Ecohydrology
January 2020, Volume 13 Issue 1 Pages e2150 (19p.)
https://doi.org/10.1002/eco.2150
https://archimer.ifremer.fr/doc/00599/71110/

Archimer
https://archimer.ifremer.fr

Stygofaunal community trends along varied rainfall conditions: Deciphering ecological niche dynamics of a shallow calcrete in Western Australia

Saccò Mattia [1, *] , Blyth Alison J. [1], Humphreys William F. [2, 3], Karasiewicz Stephane [4], Meredith Karina T. [5], Laini Alex [6], Cooper Steven J.B. [7, 8], Bateman Philip W. [9], Grice Kliti [1]

[1] WA-Organic Isotope Geochemistry Centre, The Institute for Geoscience Research, School of Earth and Planetary SciencesCurtin University Perth Western Australia 6102 ,Australia
[2] Collections and Research Centre Western Australian Museum Welshpool Western Australia 6986 , Australia
[3] School of Biological Sciences University of Western Australia Crawley Western Australia 6009, Australia
[4] Centre de Brest, Unité Dynamiques des Écosystèmes Côtiers, Laboratoire D'écologie Pélagique, Département Océanographie et Dynamique des EcosystèmesIFREMER Plouzané F-9280, France
[5] Australian Nuclear Science and Technology Organisation (ANSTO) Locked Bag 2001 Kirrawee DC NSW 2232 , Australia
[6] Department of Chemistry, Life Sciences and Environmental Sustainabilit University of Parma Viale G. P. Usberti 33/A Parma 43124 ,Italy
[7] Australian Centre for Evolutionary Biology and Biodiversity, School of Biological Sciences University of Adelaide Adelaide South Australia 5005 ,Australia
[8] Evolutionary Biology Unit South Australian Museum North Terrace Adelaide South Australia 5000, Australia
[9] Behavioural Ecology Lab, School of Molecular and Life Sciences Curtin University Perth Western Australia 6102, Australia

* Corresponding author : Mattia Saccò, email address : mattia.sacco@postgrad.curtin.edu.au

Abstract :

Groundwaters host highly adapted fauna, known as stygofauna, which play a key role in maintaining the functional integrity of subterranean ecosystems. Stygofaunal niche studies provide insights into the ecological dynamics shaping the delicate balance between the hydrological conditions and community diversity patterns. This work aims to unravel the ecological trends of a calcrete stygofaunal community, with special focus on niche dynamics through the Outlying Mean Index analysis (OMI) and additional calculation of Within Outlying Mean Indexes (WitOMI), under three rainfall regimes. Temperature and pH changed significantly among different rainfall conditions ($P < .001$), and together with salinity were the most influential drivers in shaping stygofaunal assemblages. These environmental conditions, linked with nutrient fluctuations in the groundwater, constrained changes in niche occupation for water mites, two species of beetles and juvenile amphipods (OMI analysis, $P < .05$). The WitOMI analysis revealed differential subniche breadths linked with taxa-specific adaptations after different rainfall conditions. Our results indicate that stygofaunal niches are closely linked to the hydrodynamic conditions influenced by different rainfall regimes. Further long-term investigations, incorporating broader ecological perspectives,

Please note that this is an author-produced PDF of an article accepted for publication following peer review. The definitive publisher-authenticated version is available on the publisher Web site.

will help to understand the impacts associated with climate change and anthropogenic pressures on one of the most threatened ecosystems in the world.

Keywords : stygofauna, ecological niche, groundwater, calcrete, rainfall, WitOMI

Please note that this is an author-produced PDF of an article accepted for publication following peer review. The definitive publisher-authenticated version is available on the publisher Web site.

1 | INTRODUCTION

The study of ecological dynamics in groundwaters is notoriously complex (Gibert, Stanford, Dole-Olivier, & Ward, 1994; Dole-Olivier, Malard, Martin, Lefébure, & Gibert, 2009; Steube, Richter, & Griebler, 2009). This is primarily because the geological and physical conditions (occurrence of interstitial spaces, voids) of an aquifer, where the groundwater resides, create a complex abiotic structure (Danielopol, Griebler, Gunatilaka, & Notenboom, & 2003). Additionally, hydrological processes, together with chemical mechanisms, can have significant impacts on the environmental conditions shaping the subsurface faunal community (Galassi et al., 2017).

Aquifer recharge, or replenishment, plays a key role in maintaining ecological balance in groundwaters (Humphreys, 2008). As the absence of light limits autochthonous carbon production (Humphreys, 2006), groundwater recharge provides an essential input of resources needed to support the stygofaunal community (Meyer, Meyer, & Meyer, 2003; Konrad, Brasher, & May, 2008). Recharge-related changes in water quality, such as increased oxygen levels (e.g. Hakenkamp & Palmer, 2000) and nutrient availability (Datry, Malard & Gibert, 2005) constitute vital drivers in shaping biotic assemblages in groundwaters (Hahn, 2006; Reiss et al., 2019).

Generally, rainfall patterns and aquifer characteristics such as permeability and porosity control groundwater recharge processes (Berkowitz & Balberg, 1993). Recharge dynamics are particularly influenced by climate variability (Scanlon, Healy, & Cook, 2002), land use/land cover (e.g. Niemiller & Taylor, 2019) and thickness of the vadose zone (e.g. Manna et al., 2019). These factors, together with the position of the groundwater in the landscape, influence the hydrogeochemical mechanisms defining the interactions with the aquifer (e.g. Arnold, Allen, & Bernhardt, 1993; Rau et al., 2017).

Worldwide, climate change is predicted to result in profound shifts in weather patterns (Stocker & Raible, 2005), and several studies over the last two decades have predicted negative impacts on groundwater (e.g. Eckhardt & Ulbrich, 2003; Holman, 2006; Green et al., 2011). In Australia, where climate is strongly influenced by the El Nino-Southern Oscillation (ENSO) and Indian Ocean Dipole (IOD), global warming is predicted to trigger high rates of biodiversity loss and habitat fragmentation (Hughes, 2003; Horwitz et al., 2008). The vast array of Australian groundwater habitats host vulnerable aquatic fauna, termed stygofauna (Humphreys, 2006; Humphreys, 2019), which are expected to face major threats in response

to rapidly changing regional climates (Davis, Pavlova, Thompson, & Sunnucks, 2013). Recent investigations have linked artificial warming to increased biodiversity loss rates (e.g. Brielmann, Griebler, Schmidt, Michel, & Lueders, 2009), while increased rainfall variability will affect recharge patterns (e.g. Rosenberger al., 1999; Hendrickx & Walker, 2017). A comprehensive understanding of the functional ecology and ecosystem dynamics in groundwater systems is therefore urgently needed in order to understand the threat posed by climatic change (Mammola et al., 2019).

Over the past two decades, the incorporation of multidisciplinary approaches, such as biogeochemistry, microbiology and genetics, into groundwater ecology has helped define the vital role played by stygofauna in the conservation of subsurface environmental conditions (Hancock, Boulton, & Humphreys, 2005). Stygofaunal activities such as grazing, burrowing or bioturbating help maintain the hydraulic connectivity between aquifers and surface environments (Murray, Zeppel, Hose, & Eamus, 2008). Stygobionts also interact with microbes, vital actors in shaping groundwater biogeochemical cycling of nutrients (Griebler & Lueders, 2009), and their consumption and excretion of organic matter regulates the proliferation of microbial biofilms (Mermillod-Blondin & Rosenberg 2006; Schmidt, Cuthbert, & Schwientek, 2017). As a result, groundwater functional integrity is dependent on the health of its stygofaunal community assemblages (Boulton, Fenwick, Hancock, & Harvey, 2008).

The ecological niche of a species reflects the set of required resources, encountered abiotic conditions and biotic interactions that enable its persistence through time and space (Chase & Leibold, 2003). During the last century, many definitions of this central concept in ecology have been proposed, with the Hutchinsonian niche (Hutchinson, 1957) being one of the most widely employed. A Hutchinsonian niche is a multi-dimensional volume (Levin et al., 2009) where habitat conditions and resources influence population dynamics, namely birth and death rates (Holt, 2009). This definition led to the formulation of vital concepts such as niche breadth (the conceptual inverse of niche specialization) and niche partitioning (coexisting species occupying different ecological niches) (Colwell & Futuyma, 1971). Due to the urgent need to predict ecological patterns under rapidly changing environmental conditions such as climate change (Soberón, 2007), re-evaluation of Hutchinson's approach has led to recent upsurges in ecological niche research (e.g. Chase & Leibold, 2003; Holt, 2009; Pironon et al., 2017).

The measurement of ecological niches faces two major challenges (Peterson, Papeş, & Soberón, 2008). On one side, the characterization of the abiotic conditions is strictly dependent

on the accuracy of sampling procedures (Karasiewicz, Dolédec, & Lefebvre, 2017). On the other, the understanding of the role of biotic interactions still remains poor in many ecosystems (Soberón & Nakamura, 2009). These obstacles grow exponentially when groundwater environments are considered, due to the poor accessibility of these systems (Halse et al., 2014), and the sparse knowledge of stygofaunal population dynamics (Guzik et al., 2011) and biotic interactions between stygofauna and microbial communities (Schmidt et a., 2017). As a result, studies of ecological niches in groundwater environments are scant, with the majority being surveys at a regional scale carried out in Europe (Dole-Olivier et al., 2009; Galassi, Stoch, Fiasca, Di Lorenzo, & Gattone, 2009; Martin et al., 2009).

In Australia, the calcrete aquifers of the Yilgarn region (WA) harbour a myriad of short-range endemic invertebrate species (Humphreys, 2001). This area, together with the Pilbara, is a global diversity hotspot that hosts one of the highest stygofaunal densities in the world (Humphreys, Watts, Cooper, & Leijs, 2009; Guzik et al., 2011). However, this diversity is largely undescribed (80% Western Australia stygofauna are unidentified taxa, see Guzik et al., 2011), which creates a major obstacle for the investigation of the biological dynamics shaping stygofaunal communities. Consequently, despite the urgent need for a detailed understanding of the ecological patterns in the region (Humphreys, 2006), research is still in its infancy.

The Sturt Meadows calcrete aquifer in Western Australia provides a unique opportunity to investigate the linkage between hydrogeochemical conditions and taxa-specific ecological niche dynamics. The fauna of this relatively pristine shallow groundwater has been the subject of genetic and taxonomic studies during the last 15 years (e.g. Leys, Watts, Cooper, & Humphreys, 2003; Allford, Cooper, Humphreys, & Austin, 2008; Cooper, Saint, Taiti, Austin, & Humphreys, 2008; Guzik, Cooper, Humphreys, & Austin, 2009; Bradford, Adams, Humphreys, Austin, & Cooper, 2010; Bradford et al., 2013). It therefore provides a well-defined stygofaunal community on which to base studies linking the fauna to broader biotic and abiotic dynamics. Hyde, Cooper, Humphreys, Austin, and Munguia (2018) investigated diversity patterns within the invertebrate assemblages using data from a six years collection period. Here we extend this research by comparing the environmental parameters and the stygofaunal distributions from three sampling campaigns undertaken during two contrasting rainfall periods (defined as low rainfall and high rainfall, see Hyde et al., 2018). We aim to: 1) test the importance of physicochemical and environmental parameters as species distributions' descriptors in calcrete aquifers; 2) determine the linkage between the changes in potential

aquifer recharge events and stygofaunal assemblages 3) identify potential shifts in ecological niche occupation within the invertebrate community in the calcrete aquifer.

2 | METHODS

2.1 | Study site

FIGURE 1 HERE

The field work was carried out at the Sturt Meadows calcrete aquifer (28˚41‘S 120˚ 58‘E) located on Sturt Meadows pastoral station, Western Australia, ~42 km from the settlement of Leonora (833 km northeast of Perth, Figure 1a). The surface vegetation is dominated by open Acacia woodlands, primarily *Acacia aneura* (F.Muell. ex Benth.). The understorey is herbaceous mixed with saltbush shrubs and grasses. The area experiences combined grazing pressure from domestic stock, feral animals and macropods. The average monthly rainfall of the area ranges from 6.9 mm in September to 30.6 mm in March (data from the Australian Bureau of Meteorology (BoM)), and the daily average temperature varies between 37°C (January) and 18.4°C (July). The average pan evaporation of 2400 mm per year exceeds the average rainfall (200 mm per annum, BoM).

The Sturt Meadows aquifer occurs in a calcrete deposit formed in a palaeodrainage channel located in the northeast Yilgarn, the largest Archean craton in Australia (Figure 1a). The surrounding area is dominated by Quaternary surficial unconsolidated deposits (Figure 1b). The calcrete systems of this region formed as secondary sedimentary deposits via precipitation of calcium carbonate in the groundwater flowpath of palaeodrainage channels during the Late Eocene to Early Oligocene (37–30 Mya) (Morgan, 1993). The groundwater within the Sturt Meadows calcrete has a strong northeast to southwest biogeochemical gradient that is comparable to estuarine habitats (Humphreys et al., 2009).

Groundwater was accessed through a grid of shallow bores, initially drilled for mineral exploration, which comprises 115 bore holes of between 5-11 m in depth forming a 1.4 km X 2.5 km (3.5 km^2) area (Figure 1c). These bores are unlined, except within about 0.5 m from the surface, where they are lined with PVC pipe, to stabilise the surface, and capped (Allford et al., 2008). Two sub-grids can be identified: the northern area (bores separated by 100 m in each direction) and the southern portion (bores separated by 100 m EW and 200 m NS). According to previous investigations of the depth and lithography of the calcrete (Bradford et al., 2013), the study area can be divided into two major geological zones (A1 and A2, the deepest calcrete

intersected with clayey material), a clay bar (CD) and two minor zones (A3 and A4 (together with A5), the shallowest calcretes overlying clay) (Figure 1c). Previous studies at the Sturt Meadows aquifer have found a multitude of endemic invertebrate species, including both stygobitic (e.g. Bradford et al., 2010) and troglobitic (e.g. Barranco & Harvey 2008; Javidkar, Cooper, King, Humphreys, & Austin, 2015) taxa.

2.2 | Samples and data collection

Preliminary analysis of historical data (eleven sampling campaigns, see Hyde et al., 2018) was undertaken on the hydrological (i.e water level fluctuations, water physicochemical parameters) and stygofaunal distribution patterns throughout the bore grid. Thirty boreholes (six samples from A1, seven from A2, seven from CD, five from A3 and five from A4, Figure 1c) were then selected by simple random sampling among the most reliable (i.e. lowest risk of drying, broadest ranges of stygofaunal abundances) and most representative (i.e. widest ranges of stygofaunal diversity and water level changes) bores. The *in situ* sampling campaign involved collecting stygofauna and water physicochemical parameters from each bore, while samples for nutrient analysis were collected from bores J7 (zone A1), W4 (zone A2) and D13 (ZONE CD).

A weather station with rain gauge was installed near bore E7 (Figure 1c, in blue) to monitor rainfall events and changes in the groundwater water level *via* a differential pressure water level sensor. Daily data of both parameters were recorded for the period ranging from 18/06/2017 to 17/06/2018 (Figure 2a). By following the Sturt Meadows's rainfall periods categorization proposed by Hyde et al. (2018), three sampling campaigns were carried out. Two were associated with low rainfall periods (LR: <10 mm of rain during the 30 days prior to sampling, Figure 2b and c): LR1 on the 26/07/2017 (4.4 mm of cumulative rainfall) and LR2 on the 7/11/2017 (0.8 mm of cumulative rainfall). A final sampling trip associated with a higher rainfall event (HR: >30 mm of rain in the previous 30 days, Figure 2d) was carried out on the 17/03/2018 (37.8 mm of cumulative rainfall).

Temperature, pH, ORP, salinity, DO, and depth were measured *in situ*, using portable field measurement equipment (Hydrolab Quanta Multi-Probe Meter®), for all the bores sampled in this study. Water samples for nutrient analysis from the bores J7 (zone A1), W4 (zone A2) and D13 (ZONE CD) were collected by using a 2 L bailer during the sampling campaigns corresponding to LR2 and HR. Bailers were washed with bleach and rinsed with distilled water.

Standard pre-purging of the bores was not conducted to preserve in-site specific hydrogeological dynamics and avoid increased sample turbidity, as recommended by Gray et al. (2016) for shallow aquifers in this arid region.

Groundwater samples were stored in 1 L high density polyethylene (HDPE) bottles, immediately frozen and kept at –20°C until further analyses. In the laboratory, nitrites (HACH kit, Method 10207), nitrates (HACH kit, Method 10020), ammonia (HACH kit, Method 8038), phosphates (HACH kit, Method 8048), sulphates (HACH kit, Method 8051) and total alkalinity (CaCO3 mg/L, titration method) were analysed. These parameters were measured to test nutrient concentrations in the system, and detect fluctuations linked with potential recharge processes.

Adult and larval stygofaunal specimens were collected by haul netting, with five hauls of a weighted plankton net with a mesh size of 100 µm (Allford et al., 2008) through the water column in each of the thirty bores sampled. All biological samples were kept frozen (–20°C) in darkness until further processing in the laboratory where individual organisms were counted and identified to the lowest taxonomic level via optical microscopy and reference to specific taxonomic keys (e.g. Alarie, Michat, & Watts, 2009; Watts & Humphreys, 2006; King, Bradford, Austin, Cooper, & Humphreys, 2012).

FIGURE 2 HERE

2.3 | Statistical analysis

Physicochemical water parameters - 90 samples in total - were compared across the three rainfall events and the geological zones using a factorial ANOVA. Tukey's *post hoc* comparisons were used to test pairwise interactions. Nutrients (nitrites, nitrates, ammonia, phosphate and sulphates) and alkalinity trends between LR2 and HR were tested *via* ANOVAs. Kruskal-Wallis with Dunn's *post hoc* analyses were run to test the same patterns for the number of individuals, α-diversity, Shannon diversity index (H) and Buzas and Gibson's evenness (E).

To investigate niche overlap and co-occurrence, we calculated Pianka's index of niche overlap and the species segregation (co-occurrence) by mean of the Stone and Robert's C-scores (package 'EcoSimR' in R software version 3.5.1). Pianka's index (Levins, 1968) calculates the mean overlap of all possible species pairs. It ranges from 0 (no overlap) to 1 (complete overlap) and allows detection of niche overlaps at basic community-levels in groundwaters (Fattorini et

al., 2017). C-scores enables comparison of randomness across sampling events, with lower average index values indicating higher probability that the presence of one species might be affected by the distribution of the others (Ulrich & Gotelli, 2007). While abundance data is employed for the analysis of niche overlapping (*via* Pianka's index), C-scores (randomness of the species assemblages) are calculated by using a presence/absence data matrix. Overall, we employed both indices as descriptive tools to compare potential changes in the community niche occupations across the rainfall regimes (LR1, LR2 and HR).

We investigated the ecological niches by means of Outlying Mean Index (OMI) analysis (Dolédec, Chessel, & Gimaret-Carpentier, 2000), package'ade4' in R software version 3.5.1. The OMI analysis required the stygofaunal abundance table and the environmental matrix containing the values of the hydrological parameters. Details about the mathematical insights associated with the analysis can be found in Dolédec et al. (2000).

OMI analyses provide the position of each taxonomic group in a two-dimensional Euclidean space by decomposing species distribution patterns into marginality, tolerance and residual tolerance. The marginality of a species is defined as the squared Euclidean distance between the mean conditions used by the species and the average environmental conditions (*G*) (Dolédec et al., 2000). Species are positioned according to their respective deviation to a theoretical ubiquitous species. Species with high marginality values indicate that they occur in less common habitats compared to the rest of the community. Contrarily, low marginality values express a common occurrence of the species within the environment studied. The tolerance, or niche breadth, represents the variance of the environmental condition used by the species. A species can be considered generalist or specialist to the environment studied by having a high or low tolerance value respectively. The residual tolerance is the quantification of the species niche variability not incorporated in the marginality axes. The niche statistical significance is tested using Monte Carlo permutations by comparing the observed marginality with 1000 simulated marginalities.

Specific seasonal shifts linked with the rainfall patterns were detected by calculating Within Outlying Mean Indexes (WitOMI, 'subniche' package in R version 3.5.1, Karasiewicz et al., 2017). The WitOMI indexes use the environmental space created by the OMI analysis and integrates the characteristics of the K-select analysis (Calenge, Dufour, & Maillard, 2005), enabling niche breakdown into subniches linked with temporal subsets. As a result, interactions such as competition or predation are deciphered at both population and individual scales, and

can be further linked with community responses to changes in environmental conditions (Karasiewicz et al., 2017). The subniche parameters are similar to the niche equivalents calculated in the OMI analysis, with the difference that they consider one subset at a time. The environmental subsets of this study were defined by the three rainfall conditions LR1, LR2 and HR. Similarly to the OMI analysis, subniche parameters for each species (compared to *G*) were calculated (WitOMI*G*), and additionally compared to the mean environmental condition within a specific subset (G_K) (WitOMIG_K). OMI and WitOMI analytical details for the present study are illustrated in Figure 3.

FIGURE 3 HERE

3 | RESULTS

3.1 | Environmental gradients

Figure 2 illustrates the annual water level and rainfall trends at Sturt Meadows. Over one year, the water table dropped from 4.4 to 3.6 m. A previous survey in 2006 from the same bore (Hyde et al., 2018) indicated initial and final water levels of 5.26 m and 5.12 m respectively. Rainfall during our study (171.8 mm) was below the mean annual rainfall for Sturt Meadows (222.7 mm; 100 years of data, Australian Bureau of Metereology). Together, this suggests that Sturt Meadows calcrete groundwater level is in a recessional state (Figure 2a).

Table 1 summarises the mean values of the hydrochemical parameters across the three rainfall events. Temperature of the groundwater was significantly higher during the HR period (pairwise comparisons: LR1 vs HR: $P < 0.001$, LR2 vs HR: $P < 0.001$). pH was significantly higher during LR2 when compared with LR1 and HR ($P < 0.001$), and this pattern is linked with interactions between space (geological zones) and time (rainfall regimes) ($P < 0.001$, Table 2).

Salinity ($P<0.05$), DO ($P < 0.005$) and depth ($P<0.05$) changed significantly between geological zones. Salinity (Tukey's test, *A4 vs A2*, $P < 0.05$) was significantly higher downstream (zone A4) than upstream (zone A2), and deeper calcretes (zone A1) had higher oxygen concentrations when compared with the clay bar area (zone CD) (Tukey's test, *A1 vs CD*, $P < 0.05$) (Table 2). These patterns are in accordance with Humphreys et al. (2009) and confirm the influence of a northeast to southwest biogeochemical gradient along the borehole grid. Total alkalinity increased in groundwater after rainfall (*LR2 vs HR*, $P< 0.001$), ammonia

increased ($P< 0.001$) and phosphates decreased ($P< 0.001$). All the other nutrients (sulphates, nitrites and nitrates) revealed steady non-significant trends in time and space (geological zones) (Table 1).

TABLE 1 AND TABLE 2 HERE

3.2 | Stygofaunal trends

The collected stygofauna comprised 7424 individuals from 11 taxa belonging to five different Classes: Oligochaeta (Family Tubificidae (Vejdovský 1884)), Subcohort Hydrachnidia, Maxillopoda (Order Harpacticoida (G. O. Sars, 1903) and Cyclopoida (Burmeister, 1834)), Malacostraca (Family Chiltoniidae (Barnard, 1972) (juveniles) and species *Scutachiltonia axfordi* (King, 2012), *Yilgarniella sturtensis* (King, 2012) and *Stygochiltonia bradfordae* (King, 2012)) and Insecta (species *Paroster macrosturtensis* (Watts & Humphreys, 2006), *Paroster mesosturtens*is (Watts & Humphreys, 2006) and *Paroster microsturtensis* (Watts & Humphreys, 2006) and respective larvae). Table 3 illustrates taxa codes and abundances for each bore and geological zone within the three sampling campaigns.

TABLE 3 HERE

Overall, the most abundant taxa were cyclopoids (C) and harpacticoids (H), accounting for 49.99% and 28.27% of the total respectively, followed by the amphipod *Y. sturtensis* (AM2, 4.36%), water mites (OR, 2.95%), the amphipod *S. axfordi* (AM1, 2.8%), juvenile amphipods Chiltoniidae (AMJ, 2.76%) and the beetles *P. mesosturtens*is (M, 2.61%), *P. microsturtensis* (S, 2.42%) and *P. macrosturtensis* (B, 2.22%). The remaining taxa (AM3, Slv, Blv, Tu and Mlv) accounted for 2.6% of the total. The abundance of copepods (H and C), tubificids (TU), juvenile amphipods (AMJ) and beetles (B, M and S) was higher during HR compared with LR1 and LR2, whilst adult amphipods (AM1, AM2 and AM3) were more abundant during LRs (LR1 and LR2) compared to HR. None of these differences were statistically significant. Statistical analysis confirmed that abundances of tubificids (TU, Kruskal-Wallis test, $\chi^2= 6.7698$, $P < 0.05$), water mites (OR, Kruskal-Wallis test, $\chi^2= 7.8973$, $P < 0.05$) and *S. bradfordae* amphipods (AM3, Kruskal-Wallis test, $\chi^2= 9.2196$, $P < 0.05$) changed significantly among rainfall regimes. However, pairwise comparisons between rainfall events showed significant (decreasing) patterns only for the latter two (OR: Dunn's test, *LR2 vs HR*, $Z = -2.802977$, $P < 0.05$; AM3: Dunn's test, *LR2 vs HR*, $Z = -2.974035$, $P < 0.05$).

The number of individuals was higher, but not statistically significant, during the HR period (2875) when compared with LR1 (2152) and LR2 (2397), while the average number of taxa per bore was similar, ranging from 6.17 ± 2.35 (LR1) to 6.53 ± 2.71 (LR2). The Shannon diversity index calculated per each bore was higher on average within low rainfall campaigns (S_{LR1} = 1.20 ± 0.48 and S_{LR2}= 1.22 ± 0.48 vs S_{HR} = 1.13 ± 0.33) and average evenness remained steady – with a slight but not statistically significant decrease from LR1 to HR – across the three sampling events (E_{LR1} = 0.61 ± 0.15; E_{LR2} = 0.60 ± 0.18; E_{HR} = 0.57 ± 0.19). None of the diversity patterns commented above revealed significant results. The community was not distributed differently across the five geological areas and the number of individuals, taxa, Shannon and Evenness indexes did not change significantly according to the geological zones across the different rainfall periods.

3.3 | Ecological niche analysis

3.3.1 | Overlap, co-occurrence and realized niches

Overall observed niche overlap was 28% for LR1, 27% for LR2 and 32% for HR. This increase was paralleled by a decrease in co-occurrence (C-scores): from C= 22.736 for LR1, to C= 16.71 for LR2 and C= 15.36 under HR conditions (Supplementary Table 1).

The first two OMI axes accounted for 76.30% of the explained variability, with OMI axis one representing 56.55% and OMI axis two 19.74% of the variance. The average marginality of the theoretical ubiquitous species was significant ($P < 0.05$; 1000 Monte Carlo permutations), suggesting an influence of the environmental conditions on the stygofaunal community assemblages (Table 4). Temperature ($P < 0.001$) and pH ($P < 0.001$) for LR2 and HR, and temperature ($P < 0.001$) and salinity ($P < 0.05$) for LR1 were the most influential environmental parameters on the taxa's realized niches (Figure 4).

FIGURE 4 HERE

TABLE 4 HERE

The subsets of the environmental conditions associated with each rainfall regime were statistically different from the origin (the realized environmental space) (LR1: $P < 0.001$; LR2: $P < 0.001$; HR: $P < 0.001$; 1000 Monte Carlo permutations, Table 4), confirming the three regimes are associated with differential habitat settings. Overall, stygofauna showed less

scattered distributions along the environmental gradients during HR conditions (Figure 5). All the taxa showed low OMI values indicating a common use of the habitat (OMI<2). Four taxa (28.5%) out of 14 had a significant niche (OMI, $P < 0.05$, Table 4): water mites (OR), juvenile amphipods (AMJ*), P. macrosturtensis* (B) and *P. microsturtensis* (S). Water mites had the highest marginality, (OMI=1.09), and tolerance (TOL= 3.06), while AMJ and B had similar marginality (OMI=0.51 and OMI=0.46) and tolerance (TOL=1.13 and TOL=1.20) values. S had very low marginality (OMI=0.34), indicating a use of the available habitat which is ubiquitous to the community object of study. Overall, during low rainfall conditions (LR1 and LR2) the sub-environmental conditions revealed lower constraint on the species realized niches than for HR (Figure 5).

FIGURE 5 HERE

3.3.2 | Subniche trends

All the WitOMI were significant (Supplementary Table 2) and the observed changes in realized subniche occupations are linked with the differences between rainfall regimes' subset average environmental conditions (red dots in Figure 5). The significant taxa (B, AMJ, OR and S) occupied smaller ecological niches during HR relative to the other rainfall regimes (see Table 4 for taxa tolerance (Tol) values).

Apart from water mites (Figure 6a), realized subniches were wider for HR than during LR2, and showed the biggest departures from the average conditions (Figure 6b, c and d). Water mites (OR) and *P. microsturtensis* (S) occupied wider realized subniches than *P. macrosturtensis* (B) and juvenile amphipods (AMJ), and during the HR regime the latter three species occupied more similar niches than that used by the stygofaunal assemblage if compared with the two low rainfall periods (Figure 5 and Figure 6). Aside from water mites, the other taxa occupied common environments (OMI values below one, Table 4) and they used more marginal habitats (higher WitOMI*G* values) under HR conditions than during LR regimes (lower WitOMI*G* values, Table 4). Concurrently, all the significant taxa decreased their tolerance under HR conditions (Table 4).

In contrast, the average positions of the two most widely distributed taxa, the cyclopoid and harpacticoid copepods, along the first two axes of OMI analysis (Supplementary Figure 1) were closest to the origin of the axis, indicating wide ecological tolerance to environmental variation. Considering each rainfall category separately, water mites were the only taxon group that revealed higher marginality under HR (WitOMIG_K=2.36) when compared with LR1

(WitOMIG_K=0.59) and LR2 (WitOMIG_K=0.67). In contrast, marginalities of amphipod juveniles (AMJ) and *P. microsturtensis* (S) plummeted under HR conditions, and marginality values for *P. macrosturtensis* (B) were 1.05 (LR1), 0.23 (LR2) and 0.54 (HR) (Supplementary Table 2).

FIGURE 6 HERE

4 | DISCUSSION

4.1 | Environmental gradients

Water level monitoring of the groundwater at Sturt Meadows was conducted under dry conditions when compared with previous years (see Hyde et al., 2018). Such conditions are not unexpected, given that the Australian climate regimes, the driest inhabited continent on earth, have erratic and largely unpredictable patterns (Buys, Miller, & van Megen, 2012). Groundwater recharge is controlled by climatic conditions such as rainfall intensity and frequency, together with water exchange between aquifers and catchments (Datry et al., 2005). With this in mind, the sampling design for the present study not only focused on specific rainfall periods that maybe linked to groundwater recharge into the aquifer, but also incorporated a broad approach involving the yearly long term cyclic alternation between the wettest (January (26.3), February (29.2) and March (30.6), average monthly rainfall (mm) within parentheses; from BoM) and driest period (from July (17) to December (15.6)) (Figure 2a).

Soil conditions such as temperature and moisture are strongly linked with rainfall events (Maxwell, Chow & Kollet, 2007), and play a key role in aquifer recharge (e.g. Edmunds & Wright, 1979; Wooding, Tyler, & White, 1997; Nasta, Adane, Lock, Houston, & Gates, 2018). Although water levels did not respond to the low rainfall amounts, we did see a change in groundwater chemistry. While pH significantly decreased, temperature increased after rainfall (i.e. under HR conditions). A possible explanation for these trends is that at Sturt Meadows the surface soil layers can reach temperatures of up to 70 °C (Saccò unpublished data). Therefore, when the limited rainfall does occur at the site, it infiltrates the soil zone and the water becomes warmer and more acidic suggesting a recharge pulse has indeed migrated to the water table through either diffuse or point sources.

433 This change also links with changes in nutrient concentrations. The significant increase in
434 ammonia is most likely derived from the dissolution and overland transport of animal waste
435 (Bradford et al., 2013; over 1000 bovines feed on the surficial saltbush) across the study site
436 after increased rainfall, and subsequent migration to the groundwater (Kendall, 1998; Mallin
437 & Cahoon, 2003). However, nitrate concentration showed no significant change, suggesting
438 that microbial processing may play an important role in the biogeochemical patterns of the
439 system. Further investigations involving isotopic fingerprinting would allow more refined
440 elucidation of nutrients flows at Sturt Meadows.

441 Concurrently, the inflow of acidic water under HR conditions (pH = 6.7) has the potential to
442 access phosphorus from its insoluble forms (Cook & Heizer, 1965) and as a result, phosphates
443 significantly decrease in concentration relative to LR2. The groundwater level remained steady
444 across all rainfall periods, and together with ORP, was not a significant driver in determining
445 the stygobiotic dynamics at Sturt Meadows. The DO concentration was higher under HR
446 conditions, suggesting that dissolved oxygen is transported through the aquifer, most likely via
447 advection (Malard & Hervant, 1999). Overall, these shifts in water chemistry and nutrient
448 availability might be responsible for driving ecological changes in the stygofaunal community
449 (Humphreys, 2008; Datry et al., 2005).

450 Local geology (denoted as the five different geological zones) was not found to be a significant
451 driver shaping the stygofaunal assemblages and it was finally discarded for our OMI analysis.
452 This does not rule out geology being significant in notably different contexts (e.g. karst vs
453 alluvial sediments, or areas with hydrocarbons). It is important to emphasise that our study
454 focused on a considerably smaller scale compared with those more extensive investigations of
455 marginality at regional scales (Dole-Olivier et al., 2009; Galassi et al., 2009).

456

457

458 **4.2 | Stygofaunal patterns**

459 Although stygofaunal diversity did not change significantly after the three rainfall periods,
460 some interesting distribution patterns can be unveiled. Copepods (C and H) were the most
461 abundant taxa, and increased their number under HR. Hyde et al. (2018) showed similar
462 patterns for the same system, suggesting that HRs might increase the hydrological connectivity,
463 together with increased nutrient availability, throughout the calcrete at Sturt Meadows and

trigger an overall increase in copepods' population sizes. Galassi et al. (2009) reported colonization processes by free-swimming cyclopoids in low water velocity karst systems coupled with harpacticoid dominance in interstitial voids within the sediment (also in Galassi & Laurentiis, 2004; Di Lorenzo et al., 2005). However, our results indicated that among the copepods, cyclopoid populations increased more markedly with increasing rainfall than the harpacticoids, although the differences within these two groups were not significant. This suggests that species-specific and scale-specific patterns may play a role in the observed patterns.

Copepods, together with amphipods, graze biofilms and assimilate microbially-derived DOC and POM, fuelling carbon transfers along the trophic chain and maintaining the biochemical flows in groundwater (Humphreys, 2006; Tomlinson & Boulton, 2010; Galassi et al., 2017). Subterranean amphipods display opportunistic trophic habits characterized by a wide range of feeding modes (filter feeders, scrapers, scavengers, predators) (Hutchins, Schwartz, & Nowlin, 2014). Hartland, Fenwick, and Bury (2011) suggest that switches of feeding modes according to environmental conditions could explain the dominance of amphipods in oligotrophic groundwaters. At Sturt Meadows, abundances of adult amphipods (AM1, AM2 and AM3) showed decreases through the three rainfall regimes (from LR1 to HR).

These results are likely to be linked with differential resource availability after rainfall periods, together with predatory pressures from higher trophic levels. Previous DNA analyses on beetles (B, M and S) at Sturt Meadows provided evidence of amphipods and copepods as prey items (Bradford, 2010). Our results show that adult beetles increased their abundances through the rainfall regimes (from LR1 to HR), indicating similar potential predator-prey interactions. Abundances of beetle larvae (Blv, Mlv and Slv) were very low, indicating they may occupy different trophic niches to the adults, as suggested by Bradford et al. (2013).

Water mites (OR) did not follow a specific trend, and were most widely distributed (15 bores out of 30) and abundant (149 in total) in the LR2 rainfall period, suggesting that limited rainfall, or stable groundwater conditions, provide the most favourable environmental conditions for this taxonomic group (Irmler, 2004). In contrast, oligochaete tubificids (TU) were most abundant after rainfall, i.e. under the HR regime. As reported by Learner, Lochhead, and Hughes (1978), high water temperatures stimulate rates of growth and asexual reproductions in oligochaetes, and these conditions are likely to be responsible for the population increases observed at Sturt Meadows.

4.3 | Ecological niche interactions

The environmental settings resulting from different rainfall events revealed diverse ecological niche patterns, suggesting specific biotic adaptations to the changes in hydrogeochemistry in the aquifer. Overall, species increased their niche overlaps under HR conditions. Increased rainfall recharge over the site was linked with more suitable environmental conditions and increased overlaps in the use of resources. Concurrently, lower species segregations (C-scores) also indicated that existing interspecific interactions were strengthened under HR conditions. In line with the dynamic reported by Fattorini et al. (2017) in a different context, this result indicates species 'displacements' linked with differential subsurface conditions. Further, WitOMI analysis confirmed a shift in ecological niche occupation (between low rainfall (LR1 and LR2) compared to higher rainfall conditions (HR)) for OR, AMJ, B, and S. Overall, the patterns revealed by AMJ, B and S seem to be driven by dissolved oxygen flux after rainfall (from LR1 and LR2, to HR).

Several studies (e.g. Hakenkamp & Palmer 2000; Dumas, Bou, & Gibert, 2001; Hahn, 2006) have shown the importance of oxygen gradients in shaping stygofaunal assemblages. Dole-Olivier et al. (2009) concluded that the main drivers shaping stygofaunal biodiversity patterns in the French Jura region were salinity and high dissolved oxygen in waters. This is consistent with our results, where oxygenated conditions and higher resource availability (higher marginality values, Table 4) were found to be a key factor in shaping shifts in niche occupation. Simultaneously, the four taxa reduced their niche breadths (tolerance) under HR conditions and this, associated with increased levels of specialization (Karasiewicz et al., 2017), seems to indicate biotic strategies driven by higher levels of adaptations to the environmental conditions.

Amphipod juveniles (AMJ) revealed coupled ecological patterns with *P. macrosturtensis* (B) and *P. microsturtensis* (S) (Figure 6b, d and c). Our results suggest that the beetles' predatory pressures, reported at Sturt Meadows by Bradford et al. (2013), are predominantly exerted on adult amphipods. In line with this axiom, Sudo and Azeta (1992) suggest that amphipod juveniles in benthic and pelagic environments might be protected from predation because of their small size. In groundwaters, where interstitial voids shape stygofaunal distributions within the aquifer matrix (Hose & Stumpp, 2019), small bodies might represent a practical advantage in avoiding predation. Concurrently, adults usually display greater activity than juveniles, including mate searching behaviour, and this may expose them to more frequent interception by active predators like *P. microsturtensis* or *P. macrosturtensis* (Strong, 1972; Peer,

Linkletter, & Hicklin, 1986; Conlan, 1994). However, while reported for surface freshwater environments (e.g. McGrath, Peeters, Beijer, & Scheffer, 2007), there is a lack of evidence for these patterns in groundwaters and they need to be empirically tested.

In another study, Bradford (2010) found discordant diversity patterns among the two sympatric sister species *P. microsturtensis* and *P. macrosturtensis*, and suggested an ecological niche partitioning process occurs within the calcrete environment. Our results illustrate a substantial overlap between *P. macrosturtensis* (B) and *P. microsturtensis* (S) in ecological niche occupation through the three rainfall periods (Figure 6d and c). These patterns suggest that the shifts in environmental conditions, and therefore resource availability, provided by rainfall (HR) do not shape differential inter-specific competition. As suggested by Allford et al. (2008), vertical partitioning down the water column, as a result of contrasting oxygen requirements (Jones, Cooper, & Seymour, 2019), is likely to play an important role in beetles' interactions at Sturt Meadows, and further investigations will help unravel these ecological dynamics.

Unlike the other significant taxa, water mites showed contrasting ecological shifts within LR regimes (LR1 and L2), and depth was an influential environmental factor for their biotic assemblages during LR2 (Figure 6a). In shallow aquifers, deeper and more oxygenated water columns can provide mites with more accessible and heterogeneous habitats (i.e. voids) that might contain adult beetles to parasitize. Dystiscids host suitable sites such as thorax or abdomen or wings for acari larval attachments (e.g. Smith & Oliver, 1986; Mortazavi, Hajiqanbar, & Lindquist, 2018). However, given the low and scattered abundances of this group, conclusions about specific population dynamics at Sturt Meadows are at risk of bias. Moreover, despite their morphological and physiological adaptations to the aquatic environment (Schatz & Behan-Pelletier, 2008) and their occurrence in the hyporheic zone (Williams, 1993; Irmler, 2004), oribatid mites are unstudied in the region and their adscription to stygofauna is still uncertain. Further species-specific investigations involving different habitats and locations from expanded research areas will allow a better comprehension of the ecological dynamics of this group in calcrete systems (i.e. Sabatino, Cicolani, & Gerecke, 2003).

Overall, residual tolerance values for all the taxa (Table 4) indicate that there are environmental drivers present that are not being captured in our data. These results are in line with those presented by other groundwater 'OMI analysis' investigations in Europe (PASCALIS project, Dole-Olivier et al., 2009; Galassi et al., 2009; Martin et al., 2009). Indeed, the quantification

of the ecological niches represents one of the major keystones in ecology (Miklos, 1959; Larson, Holden, & Usio, 2010). Several models have attempted to unravel ecological niche determinations *via* different approaches, namely, experimental (e.g. Moore, 2009), mechanistic (e.g. Kearney et al., 2008) and statistical (e.g. Peterson, 2001), and all of them require the definition of 'environmental' conditions (Holt, 2009).

In groundwaters, the habitat characterisation usually includes hydrological and geological parameters coupled with water chemistry parameters (e.g. Datry et al., 2005; Martin et al., 2009; Galassi et al., 2017). Dole-Olivier et al. (2009) suggest that the incorporation of factors like food availability, habitat fragmentation and biotic interactions would considerably improve the analysis of niche dynamics. However, the reduced accessibility of the majority of subsurface ecosystems is a crucial limiting factor for the integration of broader sampling techniques (Allford et al., 2008). Therefore, additional perspectives, such as the integration of genetic and geochemical information, need to be brought to the field. Metagenomics (branded environmental DNA, eDNA) is an emerging and extremely powerful biomonitoring tool able to unravel stygofaunal functions and biotic community structures (Niemiller et al., 2018). Providing the crucial linkage between the stygofaunal and microbial communities, this technique has the potential to unravel crucial biochemical mechanisms that will provide novel insights into groundwater ecology investigations.

Given the recent advances towards a multidisciplinary focus (e.g. Hancock et al., 2005; Danielopol & Griebler, 2008; Humphreys, 2008; Murray et al., 2008; Steube et al., 2009; Fleckenstein, Krause, Hannah, & Boano, 2010), the field of groundwater ecology can benefit from the incorporation of designs from different disciplines such as hydrology, isotope geochemistry and genetics (Saccò et al., 2019).

Overall, the stygofaunal community at Sturt Meadows aquifer displayed broad ecological tolerances, tendencies in line with several other investigations (e.g. Martin et al., 2009; Schulz, Steward & Prior, 2013). Our results show that ecological shifts are driven by the influx of dissolved oxygen from rainfall. However, climate change, linked with anthropogenic pressures such as overexploitation and contamination, puts at risk the maintenance of the delicate ecological balance sustaining these communities (Spangler & Hahn, 2018). Further investigations involving whole-system approaches, considering spatiotemporal ecological dynamics of the aquatic fauna and their linkage to microbial assemblages (e.g. Žutinić et al., 2018), are needed (Datry et al., 2005) to address how our indisputably changing climate will

affect the aquatic biota in groundwaters, one of the most distinct and understudied ecosystems on Earth.

ACKNOWLEDGEMENTS

We wish to acknowledge the traditional custodians of the land at Sturt Meadows, the Wongai people. We pay respect to their Elders past, present and emerging, and their continuing connection to land, waters and culture in the Yilgarn region.
This research was funded by Australian Research Council linkage grants (LP140100555) with industry partners, the Western Australian Museum, the South Australian Museum, Rio Tinto Biota Environmental Sciences, Bennelongia Environmental Consultants and the Department of Biodiversity, Conservation and Attractions (WA). Saccò is supported by a Curtin International Postgraduate research Scholarship (CIPRS) and an AINSE postgraduate sholarship (PGRA). Blyth was supported by an AINSE Fellowship (2012-2018) The authors thank Dr Elizabeth Ooi, Bernardo Dewey and Lucila López Gibson for their amazing attitude and crucial assistance with field collections. Flora, Peter and Paul Axford of the Sturt Meadows pastoral station are thanked for their kindness and generosity in providing both accommodation and access to their property.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY

The data that support the findings of this study are available on request from the corresponding author. Restrictions apply to the availability of these data, which were used under license for this study. Data are available from the authors with the permission of Prof Steve Cooper and Prof Andy Austin from the University of Adelaide.

REFERENCES

Alarie, Y., Michat, M. C., & Watts, C. H. (2009). Larval morphology of Paroster Sharp, 1882 (Coleoptera: Dytiscidae: Hydroporinae): reinforcement of the hypothesis of monophyletic origin and discussion of phenotypic accommodation to a hypogaeic environment. *Zootaxa*, 2274, 1-44

Allford, A., Cooper, S. J., Humphreys, W. F., & Austin, A. D. (2008). Diversity and distribution of groundwater fauna in a calcrete aquifer: does sampling method influence the story? *Invertebrate Systematics,* 22 (2), 127-138.

Arnold, J. G., Allen, P. M., & Bernhardt, G. (1993). A comprehensive surface-groundwater flow model. *Journal of hydrology,* 142 (1-4), 47-69.

Australian Government Bureau of Meteorology, 2018. Daily rainfall at Sturt Meadows available on line: http://www.bom.gov.au/jsp/ncc/cdio/weatherData/av?p_nccObsCode=136&p_display_type=dailyDataFile&p_startYear=&p_c=&p_stn_num=012176, Accessed date: December 2018

Barranco, P., & Harvey, M. S. (2008). The first indigenous palpigrade from Australia: a new species of Eukoenenia (Palpigradi: Eukoeneniidae). *Invertebrate Systematics*, 22 (2), 227-233.

Berkowitz, B., & Balberg, I. (1993). Percolation theory and its application to groundwater hydrology. *Water Resources Research,* 29 (4), 775-794.

Boulton, A. J., Fenwick, G. D., Hancock, P. J., & Harvey, M. S. (2008). Biodiversity, functional roles and ecosystem services of groundwater invertebrates. *Invertebrate Systematics,* 22 (2), 103-116.

Bradford, T. M. (2010). *Modes of speciation in subterranean diving beetles from a single calcrete aquifer in Central Western Australia* (Doctoral dissertation).

Bradford, T., Adams, M., Humphreys, W. F., Austin, A. D., & Cooper, S. J. (2010). DNA barcoding of stygofauna uncovers cryptic amphipod diversity in a calcrete aquifer in Western Australia's arid zone. *Moecularl Ecology Resources,* 10 (1), 41-50.

Bradford, T. M., Adams, M., Guzik, M. T., Humphreys, W. F., Austin, A. D., & Cooper, S. J. (2013). Patterns of population genetic variation in sympatric chiltoniid amphipods within a calcrete aquifer reveal a dynamic subterranean environment. *Heredity,* 111 (1), 77-85.

Brielmann, H., Griebler, C., Schmidt, S. I., Michel, R., & Lueders, T. (2009). Effects of thermal energy discharge on shallow groundwater ecosystems. *FEMS Microbiology Ecology* 68 (3), 273-286.

669 Buys, L., Miller, E., & van Megen, K. (2012). Conceptualising climate change in rural
670 Australia: community perceptions, attitudes and (in) actions. *Regional Environmental*
671 *Change,* 12 (1), 237-248.

672 Calenge, C., Dufour, A. B., & Maillard, D. (2005). K-select analysis: a new method to
673 analyse habitat selection in radio-tracking studies. *Ecological modelling*, 186 (2), 143-153.

674
675 Chase, J. M., & Leibold, M. A. (2003) 'Ecological niches: linking classical and contemporary
676 approaches.' (University of Chicago Press)

677
678 Colwell, R. K., & Futuyma, D. J. (1971). On the measurement of niche breadth and overlap.
679 *Ecology,* 52 (4), 567-576.

680
681 Conlan, K. (1994). Amphipod crustaceans and environmental disturbance: a review. *Journal*
682 *of Natural History,* 28 (3), 519-554.

683
684 Cook, S. F., & Heizer, R. F. (1965). *Studies on the Chemical*
685 *Analysis of Archaeological Sites, Vol. 2*. University of California
686 Publications in Anthropology.
687
688 Cooper, S. J., Saint, K. M., Taiti, S., Austin, A. D., & Humphreys, W. F. (2008).
689 Subterranean archipelago: mitochondrial DNA phylogeography of stygobitic isopods
690 (Oniscidea: Haloniscus) from the Yilgarn region of Western Australia. *Invertebrate*
691 *Systematics,* 22 (2), 195-203.

692
693 Danielopol, D. L., & Griebler, C. (2008). Changing Paradigms in Groundwater Ecology -
694 from the 'Living Fossils' Tradition to the 'New Groundwater Ecology'. *International Review*
695 *of Hydrobiology,* 93 (4-5), 565-577.

696
697 Danielopol, D. L., Griebler, C., Gunatilaka, A., & Notenboom, J. (2003). Present state and
698 future prospects for groundwater ecosystems. *Environmental conservation*, 30 (2), 104-130.

699
700 Datry, T., Malard, F., & Gibert, J. (2005). Response of invertebrate assemblages to increased
701 groundwater recharge rates in a phreatic aquifer. *Journal of the North American*
702 *Benthological Society*, 24 (3), 461-477.

703
704 Davis, J., Pavlova, A., Thompson, R., & Sunnucks, P. (2013). Evolutionary refugia and
705 ecological refuges: key concepts for conserving Australian arid zone freshwater biodiversity
706 under climate change. *Global change biology,* 19 (7), 1970-1984.

707
708 Di Lorenzo, T., Cipriani, D., Bono, P., Rossini, L., De Laurentiis, P., Fiasca, B., ... Galassi,
709 D. (2005). Dynamics of groundwater copepod assemblages from Mazzoccolo karstic spring
710 (central Italy). *Meiofauna Marina,* 14, 97-103.

711

Dole-Olivier, M. J., Malard, F., Martin, D., Lefébure, T., & Gibert, J. (2009). Relationships between environmental variables and groundwater biodiversity at the regional scale. *Freshwater Biology*, 54 (4), 797-813.

Dolédec, S., Chessel, D., & Gimaret-Carpentier, C. (2000). Niche separation in community analysis: a new method. *Ecology*, 81 (10), 2914-2927.

Dumas, P., Bou, C., & Gibert, J. (2001). Groundwater macrocrustaceans as natural indicators of the Ariege alluvial aquifer. *International Review of Hydrobiology: A Journal Covering all Aspects of Limnology and Marine Biology*, 86 (6), 619-633.

Eckhardt, K., & Ulbrich, U. (2003). Potential impacts of climate change on groundwater recharge and streamflow in a central European low mountain range. *Journal of Hydrology*, 284 (1-4), 244-252.

Edmunds, W., & Wright, E. (1979). Groundwater recharge and palaeoclimate in the Sirte and Kufra basins, Libya. *Journal of Hydrology*, 40 (3-4), 215-241.

Fattorini, S., Lombardo, P., Fiasca, B., Di Cioccio, A., Di Lorenzo, T., & Galassi, D. M. (2017). Earthquake-related changes in species spatial niche overlaps in spring communities. *Scientific Reports*, 7 (1), 443.

Fleckenstein, J. H., Krause, S., Hannah, D. M., & Boano, F. (2010). Groundwater-surface water interactions: New methods and models to improve understanding of processes and dynamics. *Advances in Water Resources*, 33 (11), 1291-1295.

Galassi, D. M., Fiasca, B., Di Lorenzo, T., Montanari, A., Porfirio, S., & Fattorini, S. (2017). Groundwater biodiversity in a chemoautotrophic cave ecosystem: how geochemistry regulates microcrustacean community structure. *Aquatic ecology*, 51 (1), 75-90.

Galassi, D. M. P., & Laurentiis, P. D. (2004). Little-known cyclopoids from groundwater in Italy: re-validation of Acanthocyclops agamus and redescription of Speocyclops italicus (Crustacea, Copepoda, Cyclopoida). *Vie et milieu*, 54 (4), 203-222.

Galassi, D. M., Stoch, F., Fiasca, B., Di Lorenzo, T., & Gattone, E. (2009). Groundwater biodiversity patterns in the Lessinian Massif of northern Italy. *Freshwater Biology*, 54 (4), 830-847.

Gibert, J., Stanford, J. A., Dole-Olivier, M. J., & Ward, J. V. (1994). 1 - Basic Attributes of Groundwater Ecosystems and Prospects for Research. In Groundwater Ecology. (Eds. J Gibert, DL Danielopol and JA Stanford) pp. 7-40. (Academic Press: San Diego)

Gray, D. J., Noble, R. R., Reid, N., Sutton, G. J., & Pirlo, M. C. (2016). Regional scale hydrogeochemical mapping of the northern Yilgarn Craton, Western Australia: a new technology for exploration in arid Australia. *Geochemistry: Exploration, Environment, Analysis*, 16 (1), 100-115.

Green, T. R., Taniguchi, M., Kooi, H., Gurdak, J. J., Allen, D. M., Hiscock, K. M., … Aureli, A. (2011). Beneath the surface of global change: Impacts of climate change on groundwater. *Journal of Hydrology,* 405 (3-4), 532-560.

Griebler, C., & Lueders, T. (2009). Microbial biodiversity in groundwater ecosystems. *Freshwater Biology* 54, 649-677.

Guzik, M. T., Austin, A. D., Cooper, S. J., Harvey, M. S., Humphreys, W. F., Bradford, T., … Muirhead, K. A. (2011). Is the Australian subterranean fauna uniquely diverse? *Invertebrate Systematics,* 24 (5), 407-418.

Guzik, M. T., Cooper, S. J. B., Humphreys, W. F., & Austin, A. D. (2009). Fine- scale comparative phylogeography of a sympatric sister species triplet of subterranean diving beetles from a single calcrete aquifer in Western Australia. *Molecular Ecology,* 18 (17), 3683-3698.

Hahn, H. J. (2006). The GW-Fauna-Index: A first approach to a quantitative ecological assessment of groundwater habitats. *Limnologica-Ecology and Management of Inland Waters,* 36 (2), 119-137.

Hakenkamp, C. C., & Palmer, M. A. (2000). The ecology of hyporheic meiofauna. *Streams and ground waters,* 307-336.

Halse, S., Scanlon, M., Cocking, J., Barron, H., Richardson, J., & Eberhard, S. (2014). Pilbara stygofauna: deep groundwater of an arid landscape contains globally significant radiation of biodiversity. *Records of the Western Australian Museum,* 78, 443-483.

Hancock, P. J., Boulton, A. J., & Humphreys, W. F. (2005). Aquifers and hyporheic zones: Towards an ecological understanding of groundwater. *Hydrogeology Journal,* 13 (1), 98-111.

Hartland, A., Fenwick, G. D., & Bury, S. J. (2011). Tracing sewage-derived organic matter into a shallow groundwater food web using stable isotope and fluorescence signatures. *Marine and Freshwater Research,* 62 (2), 119-129.

Hendrickx, J. M., & Walker, G. R. (2017). Recharge from precipitation. In *Recharge of phreatic aquifers in (semi-) arid areas* (pp 19-111). Routledge

Holman, I. P. (2006). Climate change impacts on groundwater recharge-uncertainty, shortcomings, and the way forward? *Hydrogeology journal,* 14 (5), 637-647.

Holt, R. D. (2009). Bringing the Hutchinsonian niche into the 21st century: ecological and evolutionary perspectives. *Proceedings of the National Academy of Sciences,* 106 (Supplement 2), 19659-19665.

Horwitz, P., Bradshaw, D., Hopper, S., Davies, P., Froend, R., & Bradshaw, F. (2008). Hydrological change escalates risk of ecosystem stress in Australia's threatened biodiversity hotspot. *Journal of the Royal Society of Western Australia,* 91, 1.

Hose, G. C., & Stumpp, C. (2019). Architects of the underworld: bioturbation by groundwater invertebrates influences aquifer hydraulic properties. *Aquatic Sciences*, 81 (1), 20.

Hughes, L. (2003). Climate change and Australia: trends, projections and impacts. *Austral Ecology*, 28 (4), 423-443.

Humphreys, W. F. (2001). Groundwater calcrete aquifers in the Australian arid zone: the context to an unfolding plethora of stygal biodiversity. *Records of the Western Australian Museum*, 64 (Supplement), 63-83.

Humphreys, W. F. (2006). Aquifers: the ultimate groundwater-dependent ecosystems. *Australian Journal of Botany*, 54 (2), 115-132.

Humphreys, W. F. (2008). Hydrogeology and groundwater ecology: Does each inform the other? *Hydrogeology Journal*, 17 (1), 5-21.

Humphreys, W. F. (2019). Biodiversity patterns in Australia. In *Encyclopedia of Caves* (pp. 109-126). Academic Press.

Humphreys, W., Watts, C., Cooper, S., & Leijs, R. (2009). Groundwater estuaries of salt lakes: buried pools of endemic biodiversity on the western plateau, Australia. *Hydrobiologia*, 626 (1), 79-95.

Hutchins, B. T., Schwartz, B. F., & Nowlin, W. H. (2014). Morphological and trophic specialization in a subterranean amphipod assemblage. *Freshwater Biology*, 59 (12), 2447-2461.

Hutchinson, G. (1957). Concluding remarks Cold Spring Harbor Symposia on Quantitative Biology, 22, 415–427. *GS SEARCH*.

Hyde, J., Cooper, S. J., Humphreys, W. F., Austin, A. D., & Munguia, P. (2018). Diversity patterns of subterranean invertebrate fauna in calcretes of the Yilgarn Region, Western Australia. *Marine and Freshwater Research*, 69 (1), 114-121.

Irmler, U. (2004). Long-term fluctuation of the soil fauna (Collembola and Oribatida) at groundwater-near sites in an alder wood. *Pedobiologia*, 48 (4), 349-363.

Kendall, C. (1998). Tracing nitrogen sources and cycling in catchments. In *Isotope tracers in catchment hydrology* (pp. 519-576). Elsevier.

Javidkar, M., Cooper, S. J., King, R. A., Humphreys, W. F., & Austin, A. D. (2015). Molecular phylogenetic analyses reveal a new southern hemisphere oniscidean family (Crustacea: Isopoda) with a unique water transport system. *Invertebrate Systematics*, 29 (6), 554-577.

Jones, K. K., Cooper, S. J., & Seymour, R. S. (2019). Cutaneous respiration by diving beetles from underground aquifers of Western Australia (Coleoptera: Dytiscidae). *Journal of Experimental Biology*, 222 (7), jeb196659.

Karasiewicz, S., Dolédec, S., & Lefebvre, S. (2017). Within outlying mean indexes: refining the OMI analysis for the realized niche decomposition. *PeerJ,* 5, e3364.

Kearney, M., Phillips, B. L., Tracy, C. R., Christian, K. A., Betts, G., & Porter, W. P. (2008). Modelling species distributions without using species distributions: the cane toad in Australia under current and future climates. *Ecography,* 31 (4), 423-434.

King, R. A., Bradford, T., Austin, A. D., Cooper, S. J. B., & Humphreys, W. F. (2012). Divergent molecular lineages and not-so-cryptic species: the first descriptions of stygobitic chiltoniid amphipods (Talitroidea: Chiltoniidae) from Western Australia. *Journal of Crustacean Biology*, 32 (3), 465-488.

Konrad, C., Brasher, A., & May, J. (2008). Assessing streamflow characteristics as limiting factors on benthic invertebrate assemblages in streams across the western United States. *Freshwater Biology,* 53 (10), 1983-1998.

Larson, E. R., Olden, J. D., & Usio, N. (2010). Decoupled conservatism of Grinnellian and Eltonian niches in an invasive arthropod. *Ecosphere*, 1 (6), 1-13.

Learner, M., Lochhead, G., & Hughes, B. (1978). A review of the biology of British Naididae (Oligochaeta) with emphasis on the lotic environment. *Freshwater Biology,* 8 (4), 357-375.

Levin, S. A., Carpenter, S. R., Godfray, H. C. J., Kinzig, A. P., Loreau, M., Losos, … Wilcove, D.S. (2009) 'The Princeton guide to ecology.' (Princeton University Press)

Levins, R. (1968). *Evolution in Changing Environments – Some Theoretical Explorations* (Princeton University Press).

Leys, R., Watts, C. H., Cooper, S. J., & Humphreys, W. F. (2003). Evolution of subterranean diving beetles (Coleoptera: Dytiscidae Hydroporini, Bidessini) in the arid zone of Australia. *Evolution,* 57 (12), 2819-2834.

Malard, F., & Hervant, F. (1999). Oxygen supply and the adaptations of animals in groundwater. *Freshwater Biology,* 41 (1), 1-30.

Mallin, M. A., & Cahoon, L. B. (2003). Industrialized animal production—a major source of nutrient and microbial pollution to aquatic ecosystems. *Population and Environment,* 24 (5), 369-385.

Mammola, S., Piano, E., Cardoso, P., Vernon, P., Domínguez-Villar, D., Culver, D. C., ... & Isaia, M. (2019). Climate change going deep: the effects of global climatic alterations on cave ecosystems. *The Anthropocene Review*, 2053019619851594.

Manna, F., Murray, S., Abbey, D., Martin, P., Cherry, J., & Parker, B. (2019). Spatial and temporal variability of groundwater recharge in a sandstone aquifer in a semiarid region. *Hydrology and Earth System Sciences*, 23 (4), 2187-2205.

Martin, P., De Broyer, C., Fiers, F., Michel, G., Sablon, R., & Wouters, K. (2009). Biodiversity of Belgian groundwater fauna in relation to environmental conditions. *Freshwater Biology*, 54 (4), 814-829.

Maxwell, R. M., Chow, F. K., & Kollet, S. J. (2007). The groundwater–land-surface–atmosphere connection: Soil moisture effects on the atmospheric boundary layer in fully-coupled simulations. *Advances in Water Resources*, 30 (12), 2447-2466.

McGrath, K. E., Peeters, E. T., Beijer, J. A., & Scheffer, M. (2007). Habitat-mediated cannibalism and microhabitat restriction in the stream invertebrate Gammarus pulex. *Hydrobiologia*, 589 (1), 155–164.

Meyer, A., Meyer, E. I., & Meyer, C. (2003). Lotic communities of two small temporary karstic stream systems (East Westphalia, Germany) along a longitudinal gradient of hydrological intermittency. *Limnologica - Ecology and Management of Inland Waters*, 33 (4), 271-279.

Mermillod-Blondin, F., & Rosenberg, R. (2006). Ecosystem engineering: the impact of bioturbation on biogeochemical processes in marine and freshwater benthic habitats. *Aquatic sciences*, 68, 434-442.

Miklos, F. D. U. (1959). Notes on the Ecological Concepts of Habitat, Biotope and Niche. *Ecology*, 40 (4), 725-728.

Moore, K. A. (2009). Fluctuating patch boundaries in a native annual forb: the roles of niche and dispersal limitation. *Ecology*, 90 (2), 378-387.

Morgan, K. H. (1993). Development, sedimentation and economic potential of palaeoriver systems of the Yilgarn Craton of Western Australia. *Sedimentary Geology*, 85, 637-656.

Mortazavi, A., Hajiqanbar, H., & Lindquist, E. E. (2018). A new family of mites (Acari: Prostigmata: Raphignathina), highly specialized subelytral parasites of dytiscid water beetles (Coleoptera: Dytiscidae: Dytiscinae). *Zoological Journal of the Linnean Society*, 184 (3), 695-749.

Murray, B. B. R., Zeppel, M. J. B., Hose, G. C., & Eamus, D. (2008). Groundwater-dependent ecosystems in Australia: It's more than just water for rivers. *Ecological Management & Restoration*, 4 (2), 110-113.

Nasta, P., Adane, Z., Lock, N., Houston, A., & Gates, J. B. (2018). Links between episodic groundwater recharge rates and rainfall events classified according to stratiform-convective storm scoring: A plot-scale study in eastern Nebraska. *Agricultural and Forest Meteorology*, 259, 154-161.

Niemiller, M. L., Porter, M. L., Keany, J., Gilbert, H., Fong, D. W., Culver, D. C., ... & Taylor, S. J. (2018). Evaluation of eDNA for groundwater invertebrate detection and

monitoring: a case study with endangered Stygobromus (Amphipoda: Crangonyctidae). *Conservation genetics resources*, 10 (2), 247-257.

Niemiller, M. L., & Taylor, S. J. (2019). Protecting cave life. In *Encyclopedia of Caves* (pp. 822-829). Academic Press.

Peer, D. L., Linkletter, L. E., & Hicklin, P. W. (1986). Life history and reproductive biology of Corophium volutator (crustacea: amphipoda) and the influence of shorebird predation on population structure in chignecto bay, bay of fundy, Canada. *Netherlands Journal of Sea Research*, 20 (4), 359-373.

Peterson, A. T. (2001). Predicting SPECIES'Geographic Distributions Based on Ecological Niche Modeling. *The Condor*, 103 (3), 599-605.

Peterson, A. T., Papeş, M., & Soberón, J. (2008). Rethinking receiver operating characteristic analysis applications in ecological niche modeling. *Ecological Modelling*, 213 (1), 63-72.

Pironon, S., Villellas, J., Thuiller, W., Eckhart, V. M., Geber, M. A., Moeller, D. A., & García, M. B. (2017). The 'Hutchinsonian niche' as an assemblage of demographic niches: implications for species geographic ranges. *Ecography*, 41 (7), 1103-1113.

Rau, G. C., Halloran, L. J., Cuthbert, M. O., Andersen, M. S., Acworth, R. I., & Tellam, J. H. (2017). Characterising the dynamics of surface water-groundwater interactions in intermittent and ephemeral streams using streambed thermal signatures. *Advances in water resources*, 107, 354-369.

Reiss, J., Perkins, D. M., Fussmann, K. E., Krause, S., Canhoto, C., Romeijn, P., & Robertson, A. L. (2019). Groundwater flooding: Ecosystem structure following an extreme recharge event. *Science of The Total Environment*, 652, 1252-1260.

Rosenberg, N. J., Epstein, D. J., Wang, D., Vail, L., Srinivasan, R., & Arnold, J. G. (1999). Possible impacts of global warming on the hydrology of the Ogalla aquifer region. *Climatic change*, 42 (4), 677-692.

Sabatino, A. D., Cicolani, B., & Gerecke, R. (2003). Biodiversity and distribution of water mites (Acari, Hydrachnidia) in spring habitats. *Freshwater Biology*, 48 (12), 2163-2173.

Saccò, M., Blyth, A., Bateman P. W., Hua, Q., Debashish, M., White, N., … W.Grice, K. (2019). New light in the dark -a proposed multidisciplinary framework for studying functional ecology of groundwater fauna, *Science of the Total Environment*, https://doi.org/10.1016/j.scitotenv.2019.01.296 (in press).

Scanlon, B. R., Healy, R. W., & Cook, P.G. (2002). Choosing appropriate techniques for quantifying groundwater recharge. *Hydrogeology Journal*, 10 (1), 18-39.

Schatz, H., & Behan-Pelletier, V. (2008). Global diversity of oribatids (Oribatida: Acari: Arachnida). *Hydrobiologia*, 595(1), 323-328.

Schmidt, S. I., Cuthbert, M. O., & Schwientek, M. (2017). Towards an integrated understanding of how micro scale processes shape groundwater ecosystem functions. *Science of the Total Environment*, 592, 215-227.

Schulz, C., Steward, A., & Prior, A. (2013). Stygofauna presence within fresh and highly saline aquifers of the border rivers region in southern Queensland. *Proceedings of the Royal Society of Queensland*, 118, 27.

Smith, I. M., & Oliver, D. R. (1986). Review of parasitic associations of larval water mites (Acari: Parasitengona: Hydrachnida) with insect hosts. *The Canadian Entomologist*, 118 (5), 407-472.

Soberón, J. (2007). Grinnellian and Eltonian niches and geographic distributions of species. *Ecology Letters*, 10 (12), 1115-1123.

Soberón, J., & Nakamura, M. (2009). Niches and distributional areas: concepts, methods, and assumptions. *Proceedings of the National Academy of Sciences*, 106 (2), 19644-19650.

Spangler, C., & Hahn, H. J. (2018). Thermostress: Ökologisch gegründete, thermische Schwellenwerte und Bewertungsansätze für das Grundwasser (Ecological based temperature thresholds and ecosystem assessment schemes for groundwater). *Korrespondenz Wasserwirtschaft*, 9, 521-525

Steube, C., Richter, S., & Griebler, C. (2009). First attempts towards an integrative concept for the ecological assessment of groundwater ecosystems. *Hydrogeology Journal*, 17 (1), 23-35.

Stocker, T. F., & Raible, C. C. (2005). Climate change: water cycle shifts gear. *Nature*, 434 (7035), 830.

Strong, D. R. (1972). Life History Variation Among Populations of an Amphipod (Hyalella Azteca). *Ecology*, 53 (6), 1103-1111.

Sudo, H., & Azeta, M. (1992). Selective predation on mature male Byblis japonicus (Amphipoda: Gammaridea) by the barface cardinalfish, Apogon semilineatus. *Marine Biology*, 114 (2), 211-217.

Tomlinson, M., & Boulton, A. J. (2010). Ecology and management of subsurface groundwater dependent ecosystems in Australia a review. *Marine and Freshwater Research*, 61 (8), 936-949.

Ulrich, W., & Gotelli, N. J. (2007) Disentangling community patterns of nestedness and species co-occurrence. *Oikos* 116, 2053–2061.

Watts, C. H. S., & Humphreys, W. F. (2006). Twenty-six new dytiscidae (Coleoptera) of the genera Limbodessus Guignot and Nirripirti Watts & Humphreys, from underground waters in Australia. *Transactions of the Royal Society of South Australia*, 130 (1), 123-185.

Williams, D. D. (1993). Changes in freshwater meiofauna communities along the groundwater-hyporheic water ecotone. *Transactions of the American Microscopical Society*, 181-194.

Wooding, R. A., Tyler, S.W., & White, I. (1997). Convection in groundwater below an evaporating Salt Lake: 1. Onset of instability. *Water Resources Research*, 33 (6), 1199-1217.

Žutinić, P., Petrić, I., Gottstein, S., Gligora Udovič, M., Kralj Borojević, K., Kamberović, J., … Ternjej, I. (2018). Microbial mats as shelter microhabitat for amphipods in an intermittent karstic spring. *Knowledge & Management of Aquaict Ecosysemst,*419, 7.

TABLE 1 Mean values of the hydrochemical parameters. PSS, Practical Salinity Scale; DO, Dissolved Oxygen; ORP, oxidation reduction potential; *, parameters showing significant trends within rainfall periods (LR1/LR2 and HR; results of the physicochemical pairwise comparisons are illustrated as 'a' and 'b')

Rainfall period	Temperature* (°C)	pH*	Salinity (PSS)	DO (mg L^{-1})	ORP	Depth (m)	NO_2^- (mg L^{-1})	NO^{3-} (mg L^{-1})	NH_3* (mg L^{-1})	PO_4^{3-}* (mg L^{-1})	SO_4^{2-} (mg L^{-1})	Alkalinity* (mg $CaCO_3$ L^{-1})
LR1	22.9 ± 1.7[a]	7.3 ± 0.3[a]	14.4 ± 4.9	3.7 ± 2.2	85.1 ± 126.6	3.6 ± 2.2	n/a	n/a	n/a	n/a	n/a	n/a
LR2	23.3 ± 0.7[a]	7.7 ± 0.5[b]	15.9 ± 3.2	3.7 ± 1.8	74.2 ± 91.6	3.5 ± 2.3	0.01 ± 0	0.81 ± 0.17	1.74 ± 1.10	0.14 ± 0.05	102.56 ± 4.56	205.11 ± 36.61
HR	26.2 ± 0.5[b]	6.7 ± 0.2[a]	16.6 ± 2	4.1 ± 1.5	88.3 ± 33.3	3.3 ± 2.3	0.01 ± 0	0.78 ± 0.11	3.44 ± 0.83	0.05 ± 0.02	103.33 ± 6.48	291.33 ± 28.93

TABLE 2 Results of ANOVA using rainfall periods (LR1, LR2 and HR), geological zone (A1, A2, A3, A4, CD) and their interaction as factors. Significant results are highlighted in bold. d.f. degrees of freedom; PSS, Practical Salinity Scale; DO, Dissolved Oxygen

	RAINFALL PERIOD			ZONE			ZONE*RAINFALL PERIOD		
	d.f.	F	*P*	d.f.	F	*P*	d.f.	F	*P*
Temperature (°C)	2	82.94	< **0.001**	4	2.19	0.08	8	1.19	0.32
pH	2	89.24	< **0.001**	4	1.72	0.16	8	3.06	< **0.005**
Salinity (PSS)	2	2.94	0.06	4	2.73	< **0.05**	8	0.27	0.97
DO (mg L^{-1})	2	0.64	0.53	4	4.94	< **0.005**	8	0.63	0.75
ORP (mV)	2	0.18	0.83	4	1.08	0.37	8	0.40	0.92
Depth (m)	2	0.12	0.88	4	3.20	< **0.05**	8	0.06	1.00

TABLE 3 Species matrix of Sturt Meadows stygofaunal taxa. Abbreviations: ID: Taxonomic Code, LR1: 1st low rainfall period, LR2: 2nd low rainfall period, HR: high rainfall period. Results from 30 bores are displayed (A8 to F13)

RAINFALL PERIOD		LR1																												
GEOLOGICAL ZONES		A1						A2							A3				CD							A4				
TAXON	ID	A8	C5	F4	H5	J7	N4	L4	S2	U2	W2	Q2	W3	W4	C1	D1	E1	G1	H2	A11	D13	E10	E11	Q3	Q4	S8	B13	C13	E12	E13	F13
Tubificidae	TU														2															
Oribatida	OR			2			2					1	1									1		1		3		1	1	2
Harpacticoida	H	1	3	9	17	18	5	7	13	17	12		8	27	5	15	12	3	72	21	8	19		2	8	27	7	1		6	11
Cyclopoida	C	18	6	1	11	16	36	8	9	14	2	5	3	3	2	6	1	5	131	92	1	33	11	19	14	55	2	269	3	26	1
Chiltoniidae (juveniles)	AMJ				15	1		3	15	4	5			1					4								3			6	11
Scutachiltonia axfordi	AM1		19	1	14		13		1	7		1		1		1		1	9	4	5		2					6		7
Yilgarniella sturtensis	AM2		1	1	23		13		21		2		2	2				3	2	2	7	2		2			3			
Stygochiltonia bradfordae	AM3			1	3				6	3			1					1	4			2							2	
Paroster macrosturtensis	B		1		1	1							1				1		2								1		11	1	6
Paroster mesosturtensis	M			3	1		3		9	1	1		1	1	4	1	4	1	3	9	1				1				1	9	
Paroster microsturtensis	S		1		2		1		6							3	2	1	1		6			1					1	5	1
Paroster macrosturtensis larvae	Blv		1										1				1	1	2	1	1									1	
Paroster mesosturtensis larvae	Mlv								1																		1				
Paroster microsturtensis larvae	Slv		1						1	1	1		3			1	1	1	1		2		2	1	1				1	2
		LR2																												
Tubificidae	TU																								1					
Oribatida	OR			1			2		1	1	8	123		3		1		1		1		2			2		1		1	1	
Harpacticoida	H	82	26	5	12	2	18	2	8	2	17	7	4	29	259	7	22		11	6	1	2	3	34	3	3	12		1	2	1
Cyclopoida	C	86	29	22	24	4	16	2	16	129	7	97	87	19	68	16	31	4	22	35	31	9	29	66	1		6	33	7	81	38
Chiltoniidae (juvelines)	AMJ	32	6		1	1													6	1		1							2	5	
Scutachiltonia axfordi	AM1	3	1		3	1	1		1	6	1		12	4			2	2		2				1			8	1	9	1	1
Yilgarniella sturtensis	AM2	28	11		1	2				6		2	11	2		2	6		4	6	1	5		1	1		4		6	4	2
Stygochiltonia bradfordae	AM3	2	2		1	1	3			1			1				1	1	2	2	1	5	2	1				2	2		1
Paroster macrosturtensis	B	1				2			2	1	2		7					3		3	1						4		7	3	9
Paroster mesosturtensis	M	3				1	1			7		3	6	2	2		3			5	22						1			2	
Paroster microsturtensis	S	1				1	3	1			1		7	1		1				1	17						7		2	3	
Paroster macrosturtensis larvae	Blv		1				1						3							6							1				
Paroster mesosturtensis larvae	Mlv																				2										
Paroster microsturtensis larvae	Slv	1	2			3						2	1			2	1			5	6			4			2			1	
		HR																													
Tubificidae	TU	14	1			1													3			1									1
Oribatida	OR											29						2	3											3	
Harpacticoida	H	64	44	13	1	32	17	8	4	83	6		5	4	3	7	154	11	19	13	12	2	26	9	2	13	8	3	7	14	4
Cyclopoida	C	54	91	23	17	22	12	4	8	19	4	2	23	13	15	2	87	1	29	148	77	66	76	15	11	16	137	41	39	36	47
Chiltoniidae (juveniles)	AMJ	7	8		14	2				3	1			1			8		2	6	2		1			3	2		2	7	4
Scutachiltonia axfordi	AM1				2		4			13			2	2			2	1	1	1			1				4	2	8		4
Yilgarniella sturtensis	AM2	2	3		9	4	2			12			7	1			13		2	4		3	4				2	8		5	7
Stygochiltonia bradfordae	AM3		1							1										1		8						7		1	
Paroster macrosturtensis	B	4			3					3	9		28			3				6			1	3			1		2	5	8
Paroster mesosturtensis	M	7	2		6	1	3	1	1	1		6	4	2	1		1		1	11		1	6	1	1		4	1		11	
Paroster microsturtensis	S	22	3	3	6	1	3			14	1	3	9	7				1		8			2				7		2	6	5
Paroster macrosturtensis larvae	Blv									1				1			1		1		2									1	
Paroster mesosturtensis larvae	Mlv																			2		1						1			
Paroster microsturtensis larvae	Slv		1																	2	1						2			4	

TABLE 4 Result of OMI and WitOMI analyses. OMI, Outlying Mean Index; WitOMI*G*, marginalities from the average habitat condition *G*, Tol, tolerance; Rtol, residual tolerance; $\bar{x}$, average marginality

Rainfall period	All				LR1			LR2			HR		
ID	OMI	Tol	Rtol	*P*	WitOMI*G*	Tol	Rtol	WitOMI*G*	Tol	Rtol	WitOMI*G*	Tol	Rtol
TU	2.00	0.86	7.12	0.16									
OR	1.09	3.06	5.07	< **0.05**	2.00	3.73	5.81	2.69	1.61	5.37	3.42	0.20	1.46
H	0.04	2.25	5.35	0.13									
C	0.02	1.92	6.07	0.12									
AMJ	0.51	1.13	3.79	< **0.05**	0.70	0.43	6.04	0.85	0.76	1.82	2.77	0.13	2.38
AM1	0.13	1.03	4.89	0.34									
AM2	0.16	0.92	4.95	0.12									
AM3	0.24	1.47	5.01	0.27									
B	0.46	1.20	3.49	< **0.05**	1.32	1.70	2.35	1.15	0.63	2.94	3.11	0.38	1.88
M	0.11	1.18	6.94	0.32									
S	0.34	1.10	5.06	< **0.05**	1.85	1.41	6.62	1.54	0.75	3.31	2.60	0.18	2.59
Blv	0.30	0.58	4.84	0.55									
Mlv	1.96	0.77	5.19	0.17									
Slv	0.20	1.14	5.68	0.37									
$\bar{x}$ (*P*)				< **0.05**	< **0.001**			< **0.001**			< **0.001**		

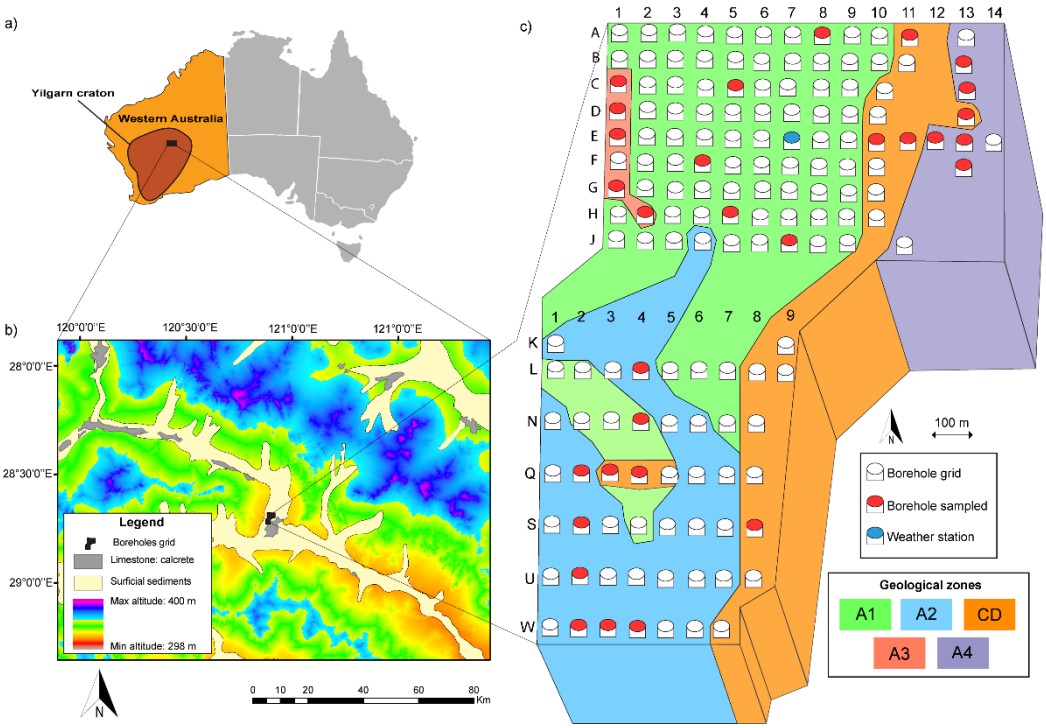

FIGURE 1 Sturt Meadow calcrete: a) location within the Yilgarn craton region, b) elevation map showing the surficial sediments and the calcretes nearby the borehole grid and c) grid map showing the location of the boreholes sampled, the weather station at bore E7 and the five geological zones (A1, A2, A3, A4 and CD).

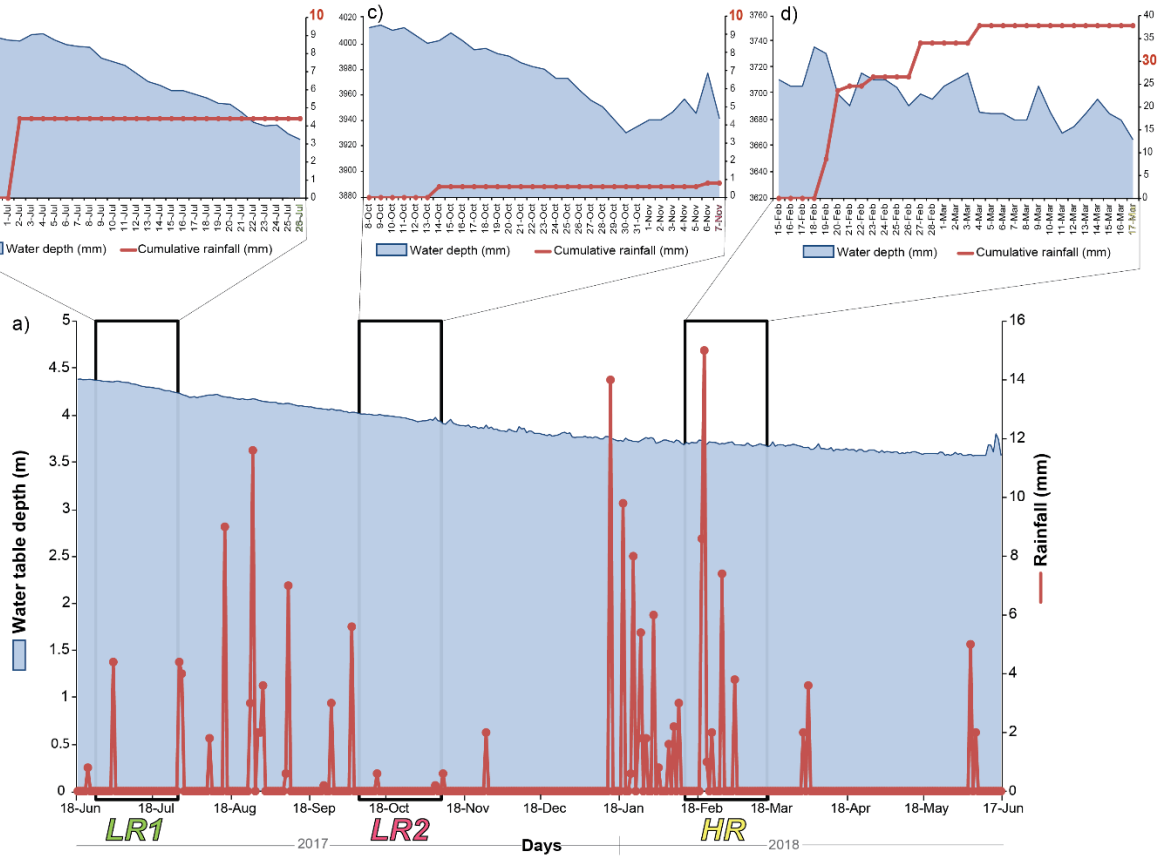

FIGURE 2 Weather station data from Sturt Meadows aquifer (bore E7) water level (in mm, in light blue) and the rainfall events (in mm, in red): a) annual evolution (1 year: from 18/06/2017 to 17/06/2018), b) cumulative rainfall and water depth trend for the 30 days before sampling campaign LR1 (sampling date: 26th of July 2017), c) cumulative rainfall and water depth trend for the 30 days before sampling campaign LR2 (sampling date: 7th of November 2017) and d) cumulative rainfall and water depth trend for the 30 days before sampling campaign HR (sampling date 17th of March 2018). Red numbers in b), c) and d) refer to the category thresholds established by Hyde et al. (2018)

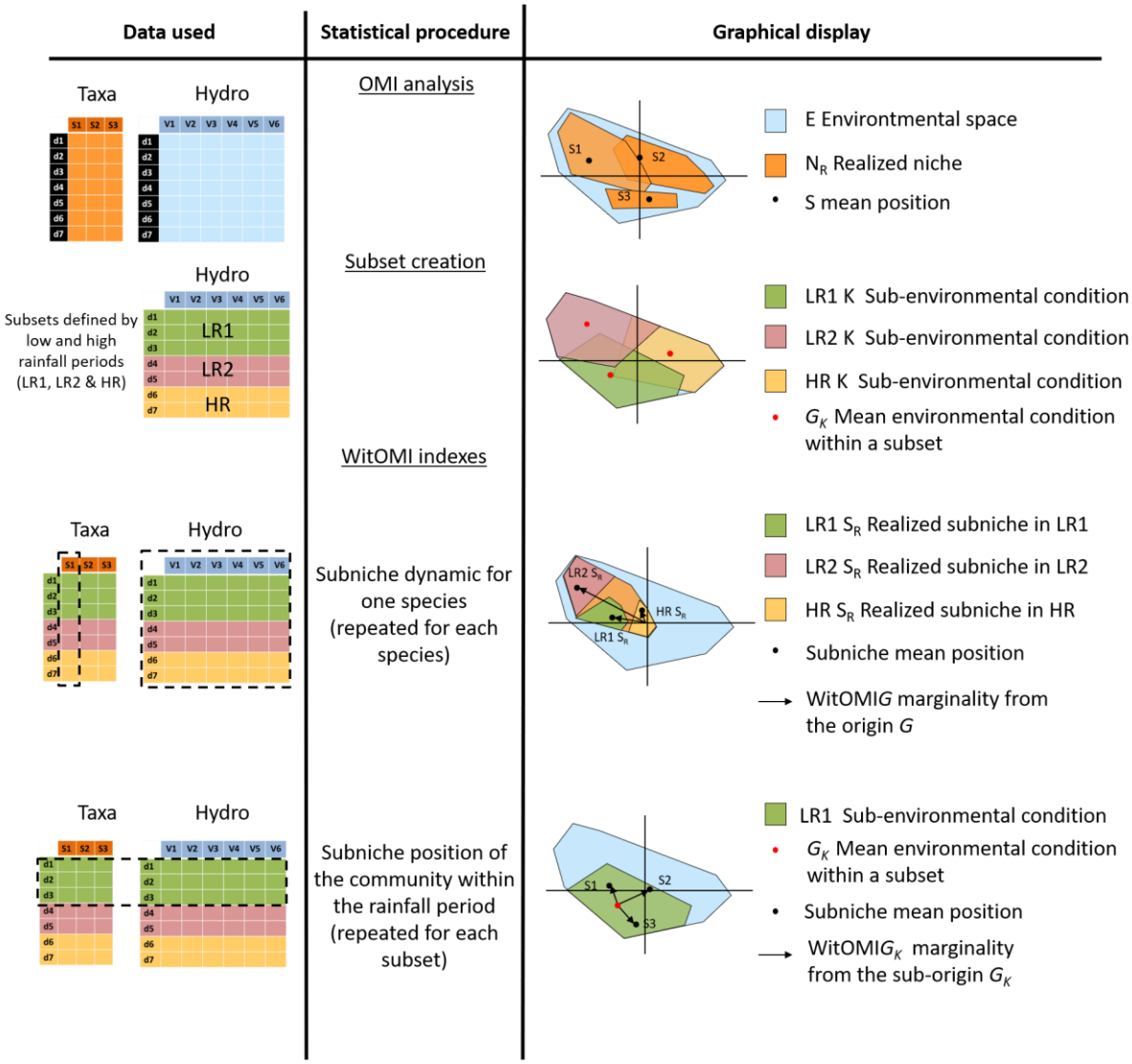

FIGURE 3 Data sets, statistical procedures and graphical displays of the OMI and WitOMI analyses carried out in this study. Taxa: table containing stygofaunal abundance data; Hydro: table containing data from environmental parameters.

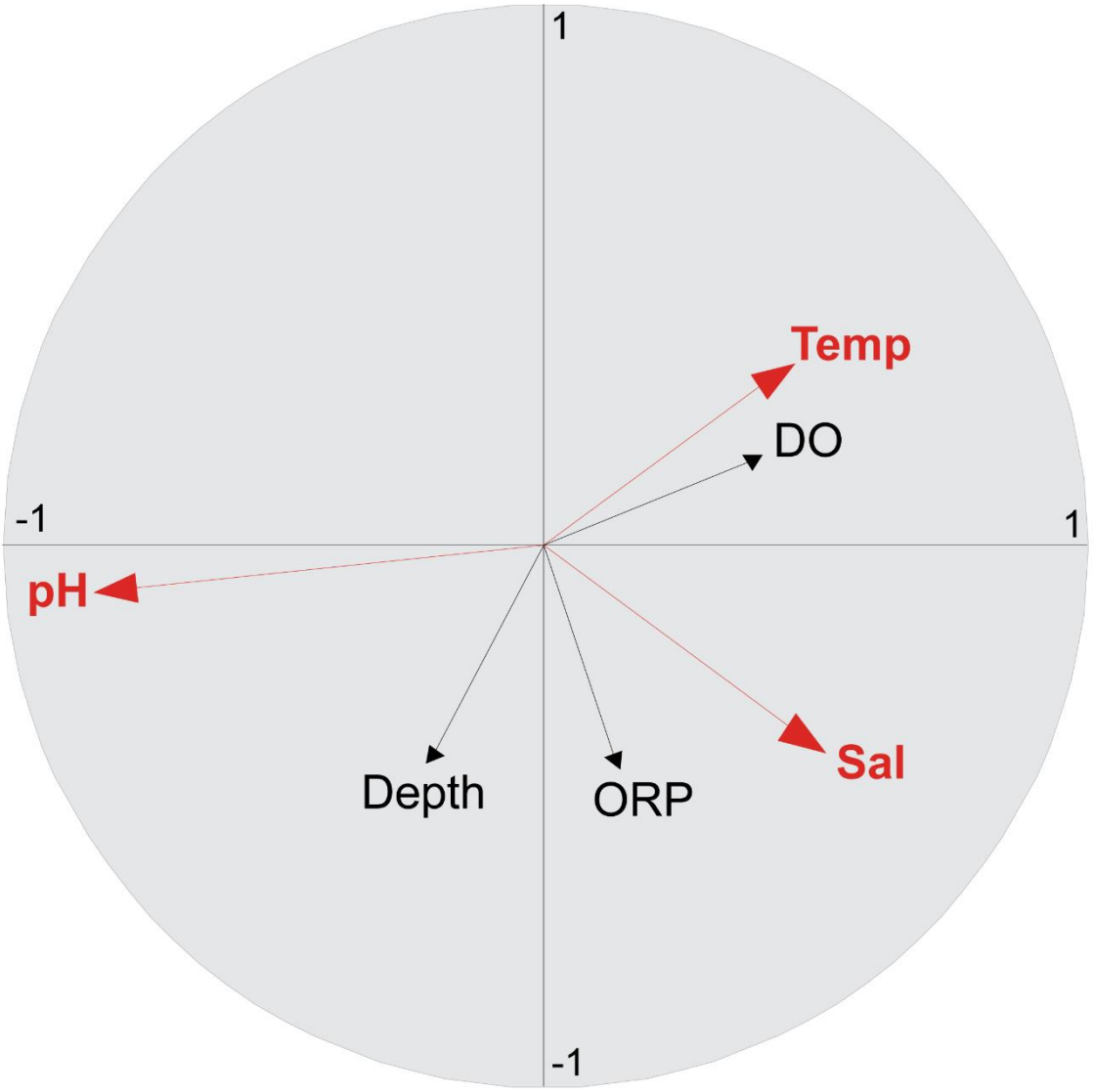

FIGURE 4 Results of the canonical weights of 6 environmental variables extracted by OMI analysis (axis 1 and axis 2, 76.30% of the variability in the data set). The significant environmental variables are in red. Temp, Temperature; DO, Dissolved Oxygen; Sal, Salinity; ORP, Oxidation Reduction Potential.

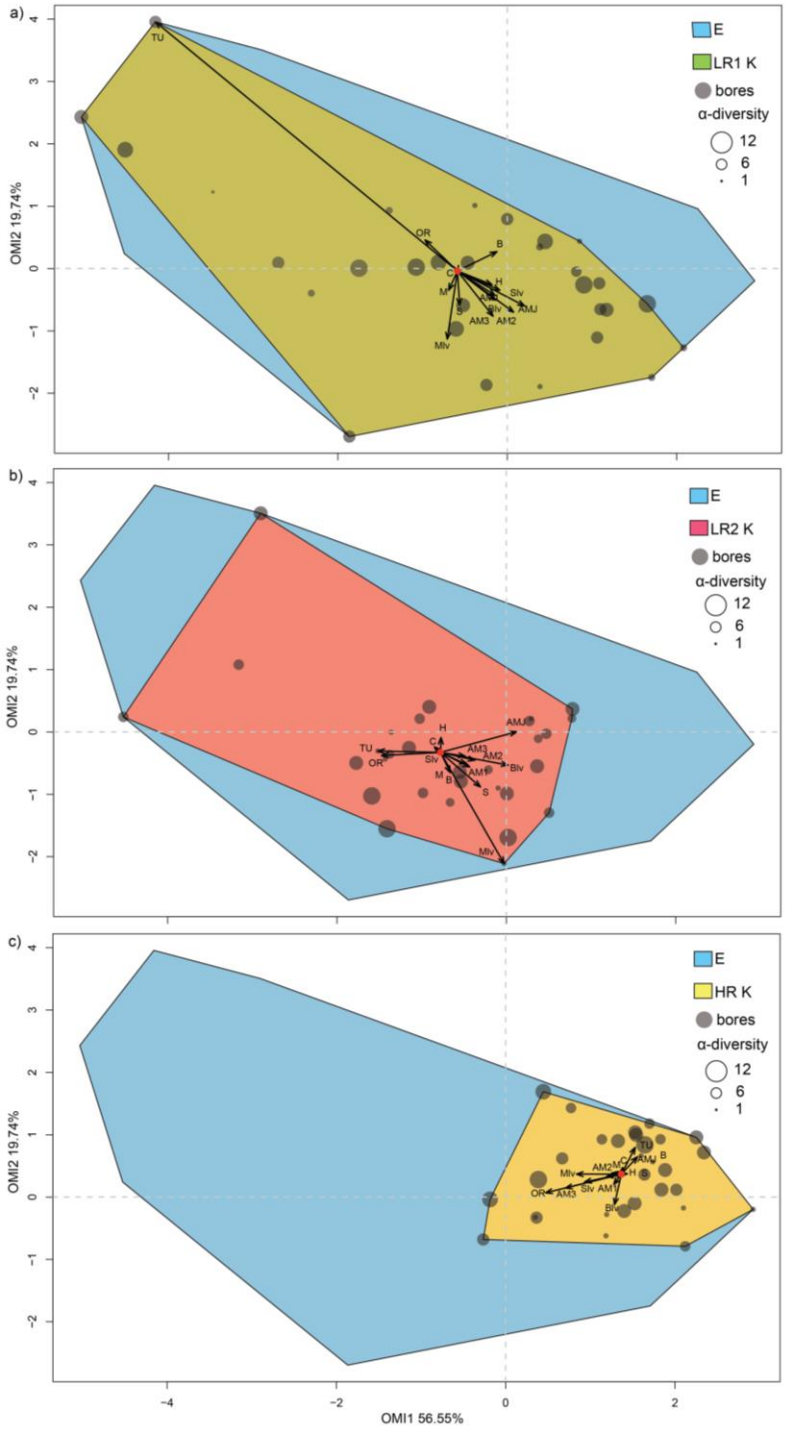

FIGURE 5 Rainfall periods (LR1, LR2 and HR) subset's constraints of habitat conditions (green, red and yellow polygons respectively), found within the overall available habitat conditions (light blue polygons). