteus spp. Bacteria in Natural Environments. Microb. Ecol. 2016, 72, 741–758. [CrossRef] [PubMed]

79. Chaparro-Acúa, 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. Ghabadi Nia, M.; Rahimi, H.; Sohrabi, T.; Naseri, A.; Tofigh, 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. 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]

84. 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]

85. Danhorn, T.; Fuqua, C. Biofilm Formation by Plant-Associated Bacteria. Annu. Rev. Microbiol. 2007, 61, 401–422. [CrossRef] [PubMed]

86. Meza, A.; Rojas, P.; Cely-Veloz, W.; Guerrero-Perilla, C.; Coy-Barrera, E. Application of Helminth Ova Infection Dose Curve to Estimate the Risks Associated with Irrigation Water, Packing Shed Equipment, and Fresh Produce in Texas. J. Food Prot. 2005, 68, 70–79. [CrossRef]

87.江, 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]

88. 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]

89. Suress, 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]

90. Gerbal, C.; Smith, J. Sources of Pathogenic Microorganisms and Their Fate During Land Application of Wastes. J. Environ. Qual. 2005, 34, 42–48.

91. 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]

92. 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]

93. 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]

94. Híbing, M.; Fuqua, C.; Parsek, M.; Peterson, S. Bacterial Competition: Surviving and Thriving in the Microbial Jungle. Nat. Rev. Microbiol. 2009, 8, 15–25. [CrossRef]