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Fish-processing effluent discharges influenced physicochemical properties and prokaryotic community structure in arid soils from Patagonia.

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Abstract

Along the Patagonian coast, there are processing factories of marine products in land that produce fish-processing effluents. The aim of the present study was to assess the physicochemical properties and the prokaryotic community composition of soils receiving fish-processing effluent discharges (effluent site-ES), and to compare them with those of unaltered soils (control site-CS) in the arid Patagonian steppe. We analyzed soil prokaryotic communities (using amplicon-based sequencing of 16S rRNA genes), soil physicochemical properties and fish-processing effluent characteristics. Soil moisture, electrical conductivity (EC), total and inorganic C were significantly higher in ES than in CS ($p < 0.05$). Effluent discharges induced a decrease in the total number of Operational Taxonomic Units (OTUs) and in the Shannon diversity index ($p = 0.0009$ and 0.01, respectively) of soil prokaryotic community. Proteobacteria, Actinobacteria and Acidobacteria were the dominant phyla in CS, while ES soil showed a more heterogeneous composition of phyla. Linear discriminant analysis (LDA) effect size (LEfSe) analysis showed that fish-processing effluent discharges promoted an enrichment of Firmicutes and Bacteroidetes, which are active contributors to organic matter mineralization, along with a decrease of oligotrophic phyla such as Acidobacteria, Chloroflexi, Armatimonadetes and Nitrospirae, commonly found in nutrient-poor arid soils. The concentrations of inorganic C and ammonium, the EC and the soil moisture explained 73% of the total variation within the community composition. Due to its salinity and nutrients, fish-processing effluents have potential mainly for native salt-tolerant plant irrigation, however the impacts of soil prokaryotic community shifts over plant growth remain to be determined.

Keywords

Industrial wastewater, Prokaryotic diversity, Soil nutrients, Soil C, Salinity, V4 region of 16S rRNA gene

1. Introduction

Drylands are of vital importance to our planet, not only because they are largely expanded (occupying about 40% of the total land area of the Earth) but also because they provide much of the world's grain and livestock (FAO, 2008). In these regions, the erratic and discontinuous input of precipitations together with the scarcity of nutrients, especially nitrogen, limit the primary productivity (Whitford and Duval, 2019). The lack of sufficient available water in arid and semi-arid regions leads to the consideration of non-conventional water resources (e.g. wastewater reuse) as an alternative to partially meet the water demands. Therefore, irrigation with treated wastewater contributes with water requirements for agriculture and landscaping in many countries (Becerra-Castro et al., 2015).

Fishing industry is currently increasing, reaching a total world production of 171 million tons in 2016 (FAO, 2018). Fish processing is characterized by high water consumption, which results in high wastewater production (de Melo Ribeiro and Naval, 2019). Although the quantity of fish-processing wastewater varies according to the processed species, the adopted technology and the manufactured product (Guimarães et al., 2018), it has been estimated that approximately 11 and 15 m^3 of water are consumed to process a ton of fish and shrimp, respectively (de Melo Ribeiro and Naval, 2017). Fish-processing wastewater is characterized by high Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Total Suspended Solids (TSS), resulting from the complex mixture of organic substances present in the effluents (Muthukumaran and Baskaran, 2013). Moreover, fish-processing wastewater may contain significant amounts of salts, oils, greases and nitrogen (Tay et al., 2006; Mseddi et al., 2014). After an appropriate treatment, it is possible to reuse fish-processing effluents for some industrial processes (de Melo Ribeiro and Naval, 2019; Guimarães et al., 2018). Treated fish-processing effluents also have the potential to be reused for irrigation in agriculture and landscaping, as they contain organic substances and nutrients that could be assimilated by plants and soil microorganisms. Nevertheless, the recovery and reuse of water and nutrients from such effluents has been scarcely explored. There are some evidences that wastewater from fish-processing industries has high potential to be reused as a liquid fertilizer in agriculture (Muthukumaran and Baskaran, 2013; Ching and Redzwan, 2017). Aerobic biodegradation of (8-folds diluted) fish-meal wastewater resulted in amino acid levels in the final broth comparable to those in a commercial fertilizer, and when more diluted (32-folds) wastewater was used, phytotoxicity of the biodegraded final broth was reduced, suggesting a potential of the fish-meal wastewater for fertilizer production (Kim et al., 2007). After treating wastewater from a fish-canning factory by coagulation/flocculation, Fahim et al. (2001) suggested that the final effluent, if not discharged to the area sewer, was safe to be used under controlled conditions in some irrigation applications or forestry projects at the desert area surrounding the factory.

Even though one of the mayor concerns about wastewater reuse for irrigation is the risk associated with the introduction of human pathogens to soil and crops, its impact on soil properties and on microbial communities, which are involved in important soil processes such as organic matter decomposition and nutrient cycling, must be also considered (Lüneberg et al., 2018). If not properly controlled, wastewater irrigation may induce an excessive soil organic matter and nutrient supply (e.g. nitrogen and phosphorous), changes in soil pH, insertion of exogenous microorganisms and soil salinization/sodification (Delvaux Silva et al., 2016). For example, nutrient input through wastewater irrigation may have a fertilizer effect in agriculture and enhance the metabolic activity of soil microorganisms (Durán-Álvarez and Jiménez-Cisneros, 2014; Delvaux Silva et al., 2016). However, a long-term excessive nutrient application may lead to soil eutrophication (Mikkelsen et al., 1997). Moreover, salts in wastewater may negatively affect soil porosity and water holding capacity, and consequently produce detrimental effects on soil microorganisms and plants (Hussain et al., 2019). Thus, fish-processing wastewater reuse could contribute to cope with the problem of water and nutrient scarcity, particularly in arid ecosystems, but its effect on soil fertility needs to be better characterized.

