Earthquakes trigger the loss of groundwater biodiversity

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Earthquakes are among the most destructive natural events. The 6 April 2009, 6.3-Mw earthquake in L'Aquila (Italy) markedly altered the karstic Gran Sasso Aquifer (GSA) hydrogeology and geochemistry. The GSA groundwater invertebrate community is mainly comprised of small-bodied, colourless, blind microcrustaceans. We compared abiotic and biotic data from two pre-earthquake and one post-earthquake complete but non-contiguous hydrological years to investigate the effects of the 2009 earthquake on the dominant copepod component of the obligate groundwater fauna. Our results suggest that the massive earthquake-induced aquifer strain biotriggered a flushing of groundwater fauna, with a dramatic decrease in subterranean species abundance. Population turnover rates appeared to have crashed, no longer replenishing the long-standing communities from aquifer fractures, and the aquifer became almost totally deprived of animal life. Groundwater communities are notorious for their low resilience. Therefore, any major disturbance that negatively impacts survival or reproduction may lead to local extinction of species, most of them being the only survivors of phylogenetic lineages extinct at the Earth surface. Given the ecological key role played by the subterranean fauna as decomposers of organic matter and “ecosystem engineers”, we urge more detailed, long-term studies on the effect of major disturbances to groundwater ecosystems.

Although groundwater is predominantly viewed as a life-devoid water reservoir, there is a unique, stable animal community which lives in subterranean waters1–3. Life at extremes? Not for groundwater animals, which have developed adaptive traits as long-term groundwater dwellers4. They are small-sized, colourless, blind, with low metabolic rate and fecundity, features which allow survival in total darkness, lack of photosynthesis, low food availability, and strict dependence on the organic matter entering groundwater from the surface5,6. Crustaceans dominate in groundwater communities2, and most of them are minute copepods6 (Fig. 1a), followed by ostracods7 and larger-bodied amphipods6.

More than 90% of the groundwater fauna is composed of local endemics, groundwater-exclusive taxa (from species through class) that significantly expand the phylogenetic global animal biodiversity5,6,8,9. Despite its uniqueness, and a timid increase in presence within the scientific literature on inland waters, the groundwater fauna remains poorly investigated and the least known component in global biodiversity assessments6,10 being hidden, invisible, and often unapproachable. Several groundwater taxa are living fossils, the only remnants of very ancient evolutionary lineages disappeared from the Earth surface, but preserved underground and at high risk of extinction8,12. Groundwater fauna importance in the overall freshwater biodiversity goes beyond its uniqueness. Obligate-groundwater species (i.e. stygobites, which complete their life cycle in groundwater, being unable to reproduce and survive in surface-water habitats) play key roles in organic matter recycling12,13 and as “ecosystem engineers” because the burrowing life style of worm-like elements allows the oxygenation of the minute voids among sediment particles14. Groundwater invertebrates may also serve as early warning systems in monitoring groundwater quality and quantity, being sensitive to groundwater contamination15–17 and withdrawal18,19, because they respond to such disturbances as much as surface-water species do. However, a knowledge vacuum still surrounds the response of groundwater fauna to both human-induced16 and natural drivers, earthquakes holding the primacy among the latter.

Earthquakes are among the most destructive natural events20. Their effects on underground hydrology are well known, and are associated with the potential loss of groundwater supply for human use21. Hydrological responses depend on several factors, such as earthquake magnitude, distance from the epicentre, and local geological conditions21–24. The mainshock of the L’Aquila earthquake on 6 April 2009 (Mw 6.3) dramatically changed the...
groundwater flow of the Gran Sasso Aquifer (GSA) and consequently of the Tirino River valley, where most of total aquifer discharge is located (Fig. 2). A strong base-flow component and the presence of no-flow limits defined by aquitards (constant head conditions) determine a steady water table at the Tirino Springs (TS), historically characterised by limited seasonal variations in springtime recharge, summer peak and autumn/winter shortage. Short-term earthquake effects on GSA hydrology were determined by a pore pressure increase due to aquifer deformation. This short-term process triggered a medium-term response in the fractured aquifer: an earthquake-induced increase in bulk hydraulic conductivity in the recharge area, near the earthquake-ruptured fault zone, attributable to fracture clearing and/or microcrack formations. The water table in December 2012 was still at least 1 m higher than in the two years after the mainshock. A partial return toward pre-earthquake discharge levels started only in 2013 (Supplementary Fig. 1). The increased post-seismic discharge at TS was evident already in May 2009, reaching out-of-range values (~80% by individual-based density). The remainder of the meiofauna collected at TS included nematodes (~14%), oligochaetes (~3%), ostracods (~1%), halacarid mites (~1%), gastropods (~1%), together with a small fraction of macroinvertebrates, such as amphipods and chironomid larvae (~3%).

Results
Changes in groundwater environmental descriptors. Univariate nonparametric tests returned significant 1997–2005 vs. 2012 differences for most of the environmental parameters analysed. Post-
seismic groundwater pH was significantly lower and Ca$^{2+}$ and dissolved oxygen (DO) concentrations were significantly higher than pre-seismic levels (Fig. 3). Electrical conductivity increased in September 2009 (up to 580 $\mu$S cm$^{-1}$), but returned rapidly to pre-earthquake values (Fig. 3). Very fine sand and particulate organic matter (POM) significantly increased in 2012 at the TS outlets (Fig. 3).

Water temperature at the TS sampling sites remained roughly constant throughout the sampling period, with median values at around 11°C (Supplementary Fig. 2). Post-seismic nitrate concentrations were significantly lower than pre-seismic values (Supplementary Fig. 2). Sulphate concentrations in 2012 returned to 1997 values after a transient peak in 2005 (Supplementary Fig. 2). Except for very fine sand (Fig. 3), none of the grain size classes sampled at TS changed significantly across sampling years, though the finest classes (medium and fine sand, and silt and clay) tended to increase after the earthquake, albeit qualitatively (Supplementary Fig. 2). The raw abiotic data set is in Supplementary Table 1.

**Effects on groundwater biodiversity.** Total groundwater copepod abundance declined significantly after the earthquake (Fig. 3). Though statistical separation remained incomplete, groundwater species richness also exhibited a post-seismic negative trend (Fig. 3). Community patterns for non-obligate groundwater copepods (i.e., species which live in spring habitats because cold stenothermic or generalists, and species originally coming from surface water in recharge areas and “leaked” underground) did not change significantly after the earthquake (Fig. 3). Though both non-obligate and obligate groundwater species were found at 26 out of the 32 sampling sites in 2012, such numbers represented a decrease for the stygofauna and an increase for non-obligate groundwater copepods (Table 1). The response at species level was varied for surface-water taxa, with six (~46%) species decreasing and five (~38%) species increasing in site-specific occurrence in 2012, while seven of the nine obligate groundwater species (including all the three most common pre-earthquake species) had decreased significantly in post-earthquake site-specific occurrence (Table 1).

Abundances of the 22 copepod species that we found at the 32 sampling sites declined dramatically after the earthquake (Supplementary Table 2). Pre- vs. post-earthquake differences in species abundance were highly significant (Supplementary Table 3; pairwise tests after significant one-way PERMANOVA: 1997 vs. 2012: $P = 0.0009$; 2005 vs. 2012: $P = 0.0015$), while differences between the two pre-seismic samplings remained non-significant (1997 vs. 2005: $P = 0.5131$). Obligate groundwater species mostly accounted for the dramatic post-earthquake decrease in faunal abundance (pairwise tests after significant one-way PERMANOVA: 1997 vs. 2012: $P = 0.0007$; 2005 vs. 2012: $P = 0.0012$; 1997 vs. 2005: $P = 0.7450$), with the contribution from non-obligate groundwater species remaining non-significant (one-way PERMANOVA: $P = 0.1379$) (Supplementary Table 3).

The groundwater species *Nitocrella pescei*, *Diacyclops paolae*, *Parastenocaris lorenzae* and *Elaphoidella mabelae* cumulatively provided 90% of the discrimination between pre- vs. post-earthquake assemblages (Supplementary Table 4). *N. pescei* contributed the most (41.7%) to the pre- vs. post-dissimilarity by declining by ~2.5× after the earthquake (Fig. 4, Supplementary Table 4). The other three most common species contributed further to pre- vs. post-seismic dissim-
illarity to a cumulative ~50%, though *D. paolae* and *P. lorenzae* decreased in abundance by only 0.5× and *E. mabelae* had slightly increased by 2012 (Table 1, Supplementary Table 4, Fig. 4). The vast majority of the remaining groundwater species became rare to very rare in 2012 (Table 1, Supplementary Table 2).

**Discussion**

The mainshock of the L’Aquila earthquake of 6 April 2009 markedly changed the Gran Sasso groundwater flow and consequently the discharge at the main spring sites. Medium-term local effects included an anomalous increase in TS discharge through 2012 (Supplementary Fig. 1). The TS discharge gradually returned toward pre-seismic values only in summer 2013 (Supplementary Fig. 1), consistent with model predictions.

Pre- vs. post-earthquake changes in groundwater physico-chemistry were observed at the TS main outlets. Post-seismic water pH (Fig. 3) decreased as a reflection of an increase of deeply-derived CO\(_2\) along the main faults during the mainshock. The post-seismic decrease in pH was accompanied by a significant increase in Ca\(^{2+}\) concentration from calcite dissolution (Fig. 3). Electrical conductivity increased in September 2009 as a result of the clearing of previously obstructed fractures from which high residence-time ion-enriched water flushed out after crustal stress and ground shaking (Fig. 3). Concentrations of very fine sand and POM increased at the main TS outlets (Fig. 1b–d, Fig. 3) due to the massive dewatering of the conductive systems which emptied the annexed fractures in the aquifer. Dissolved oxygen (DO) concentration increased significantly after the earthquake (Fig. 3), as expected for the increase in fast groundwater flowpaths in the aquifer due to a post-seismic higher hydraulic conductivity.

The decrease in post-seismic nitrate concentrations (Supplementary Fig. 2) was most likely due to a markedly lower human presence in the catchment area following the post-earthquake temporary evacuation of towns and farms. Higher sulphate concentrations in 2005 (Supplementary Fig. 2) were related to the dual groundwater flow system feeding the TS. The seasonal contribution from local heavy rainfall led to an above-average recharge rate in 2005, and to an enrichment in SO\(_4^{2-}\) due to a more intense interaction with the Triassic evaporite bedrocks along the local and most superficial groundwater flowpath. Conversely, the highest post-seismic groundwater contribution was from the deepest, base-flow fractured aquifer, described by large karstic conduits and the annex capacitive subsystem. Absence of temporal changes for most such variables and the overall low values for nitrates and sulphates (Supplementary Fig. 2) suggest a limited (if any) influence of such variables on the observed changes in aquifer-dwelling biota.

Stygobiotic copepods responded negatively to changes in the groundwater hydrodynamics triggered by the earthquake (Table 1, Figs. 3, 4, Supplementary Table 3). However, the relative contributions of the single-event massive mainshock (from which the stygofauna had yet to recover as of late 2012) or from the post-mainshock diminished drift (due to the overall initial crash in stygobiobtic abundance) remain unknown. Interestingly enough, our earthquake-targeting investigation has led to a noteworthy corollary finding: though most species within the harpacticoid genus *Elaphoidella* are true stygobites, the slight but significant post-earthquake increase...
in E. mabelae presence (Table 1, Fig. 4) supports the view of an intermediate stygo- and crenobiotic ecology of this species.37

Though a few species can cope with the challenging fast-flowing conduit system of karstic aquifers, the dendritic fracture network preferentially allows the establishment of permanent (in ecological terms) invertebrate communities.38,39 The low flow rate in such fracture “chambers” (Fig. 1b–d) allows the storage of fine sediments, which form the physical substrate for burrowing animals, and of POM, which constitutes the bulk of the food. Open-water swimmers also avoid massive drifting (Fig. 1b).

Based on our limited dataset of three non-contiguous sampling years, and considering the relatively stable pre-earthquake groundwater faunal patterns, we propose that the earthquake flushed out groundwater invertebrates, very fine sand and POM at the aquifer discharge sites (Fig. 1c). The massive earthquake-induced increase in hydraulic conductivity and the consequent aquifer dewatering (Fig. 1e–g) thus acted as what we call a biotrigger. Immediately after the mainshock, groundwater communities were stranded in springbed sediments, where they survived and maintained the pre-earthquake species richness albeit at lower abundances, a condition expected if individuals of fracture-dwelling species were massively flushed out (Table 1). Neither the post-seismic higher DO nor the higher POM availability at the TS outlets favoured the survival and especially the reproduction of groundwater species, and the contribution of post-earthquake drift for groundwater species was still negligible as of 2012. Stygobite numerical losses in the aquifer fractures were not consistently or fully counterbalanced by conspecifics coming from their original habitat (i.e. small and large fractures eventually less affected by the aquifer dewatering). Progressively decreasing abundances of obligate groundwater species (i.e., stygobites) were found at the TS outlet, because the aquifer became almost totally deprived of animal life, no longer replenishing groundwater populations normally flushing out at the spring outlets (Fig. 1c, d). Abundance and species richness of non-obligate groundwater species remained unchanged (Fig. 3), suggesting that the earthquake had an impact only on aquifer-dwelling microinvertebrates. We therefore believe that the main driver of the observed crash in post-earthquake biodiversity occurred inside the aquifer.

Table 1 | Changes in frequency of occurrence of copepods in the Tirino Springs (TS). Number of sites per sampling year at which the two major groups of obligate groundwater (GW) and non-obligate groundwater (nGW) species, and all individual species, were recorded as present. Significant departures from expected values in 2012 (according to Pearson adjusted residuals following significant contingency-table, Pearson-distance based tests of independence on coded data: groups: \(X^2 = 9.627, df = 2, P = 0.008\); species: \(X^2 = 5507.511, df = 42, P < 0.0001\)) are highlighted with up and down arrows, representing significant increases or decreases, respectively; * = most common pre-seismic species

| ecological category | taxon                                      | number of sites of occurrence |
|---------------------|--------------------------------------------|-------------------------------|
|                     |                                            | 1997 | 2005 | 2012 | 2005-to-2012 trend |
| GW                  | all species                                | 31   | 30   | 26   | ↓                |
| GW                  | Diacyclops italicus                        | 2    | 0    | 0    | ↓                |
| GW                  | Diacyclops paolae*                         | 17   | 18   | 13   | ↓                |
| GW                  | Pseudaecotoma reductum                     | 1    | 1    | 0    | ↓                |
| GW                  | Nitocrella kunzi                           | 5    | 2    | 1    | ↓                |
| GW                  | Nitocrella pescei*                         | 24   | 26   | 17   | ↓                |
| GW                  | Parmaseudolentamesochra italic             | 6    | 2    | 2    | ↓                |
| GW                  | Elaphoidella mabelae                       | 4    | 7    | 12   | ↓                |
| GW                  | Parastenocaris lorenzoe*                   | 16   | 14   | 9    | ↓                |
| GW                  | Simplicaria lethaea                        | 4    | 1    | 2    | ↓                |
| nGW                 | all species                                | 28   | 27   | 26   | ↓                |
| nGW                 | Eucyclops serrulatus                       | 5    | 7    | 12   | ↓                |
| nGW                 | Paracyclops fimbriatus                     | 11   | 9    | 4    | ↓                |
| nGW                 | Microcyclops varicans                      | 1    | 3    | 2    | ↓                |
| nGW                 | Nitokra hibernica                          | 2    | 3    | 11   | ↓                |
| nGW                 | Astheylla crassa                           | 5    | 9    | 5    | ↓                |
| nGW                 | Bryocamptus minutus                        | 0    | 1    | 0    | ↓                |
| nGW                 | Bryocamptus zschokkei                      | 7    | 5    | 6    | ↓                |
| nGW                 | Bryocamptus pygmaeus                       | 9    | 8    | 6    | ↓                |
| nGW                 | Bryocamptus tychlops                       | 7    | 14   | 9    | ↓                |
| nGW                 | Bryocamptus echnatus                       | 19   | 23   | 20   | ↓                |
| nGW                 | Moraria poppei                             | 1    | 10   | 7    | ↓                |
| nGW                 | Pesceus schmeili                           | 13   | 16   | 10   | ↓                |
| nGW                 | Epactophanes richardi                      | 1    | 2    | 0    | ↓                |
How much time is needed for the re-colonisation of the Gran Sasso aquifer by groundwater species, allowing the re-establishment of viable microcrustacean populations? We think that such a process will take a very long time, probably several decades, much longer than the return to the pre-earthquake aquifer recharge/discharge cycle and geochemistry. Groundwater communities are notorious for their low resilience\(^5\). Virtually all species have low reproductive rates, long life spans (up to 3.5 years for groundwater copepods vs. \( \leq 1 \) year for surface-water relatives), and long post-embryonic development times before reaching sexual maturity\(^6\). The potential for replacement by other groundwater species is very low, aquifer communities being characterised by low community redundancy (although omnivory is claimed as a relatively frequent functional trait in the oligotrophic groundwater\(^4\), an ecological statement awaiting confirmation).

Our investigation is based on a limited dataset of three complete but non-contiguous hydrological years, so that a precise quantification of background variability ("noise") was not possible. Though some abiotic variables were fully comparable in the two pre-earthquake years (e.g., pH, Ca\(^{2+}\), POM and DO in Fig. 3), others were not (electrical conductivity in Fig. 3 and SO\(_4^{2-}\) in Supplementary Fig. 2). Also, some site-specific spatial variability occurred (e.g., electrical conductivity slightly higher at site 8 in Supplementary Table 1), suggesting the existence of some background variability in hydrogeology. However, all statistical analyses pointed to a pre-\(t\)-earthquake variation higher than within pre-earthquake variation (e.g., Friedman tests in Fig. 3 and all multivariate biological tests). Therefore, we believe that our results remain valid and robust as a first look into the effects of the major single-event disturbance such as an earthquake on groundwater ecosystems, but we urge more detailed, long-term studies on the topic. Despite the limited dataset, our results strongly suggest that a catastrophic natural disturbance such as a major earthquake seems to negatively impact the survival or reproduction of aquifer-dwelling stygobites, with a potential detrimental effect on groundwater biodiversity.

Methods

Sampling and sample processing: The Tirino Springs (TS) were selected as the study site because a natural entrance to the aquifer (e.g., caves) is not available in the Gran Sasso karst. In such cases, sampling is carried out at spring outlets\(^7,8\), or through artificial boreholes\(^1\). Most of the Gran Sasso Aquifer (GSA) discharge occurs through springs at the aquifer boundary (Fig. 2), and the TS receive \( \approx 65\% \) of the aquifer discharge\(^9\). We sampled the TS through bedrock fractures in the springbed, deep into the fracture zone where the springbed itself is incised into the carbonate bedrock, or stranded in the alluvial aquitards. Stygobites depend on groundwater to complete their life cycle, as they cannot reproduce in other environments. Species were characterised as stygobites on the basis of well-established morphological, physiological and ecological features\(^10,11\). Stygobites are frequently found in spring habitats where they can temporarily survive after being drifted from their original groundwater habitats\(^12,13\). Surface-water species are also found along with stygobites, either flushed out from the aquifer where they enter from recharge areas, or because they live in springs, as crenobionts or surface-water habitat generalists\(^14\).

Abiotic and biological variables were determined at eight sampling sites at the TS adopting a random sampling method with four seasonal replicates per site (Jan–Feb, Mar–Apr, Jul–Aug, Sep–Oct) in three consecutive years: 1997 and 2005 (before the earthquake) and 2012 (after the earthquake). Samples were collected with a Bou-Rouch pump\(^15\) and mobile pipes hammered at each sampling site. Samples were composite from 0.3, 0.7, and 1.5 m below the springbed. Since both sample size and pumping rate may affect estimates of faunal density for a range of different sediment types, all samples were maintained constant pumping rates and sample volume sizes throughout the surveys. Sufficient samples would detect spatial variation in invertebrate composition among sites with different sediment texture, sample size affecting mainly large-sized invertebrates like insect larvae\(^16\), a sample size of 20 L was adopted for density estimates, thus including abundance-related rarity\(^17\) of groundwater communities. The copepods were used as the target biological variable being by far the most abundant and species-rich groundwater group (~80% by individual-based density).

Meiofauna was extracted by filtering the 20-L samples through a hand net (mesh size = 60 \(\mu\)m). Faunal samples were preserved in an ethyl alcohol solution at 80%.

Statistical analysis: The sampling design was fully balanced, thus maximising statistical power and reliability\(^18\). Coleman rarefaction curves run for biological data were used for sampling effort comparisons for all sampling years (Supplementary Table 5, Supplementary Fig. 3). Coleman rarefaction curves were constructed with EstimateS\(^9\) using resampling with replacement based on 50 iterations. Between-years comparisons for abiotic and biological variables were tested with non-parametric Friedman tests, a block-based variant of the Kruskal–Wallis test, also based on ranks, and used when \( k \geq 5\)\(^19\). Sampling sites within sampling years were used as blocks, resulting in triple-based paired comparisons (simultaneous 1997 vs. 2005 vs. 2012 comparisons). The paired-sample, rank-based approach was chosen also to minimise the impact of the inherent between-site variability, especially for some variables (e.g., grain size composition of sediments). Sample size (n) was 32 for all variables in each year, except for grain size, for which \( n = 8\). Friedman tests were strongly robust to the relatively low-sampling grain size categories\(^20\). However, the Friedman test is considered a robust technique as long as the assumption of homoscedasticity is met\(^21\). The Friedman test was applied only to those variables with homogeneous variances tested with a two-way Cochran C test\(^22\) (\( P < 0.05\)) for all abiotic variables and major biological groups; Supplementary Table 6). P-values for Friedman tests were determined asymptotically. A Nemenyi paired-sample multiple comparison test was performed for statistically significant Friedman tests to identify pairwise year-\(t\)-year trends. Partly to counterbalance the possible slight liberalism of Bonferroni correction, a Bonferroni correction was applied to all tests (whose original significance threshold was set at \( P < 0.05\), leading to a Cochran-Friedman-Nemenyi experiment-wide significance threshold of \( P < 0.016\). Cochran, Friedman, and Nemenyi tests were performed using XLSTAT\(^23\) (Addinsoft\(^\text{TM}\) XLSTAT v. 2013.6.01, Paris, France, 2014).

Copepod assemblages before and after the earthquake were examined using non-parametric PERMANOVA tests. Following significant PERMANOVA results, patterns of obligate groundwater copepods were examined using a HACH DR 2000 spectrophotometer (Hach Co., Loveland, CO, U.S.A.). Particulate organic matter (POM) was quantified on 20-L samples of all fauna, oven-drying at 105 °C for 24 h, and weighing in anhydrous conditions. The dry-weighed samples were ashed at 540 °C and re-weighed to determine POM concentration as the difference between dry and ash mass. The grain size composition of springbed sediments was measured only once per year at each spring site, as sediment transport is negligible throughout the spring system. A major indication of springbed sedimenting also would have altered habitat features and hence species abundance and distribution across sites. The mass of eight granulometric classes was determined at each site on the fractional dry-sieving of dissected samples through a stack of sieves according to the Wentworth\(^24\) scale: median gravel (4.8–4 mm), fine gravel (2.4–4 mm), very coarse sand (1.2–2 mm), coarse sand (0.5–1.5 mm), medium sand (0.25–0.5 mm), fine sand (0.125–0.25 mm), very fine sand (0.063–0.125 mm), silt and clay \((<0.063 \text{ mm})\).

Copepods were used as the target biological variable because they represent an easily sampled and quantified group of organisms that is representative of the water column. The copepods were used as the target biological variable because they represent an easily sampled and quantified group of organisms that is representative of the water column.
Levene test for homogeneity of variances was carried out before PERMANOVA (Supplementary Table 3). All multivariate analyses were done using PRIMER v6 (PRIMER-E, Plymouth, UK, 2006) and R software (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria, 2008).

1. Holsinger, J. R. Troglobites: the evolution of cave-dwelling organisms. Am. Sci. 76, 147–153 (1988).
2. Gibert, J. & Deharyana, L. Subterranean ecosystems: a truncated functional biodiversity. Bioscience 52, 473–481 (2002).
3. Gibert, J., Danielopol, D. L. & Stanford, J. A. Eds. Studies in Crenobiology: the Biology of Springs and Springbrooks. (Backhuys Publishers, Leiden, 1988).
4. Barquín, J. & Scarsbrook, M. Management and conservation strategies for coldwater springs. Aquatic Conserv: Mar. Freshw. Ecosyst. 18, 580–591 (2008).
5. Boulton, A. J., Dole-Olivier, M.-J. & De Laurentis, P. Phylogeny and biogeography of the genus Pseudocamptus, and description of P. minuta sp. n. (Copepoda, Copepoda, Cypridinidae). Pol. J. Limnol. 28, 289–303 (1999).
6. Kröbel, K. L. & Hose, G. C. A tiered framework for assessing groundwater invertebrates. Invertebr. Ecol. 22, 103–116 (2009).
7. Purvis, A., Agapow, P.-M., Gittleman, J. L. & Mace, G. M. Nonrandom extinction of vertebrates: insights from habitats with low thermal seasonality. Global Ecol. Biogeogr. http://onlinelibrary.wiley.com/doi/10.1111/geb.12200/pdf (2014) Date of access: 01/07/2014.
8. Dole-Olivier, M.-J., Fenwick, G. D., Hancock, P. J. & Harvey, M. S. Biodiversity, ecosystem functions and ecosystem services of groundwater invertebrates. Invertebr. Ecol. 22, 103–116 (2009).
9. Montgomery, D. R. & Manga, M. Streamflow and water well responses to earthquakes. Science 300, 2047–2049 (2003).
10. Chen, J. S. & Wang, C.-Y. Rising springs along the Silk Road. Geology 37, 243–246 (2009).
11. Wang, C.-Y. & Manga, M. Hydrologic responses to earthquakes – a general model. Geofluids 10, 206–216 (2010).
12. Manga, M. & Rowland, J. C. Response of Alum Rock springs to the October 30, 2007 earthquake and implications for the origin of increased discharge after earthquakes. Geofluids 9, 237–250 (2009).
13. Admoffi Falcone, R. et al. Changes on groundwater flow and hydrochemistry of the Gran Sasso carbonate aquifer after 2009 L’Aquila earthquake. Ital. J. Geosci. (Boll. Soc. Geol. It.) 131, 459–474 (2012).
14. Amoruso, A. et al. Impact of the 6 April 2009 L’Aquila earthquake on groundwater flow in the Gran Sasso carbonate aquifer, Central Italy. Hydrof. Process. 25, 1754–1764 (2011).
15. Amoruso, A., Crescentini, L., Petitta, M. & Tallini, M. Parisonsionous recharge/ discharge modeling in carbonate fractured aquifers: The groundwater flow in the Gran Sasso aquifer (Central Italy). J. Hydrof. 476, 136–146 (2013).
16. Amoruso, A., Crescentini, L., Martino, S., Petitta, M. & Tallini, M. Correlation between groundwater flow and deformation in the fractured carbonate Gran Sasso aquifer (Central Italy). Water Resour. Res. 50, http://onlinelibrary.wiley.com/doi/10.1002/2014WR014491/(2014) Date of access: 01/07/2014.
17. Tallini, M. et al. Isotope hydrology and geochemical modeling: new insights into the recharge processes and water–rock interactions of a fissured carbonate aquifer (Gran Sasso, central Italy). Environ. Earth Sci. http://link.springer.com/article/10.1007%2Fs12665-014-3364-9 (2014) Date of access: 01/07/2014.
18. Boulton, A. J., Dole-Olivier, M.-J. & De Laurentis, P. Effects of sample volume and taxonomic resolution on assessment of hyporheic assemblage composition sampled using a Bou-Rouch pump. Arch. Hydrof. 159, 327–355 (2004).
19. Underwood, A. J. A new approach for non-parametric multivariate analysis of variance. (Cambridge Univ. Press, Cambridge, UK, 1997).
20. Quinn, G. P. & Keough, M. J. Experimental design and data analysis for biologists. (Cambridge Univ. Press, Cambridge, UK, 2002).
21. Colwell, R. K. EstimateS: statistical estimation of species richness and shared species from samples. Version 9, User’s Guide and application published at: http://viceroy. eeb.uconn.edu/estimates (2013) Date of access: 01/07/2014.
22. Zar, J. H. Biostatistical analysis. 5th ed. (Prentice Hall/Pearson, Upper Saddle River, NJ, 2009).
23. Iram, U. R. E. Scrutiny of variance results for outliers: Cochran’s test optimized. Anal. Chim. Acta 659, 68–84 (2010).
24. Anderson, M. J. A new method for non-parametric multivariate analysis of variance. Austral. J. Ecol. 36, 32–46 (2001).
25. Clarke, K. R. Non-parametric multivariate analysis of changes in community structure. Austral. J. Ecol. 18, 117–143 (1993).

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Author contributions

D.M.P. had the idea; B.F. and A.D.C. designed the sampling method and analyzed the samples; D.M.P. and B.F. identified the species; P.L., B.F. and T.D.L. performed the statistical analyses; P.L. and P.D.C created the figures; M.P. wrote the hydrogeological setting of the study area. All authors discussed the results and contributed to writing the paper.
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