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# Relationships among invertebrate communities and groundwater properties in an unconfined aquifer in Argentina

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The paper evaluates the relationships among invertebrate communities, land uses and chemical and microbiological groundwater properties, in a loessic unconfined aquifer in Argentina. Two surveys were conducted and seven wells were selected based on land use and unsaturated zone (USZ) thickness. Groundwater was characterized mainly as freshwater of sodium bicarbonate type. Invertebrates collected belong to Crustacea, Acari, Insecta, Collembola, Oligochaeta, Collembola, Paurododa, and Nematoda. Crustacea was the most abundant group. The wells differed in terms of invertebrate composition and abundance. The lowest abundance was observed at sites with thickest USZ. No direct relationship was found between invertebrate abundance and any particular physico-chemical parameter. In the only well where bacteria were detected, total invertebrate abundance, especially of copepods, was high in both surveys. Both USZ thickness and land use have a significant influence on abundance and composition of invertebrate communities in terms of organic matter inputs into the aquifer.

*Keywords:* Groundwater; Ecosystem; Crustacea; Land-use; Indicators

## Introduction

Underground systems are complex [1] and ecosystem functioning depends on complex interactions between physico-chemical and biological factors [2]. The base of the groundwater food web is represented by heterotrophic microorganisms [3], which generate energy through redox reactions and consumption and transformation of organic compounds [4,5]. In the past, microorganisms and invertebrates were often considered separately. Today, they are recognised to share complex biological interactions [6].

Because groundwater ecosystems are characterized by constantly dark environments, photosynthesis does not occur *in situ*; organic matter standing stocks are low relative to surface ecosystems and biological productivity in the subsurface is often limited by organic matter availability [7]. Faunal assemblages reflect certain hydrological, geomorphological, physical and chemical processes [8] and ecologically significant changes in inputs of energy, nutrients, water or contaminants may change faunal community composition [9].

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Griebler et al. hypothesised that because of the relative invariant and predictable physical–chemical conditions in groundwater, ecosystems communities or individual organisms should be highly sensitive to environmental changes [10]. Thus, these researchers noted the potential value of groundwater invertebrates as indicators of water quality [11–14].

Invertebrates have immediate application as indicators of the life-supporting capacity of the groundwater ecosystem because of their short-to-medium term response to a broad spectrum of pollutants over a range of concentrations and time distributions [15]. Sewage-polluted sites have been shown to undergo significant changes in composition and relative abundance with respect to uncontaminated sites [16].

Thus, dissolved organic carbon input in groundwater is expected to stimulate the production of microbial biofilms, which are then grazed by invertebrates [17]. The amount of organic matter reaching the groundwater table depends on dissolved organic carbon production in the surface environment, water infiltration rate, and significance of adsorption and biodegradation of dissolved organic carbon in the vadose zone [18]. There is evidence that inputs of carbon and nutrients into groundwater ecosystems cause increased microbial activity and fauna biomass [7,19–22]. Consequently, shifts in the composition and structure of biotic assemblages may be expected in areas where agricultural practices influence groundwater quality and quantity and that, further, biota may differ in areas under different practices [23].

On the other hand, dissolved oxygen (DO) concentration also strongly influences diversity and density of invertebrate assemblages in groundwater [18] and is one of the prime ecological factors governing the occurrence and spatio-temporal distribution of hypogean animals [24]. Other variables that determine community composition are salinity, temperature, hydraulic conductivity, aquifer porosity, and water table depth [25]. Therefore, monitoring changes in composition of groundwater communities can be a useful tool in assessing changes in hydrology and water quality [8,10].

Taking into account the great importance of community studies of groundwater, the aim of the present investigation was to evaluate the relationships among invertebrate communities, land uses and chemical and microbiological groundwater properties in a piedmont loessic unconfined aquifer in La Colacha stream basin (Córdoba, Argentina).

## Material and methods

### *Study area*

The study area is located in La Colacha Stream basin, north-west of the department of Río Cuarto, Córdoba, Argentina, and covers an area of 195 km<sup>2</sup> (figure 1). The geological faults and lineaments have generated differentially elevated tilted blocks that determined different depths in the bedrock as well as variable thickness of alluvial and aeolian deposits lying on the rock. The area is covered by sandy-silty loessic aeolian sediments from the Holocene and Upper Pleistocene, and fine-grained subordinate alluvial and colluvial sediments of the Holocene [26].

Two large hydrogeological environments can be distinguished in the basin: a fractured rocky environment and a porous sedimentary one. In almost the entire basin the bedrock serves as the base of the sedimentary unconfined aquifer. Mean hydraulic conductivity of the aquifer in the porous sedimentary environment is 10<sup>-6</sup> to 10<sup>-4</sup> cm sec<sup>-1</sup> and the storage coefficient is in the order of 0.05–0.1. Transmissivity (1–60 m<sup>2</sup> day<sup>-1</sup>) is conditioned by the great variability of saturated thickness [26]. The water table surface is slightly

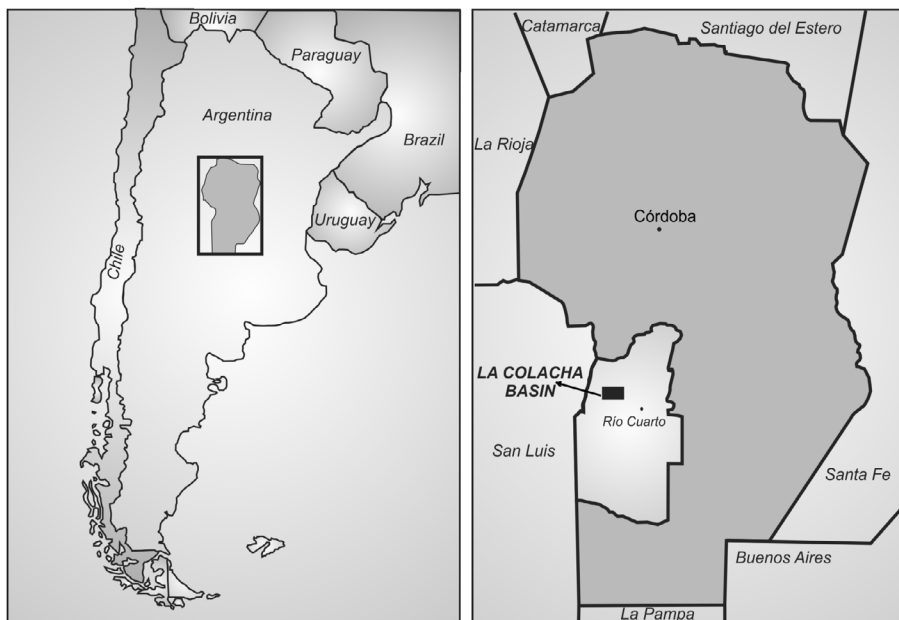


Figure 1. Location of the study area.

undulating (figure 2). The preferential recharge area of the aquifer is located to the west of the basin (figure 2). Groundwater flows in three directions: N–S in the eastern portion of the basin; W–E in the south and NW–SE in the central sector (figure 2) [26]. The basin is

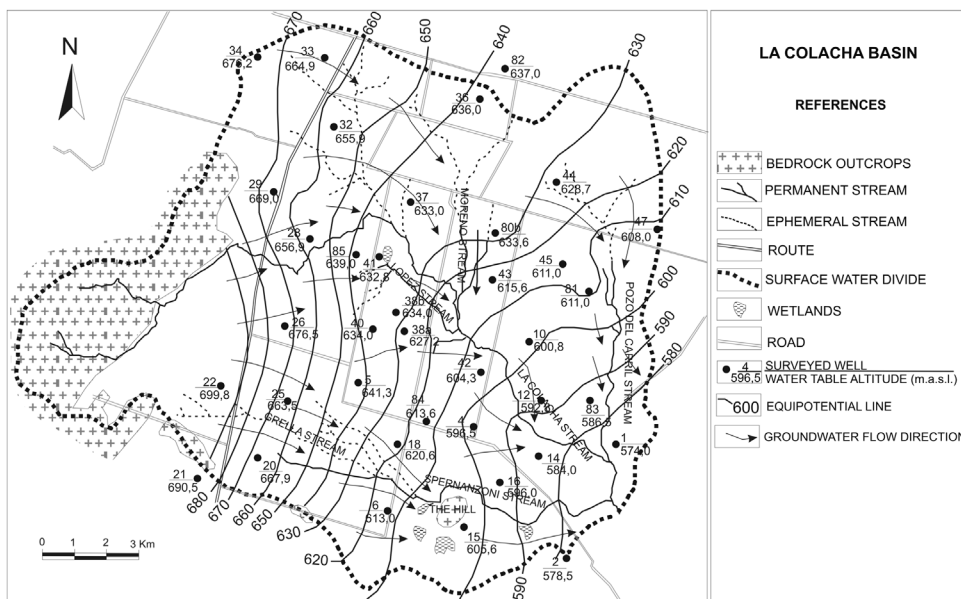


Figure 2. Equipotential lines and groundwater flow lines (unconfined sedimentary aquifer).

characterized by gaining streams, especially in the lower reaches of the courses, because of unconfined aquifer supply. The water table exhibits a wide range of depths (figure 3): between 17 and 40 m in the north of the basin, less than 3 m in the south, and between 3 and 20 m in the central-southern area. This wide range is related to the geological structural control on the relief [26].

### Methodology

Climatic data (1994–2008 series), obtained from La Aguada meteorological station, were analysed. Mean temperature and precipitation values were calculated and their temporal distribution was evaluated. Soil water balances were performed to calculate Actual Evapotranspiration (AET), water deficit and surplus. Potential Evapotranspiration (PET) values were obtained from Blarasin et al. [26]. AET was obtained with a time-series water balance using the PDIMES software [27]. Soil storage value was 150 mm, corresponding to fine sandy-loam soils and fully developing crops, with 1 m-rooting depth.

Sites suitable for groundwater sampling and invertebrate collection were selected based on hydrogeological data, mainly thickness of the unsaturated zone (USZ) and land use in the surrounding area. Seven wells (W) were sampled (table 1 and figure 4) in June 2007 (low water period) and April 2008 (end of rainy season). The first sampling included the sites W1, W86, W87, W80b, W36, whereas W15 and W47 were incorporated in the second sampling (figure 4) so as to have pairs of wells of similar USZ thickness and different surrounding land use.

In each well, the following parameters were determined *in situ*: pH, temperature, electrical conductivity (EC), and DO. Water samples were collected for further laboratory physico-chemical analysis of major ions ( $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ )

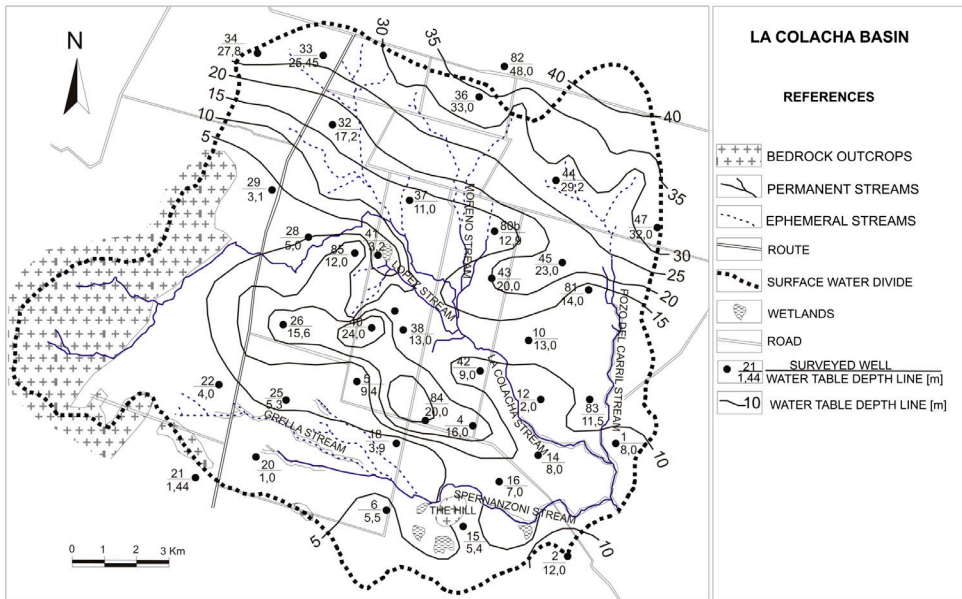


Figure 3. Water table depth lines (unconfined sedimentary aquifer).

Table 1. Sampled wells: main characteristics and related land use.

Well	Total depth of well (meters)	Unsaturated zone (meters)	Surrounding land use
W1	1.00	0.50	Wetland; cattle occasionally
W86	11.00	5.00	Cow and pig pens
W15	13.00	5.40	Garden, family home
W87	18.00	12.50	School; schoolyard
W80b	19.00	12.80	Intensive breeding pigs
W36	43.00	33.50	Cow and pig pens
W47	52.00	32.00	Park; cattle occasionally

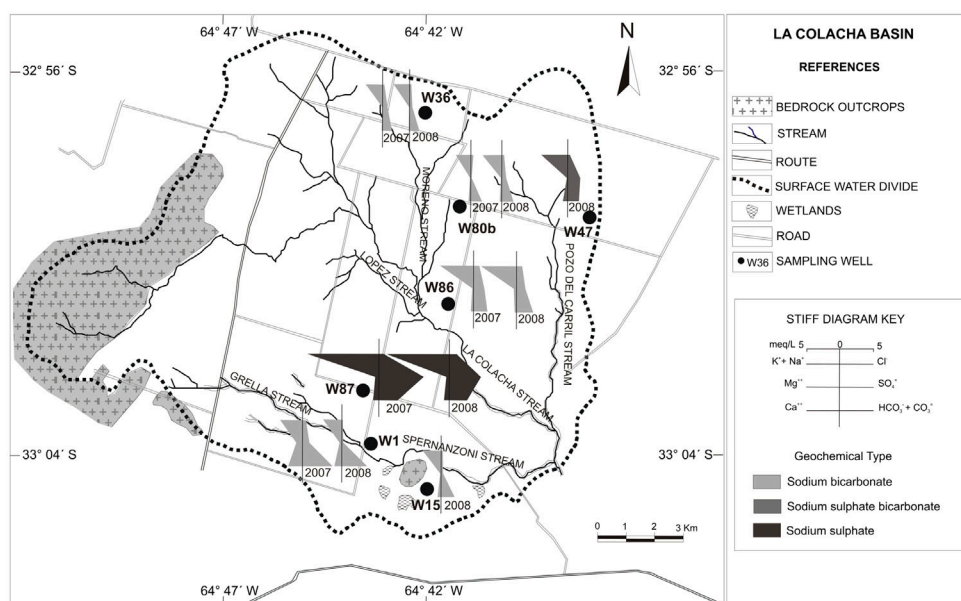


Figure 4. Sampling sites and geochemical groundwater type (unconfined sedimentary aquifer).

and  $\text{NO}_3^-$ . Samples also were collected in sterile containers for microbiological analyses: total microbial count (TMC), total coliform count (TC) and presence or absence of *Escherichia coli* and *Pseudomonas aeruginosa*.

For invertebrate sampling, two 20-L samples were taken from each well. In the field, water was passed through a 63  $\mu\text{m}$  sieve, the appropriate mesh size for retention of invertebrates [28]. The retained material was washed and placed in plastic containers. After that, the samples were preserved in the laboratory in 70% ethyl alcohol.

### Data analyses

Invertebrate abundance was compared among wells with an ANOVA, followed by an a posteriori Fisher's LSD test [29]. The analyses were performed for the variables Total abundance of Invertebrates, Acari, Copepoda and Crustacea, for which there were sufficient data. Differences between the sampling years within a site were evaluated with a *T* test [29]. Normality assumption was confirmed before the analyses by means of a

modified Shapiro–Wilk test [30]. The original Shapiro–Wilk test uses the null hypothesis principle to check whether a given sample came from a normally distributed population, but its application is limited up to  $n = 50$ . The study by Rahman and Govindarajulu [30] modifies the test, such that it can be extended for all sample sizes. Only the variable abundance of Copepoda had to be square-root transformed. Finally, a Detrended Correspondence Analysis (DCA) [31] was performed to evaluate similarity of wells based on all the invertebrate groups analysed. This indirect gradient analysis maximizes the separation between sites along ordination axes based on species composition, and has proven to be a powerful tool for detecting patterns in communities that reflect underlying environmental gradients [31]. The analyses were performed using InfoStat 2012 [32] and CANOCO [33] software.

## Results

### *Hydrometeorology*

Mean temperature was 16.5 °C; minimum and maximum values were recorded in July (9.2 °C) and January (22.8 °C), respectively. Precipitation increases in September, with maximum records in January (about 150 mm). In May, the precipitation begins to decrease with the lowest values recorded in June, July, and August (10 mm). Mean annual precipitation was 775 mm (figure 5); alternating humid and dry periods around the mean with a seasonal trend in precipitation records. The driest and wettest years of the series were 2003 (617 mm) and 1998 (>1000 mm), respectively (figure 5). In 2007 and 2008, the invertebrate sampling years, the annual precipitation was 833 and 717 mm, respectively.

Based on results of the water balance performed for the data series analysed, three water surplus periods were identified (figure 6): 1998–2001, 2004–2005 and 2007. Figure 7 shows the precipitation, PET, AET, and water surplus corresponding to the two last years of the series. The highest precipitation values were recorded in February 2007 and January 2008, whereas AET was above 130 mm in January of both years. Surplus amounted to almost 57 mm, distributed between February and March 2007. There was no water surplus in the rest of the period analysed.

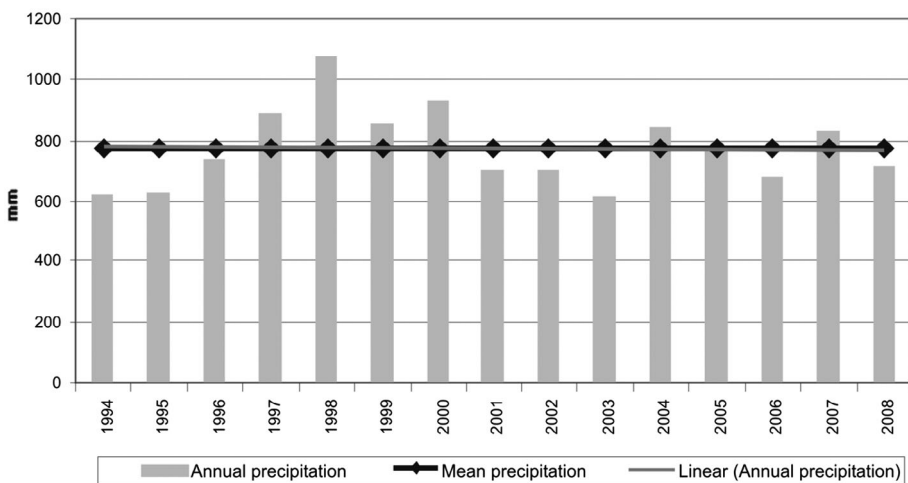


Figure 5. Chronological curve of annual precipitation. La Aguada Series 1994–2008.

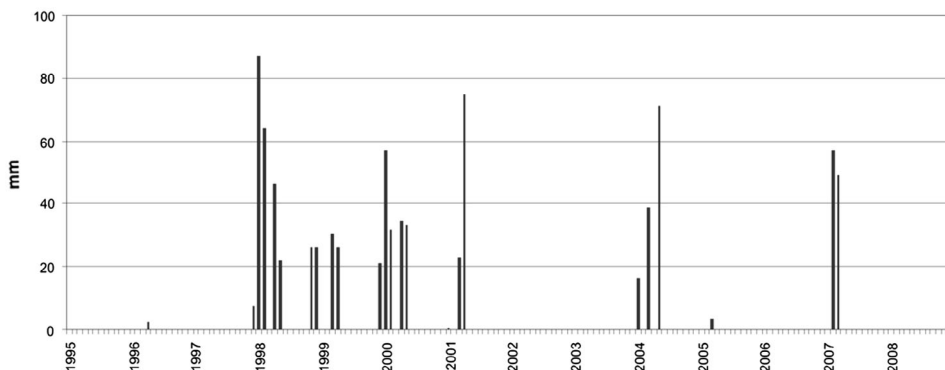


Figure 6. Water surplus estimated with water soil budget. La Aguada Series (1994–2008).

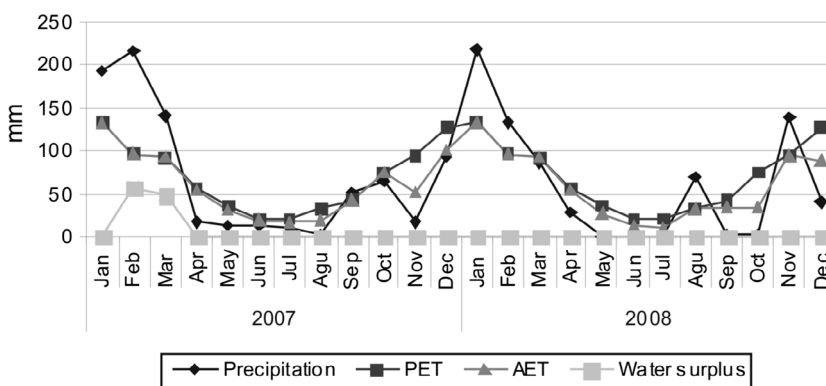


Figure 7. Precipitation, Potential Evapotranspiration (PET), Actual Evapotranspiration (AET) and water surplus. La Aguada Series (2007–2008).

**Physico-chemical groundwater characteristics**

Table 2 presents results of the physico-chemical parameters measured *in situ* and  $\text{NO}_3^-$  concentrations for each well. The lowest temperature value was recorded in W1 in the 2007 sampling and the highest value was detected in W36 in the 2008 sampling. DO concentrations ranged between 2.2 and 6.5  $\text{mg l}^{-1}$ , and pH values were above 7. Groundwater is fresh, except in W87, which exhibited the highest salt concentrations (brackish waters). Groundwater is of sodium bicarbonate type, except in W87 and W47, where it is of sodium sulphate and sodium sulphate bicarbonate type, respectively (figure 4).  $\text{NO}_3^-$  was detected in all the samples analysed, with values ranging between 1 and 190  $\text{mg l}^{-1}$ .

**Water microbiology**

Table 3 presents results of TMC, TC, and presence or absence of *E. coli* and *P. aeruginosa* for each well and sampling date. The highest TMC values in the 2007 sampling were detected in W36, whereas in the 2008 sampling, values in that well were 0. In the 2008 sampling, the highest values were recorded in W86. The only well with presence of



Table 2. Physical chemical parameters measured in situ and  $\text{NO}_3^-$  in each sampling point and year.

	W1		W86		W15	W87		W80b		W36		W47
	2007	2008	2007	2008	2008	2007	2008	2007	2008	2007	2008	2008
T° (°C)	12.5	18.9	17.2	22.5	19.2	17.8	20.6	19.4	19.6	19.6	25.4	22.5
DO (mg/L)	3.25	2.22	3.5	2.52	4	3.45	4.25	3.74	6.5	4.43	3.12	3.1
pH	7.33	7.28	7.93	7.79	7.56	7.74	7.66	7.79	7.57	8.36	8.25	7.85
EC	2,140	1,567	1,965	2,030	1,087	4,150	3,670	1,108	1,071	895	833	1,569
$\text{NO}_3^-$ (mg/L)	*	1	125	190	48	25	12	25	14	10	7	12

\*No data.

Table 3. Groundwater microbiological characteristics in each sampling point and year. TMC: total microbial count; TC: total coliform count.

	W1		W86		W15		W87		W80b		W36		W47	
	2007	2008	2007	2008	2008	2008	2007	2008	2007	2008	2007	2008	2007	2008
TMC (UFC/ml)	*	$1.4 \times 10^3$	$5 \times 10^3$	0	$2 \times 10^3$	0	0	$4 \times 10^3$	$1.5 \times 10^3$	$4 \times 10^2$	$2 \times 10^4$	0	$1 \times 10^2$	0
TC (NMP/100 ml)	*	>2400	3	0	43	0	9	23	1100	93	93	0	0	0
<i>Escherichia coli</i>	*	P	P	P	A	A	A	A	A	P	A	P	A	A
<i>Pseudomonas aeruginosa</i>	*	P	A	A	P	A	A	P	A	A	P	A	P	P

\*No data.

P: Presence.

A: Absence.

Table 4. Invertebrate abundance for each sampling point and year.

TAXÓN	CODE	W1		W86		W15		W87		W80b		W36		W47												
		2007	2008	2007	2008	2008	2008	2007	2008	2007	2008	2007	2008	2007	2008											
<b>Crustacea</b>																										
Copepoda	Cope	5	a	71	B	39	a	4	a	27	AB	23	a	187	C	812	C	812	b	12	A					
Cladocera	Clad	5	ab	58	B	39	c	4	ab	27	AB	23	bc	187	C	698	C	698	d	12	AB					
Ostracoda	Ostr			13												25		25								
Amphipoda	Amph															4		4								
<b>Acari</b>																72		72			2	9				
Mesostigmata	Meso	29		1		2		15		2		56										1				
Oribatida	Orib	13		1																						
Astigmata	Asti	8						1		1		3										2				
Prostigmata	Pros	1						14		1		53														
<b>Collembola</b>																										
Symphyleona	Coll	6	252	1		2				1		1				72					1	8				
Arthropleona		2	4	1																		1				
<b>Insecta</b>																										
Larvae	Ins.Lary	4	248	1						1		1										1				
Adults	Ins.Adu	1	6	3						1						7		7				2				
<b>Oligochaeta</b>																										
<b>Pauropoda</b>																										
<b>Nematoda</b>																										
<b>Total abundance</b>																										
		7	293	a	76	B	41	a	19	a	30	AB	80	a	187	C	6179	C	6179	b	15	AB	3	a	12	a

Capital letters indicate significant differences ( $p < 0.05$ ) between sites for 2007 and lower case letters indicate significant differences between sites for 2008 ( $p < 0.05$ ).

microorganisms in both samplings was W80b. W47, one of the deepest wells, exhibited very low TMC values; but the presence of *P. aeruginosa* was observed. The highest TC values were detected in W80b and W1 in the 2007 and 2008 samplings, respectively. The presence of *E. coli* was detected in W1, W80b and W86; in the last well, *E. coli* was detected in both samplings. *P. aeruginosa* was observed in all wells, except in W86 and W80b.

**Invertebrates**

Table 4 presents the abundance of each taxon in each well and on each sampling date and ANOVA results for the variables analysed. In both samplings, the highest total abundance of invertebrates, Crustacea and Copepoda were recorded in W80b ( $p < 0.05$ ). Cladocera was observed in two wells, W86 (2007 sampling) and W80 (2008 sampling). Well W80b was the only one with presence of individuals belonging to Ostracoda and Amphipoda.

The highest abundance of Acari was detected in W80b in 2008. The most abundant Acari taxon was Prostigmata, which was found in all the wells, except for W36. Collembola was very abundant only in W1, being scarce in the remaining wells. Insecta was barely abundant and was observed up to a depth of 30 m. In the 2007 sampling, larvae were detected only in W86, whereas in the 2008 sampling, these organisms were found in W1 and W80b. Oligochaeta abundance was very high in W80b in the 2008 sampling.

Total abundance of invertebrates, Copepoda and Crustacea did not exhibit seasonal variation ( $p > 0.05$ ), except in W80b, where the three variables were significantly higher in 2008 than in 2007. DCA (figure 8) shows that samples collected from a single well on both years were located close to each other for W1, W80b and W36; the last well is clearly separated from the rest and is the only one where the taxon Pauropoda was observed. W80b exhibited high abundance of Copepoda and a community composed of Ostracoda, Amphipoda, Oligochaeta, and insect larvae. W15 and W87 formed a separate group, although they were not clearly associated with any taxon.

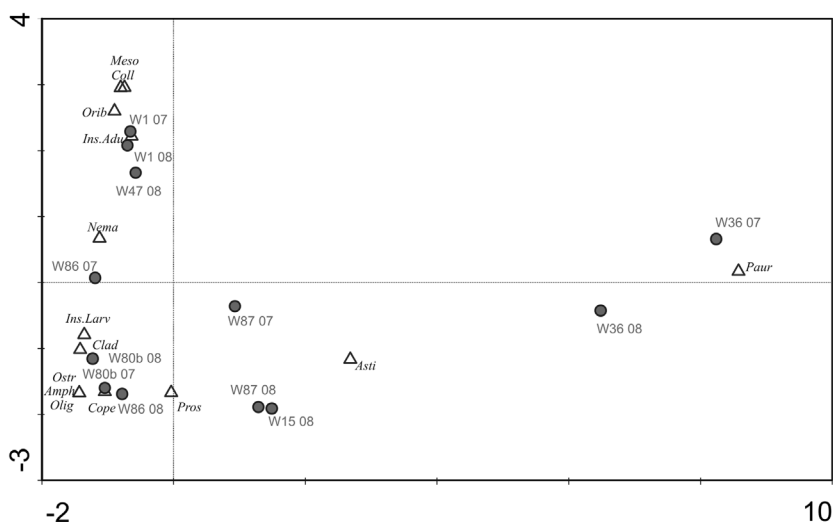


Figure 8. Detrended Correspondence Analysis based on the abundance of taxonomic groups of invertebrates. First (07) and second (08) samplings. ● Well. For abbreviations, see table 4.

## Discussion

### *Hydrometeorology*

The meteorological data show that precipitations are clearly concentrated in spring-summer. Water surplus corresponds partly to surface runoff and partly to effective infiltration that recharges the aquifer, resulting in water table rises [26]. Previous studies conducted in the basin reported rises of water table in 1995. A rising trend started in 1998, reaching the maximum value in 2002 [26]. This sequence of rises in water table is consistent with surplus periods in the series studied. Furthermore, the deficit recorded in 2008 agrees with the low precipitation which occurred in that year.

### *Physico-chemical groundwater characteristics*

In general, little variation in groundwater temperature was observed among samples owing to the moderating effect of the aquifer [34]. DO concentrations and pH values were typical for unconfined aquifers in recharge areas in this region [35,36]. According to the EC results, groundwater is fresh, resulting from recent recharge and short flow paths from the mountains. The highest EC values recorded in W87 result from the longer contact time with the loessical sediments and calcrete layers in an area with a thick USZ that delays recharge [26]. Sodium was the dominant cation in all the samples; associated with the dominance of fine-grained sediments that allow processes of cation exchange (sodium–calcium) [26]. Concentrations of  $\text{NO}_3^-$  higher than those observed under pristine conditions ( $5 \text{ mg l}^{-1}$ ) are attributed to anthropogenic pollution [36,37]. The high  $\text{NO}_3^-$  concentrations found in W86 are associated with recharge of nitrogenous organic matter discharged from a livestock pen as well as with scarce USZ thickness [35,38].

### *Water microbiology*

The highest TC values and the presence of *E. coli* and *P. aeruginosa* in W1 indicate that a 0.5 m USZ thickness is insufficient to control the amount of bacteria reaching the groundwater, as suggested by Mauclair and Gibert [39]. The presence of *E. coli* in groundwater evidences recent pollution because these bacteria have low capacity to survive outside the host [40]. The low TMC records in W47 suggest that a 30 m USZ thickness would be effective to prevent pollutants from reaching the aquifer; several works suggest that self-purification processes would occur within the first few meters of infiltration [39]. W36, with a similar USZ thickness, exhibited the highest TMC values, showing that bacterial density varies with amount of available organic matter derived from pollution sites [41]. Thus, the presence of organic matter provided by manure allows the organism to live in a somewhat isolated environment from the general soil [10,42]. Besides, elevated numbers of bacteria may directly originate from infiltrating water or may be a result of increased availability of organic carbon and nutrients [14].

### *Invertebrates*

The most abundant and widely distributed taxon was Crustacea; this finding is consistent with observations of various authors [10,11,22,25,43–48]. The high Crustacea abundance may be attributed to the lack of competitors, such as insects [43,49]. The absence of

insects leaves many habitats and potential niches empty; they may be used in a similar manner particularly by some crustacean species [49]. Adult Insecta were restricted to USZ <5 m, except for W47, whereas larvae were restricted to wells associated with pollution from livestock. These results do not agree with findings of Malard et al. [9], who indicated that no larvae were observed in most of the contaminated sites studied in France. The presence of organisms belonging to Collembola, Nematoda, Acari and Oligochaeta is consistent with findings of [10,14,45,50]. In the Crustacea taxon, Copepoda was the most common group, which is in agreement with many works [10,13,25,44–48,51,52].

DCA clearly evidenced differences in faunal composition among wells, confirming the assumption that at the microscale level, fauna shows differences in habitat preference [53].

Wells located in sectors with the thickest USZ exhibited the lowest abundances of most taxa. Comparing W86 and W36, which have similar land use and different USZ thickness, abundance of Crustacea was lower in W36, which would be associated with the lower amount of organic matter reaching the aquifer at that site because of the greater USZ thickness [18]. Furthermore, W36 differs in faunal composition from the remaining wells, showing that both abundance and assemblages change with increasing depth of the water table [18]. These results are in agreement with previous studies indicating decreases in abundance in deep ecosystems [18,54]. Comparing wells without point source pollution and with different USZ thickness (W15 and W47), no differences in total abundance were observed; but, there were differences in composition. In the well of greatest USZ thickness, no crustaceans were found, but insects were observed. Overall, and as expected, the effect of USZ thickness on abundance is more noticeable in those sites where point contamination sources exist, which supply organic matter.

Wells W80b and W87 have similar USZ thickness (12 m) but different hydrogeological conditions and land use, characteristics that are reflected in faunal composition. According to Ward et al. [55], the specific geomorphology and the hydrogeological characteristics of sites are the most important determinants of fauna distribution and abundance. The high abundance of invertebrates in W80b agrees with results reported for an aquifer in France, where the most abundant populations were observed near sites producing organic matter [18]. The entry of pollutants to W80b is associated with the increase in the number of pigs. High abundance of Oligochaeta was observed in this well. Oligochaetes have played an important role in assessment, particularly in their mass occurrences under disturbed conditions [56] and are mainly related to organic pollution because they feed on readily degradable organic matter [56]. The high density of Copepoda, along with the occurrence of Amphipoda, is consistent with values recorded in an aquifer from New Zealand, which were considered indicators of organic enrichment [11]. The copepods show marked differences in microhabitat preferences and sensitivity to anthropogenic disturbances [53,57] and the high copepod abundance may provide useful indicators of organic enrichment [11]. The presence of Ostracoda and Cladocera agrees with the works of [9,43], who found that those taxa were characteristic of contaminated sites.

Comparing W15 and W86, which have similar USZ thickness (5 m) and different land use, total abundance was higher in W86 (data not statistically confirmed). A higher abundance in W86 would be caused by the input of organic matter from livestock pens because a high number of individuals is expected in shallow aquifers that receive energy and biomass input [8]. Land use also differs between the deepest wells, W36 (cow and pig pens) and W47 (park and some cows). There were no marked differences in invertebrate abundance, however. Indeed, the effect of land use decreases with depth, because nutrient presence in the aquifer would be precluded by the great thickness of the USZ.

In general, faunal communities of all wells presented marked seasonal stability, which was evident in the close location of the two samplings of a single well on the DCA graph. These results agree with observations reported for aquifers from Spain and New Zealand, in which seasonal differences in the community were weak or undetectable [11,43]. On the other hand, the present results are not consistent with findings of Bruno and Perry, who observed seasonal differences in abundances [58].

### ***Water microbiology and invertebrates***

The increased organic carbon entering the groundwater from contamination sources stimulates biofilm formation; that, in turn, increases densities of groundwater invertebrates [59]. Although it is well known that microbial biofilms are a food source for invertebrates [17], no relationship was observed between TMC and invertebrate abundance in the present work. In the 2007 sampling, however, the highest invertebrate abundance observed in W80b was consistent with the highest TC values. In the 2008 sampling, the highest invertebrate abundance and the highest TC values were recorded in W80b and W1. These results agree with findings of Mösslacher and Notenboom, who stated that an increase in microbial production resulting from organic enrichment led to an increase in abundance of metazoans in groundwater [60]. Therefore, the invertebrates may reflect small-scale variability in chemical compounds and be good indicators for zones of increased nutrient and carbon input [61].

### ***Relationships between invertebrates, physico-chemical groundwater characteristics and hydro-meteorological issues***

There was no direct correlation between invertebrates and any of the water physico-chemical variables, in agreement with many works [10,43,44,50,61–65], where simple physical or chemical factors have shown correlation only inconsistently with the presence of the groundwater fauna. Thus, faunal distribution in the groundwater seems to be more closely related to complex interaction patterns than to individual factors [61,66].

The absence of correlation between DO and invertebrate abundance coincides with the results of several authors [24,61,64]. DO concentrations were above  $2 \text{ mg l}^{-1}$ ; according to Hahn, at concentrations above  $1 \text{ mg l}^{-1}$  there is no correlation between DO and fauna [50]. On the other hand, Danielopol, and Bruno and Perry demonstrated a positive correlation between fauna and DO [54,58], whereas Schmidt et al. and Särkkä et al. did not find DO to be a limiting factor [61,67]. In the present study, for each well, except for W1, total abundance of invertebrates increased with increasing DO concentration.

Because temperature in groundwater is less variable than in surface water, organisms are expected to be stenothermal [68]. In the present work, no correlation between temperature and invertebrate was observed. By contrast, Schmidt et al. found that temperature was related to differences between faunal groups [61]. Furthermore, salinity in groundwater exhibits wide variation and restricts biota distribution, although many invertebrates are euryhaline [68]. Although no correlation between EC and fauna abundance was detected, the well with highest EC (W87) differed in faunal composition from the rest. As indicated by Humphreys, groundwater fauna is rich in systems of pH values ranging between 7.2 and 8.2 [16]; the values reported in this study fall within that range. In addition, in this study  $\text{NO}_3^-$  concentration was not correlated to invertebrate abundance, not even the high values found in W86. These results are in agreement with findings of Di Lorenzo et al.,

who indicated that the presence of fauna was not affected at high  $\text{NO}_3^-$  values ( $56.8 \text{ mg l}^{-1}$ ) [44].

There was not a clear influence of water soil surplus, and eventually more water recharge to the aquifer, on groundwater communities between the 2 years of sampling.

## Conclusions

Although the aquifer studied is of lower hydraulic conductivity than alluvial or karst aquifers, the invertebrate abundance values found are comparable to values of those types of aquifer. To the best of our knowledge, this is the first study documenting a diverse and abundant invertebrate fauna in loessic unconfined aquifers. Therefore, those organisms would play a key role in ecosystem functioning, in decomposition and nutrient cycling, and are therefore central in the maintenance of water quality.

A clear influence of USZ thickness on community characteristics was observed. The abundance of invertebrates was lower in sites with greater USZ thickness because of the lower amount of organic matter reaching the groundwater. Furthermore, an important influence of land use on invertebrate community was observed in wells related to point contamination sources. This effect was reduced with increasing USZ thickness, which prevents nutrients from reaching the aquifer.

Besides generating greater abundance of organisms, the input of organic matter influences the presence of certain taxonomic groups, especially Copepoda, Cladocera, Ostracoda, and Oligochaeta, which might be considered biological indicators of contamination by sources that contribute organic matter. Finally, chemical characteristics of groundwater, such as EC, influenced communities, generating environments suitable for some particular groups.

It would be of great interest to conduct a new and wide groundwater survey sampling to augment and improve knowledge of the groundwater communities.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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