In Argentina, 98% of the fishing activity is focused on marine species, of which the red shrimp (*Pleoticus muelleri* Bate, 1888) is the main product (accounting for 60 % in dollars of the Argentinean fishery exports, www.argentina.gob.ar/hacienda). Along the Patagonian coast, there are processing factories of marine products in land that produce fish-processing effluents as a result of their activities. The aim of the present study was to assess the physicochemical properties and the prokaryotic community composition of soils receiving fish-processing effluent discharges near a cluster of processing industries, and to compare them with those of unaltered soils from a nearby site in the arid Patagonian steppe. To reach that goal, we characterized prokaryotic community composition using amplicon-based sequencing of 16S rRNA genes from soil samples. Additionally, we analyzed the physicochemical properties of soil and fish-processing effluent samples, and related these properties to the changes in soil prokaryotic community composition. This study contributes with baseline information regarding a potential reuse of fish-processing wastewater for irrigation.

2. Material and methods

2.1. Study area and sampling

The study was conducted in a field near a cluster of fish-processing industries in Puerto Madryn City, Chubut Province, Argentina (42° 43′ S; 65° 02′ W). There, the vegetation is distributed in patches separated by bare soil, corresponding to a shrubland of *Larrea divaricata* Cav. with perennial grasses (León et al., 1998). Mean annual temperature is 13.4 ± 0.1°C, and mean annual precipitation is 177.9 ± 9.8 mm (1971–2016 time series, INTA SIPAS, http://anterior.inta.gov.ar/region/pas/sipas2/cmp/agromet/index.html). Soils are a complex of Typic Torriorthents (Pereyra and Bouza, 2019). We selected a site which received fish-processing effluent discharges (effluent discharge site, ES) and a nearby undisturbed control site (CS). At the CS, plant cover accounted for 46 % of the soil surface (with high occurrence of *Larrea divaricata* and *Chuquiraga avellanedae* Lorentz.). In contrast, the ES presented a visible stimulation of the vegetation (plant cover 70%), prevailing *Atriplex lampa* (Moq.) Gillies ex D.Dietr. and the invasive plant *Diplotaxis tenuifolia* (L.) DC. At each site, 5 modal size (height: > 1 m, diameter 1.5–2.5 m) plant-covered patches were randomly selected and two upper soil sub-samples (0–10 cm depth and 10 cm in diameter) were collected. Soil samples were immediately transported to the laboratory at 4°C. Each set of two sub-samples was subsequently pooled, homogenized and sieved through a 2 mm mesh for further processing. In addition, a sample of the effluent discharge was also collected and transported to the laboratory (4 °C) to be characterized.

2.2. Effluent analyses

Temperature and electrical conductivity (EC) were measured *in situ* with a Hanna HI 98192 probe, while pH was determined with a Hanna pH 211 instrument (Hanna Instruments, USA). Chemical Oxygen Demand (COD) was measured using a

Photometer Hanna HI83099 (Hanna Instruments, USA), (Method EPA 410.4-Adapted; Environmental Protection Agency, 1993). Biochemical Oxygen Demand (BOD) was measured by the method 5210B of the American Public Health Association (APHA, 2017). In addition, odor (Method 2150B), color (Method 2120B, using Aquaquant 14421 color kit, Merck), turbidity (Method 2130B), total solids (Method 2540.B), oils and greases (Method 5520B), and ammonium concentration (Method 4500-NH_3 F) determinations were carried out according to APHA (2017). Nitrate concentration was measured following EPA Method 352.1 (Keith, 1996). Sodium Adsorption Ratio (SAR) was analyzed as described in US Salinity Laboratory Staff (1954). Coliforms were tested as per the standard procedures of APHA (2017). Analyses were performed in triplicate and the results averaged.

2.3. Soil analyses

Soil moisture was gravimetrically evaluated (105 °C, 48 h) and all the results were expressed on the basis of dry soil weight. Soil texture was determined by the Bouyoucos' Hydrometer method (Bouyoucos, 1962). Soil EC, pH and SAR were assessed in soil saturation extracts as described in US Salinity Laboratory Staff (1954). Total soil carbon (C) and nitrogen (N) were measured using a CN628 Carbon/Nitrogen Determinator (LECO Corporation, USA). Inorganic C concentration was determined gravimetrically, after removing soil carbonates with 3N HCl (Allison and Moodie, 1965). The concentration of soil organic C was assessed by wet combustion (Nelson and Sommers, 1996). Ammonium concentration in soil sample extracts was analyzed according to Keeney and Nelson (1982), and nitrate and nitrite concentrations as described in Shand et al. (2008). All samples were analyzed in triplicate and the results averaged.

2.4. Soil DNA extraction and sequencing

Total DNA was extracted from *ca*. 0.5 g of soil samples using the FastDNA® SPIN Kit for Soil (MP Biomedicals, USA), following the manufacturer's instructions. DNA was quantified using a Quantus™ Fluorometer and the QuantiFluor® dsDNA Dye System (Promega Corporation, USA). The amplification of the V4 region of bacterial and archaeal 16S rRNA genes from the 10 soil DNA samples was performed using the HotStarTaq *Plus* Master Mix Kit (QIAGEN, USA), barcoded primers 515F/806R (Caporaso et al., 2011), and the following amplification program: 3 min at 94 °C, 28 cycles of 30 sec at 94 °C, 40 sec at 53 °C, and 60 sec at 72 °C, and a final elongation step at 72 °C for 5 min. Purification of the PCR products was performed using calibrated Ampure XP beads, and the purified products were paired-end sequenced (2 × 300) in an Illumina MiSeq Sequencing platform at MR DNA (Shallowater, TX, USA). Raw sequences of bacterial and archaeal 16S rRNA genes were deposited in the NCBI Sequence Read Archive (SRA) database, under project accession PRJNA562709 (https://www.ncbi.nlm.nih.gov/sra/PRJNA562709).

2.5. Bioinformatic analyses

MiSeq sequencing yielded 1,396,611 raw reads from 10 soil samples. Sequences were processed using the bioinformatic software mothur v.1.39.5, following the MiSeq SOP protocol (Schloss et al., 2009; last accessed October 2018; Kozich et al., 2013). Reads were aligned to a SILVA 16S rRNA gene reference alignment (Quast et al., 2013), and trimmed to overlap the correct region of the reference alignment. Further denoising was achieved by pre-clustering sequences that differed in less than 3 nucleotides. Chimeras were detected *de novo* with the VSEARCH algorithm and removed from the dataset (Rognes et al., 2016). Sequences were classified using a Naïve Bayesian classifier (Wang et al., 2007) based on the SILVA SSU database v132 (Quast et al., 2013), and chloroplasts, mitochondria, eukaryotic sequences, and sequences not assigned at least at the domain level were removed, so that only bacterial and archaeal sequences were retained. Sequences were clustered into operational taxonomic units (OTUs) at a 3% dissimilarity level.

2.6. Data analysis

The statistical significance of the differences in soil properties between ES and CS were evaluated by the Student's t test. The relationships among soil properties were analyzed by Spearman rank-order correlation test. Statistical analyses were carried out using SPSS 7.5 package (Norušis, 1997). Diversity analyses were performed using MicrobiomeAnalyst software (Dhariwal et al., 2017). Rarefaction curves of the number of OTUs observed at different sequencing depths were obtained for each sample (Fig. S1). The Good's coverage index was calculated as a measure of the depth of sequencing effort. Alpha diversity metrics (total observed OTUs, Shannon and Simpson diversity indices) were calculated based on a subsample of 65,992 sequences to fit the size of the smallest library. Permutational analysis of variance (PERMANOVA) based on the Bray-Curtis beta diversity index were performed to compare the community structure of different samples. The relationship between the prokaryotic community composition (based on an OTU relative abundance matrix) and the environmental variables was assessed by canonical correspondence analysis (CCA), using the R package *vegan* (Ter Braak, 1986; Oksanen et al., 2019). In this constrained ordination method only the community variation that can be explained by selected environmental variables is shown (Legendre and Legendre, 2012). We selected the concentrations of inorganic C and ammonium, the EC and the soil moisture as the constrained environmental variables in the CCA analysis. The variance inflation factor calculated on these variables was < 10, showing that they contain independent information (*i.e.*, they are not redundant). The significance of the model based on the selected environmental variables was tested with permutations (number of permutations: 999; Oksanen et al., 2019). Before CCA, environmental variables measured as concentrations were log-transformed, and all environmental variables were standardized to zero mean and standard deviation of one, to avoid different measure units in the multivariate analysis. LEfSe method, based on a normalized relative abundance matrix, was applied to search for statistically different biomarkers between sites (Segata et al., 2011). Such analysis was performed by MicrobiomeAnalyst package, using a LDA threshold score of 3.5 (Cui et al., 2018) and $\alpha = 0.1$. The correlation among the bioindicator relative abundances and the soil properties was analyzed by the Spearman rank-order correlation test.

3. Results and discussion

3.1. Effects of fish-processing effluent discharge on soil properties

The characteristics of the fish-processing discharge are presented in Table 1. It showed a reddish-brown color with strong smell. The pH of the discharge was close to neutral (Table 1), as commonly observed for seafood effluents (Thomas et al., 2015). Regarding the potential reuse of fish-processing effluents as a water and nutrient source, it is interesting to compare the quality of the fish-processing discharge with recommendations for irrigation water. EC and SAR values indicate moderate soil salinization and sodification hazards (Table 1). Other parameters such as BOD_5, COD, ammonium, and oils and greases were determined at high levels (Table 1). Overall, most of the chemical properties of the fish-processing discharge showed values that would exceed those considered by most of the worldwide water quality guidelines for irrigation (Jeong et al., 2016). In addition, according to the local Chubut Province Standards, EC and SAR values of the fish-processing discharge entail mild to moderate restrictions for irrigation reuse, while BOD, oils and greases, and coliforms overcome the limits recommended by that guideline (Chubut Province, Decree N° 1540/16; Table 1).

In both ES and CS, soil texture corresponded to loamy sand (Table 2). Soil moisture, EC, total C and inorganic C were significantly higher in ES than in CS (Table 2). In ES, the increased soil salinity is possibly associated to the concentrations of soluble salts contained in the fish-processing discharge. According to the Richards diagram for classifying irrigation waters, together the EC and SAR values of the fish-processing discharge (2.8 mS cm^{-1} and 12.9, respectively) indicate a very high and a medium risk of soil salinization and sodification, respectively (US Salinity Laboratory Staff, 1954). Moreover, waters with EC values above 2.25 mS cm^{-1} only allow the growth of the most salt-tolerant crops (US Salinity Laboratory Staff, 1954). Thus, as expected due to the increased soil salinity, a shift in ES vegetation was evident, with prevalence of the ever-green shrub *A. lampa* and the perennial plant *D. tenuifolia* (Fig. S2). The former is characterized by a high tolerance to drought and soil salinity, and its foliage concentrates salts (Caraciolo Maia et al., 2002; Soteras et al., 2013). In addition, *Diplotaxis tenuifolia* has been classified as a salt tolerant species with potential as vegetable crop for saline agriculture (de Vos et al., 2013).

In agreement with dryland characteristics, ES and CS soils showed low total and organic C concentrations (Table 2). Similar values of organic C as those in this study were observed in soils from an arid ecosystem of Patagonia under grazing disturbance, whereas higher values (0.5 to 0.7%) were observed in undisturbed sites (Marcos et al., 2019). Although the sampling site from this study was not under grazing disturbance, the degree of aridity may be high as a result of other factors, such as high evapotranspiration due to wind erosion or soil topography, which in turn may be associated to the low soil organic C values. Moreover, soil organic C concentration did not differ between sites, which was likely related with an intensification of soil prokaryotic activity in ES. According to the available evidence, there are discrepancies about the effects of effluent irrigation on the soil organic C pool. In other studies, soil organic C either decreased (Jueschke et al., 2008; Tarchouna et al. 2010), increased (Rusan et al., 2007; Bedbabis et al., 2014), or remained constant (Qian and Mecham, 2005; Ibekwe et al. 2018) after wastewater irrigation, which was associated with the wastewater organic matter composition and its mineralization by the soil microorganisms. In this study, the BOD_5/COD ratio of the fish-processing discharge was equal to 0.5, which suggests a biodegradable organic matter input into ES soil (Aloui et al., 2009).

On the other hand, the soil inorganic C concentration was significantly higher in ES than in CS (Table 2). Carbonate and bicarbonate as well as calcium and magnesium are essential elements for carbonate precipitation in soils (Bai et al., 2017). Such process is also affected by soil carbon dioxide, pH, water content and temperature (Entry et al., 2004). The fish-processing discharge could be a source of soluble calcium, magnesium and bicarbonate that, under the alkaline conditions of the studied soils, would benefit the formation of carbonates at ES. In line with the results of other studies, soil inorganic C concentration positively correlated with those of soluble Ca^{2+} and Mg^{2+} (Spearman´s rho = 0.64; *p* = 0.024; considering the entire dataset), suggesting its presence in the form of soil carbonates (Plaza-Bonilla et al., 2015; Guo et al., 2016).

At CS and ES soil pH values were alkaline, agreeing with those reported for the region (Olivera et al., 2016; Marcos et al., 2019), and unaffected by the fish-processing effluent discharge (Table 2). Previous studies have shown that soil pH may remain constant after wastewater irrigation (Truu et al., 2009; Singh et al., 2012); although pH increases (Qian and Mecham, 2005; Adrover et al., 2012), and decreases (Angin et al., 2005) have also been reported. In this study, the lack of significant differences in soil pH between ES and CS may be associated with the presence of carbonates in the former site, which act as soil buffering components preventing soil acidification (Wang et al., 2015).

Similarly, in both sites, the values of total soil N were low and within the range of those reported for other Patagonian soils (Carrera and Bertiller, 2010). In other studies, total N increased (Angin et al., 2005; Rusan et al., 2007; Truu et al., 2009) or remained constant (Kang et al., 2007) even after more than 20 years of wastewater irrigation (Adrover et al., 2012). It has been also reported that in low-fertility arid environments, some shrubs like *A. lampa* respond to increases in N and water supplies by increasing their N use efficiency and biomass production (Fernández et al., 2018). The observed vegetation stimulation in ES with high prevalence of *A. lampa* may suggest that the N supplied by the fish-processing effluents could have been taken up by these shrubs and used for biomass production, instead of being accumulated in soil (Fig. S2). Moreover, the nitrate + nitrite concentration did not significantly differ between sites, likely due to its wide variability in ES soils (Table 2). Ammonium concentration also greatly varied in ES soils, thus, despite its high input through the fish-processing discharge, there was not a significant difference in its soil concentration between sites

(Table 1 and 2). Nevertheless, a significant negative correlation between soil pH and ammonium concentration was found at ES (Spearman´s rho = -0.90; p = 0.019). This result suggests that, as observed in other wastewater irrigation studies, an increase of ammonium ions could enhance nitrification rates releasing free hydrogen ions (Jemai et al., 2013).

3.2. Response of soil prokaryotic community to fish-processing effluent discharge

Across all soil samples, we obtained a total of 846,329 archaeal and bacterial high-quality sequences. Based on 97% sequence similarity and subsampling to the smallest library (65,992), sequences were clustered into 11,795 OTUs. The Good's coverage index was over 97% for all samples, indicating that the sequencing effort was sufficient to estimate their prokaryotic diversity (Table 3). The number of OTUs and the Shannon diversity (the diversity index most sensitive to rare species; Sanz and Köchling, 2019) were significantly lower (p = 0.0009 and 0.01, respectively) in ES than in CS (Table 3). In contrast the Simpson index, which is an indicator of the dominant species in the prokaryotic community, did not significantly differ between sites (p = 0.10; Table 3). These results suggest that fish-processing effluent discharges induce a decrease in soil prokaryotic richness along with a reduction of prokaryotic diversity, in particular of the rare species. Congruently, permutational multivariate analysis of variance (PERMANOVA) separated ES from CS soils (R-squared: 0.34; $p < 0.009$), possibly reflecting the effect of the fish-processing effluent discharges on the soil prokaryotic community composition. The impact of altering soil prokaryotic diversity on ecosystem functioning is not yet fully understood; while some studies suggest that loss of prokaryotic diversity may negatively affect ecosystem functions (including plant species diversity and nutrient cycling), others state that due to the high functional redundancy of prokaryotic communities, biodiversity may be a buffer that prevents losses of ecosystem functions (Bonkowski and Roy, 2005; Tardy et al., 2014). According to the available evidence, the main beneficiaries of the nutrients supplied by wastewater irrigation are the plants, which may further induce changes in the prokaryotic community through rhizodepositions (Krause et al., 2020). Consequently, whether changes in prokaryotic diversity and community composition lead to adverse or beneficial effects for plant health or microbial ecosystem services has yet to be established (Krause et al., 2020).

Taxonomic assignment of the OTUs at the phylum-level revealed the presence of 2 archaeal and 21 bacterial phyla in ES and CS soils. Figure 1 shows the phyla with a relative abundance > 0.1% in the 10 samples. The remaining phyla, grouped as “other phyla” in Figure 1, include Armatimonadetes, Chlamydiae, Cyanobacteria, Deinococcus_Thermus, Elusimicrobia, Entotheonellaeota, Fibrobacteres, Nitrospirae, Patescibacteria, Rokubacteria, FBP, BRC1and other unclassified bacteria. In CS, Proteobacteria (22%), Actinobacteria (21%) and Acidobacteria (14%) were the dominant phyla (Fig. 1), agreeing with the findings in other drylands (Zeng et al., 2017; Marcos et al., 2019). On the other hand, the dominant phyla varied among ES samples (Fig. 1). In ES.1 and ES.5 samples, Proteobacteria relative abundance was about 50%, followed by Bacteroidetes (22%) and Actinobacteria (10 %), (Fig. 1). ES.2, ES.3 and ES.4 presented a relative abundance of Proteobacteria and Actinobacteria close to those of CS samples (Fig. 1), but other dominant phyla such as Bacteroidetes (26%) and Firmicutes (12%) in ES.2 and Gemmatimonadetes in ES.3 and ES.4 (25 and 14%, respectively) were also found (Fig. 1). In addition, the relative abundance of minority phyla varied among ES samples, and also with respect to CS (Fig. 1). Such shifts in the structure of the soil prokaryotic community were significantly related to some of the soil physicochemical properties, as shown by the Canonical Correspondence Analysis (CCA), (Fig. 2 and Table S3). The concentrations of inorganic C and ammonium, the EC and the soil moisture explained 73% of the total variation within the community composition (Table S3), and the reduced model based on these environmental variables was significant ($p = 0.001$, based on permutations). Axes CCA1 and CCA2 represented respectively 38% and 29% of the constrained variability, i.e., 28% and 21% of the total variation (Fig. 2). Axis CCA1 was mainly associated with high concentrations of ammonium and inorganic C and with the prokaryotic communities from samples ES.1 and ES.5, while CCA2 was associated with high EC, inorganic C and moisture, and with the prokaryotic communities from ES.2 and ES.3 (Fig. 2). These findings suggest that the fish-processing effluent discharge through its effects on soil inorganic C and ammonium concentrations, EC and moisture could affect the prokaryotic community structure, promoting the proliferation of certain groups of prokaryotes. For example, the sample ES.2 which had the highest EC (3.38 mS/cm), also showed the highest abundance of Halobacteria, an archaeal class ubiquitously distributed in high-salt environments (Gupta et al., 2015). It still remains unknown whether these changes in the prokaryotic community composition can be harmful or beneficial for plants (Krause et al., 2020).

To further analyze the soil prokaryotic community, a linear discriminant analysis (LDA) effect size (LEfSe) was conducted to determine the prokaryotic phyla and OTUs with significant abundance differences (with a LDA threshold of 3.5) between sites (Fig. 3). According to this analysis, the phyla enriched in ES were Firmicutes and Bacteroidetes (Fig. 3a). Firmicutes relative abundance positively correlated with soil total C, inorganic C, EC and nitrate + nitrite ($p < 0.05$; Table 4), while Bacteroidetes abundance correlated with such parameters along with soil moisture and ammonium concentration ($p < 0.05$; Table 4). Guo et al. (2017) observed that Bacteroidetes, one of the main contributors to the mineralization of organic matter, increases in response to reclaimed water irrigation of soil. Pyrosequencing analysis of 16S rRNA showed that *Bacillus*-like bacteria (belonging to phylum Firmicutes) were abundant in rhizospheric and non-rhizospheric saline soils, and most of the isolated *Bacillus* strains produced hydrolytic enzymes to degrade proteins, carbohydrates and lipids (Mukhtar et al., 2018). Thus, the tolerance of these groups to soil salinity and their ability to maintain their enzyme activity could explain their prevalence in ES. On the other hand, in CS, indicator groups were assigned to Acidobacteria, Chloroflexi, Armatimonadetes, Nitrospirae, Rokubacteria, Entotheonellaeota, FBP and Elusimicrobia (Fig. 3a). The relative abundance of most of them negatively correlated with the total and inorganic soil C ($p < 0.05$; Table 4). Most notably, all bioindicator phyla in CS showed a high significant and negative correlation with soil nitrate + nitrite concentration (Table 4). This result could be related with the oligotrophic nature of phyla such as Acidobacteria, Chloroflexi and Nitrospirae, which show slow growth rates and adaptations to grow in nutrient-poor environments (Lüneberg et al., 2018). In accordance, based on the limited number of available Armatimonadetes strains, it is believed that they are also oligotrophs sensitive to nutrient-rich culture media (Lee et al., 2014). Moreover, Candidate phylum Rokubacteria (formerly known as SPAM) possesses large genomes with the potential for a versatile and generalist metabolic strategy in oligotrophic environments (Becraft et al., 2017). In this study, Acidobacteria, Chloroflexi, Armatimonadetes and Entotheonellaeota also negatively correlated with EC, and Nitrospirae negatively correlated with SAR ($p < 0.05$; Table 4). At the OTU-level, LEfSe analysis identified 11 biomarkers with significant abundance differences between sites (Fig. 3b). Biomarker OTUs from bacterial lineages enriched in CS were assigned to the family Pyrinomonadaceae (2 OTUs) and to the phyla Actinobacteria (one OTU associated with the clone MB-A2-108), and those from archaeal lineages were assigned to Euryarchaeota (one unclassified OTU and another one associated to class Thermoplasmata), (Fig. 3b). The family Pyrinomonadaceae includes species isolated from semiarid soils using low-nutrient growth media (Wüst et al., 2016). This family is a member of the class Blastocatellia, which seems to comprise slow-growing K-strategists that prefer oligotrophic growth conditions (Wüst et al., 2016). Biomarker OTUs from genera *Pedobacter* (1), *Pontibacter* (1), *Gemmatimonas* (1), *Pseudomonas* (1), *Thermomonas* (1) and an unclassified Gammaproteobacteria (1) were most numerous in ES (Fig. 3b). Such OTUs correspond to microorganisms that could adapt to changes in soil conditions resulting from the organic matter, nutrient and salt input of fish-processing effluent discharges. The biomarker OTUs detected in this study, whose relative abundances consistently differ between ES and CS prokaryotic communities, are valuable for profiling soil bacterial communities in further irrigation studies using fish-processing effluents.

Wastewater irrigation can introduce pathogens to soil posing risks to the environment and the human health (Jaramillo and Restrepo, 2017). Fecal coliforms and potential pathogenic bacteria were found in fish-processing industrial effluents (Sivaraman et al., 2016; Rodrigues et al., 2017). Moreover, OTUs associated with fecal indicator bacteria, *Clostridium*, *Nocardia* and *Mycobacterium*, which may include potential pathogenic bacteria, were present in wastewater irrigated soils (Ibekwe et al., 2018). In this study, in congruence with the high coliform counts in the fish-processing discharge (Table 1), six OTUs affiliated to the family Enterobacteriaceae (one of them belonging to the genus *Pseudocitrobacter* and the rest unclassified) were detected (relative abundances ≤ 0.2 %) in some ES samples. *Pseudocitrobacter* strains may produce NDM-1 carbapenemase, an enzyme of medical concerns as it confers resistance to carbapenem antibiotics (Kämpfer et al., 2014). Just two unclassified Enterobacteriaceae OTUs were present, in low abundances (≤ 0.01 %), in some CS samples. The abundance of *Pseudomonas,* known to include opportunistic strains, was significantly higher in ES than in CS ($p = 0.048$). No OTU associated with the genus *Clostridium* was detected, while *Mycobacterium* OTUs were present in both CS and ES samples. These findings render the fish-processing effluents inappropriate for direct irrigation reuse. To improve the quality of such effluents, an integrated process which includes a physical pretreatment followed by biological and/or physicochemical treatments is needed (Tay et al., 2006). Such treatment process should also include a water disinfection step (*e.g.* chlorination, UV disinfection, ozonation) to ensure that the microbiological quality meets the guidelines recommended for treated wastewater used in agriculture (Blumenthal et al., 2000). Thereafter, fish-processing effluents could have potential for irrigation of native salt-tolerant species such as *A. lampa*, which is important as forage plant and for the revegetation of arid lands (Fernández et al., 2018).

4. Conclusion

In this study, fish-processing effluent discharges increased the heterogeneity of soil properties, reducing prokaryotic diversity and inducing shifts in the structure of the soil prokaryotic community. Soil moisture, electrical conductivity, inorganic C and ammonium contents were not only the most affected properties by the fish-processing discharge but also the factors driving the changes of the prokaryotic community composition in soil. Particularly, fish-processing effluent discharge promoted an enrichment of Firmicutes and Bacteroidetes which are active contributors to the mineralization of organic matter, along with a decrease of oligotrophic phyla such as Acidobacteria, Chloroflexi, Armatimonadetes and Nitrospirae commonly found in nutrient-poor arid soils as those in Patagonia. Due to its salinity and nutrients, fish-processing effluents have potential as an alternative water source mainly for irrigation of salt-tolerant plants, however its quality needs to be improved through a treatment process including disinfection to avoid sanitary risks. In addition, if fish-processing effluents are used for irrigation, the impacts of soil prokaryotic community shifts over plant growth remain to be determined. To our knowledge, this is the first study on the effects of fish-processing effluents, a worldwide-produced wastewater, on the structure of the soil prokaryotic community, and in turn on soil fertility.

Declarations of interest: none

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Figure Captions

Figure 1: Relative abundance of bacterial and archaeal phyla from control (CS.1 to CS.5) and effluent (ES.1 to ES.5) sites.

Figure 2: Canonical Correspondence Analysis of prokaryotic communities from ES and CS samples and soil physicochemical properties. Arrows represent constrained explanatory variables (inorganic C and ammonium concentrations, EC and moisture).

Figure 3: Linear discriminant analysis (LDA) effect size (LEfSe) analysis of prokaryotic abundance from: a- phyla and b-OTUs in effluent (white) and control (black) sites.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Table 1: Fish-processing effluent discharge characterization. HU: Hazen Units; TON: Threshold Odor Number; NTU: Nephelometric Turbidity Units; MPN: Most probable Number.

Effluent properties	**Values**	**Guidelines for irrigation water***
pH	7.6	6.5 – 8.4
Color (HU)	< 5 (reddish-brown color)	N.R.
Odor (TON)	10,000	N.R.
Turbidity (NTU)	93.5	N.R.
Total solids (g l^{-1})	1.2	0.5 – 2.0
EC (mS cm^{-1})	2.8	1.3 – 2.9
SAR	12.9	12 - 20
BOD_5 (mg l^{-1})	701	≤ 50
COD (mg $^{-1}$)	1320	N.R.
BOD_5/COD ratio	0.5	N.R.
Oils and greases (mg l^{-1})	34.0	≤ 10
Ammonium (mg l^{-1})	62.9	N.R.
Nitrate (mg l^{-1})	2.0	≤ 133
Total coliforms (MPN 100 ml^{-1})	> 1.1×10^6	N.R.
Fecal coliforms (MPN 100 ml^{-1})	1.1×10^5	Industrial crops, pasture and trees: Spray or sprinkler ≤ 10^5 Flood/furrow ≤ 10^3

*Chubut Province guidelines for irrigation water (adapted from Ayers and Westcot (1994) and Blumenthal et al. (2000)). Values correspond to mild to moderate restrictions for irrigation reuse. N.R.: No Recommendation (parameter not specified for irrigation water by Chubut Province guidelines).

Table 2: Soil properties at control (CS) and effluent (ES) sites. Data represents mean values ± standard error. Asterisk indicates significant differences between sites: significant at $p \leq 0.05$ (*) and $p \leq 0.01$ (**) according to Student's test.

Soil properties	**CS**	**ES**	**Significance**
Soil moisture (%)	9.4 ± 0.4	12.9 ± 0.7	**
pH	8.6 ± 0.1	9.0 ± 0.5	
Sand (%)	84.3 ± 0.7	78.4 ± 2.0	*
Silt (%)	12.0 ± 0.8	15.2 ± 2.4	
Clay (%)	3.7 ± 0.4	6.6 ± 1.4	
EC (mS cm^{-1})	0.6 ± 0.2	2.0 ± 0.5	*
SAR	6.6 ± 2.5	11.3 ± 2.6	
Total C (%)	0.42 ± 0.03	0.86 ± 0.10	**
Organic C (%)	0.26 ± 0.03	0.34 ± 0.09	
Inorganic C (%)	0.10 ± 0.02	0.46 ± 0.12	*
Calcium + Magnesium (meq l^{-1})	5.11 ± 1.17	7.55 ± 1.79	
Total N (%)	0.05 ± 0.003	0.06 ± 0.01	
Ammonium (µg g^{-1} dry soil)	4.0 ± 0.3	24.8 ± 13.7	
Nitrate + Nitrite (µg g^{-1} dry soil)	4.3 ± 0.1	74.1 ± 48.2	

Table 3: Alpha-diversity metrics for samples from effluent (ES) and control (CS) sites.

Sites	OTUs	Shannon Index	Simpson Index	Coverage
CS.1	6369	7.11	0.99	97.37
CS.2	6468	6.99	0.99	97.29
CS.3	6229	7.01	0.99	97.48
CS.4	5528	6.80	0.99	97.24
CS.5	6064	7.07	0.99	97.05
ES.1	2889	5.25	0.97	98.09
ES.2	3526	5.83	0.98	98.32
ES.3	3726	6.40	0.99	98.06
ES.4	5016	6.69	0.99	97.96
ES.5	3642	5.74	0.99	98.97

Table 4: Significant Spearman coefficients between relative abundances of LEfSe bioindicator phyla and soil properties (n = 10).

Phyla	**Total C**	**Inorganic C**	**EC**	**SAR**	**Moisture**	**Ammonium**	**Nitrite +Nitrate**
Firmicutes	0.70*	0.83**	0.70*				0.74*
Bacteroidetes	0.78**	0.71*	0.72*		0.64*	0.78**	0.74*
Acidobacteria	-0.74*	-0.79**	-0.68*		-0.64*	-0.65*	-0.86**
Chloroflexi	-0.72*	-0.82**	-0.71*			-0.82**	-0.70*
Armatimonadetes	-0.77**	-0.84**	-0.69*		-0.69*		-0.92**
Nitrospirae		-0.63*		-0.65*			-0.91**
Rokubacteria	-0.64*	-0.64*					-0.86**
Entotheonellaeota	-0.76*	-0.76*	-0.68*				-0.85**
FBP		-0.77**			-0.67*		-0.83**
Elusimicrobia					-0.64*		-0.81**

Highlights

- Fish-processing effluent discharges increased soil salinity, total and inorganic C.
- Effluent discharges decreased soil microbial diversity and total OTUs.
- Effluent discharges promoted an enrichment of Firmicutes and Bacteroidetes in soil.
- Effluent discharges induced a decrease of oligotrophic phyla in soil.
- Inorganic C, ammonium, EC and soil moisture explained 73% of community variation.

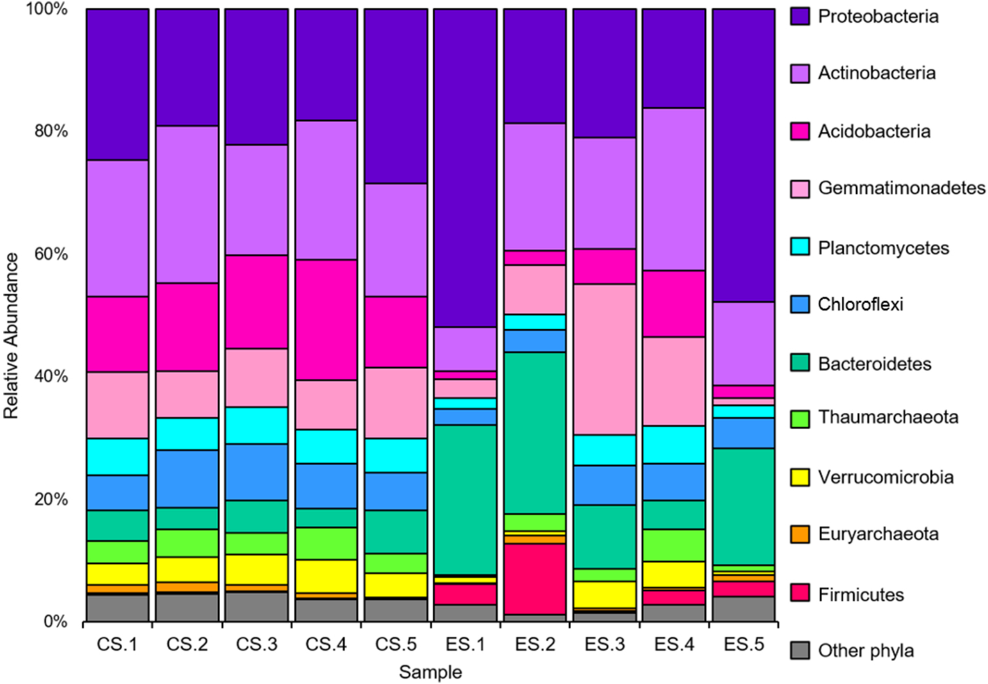
100%
80%
60%
40%
20%
0%
Relative Abundance
CS.1
CS.2
CS.3
CS.4
CS.5
ES.1
ES.2
ES.3
ES.4
ES.5
Sample
Proteobacteria
Actinobacteria
Acidobacteria
Gemmatimonadetes
Planctomycetes
Chloroflexi
Bacteroidetes
Thaumarchaeota
Verrucomicrobia
Euryarchaeota
Firmicutes
Other phyla

Figure 1

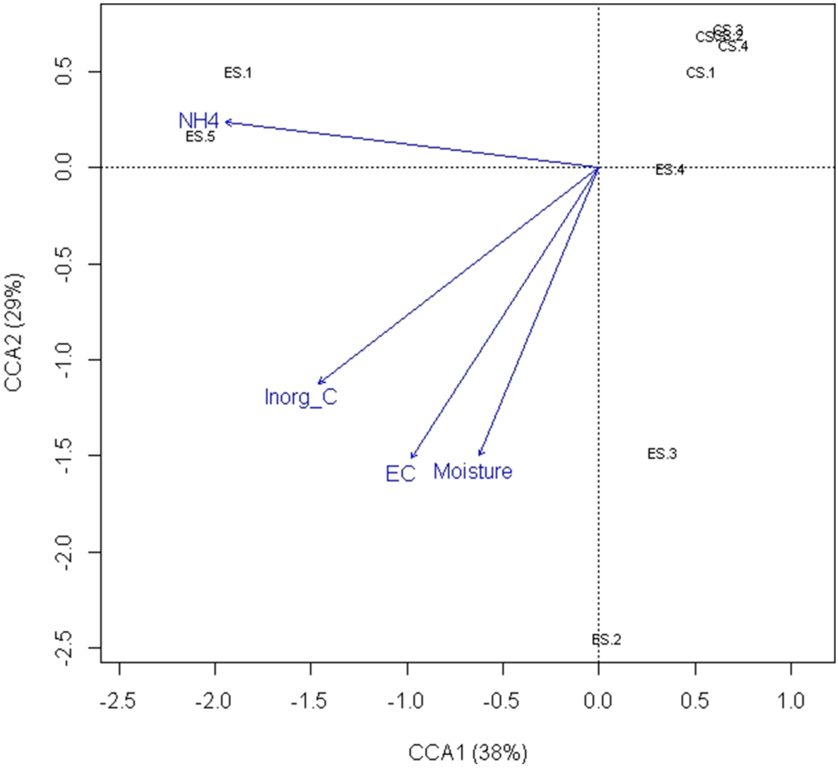
CS.3
CS.2
CS.4
CS.1
ES.1
NH4
ES.5
ES.4
Inorg_C
EC
Moisture
ES.3
ES.2
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
CCA2 (29%)
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
CCA1 (38%)

Figure 2

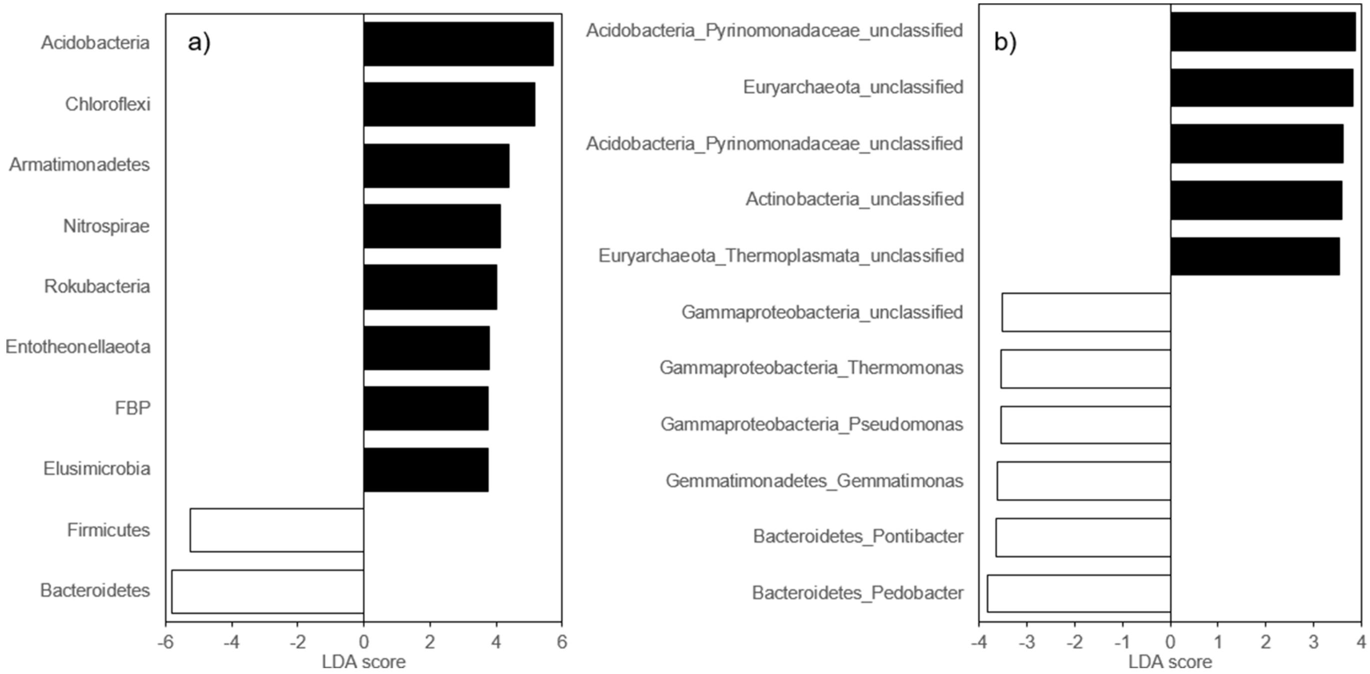
Acidobacteria
Chloroflexi
Armatimonadetes
Nitrospirae
Rokubacteria
Entotheonellaeota
FBP
Elusimicrobia
Firmicutes
Bacteroidetes
a)
-6
-4
-2
0
2
4
6
LDA score
Acidobacteria_Pyrinomonadaceae_unclassified
Euryarchaeota_unclassified
Acidobacteria_Pyrinomonadaceae_unclassified
Actinobacteria_unclassified
Euryarchaeota_Thermoplasmata_unclassified
Gammaproteobacteria_unclassified
Gammaproteobacteria_Thermomonas
Gammaproteobacteria_Pseudomonas
Gemmatimonadetes_Gemmatimonas
Bacteroidetes_Pontibacter
Bacteroidetes_Pedobacter
b)
-4
-3
-2
-1
0
1
2
3
4
LDA score

Figure 3