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Changes in the bacterial community composition of different habitats along a polluted river (Suquía River, Cordoba, Argentina)

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Abstract. The objective of the present study was to investigate the influence of environmental conditions on the bacterial community composition in water, sediment and riparian soil during different water flow periods. For this purpose, samples of three habitats (water, sediment and riparian soil) were collected from five polluted sites and one reference site along the Suquía River during high and low water flow periods. The pH, dissolved oxygen, conductivity and water temperature were measured "in situ", with total organic carbon content, nitrate and ammonia concentration being evaluated for all samples. In addition, pH, conductivity and total N were determined in the sediment and riparian soil samples, and the bacterial community composition of water, sediments and riparian soil samples was monitored using restriction fragment length polymorphism of the 16S rRNA gene. The results showed that the bacterial community composition of water was different from that of sediments or riparian soil. A redundancy analysis indicated that the changes in the bacterial community composition in the Suquía River were primarily correlated with variations in dissolved oxygen, conductivity and pH. The water bacterial community composition was very variable among sites and water flow periods, while that of sediments differed according to the water flow period, which was associated with temperature variation. Lastly, in riparian soil, differences were found in the bacterial community composition of the sites located before and after Cordoba city. Our findings suggest distinct distribution patterns in the bacterial community compositions of the three habitats evaluated.

[Keywords: organic C, lotic ecosystem, 16S rRNA gene, environmental variables]

Resumen. Cambios en la composición de la comunidad bacteriana de diferentes hábitats a lo largo de un río contaminado (Río Suquía, Córdoba, Argentina). El objetivo de este estudio fue investigar la influencia de la contaminación del Río Suquía sobre la composición de la comunidad bacteriana en agua, suelo de ribera y sedimentos en dos períodos de caudal de agua. Para ello se tomaron muestras de los tres hábitats (agua, sedimento, y suelo de ribera) en cinco sitios contaminados y un sitio de referencia a lo largo del Río Suquía en las épocas de alto y bajo caudal de agua. Se midió "in situ" el pH, el oxígeno disuelto, la temperatura y la conductividad del agua, mientras que en todas las muestras se determinó el contenido de carbono orgánico total, nitrato y amonio. Además, se midió el pH, la conductividad y el contenido de N total en sedimento y suelo de ribera. La composición de la comunidad bacteriana del agua, sedimento y suelo de ribera se analizó mediante polimorfismo de fragmentos largos de restricción del gen 16S ARNr. Los resultados mostraron que la composición de la comunidad bacteriana del agua fue diferente de la del sedimento y suelo de ribera. De acuerdo al análisis de redundancia realizado los cambios en la composición de la comunidad bacteriana en el Río Suquía fueron principalmente correlacionados con el oxígeno disuelto, la conductividad y el pH. La composición de la comunidad bacteriana en agua fue muy variable entre sitios y períodos de caudal de agua, mientras que la comunidad bacteriana de los sedimentos difiere según el período de flujo de agua asociado a la variación de temperatura. Por último, en el suelo de rivera se evidenciaron diferencias entre las comunidades bacterianas de los sitios localizadas antes y después de la ciudad de Córdoba. Estos resultados mostraron que existen diferentes patrones de distribución en la composición de la comunidad bacteriana de los tres hábitats evaluados.

[Palabras clave: C orgánico, ecosistema lótico, gen 16S ARNr, variables ambientales]

Introduction

Rivers play a role in both human life and ecological balance. As well as being used in transportation and as a drinking water source for humans, they also form the main link between terrestrial and aquatic habitats as part of the hydrological and nutrient cycles (Wetzel 2001; Barton and Northup 2011; Tiquia 2011). Microorganisms are the main drivers of nutrient cycles, because their activity can influence biogeochemical processes. Thus, any spatial or temporal change in the microbial community composition can produce changes in ecosystem processes (Strickland et al. 2009).

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Numerous studies have shown that bacterial communities are highly dynamic and can differ strongly in their response to the availability of resources such as organic carbon, nitrogen and phosphorous, as well as to the food web structure (Allison and Martiny 2008; Shade et al. 2013). Other authors have reported bacterial community shifts in relation to environmental variables in aquatic ecosystems (Powell et al. 2003; Judd et al. 2006; Bissett et al. 2007; Ikenaga et al. 2010; Fujii et al. 2012; Arroyo et al. 2015). Similarly, seasonal shifts in water column stability and water temperature can lead to an annual pattern of bacterial community variability (Kirchman et al. 2005; Ibekwe et al. 2012), with Yachi and Loreau (1999) affirming that microbial diversity has an important role in buffering environmental variability and in maintaining the ecosystem process. Higher diversity therefore increases the probability that the species of a community respond in differential ways to environmental stress.

In recent years, researchers have focused on the study of the impact of pollution on the microbial community in lotic ecosystems (Ibekwe et al. 2012; Duarte et al. 2008; Porat et al. 2010; Rubin and Leff 2007). Nevertheless, to the best of our knowledge, there are no previous investigations about the changes of the bacterial community composition (BCC) in the three abiotic components of the aquatic ecosystems. By determining the physical and chemical factors that have modified microbial community compositions, it should help to understand how microorganisms are able to tolerate different kinds of environmental changes, and increase our knowledge of microbial ecology and evolution and their effects on human health.

Molecular methods provide important tools for studying changes in microbial communities of complex ecosystems. One of these techniques is the analysis of restriction fragment length polymorphisms (RFLP) of PCR amplified bacterial 16S rRNA, which has turned out to be very useful to assess the diversity and space-time variations of microbial populations (Kimura et al. 2002; Haack et al. 2004; Ramirez Moreno et al. 2004).

The Suquía River receives pollutants from various sources, with the environmental quality of the river degrading as a consequence of an excess load of pollutants including microorganisms that can alter the nutrient and organic matter content (Merlo et al. 2011). Also, it is exposed to seasonal changes in its biological, chemical, and physical environments, which can have a significant influence on the BCC of the river. Studies have been conducted in Suquía River to evaluate the fecal indicator bacteria (Merlo et al. 2011), the diversity of the nitrate reducer and N fixing bacteria (Reyna et al. 2010; Merlo et al. 2014b) and the culturable microbial metabolic groups (Merlo et al. 2014a). However, to date, there have been no investigations on the influence of different pollutant inputs from the catchment area on BCC.

Since microbial communities play a key role in the biogeochemical cycles, it is crucial to understand the associations between the composition and diversity of microbial communities and the environmental parameters affecting this ecosystem. Therefore, the aim of this study was to determine the influence of the environmental conditions along the Suquía River on the BCC in water, riparian soil and sediment during different water flow periods. This comprehensive approach permitted a more reliable generalization of the patterns of BCC in different habitats and their link to specific environmental variables. Furthermore, to our knowledge, this is the first time that the BCC in a lotic ecosystem has been analyzed for the three habitats (water, sediment and riparian soil).

MATERIALS AND METHODS

Study area

The Suquía River of Córdoba province (Argentina) begins at the San Roque dam and flows mainly eastwards for about 200 km until emptying into Mar Chiquita lake. The watershed is located in a semi-arid region with a mean annual rainfall of between 700-900 mm, falling mainly between October and April, with mean temperatures being 10 °C in winter and 26 °C in summer. The San Roque dam forms an artificial lake where recreational activities have promoted the urbanization of the lake shorelines and their surroundings. Thirty km downstream from the dam, the Suquía River enters Córdoba city (1.29 million inhabitants), whose population in the last 20 years has almost doubled, and its growing industrialization has increased the risk of having toxic effluents discharged into the river. Near the eastern edge of the city, the Suquía River receives the sewage discharge

from the Municipal waste water treatment plant (WWTP) (Merlo et al. 2011) (Figure 1).

The flow regime of Suquía River is exclusively of pluvial origin, with a marked seasonality of the flow due to the irregular distribution of the rainfall. The water flow estimation in the high flow period (December to April), is greater than 15 $m^3 s^{-1}$, whereas in the low flow period (May to November) is only 2.7 m^3/s.

Sampling design

Five study sites were selected in the lower–middle basin of the Suquía River (Figure 1). a) Reference site (RS) (31°21´45´´ S - 64°20´99´´ W, 488 m a.s.l.). Located in La Calera city, 18.4 km downstream of San Roque dam and 18 km upstream of Córdoba city's wester limit. At this site, the river carries contaminants coming from the eutrophic San Roque dam as well as sewage discharges and urban run-off from villages further upstream (Amé et al. 2003; Galanti et al. 2013). In this river sector, there are some remaining mountain forest species such as *Schinopsis haenkeana*, *Lithraea ternifolia*, *Celtis tala*, *Prosopis* spp., *Ruprechtia apetala*, and also some exotic species (*Melia* sp., *Morus* spp., *Eucaliptus* spp., *Ulmus* spp., *Salix babilonica*). b) Site 1 (S1) (31°23'07"S - 64°14'15"W, 417 m a.s.l.). Located 17.1 km downstream from RS. This river sector receives sewage discharges from some neighborhoods' smaller treatment plants. This site also presents vegetation on its riverbanks (*Salix humboldtiana* and *Celtis tala*). c) Site 2 (S2) (31°23´82´´S - 64°14´62´´W, 393 m a.s.l.). Located in Córdoba city, 12.1 km downstream of S1. At this point, the river runs through a cement channel that replaces the natural river bed. In this segment, it is fed by La Cañada brook, which in turn is contaminated by industrial effluents, sewage waters, and run-off from the downtown commercial area (Pasquini et al. 2011). d) Site 3 (S3) (31°24'34" S - 64°10'66" W, 365 m a.s.l.). Situated 0.36 km before WWTP and 11 km downstream of S2. This site is located downstream of Córdoba city, and consequently receives different sewage discharges, urban runoff, and industrial effluents. e) Site 4 (S4) (31°26´81´´ S - 63°59´45´´ W, 430 m a.s.l.). Situated at Corazón de María village, 21 and 16 km downstream from S3 and the WWTP, respectively. This is the most degraded area of the river, and from S3 to S4 the river banks are considerably modified by sand mining (Merlo et al. 2011). In this sector vegetation including *Aspidosperma quebracho-blanco*, *Prosopis* spp., and *Celtis* spp. can be observed. f) Site 5 (S5) (31°20´29´´S - 63°36´58´´W, 243 m a.s.l.). Located in Río Primero city, 51.1 km downstream from S4. This site is in an agricultural area and the river is crossed by a heavy traffic route (Pasquini et al. 2011). The vegetation here is composed of exotic species, such as *Salix babilonica*, *Melia* sp., *Ulmus* spp. and *Morus* spp.

As there are no pristine sites, a reference site (RS) with minimal conditions of pollution was selected following Carey and Migliaccio (2009). Moreover, this allowed us to evaluate the effects due to pollution received from Cordoba city, as RS is located upstream.

Study sites were sampled in August (a low flow period, average water temperature: 15.8 °C) and February (a high flow period, average water temperature: 23.1 °C). In both these periods, five replicate points were randomly selected at each study site along a 100-m linear transect on one shoreline, and at each point, one sample of each habitat (water,

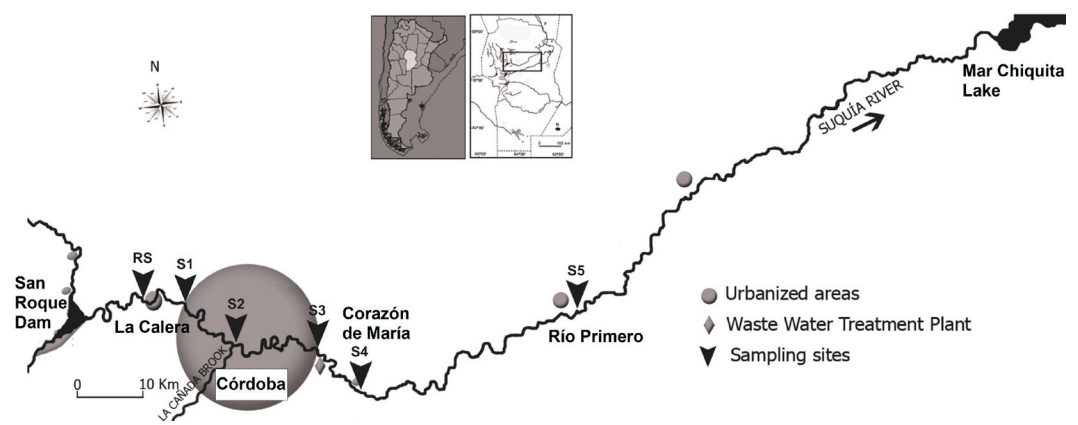

Figure 1. Study sites in the lower-middle basin of the Suquía River (Province of Córdoba, Argentina).
Figura 1. Sitios de estudio en la Cuenca media-baja del Río Suquía (Provincia de Córdoba, Argentina).

sediments and riparian soil) was collected in sterile receptacles. Water samples were taken from 10-15 cm below the surface using sterile glass bottles, and soil samples were collected from the top 20 cm and placed in a sterile plastic bag. Finally, sediment samples were obtained from the first 10 cm of the top layer using a handle dredge. The sampling of all study sites at each flow period was carried out on the same day, and samples were then immediately transported on ice to the laboratory. For chemical analysis: water samples were stored at 4 °C, and soil and sediment samples were air-dried for 24 hours, sieved through a 2-mm mesh and then stored at 4 °C. For molecular analysis: samples from each study site and each sampled period (n=5) were combined in order to obtain one sample of water, sediment and soil from each site in each period. Water samples were filtrated through polycarbonate membrane filters of 0.22 µm (Millipore) immediately after being obtained to collect microbial biomass, with four filters for each combined water sample being used. Filters, sediment and soil samples were stored at -20 °C until DNA extractions.

Physicochemical analyses

The pH, dissolved oxygen, temperature and conductivity of the water were measured "in situ" using portable equipment (WTW, Multiline F/Set 3). The following parameters were evaluated in all samples: a) total organic carbon content by wet combustion (Nelson and Sommers 1996) and b) nitrate and ammonia concentrations by colorimetric methods (Kenney and Nelson 1982; Mulvaney 1996). In addition, the pH and total N were determined in the sediment and soil samples, and conductivity was measured in soil samples according to the SSSA methodology (Klute 1986).

DNA extraction

DNA was extracted from 5 g of the combined soil or sediment samples following the method of Yeates et al. (1997). Briefly, samples were incubated with extraction buffer (100 mM Tris-HCl pH=8, 100 mM EDTA pH=8, 1.5 M NaCl), K proteinase (20 mg/mL) and SDS. Then, the supernatant was collected and incubated with 30% of polyethylene glycol in 1.6 M NaCl. The pellet was resuspended in TE (10mM Tris-HCl pH=7.5, 1 mM EDTA) and 7.5 M KAc (to a final concentration of 0.5 M) was added. The aqueous phase was sequentially extracted with an equal volume of alkaline phenol/chloroform (1:1) and chloroform/isoamyl alcohol (24:1), and the resulting aqueous phase combined with 0.6 volume of isopropanol. Precipitated DNA was washed with 70% ETOH, and resuspended in 100 µl of TE.

DNA of water samples was extracted from 4 polycarbonate membrane filters of 0.22 µm obtained for each combined sample. These filters were first incubated in 5 µL of bidistilled sterile water with agitation at 37 °C overnight to suspend bacterial cells, after which, they were discharged and the bacterial suspension was centrifuged for 2 min at 15,000 g. To carry out the DNA extraction, a methodology described by Sambrook et al. (1989) was utilized. In brief, bacterial cells were washed with 50 mM Tris-HCl pH=8, 20 mM EDTA solution and resuspended in 50 mM Tris-HCl pH=8, 2 mM EDTA solution, before being incubated with K proteinase (to a final concentration of 100 µg/mL) and SDS (to a final concentration of 0.5%). The bacterial lysate was sequentially extracted with an equal volume of alkaline phenol/chloroform/isoamyl alcohol (25:24:1) and chloroform/isoamyl alcohol (24:1), and the resulting aqueous phase was combined with 1 volume of 1/10 of 3 M NaAc (pH=6) and 2 volumes of 100% ETOH. Precipitated DNA was washed with 70% ETOH, resuspended in 100 µl of TE, and the extracted DNA was visualized by electrophoresis in a 1% (w/v) agarose gel in 1.0 × TAE buffer (40 mM Tris–acetate, 1 mM EDTA) stained with ethidium bromide. Finally, the DNA quality was evaluated by comparing the absorbance at 260 nm to 280 nm, and the DNA concentration was determined assuming that 1 unit of O.D. at 260 nm corresponded to 50 µg/mL of double strand DNA.

PCR conditions

PCR amplification of the 16S rRNA gene from 10 ng of DNA was performed using 27f (GAG TTT GAT CCT GGC TCA) and 1492r (TAC GGYTAC CTT GTT ACG ACT T) primers (Lane 1991), with the amplification being carried out in a total volume of 50 µL with 1× PCR buffer (20 mM Tris-HCl [pH 8.4], 50 mM KCl, 2.0 mM $MgCl_2$), 0.25 mM concentration of each deoxyribonucleoside triphosphate, 1 U of Paq polymerase (Stratagene), 0.3 µM of each primer, and between 5 to 25 ng of DNA. The following PCR conditions were used: a denaturation step of 5 min at 95 °C, followed by 32 cycles of denaturation at 94 °C for 30 s,

annealing at 52 °C for 30 s, and extension at 72 °C for 1 min (Vladár et al. 2008). The PCR products were visualized by electrophoresis in a 2% (w/v) agarose gel in a 1.0× TAE buffer (40 mM Tris–acetate, 1 mM EDTA) stained with ethidium bromide.

Bacterial community composition

For RFLP, the restriction enzymes AluI and HaeIII were utilized. Forty-five microliters of PCR- amplified DNA products were precipitated with two volumes of 100% cold ethanol and a one-tenth volume of 3 M NaAc (pH 5.2) for two hours at -20 °C, followed by centrifugation at 15,000 g for 15 min. The pellet was washed with 1 mL of 70% cold ethanol, and centrifuged again at 15,000 g for 15 min. Then, the pellet was suspended in 17.5 µl of nucleases free water, 1× buffer (AluI enzyme restriction buffer: 33 mM Tris-acetate pH=7,9, 10 mM MgAc, 66 mM KAc, 0.1 mg/mL BSA; HaeIII enzyme restriction buffer: 10 mM Tris-HCl pH=8,5, 10 mM $MgCl_2$, 100 mM KCl, 0.1 mg/mL BSA) and 5 U of restriction enzyme. After incubation at 37 °C for 16 h, the digested fragments were separated by electrophoresis in a 2.5% (w/v) agarose gel in 1.0× TAE buffer (40 mM Tris–acetate, 1 mM EDTA) stained with ethidium bromide. Finally, the separated fragments were visualized and photographed under UV light (High Performance Ultraviolet Transilluminator UVP, inc.).

Statistical data analysis

Statistical significances between the physicochemical characteristics of different habitats and different water flow periods were obtained by applying a Kruskal-Wallis one-way analysis of variance, using the InfoStat program (Di Rienzo et al. 2013). RFLP gel analysis was performed with InfoGen (Balzarini and Di Rienzo 2013), and similarity matrices for all pairwise combinations of RFLP profiles were constructed from the binary matrix of each habitat, using the Dice coefficient as a measure of proximity, with the distance matrix then being used as data for the cluster analysis. A redundancy analysis (RDA) was carried out to examine the main physicochemical variables affecting BCC, using a RDA procedure according to Borcard et al. (2011) (Numerical Ecology with R) with an "rda" function from the "vegan" packages of RStudio software (Version 1.0.136). Briefly, a Hellinger transformation was applied over the Y matrix (community pattern) according to Legendre and Gallagher (2001) and the X matrix (environmental variables) was standardized. Finally, the absence of collinearity ("vif.cca" function, with values <10 being accepted) was tested being not necessary rule out variables in our study.

Results

Environmental characteristics

The water environmental variables are reflected the pollution of the Suquía River, as, in general, the organic C, nutrients and conductivity showed a steady increase as the river flows through Cordoba city, while dissolved O_2 and pH decreased (Tables 1 and 2). Moreover, this pattern was more evident in low flow period (Table 1). The chemical characteristics also varied between the low and high flow periods at all sites, with an increased water flow improving water quality, as conductivity and nitrate content decreased at all sites (except for nitrate content at S4) whereas dissolved O_2 increased at S4 and S5 (Tables 1 and 2).

The physicochemical characteristics of sediments also varied significantly among sites. In the low flow period, S4 presented the highest values of organic carbon, ammonia and total N content, but the lowest value of pH (Table 1). On the other hand, in the high flow period, the organic C content was higher at RS and S1, and the total N content greater at RS. Furthermore, the pH presented the lowest values at RS, whereas the nutrient values (nitrate and ammonia) were higher at S4 (Table 2). The sediments presented fewer significant differences between periods than water. The pH was increased for almost all sites at high flow and the organic C also increased at RS, S1 and S4, along with the nitrate at RS and the total N at S1 (Tables 1 and 2).

The riparian soil of RS, S1 and S2 revealed the highest values of organic C and total N at both low and high flow periods. Moreover, the pH only varied significantly between sites at low flow period and was higher at S1, S2 and S3, with ammonia presenting significant differences at high flow and being much lower at S3. The riparian soil characteristics varied between the low and high flow periods. At high flow the pH increased in the riparian soil for all sites, and the total N increased at RS and S4 and ammonia at S2. In contrast, the conductivity decreased at S1 and also the nitrate content at S3 (Tables 1 and 2).

Bacterial community composition

The RFLP patterns obtained with the AluI enzyme did not reveal any differences

Table 1. Chemical characteristics of the Suquía River study sites at the low water flow period.

Tabla 1. Características químicas de los sitios de estudio del Río Suquía en el período de bajo caudal de agua.

Low flow	RS	S1	S2	S3	S4	S5
Water						
Organic C (mg/L)	3.28 c* (±0.19)	5.58 bc (±0.99)	12.80 ab (±4.75)	10.97 ab (±5.96)	17.19 a (±4.67)	11.97 ab* (±5.88)
Ammonia (mg/L)	0.19 bc* (±0.18)	0.10 c (±0.04)	0.72 ab (±0.90)	0.23 bc (±0.07)	15.32 a* (±0.37)	1.86 ab (±3.77)
Dissolved O_2 (mg/L)	10.01 bc* (±0.08)	10.43 bc* (±0.39)	13.29 ab* (±0.54)	16.21 a* (±0.38)	3.18 d* (±0.36)	4.36 cd* (±0.89)
pH	7.02 c (±0.11)	7.26 ab (±0.03)	7.18 b* (±0.04)	7.37 a (±0.12)	6.79 d* (±0.08)	6.77 d* (±0.14)
Conductivity (µS/cm)	215.80 c* (±1.92)	678.80 c* (±18.79)	1438.00 ab* (±29.06)	1488.60 a* (±25.48)	1411.20 ab* (±16.99)	1051.60 bc* (±225.24)
Nitrate (mg/L)	3.30 c* (±0.47)	13.30 bc* (±1.25)	37.00 ab* (±2.24)	42.90 a* (±1.08)	11.80 bc (±3.95)	31.40 ab* (±14.28)
Sediment						
Organic C (g/kg)	1.94 bc* (±1.71)	0.44 c* (±0.43)	19.08 ab (±23.65)	10.69 abc (±15.23)	25.67 a* (±22.75)	2.33 bc (±3.69)
Ammonia (mg/kg)	9.07 b (±4.87)	16.77 b (±16.93)	6.21b (±3.42)	9.29 b (±6.87)	175.14 a (±187.19)	41.26 b (±70.88)
pH	6.89 b (±0.09)	7.18 a* (±0.05)	7.24 a* (±0.19)	7.01 ab* (±0.37)	6.53 c* (±0.23)	7.06 ab* (±0.20)
Nitrate (mg/kg)	17.50 * (±5.30)	32.50 (±26.81)	366.00 (±368.02)	48.50 (±37.94)	122.25 (±142.25)	30.00 (±21.51)
Total N (mg/kg)	3232 ab (±3554)	1056 b* (±292)	4335 ab (±4801)	2400 ab (±1484)	7740 a (±6301)	1368 b (±1187)
Riparian soil						
Organic C (g/kg)	15.35 ab (±6.52)	16.31 ab (±11.09)	24.31 a (±14.24)	0.98 c (±0.54)	2.14 c (±1.65)	10.64 bc (±9.78)
Ammonia (mg/kg)	11.26 (±2.96)	7.70 (±3.36)	8.60* (±6.29)	7.89 (±3.05)	12.76 (±4.41)	7.49 (±3.72)
pH	6.96 b* (±0.29)	7.32 a* (±0.15)	7.36 a (±0.25)	7.29 a* (±0.15)	6.77 b* (±0.28)	6.83 b* (±0.14)
Conductivity (µS/cm)	736.00 (±278.13)	1517.20 * (752.02)	3912.80 (±4199.50)	1928.80 (1204.36)	2222.60 (±1767.06)	2378.60 (±2682.55)
Nitrate (mg/kg)	36.25 * (±15.91)	103.50 (±146.70)	1156.00 (±2038.26)	261.50* (±213.65)	42.50 (±26.40)	44.00 (±41.22)
Total N (mg/kg)	1860 ab* (±1520)	2820 a (±1134)	3300 a (±2741)	924 bc (±675)	672 c* (±294)	1572 ab (±702)

Mean values with different letters indicate significant differences among sampling sites (p>0.05).

* = significant differences between periods at each sampling site and habitat (p>0.05, Tables 1 and 2). RS: reference site, S1: site 1, S2: site 2, S3: site 3, S4: site 4, S5: site 5.

Valores medios con letras diferentes indican diferencias significativas entre sitios de estudio.

* = diferencias significativas entre épocas para cada sitio de muestreo y hábitat (p>0.05, Tablas 1 y 2). RS: sitio de referencia, S1: sitio 1, S2: sitio 2, S3: sitio 3, S4: sitio 4, S5: sitio 5.

among BCC for the three studied habitats, and consequently, only the RFLP patterns of the BCC obtained with HaeIII enzyme were analyzed. For sediments from RS at the high flow period, no RFLP pattern was obtained.

Eighteen fragments were obtained by the digestion with HaeIII, of which, 16 fragments were present in water samples, 9 in sediment samples and 7 in soil samples (Figure 2). Fragment number 5 was present in every sample of water, sediments and riparian soil, while fragments number 1 and 18 were only detected in water in the high flow period at S4 and S5, respectively. Eight fragments were specific to water habitat (Nº 1, Nº 3, Nº 7, Nº 8, Nº 11, Nº 12, Nº 16 and Nº 18), 5 fragments were specific to the high flow period (Nº 1, Nº 8, Nº 11, Nº 16 and Nº 18), and 3 fragments appeared in all water samples at both high and low flow periods (Nº 5, Nº 6 and Nº 7). The riparian soil and sediments revealed 3 fragments in all samples (Nº 5, Nº 9 and Nº 13), with one specific fragment for each habitat (riparian soil: N° 6 and sediment: Nº 17) (Figure 2).

Cluster and RDA analyses indicated clear differences between BCC of water and that of riparian soil or sediments (Figure 3a and Figure 4). The cluster analysis of water samples demonstrated that BCC was very different among study sites and flow periods, which can be observed in Figure 3b where only a few sites are grouped together: a) S5 and RS of the high flow period and S1 and S2 at low flow; and b) S3 and S4 of the low flow period. In contrast, cluster analysis from sediment samples separated the RFLP patterns of BCC into two clusters, with one consisting of the BCC from

Table 2. Chemical characteristics of the Suquía River study sites at the high water flow period.
Tabla 2. Características químicas de los sitios de estudio del Río Suquía en el período de alto caudal de agua.

High flow	RS	S1	S2	S3	S4	S5
Water						
Organic C (mg/L)	5.41 c* (±1.66)	12.70 ab (±15.22)	12.03 ab (±0.76)	10.05 bc (±1.06)	12.42 ab (±2.49)	27.49 a* (±8.92)
Ammonia (mg/L)	0.49 a* (±0.05)	0.12 c (±0.03)	0.40 ab (±0.10)	0.19 bc (±0.04)	1.18 a* (±0.63)	0.19 bc (±0.06)
Dissolved O2 (mg/L)	6.87 cd* (±0.03)	7.26 bc* (±0.09)	8.28 a* (±0.47)	7.60 ab* (±0.15)	4.96 d* (±0.24)	7.11 bcd* (±1.08)
pH	7.07 (±0.08)	7.20 (±0.23)	7.34 * (±0.11)	7.28 (±0.13)	7.12 * (±0.25)	7.32 * (±0.12)
Conductivity (µS/cm)	147.00 c* (±1.72)	185.94 bc* (±3.64)	506.60 a* (±11.91)	369.40 bc* (±7.44)	493.40 ab* (±11.84)	518.40 a* (±12.88)
Nitrate (mg/L)	1.76 c* (±0.81)	2.18 c* (±0.70)	9.55 ab* (±2.18)	7.05 bc* (±0.86)	10.45 ab (±1.58)	12.25 a* (±1.79)
Sediment						
Organic C (g/kg)	9.80 a* (±7.29)	10.65 a* (±9.41)	0.72 c (±0.20)	4.10 bc (±8.95)	2.01 bc* (±2.79)	8.71 ab (±8.03)
Ammonia (mg/kg)	13.82 ab (±6.33)	11.18 abc (±7.39)	3.00 c (±0.56)	10.64 bc (±13.34)	30.25 a (±22.37)	15.99 ab (±12.91)
pH	6.80 b (±0.46)	7.59 a* (±0.13)	7.65 a* (±0.06)	7.51 a* (±0.18)	7.44 a* (±0.14)	7.40 a* (±0.10)
Nitrate (mg/kg)	40.25 ab* (±16.38)	35.75 abc (±26.38)	15.25 c (±7.68)	21.00 bc (±17.40)	68.00 a (±45.29)	40.00 ab (±16.03)
Total N (mg/kg)	3360 a (±746)	2460 ab* (±1262)	948 c (±405)	2112 abc (±1785)	1620 bc (±622)	2250 abc (±1677)
Riparian soil						
Organic C (g/kg)	27.17 a (±9.44)	21.92 a (±5.84)	25.17 a (±2.22)	3.12 b (±4.49)	6.52 b (±5.83)	10.85 b (±5.73)
Ammonia (mg/kg)	15.16 a (±8.50)	11.61 ab (±3.45)	17.39 a* (±4.46)	5.22 b (±1.53)	11.79 ab (±6.66)	10.75 ab (±3.66)
pH	7.70 * (±0.15)	7.59 * (±0.17)	7.59 (±0.02)	7.67 * (±0.12)	7.51* (±0.13)	7.51 * (±0.09)
Conductivity (µS/cm)	1163.80 (±645.41)	562.80 * (±100.88)	1064.20 (±174.75)	904.00 (±197.63)	880.80 (±269.90)	919.40 (±252.34)
Nitrate (mg/kg)	82.75 * (±37.85)	59.75 (±19.27)	76.00 (±67.73)	26.00 * (±14.59)	90.25 (±67.92)	39.50 (±20.59)
Total N (mg/kg)	3960 a* (±1003)	2820 abc (±454)	3120 ab (±502)	1740 d (±684)	1920 cd* (±502)	2400 bcd (±474)

Mean values with different letters indicate significant differences among sampling sites (p>0.05).
* = significant differences between periods at each sampling site and habitat (p> 0.05, Tables 1 and 2).
RS: reference site, S1: site 1, S2: site 2, S3: site 3, S4: site 4, S5: site 5.
Valores medios con letras diferentes indican diferencias significativas entre sitios de estudio.
* = diferencias significativas entre épocas para cada sitio de muestreo y hábitat (p>0.05, Tablas 1 y 2).
RS: sitio de referencia, S1: sitio 1, S2: sitio 2, S3: sitio 3, S4: sitio 4, S5: sitio 5.

RS and S1 at the low flow period and the other grouping the bacterial community of all the other sites. Then, this latter cluster was further divided in two subclusters, which separated the samples from the different periods (Figure 3c). Finally, cluster analysis of riparian soil samples separated the RFLP patterns of the bacterial communities of RS, S1 and S2 from the S3, S4 and S5 sites at both high and low flow periods (Figure 3d).

Bacterial community and environment

The best RDA model selected four physicochemical variables (dissolved oxygen, conductivity, temperature and pH) with significant (P=0.001) capacities to explain the BCC (response matrix), with this obtained RDA model explaining 38% of the total variability of the BCC. From this percentage of variability, the first axis (RDA 1) accounted for 71.6% of the variation, and the second axis (RDA 2) 17.4%. Similarly, to cluster analysis, RDA 1 separated the bacterial communities of water from those of riparian soil and sediments. The water BCC was associated with dissolved oxygen, while most riparian soil and sediments BCC was associated with pH and conductivity, with it being possible to observe a strong correlation between these two variables. Finally, temperature showed an effect along RDA 2 and located most of the bacterial communities of the high flow period in the lower half of the RDA plot. However, this was not the main cause of the variation among BCC (Figure 4).

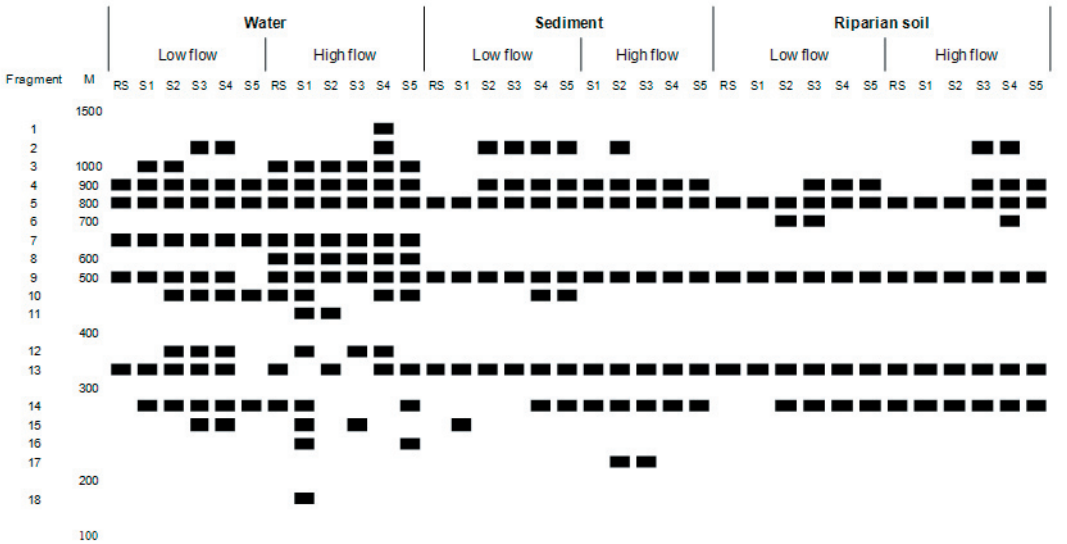

Figure 2. Schematic RFLP patterns of bacterial communities in water, sediment and riparian soil at low and high flow periods. RS: reference site; S1: site 1; S2: site 2; S3: site 3; S4: site 4; S5: site 5; L: low flow period; H: high flow period.

Figura 2. Esquema de los patrones de RFLP de las comunidades bacterianas en agua, sedimento y suelo de ribera en los períodos de bajo y alto caudal de agua. RS: sitio de referencia; S1: sitio 1; S2: sitio 2; S3: sitio 3; S4: sitio 4; S5: sitio 5; L: periodo de bajo caudal de agua; H: período de alto caudal de agua.

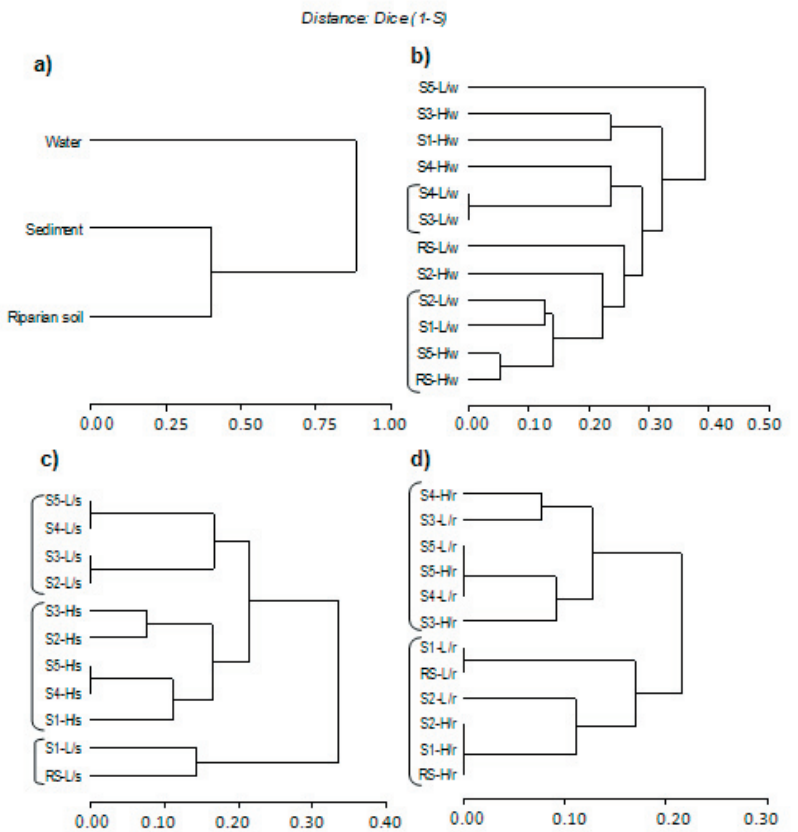

Figure 3. Cluster analyses of RFLP patterns. a) Bacterial communities from different habitats, b) bacterial community in water, c) bacterial community in sediment, and d) bacterial community in riparian soil. RS: reference site; S1: site 1; S2: site 2; S3: site 3; S4: site 4; S5: site 5; L: low flow period; H: high flow period; w: water; s: sediment; r: riparian soil.

Figura 3. Análisis de clústeres de los patrones de RFLP. a) Comunidades bacterianas de diferentes hábitats, b) comunidad bacteriana del agua, c) comunidad bacteriana del sedimento, y d) comunidad bacteriana del suelo de ribera. RS: sitio de referencia; S1: sitio 1; S2: sitio 2; S3: sitio 3; S4: sitio 4; S5: sitio 5; L: periodo de bajo caudal de agua; H: período de alto caudal de agua; w: agua; s: sedimento; r: suelo de ribera.

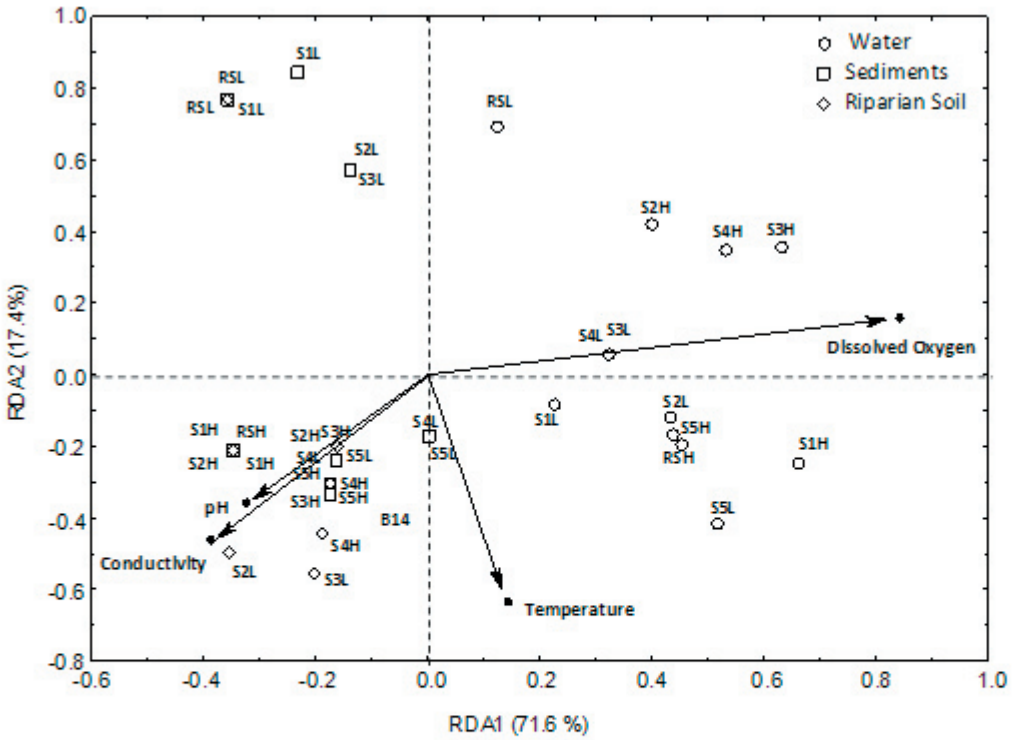

Figure 4. Redundancy analysis (RDA) showing the relationships between environmental variables and bacterial community composition. Arrows indicate the direction and magnitude of physicochemical variables associated with bacterial communities. RS: reference site; S1: site 1; S2: site 2; S3: site 3; S4: site 4; S5: site 5; L: low flow period; H: high flow period.

Figura 4. Análisis de Redundancia (RDA) que muestra la relación entre las variables ambientales y la composición de las comunidades bacterianas. Las flechas indican la dirección y la magnitud de las variables fisicoquímicas asociadas a las comunidades bacterianas. RS: sitio de referencia; S1: sitio 1; S2: sitio 2; S3: sitio 3; S4: sitio 4; S5: sitio 5; L: periodo de bajo caudal de agua; H: período de alto caudal de agua.

Discussion

Bacterial community composition

To the best of our knowledge, this is the first study describing BCC including riparian soil, sediment and water of an aquatic ecosystem. The results show that: a) the BCC of water was different from that of the sediments or riparian soil, which were found to be more similar, and b) the BCC in water was more variable whereas that of soil or sediments was more stable. Thus, it was the habitat which determined the BCC. Other studies performed on diverse aquatic ecosystems also detected bacterial community differences between sediment and water (Dillon et al. 2009; Staley et al. 2015; Ibekwe et al. 2016; Wei et al. 2016), but as few studies examined BCC in riparian soil in aquatic ecosystems, it is difficult to compare our results with these investigations.

The differences found among the BCC reflect the very different environmental conditions occurring in the three habitats. In the present study, using the RFLP technique, the RDA analysis showed dissolved oxygen, pH and conductivity to be the key factors driving the variation in BCC for the three habitats evaluated. In fact, these environmental variables have been mentioned in numerous studies as being the principal shapers of BCC in aquatic ecosystems (Ibekwe et al. 2012; Ligi et al. 2014; Schiaffino et al. 2016; Wei et al. 2016). As, the dissolved oxygen was correlated with water BCC, it seems that the oxic conditions determined that in water more aerobic bacterial develops compared with soil and sediments. In this sense, Dillon et al. (2009) detected that microbes sampled at the surface of the water column under oxic conditions consisted of many aerobic lineages, while in sediments they recovered anaerobic phylotypes. Similar results were observed by Ibekwe et al. (2012), who detected shifts in the BCC in water due to dissolved oxygen, salinity and turbidity.

The pH and conductivity, in contrast, were associated with most of the bacterial communities of the riparian soil and

sediments. Zeng et al. (2009) also reported a significant influence of pH together with total P and organic matter content on the BCC in the sediments. In other works, soil pH was a key predictor of BCC in surface soils (Bartram et al. 2014), and in agreement with our results, this was one of the variables that affected the BCC of a riverine wetland (Ligi et al. 2014), while soil salinity was one of the drivers of soil BCC in the Yellow River (Gao et al. 2015). Conversely, these results are different from those obtained by Arroyo et al. (2015), where pH was not associated with the BCC of wetland soil, with the BCC being linked to nitrogen and soil organic matter concentrations.

Despite organic matter having been described as an important driver of BCC (Powell et al. 2003; Judd et al. 2006; Bissett et al. 2007; Fujii et al. 2012; Bai et al. 2012), our RDA analysis did not demonstrate this variable to be important in accounting for the variation of the BCC in the Suquía River. In agreement with our results, Ikenaga et al. (2010) did not detect any significant relation between organic C and BCC, with this lack of association possibly being due to a differential preference of C sources by different bacterial groups (Amaral et al. 2016). However, it should be born in mind that the low resolution of the RFLP technique might mask the effect of organic C content on BCC. Although it is feasible to obtain fingerprinting of the predominant ribotypes by RFLP analysis, it should be taken into account that the cell numbers of non-dominant bacterial populations may be too low to be detected by amplification and gel visualization of restriction fragments (Ramirez Moreno et al. 2004). Related to this, as previously pointed out, the RFLP technique and other fingerprinting methods are very useful to compare differences among BCC of a great number of samples when all are processed under the same conditions (Zhang et al. 2008), but the lower resolution grade of RFLP than other fingerprinting techniques (DGGE, T-RFLP, Illumina high-throughput sequencing, etc.) must be considered when interpreting the results.

The environmental variables measured accounted only 38% of the community variations, indicating that other factors had contributed to this. Wei et al. (2016) hypothesized that sediment provides a relatively stable habitat for bacteria compared with that of the water environment. In agreement with our findings, other authors have also observed that the water bacterial communities are more affected by their surroundings than those of sediments (Wei et al. 2016; Ibekwe et al. 2016). In fact, most bacteria have the ability to adhere to the surface of sediment particles, while they are usually planktonic in fluctuating water habitats. As a result, the bacteria in sediments may propagate and assemble more easily (e.g., forming biofilms or colonies) and consequently have a greater resistance to environmental changes. Moreover, dispersal dynamic may have an important role in structuring microbial communities (Arroyo et al. 2015; Staley et al. 2015; Wei et al. 2016).

The low genetic similarities found among the analyzed communities in water indicate that the BCC was affected by the site and the sampling period. It is worth noting that the samplings in all sites at each period were performed on the same day, and thus the BCC at each site may have been affected by bacterial species coming from the surrounding land, tributaries and effluents as was observed in other aquatic environments (Chen et al. 2013; Ibekwe et al. 2016). In addition, the low similarity indexes of water BCC among the Suquía River sites suggest an important process of species substitution, which is a common ecological process that mainly occurs due to environmental shifts of a natural or anthropogenic origin, and is more pronounced in bacteria because of its high generation rate (Smith and Smith 2001; Odum 2003; Paul 2007).

Judd et al. (2006) mentioned that strong shifts in BCC may be dependent on a dormant "seed bank" of species able to utilize new organic matter compounds. Hence, the environment selects from among the phylotype groups those that could potentially occupy the new niche (Comte and del Giorgio 2010). However, this "seed bank" of species could be less effective if there has been no contact with the organic matter source beforehand. In this case, it should be noted that the input of organic matter brings new bacterial species such as those attached to the phyllosphere or in poorly treated effluents. Nevertheless, since water is a changing habitat that is very affected by its surroundings (Wei et al. 2016; Ibekwe et al. 2016), the pattern detected in water could have been a result of sampling frequency or the lower resolution of the fingerprinting technique used.

In sediments, the shifts in the BCC respond to water flow changes, which may be a result

of differences in temperature between low and high flow periods. As shown in the RDA analysis, this variable separated most of sediment samples of the high flow period from the low flow one along the second axis (Figure 4). Kara et al. (2013) suggested that in temperate regions annual cycles in temperature are an important factor controlling the diversity of aquatic microbial communities. In agreement, other studies have detected temperature to be a strong driver of changes in BCC in different seasons (Kirchman et al. 2005; Febria et al. 2010; Rösel et al. 2012; Wang et al. 2016). Moreover, it is worth noting that a previous study conducted in the Suquía River detected the removal of finer sediments by the high water velocity during the high flow period, with deposition of suspended material occurring due to the slowness of the water flow during low flow, which in turn could also have affected the BCC in both periods (Merlo et al. 2011).

Shifts in the riparian soil BCC, in contrast, responded to spatial changes, due to the first three sites differing from the three last ones in both the high and low flow periods. Riparian soil is more stable than sediments because it is less exposed to water flow erosion, but soil environmental conditions may simply be less variable on the timescale studied than the other habitats. Moreover, soil communities contain a large proportion of dormant organisms (Lennon and Jones 2011), and consequently the communities may appear to change relatively little over a given time scale. Soils also have a high spatial heterogeneity, which can mask shifts in local communities over time because of high community variability across microsites (Shade et al. 2013). Some authors (Jayakumar et al. 2009; Chen et al. 2013) have reported that when the environmental conditions for bacterial growth improves, the bacterial biomass expands, the bacterial diversity decreases and dominance increases. This process is similar to the bloom events in which few species are involved.

In conclusion, we confirmed that the BCC of water was different from that of sediment or riparian soil. Here, the BCC was significantly associated with three physicochemical parameters (dissolved oxygen, pH and conductivity); nevertheless, other aspects should also be considered to explain variations in the BCC. Our results suggest distinct distribution patterns of BCC in the three habitats evaluated. The temperature may have an effect on structuring sediment communities, since they differed according to the water flow period. Water BCC was very variable among sites and water flow period, while in riparian soils there were spatial changes, as evidenced by the differences between the bacterial communities of the sites located upstream and downstream of Cordoba city. This study contributes to the overall understanding of the assembly patterns in BCC in different habitats of lotic ecosystems and their relation to specific environmental variables.

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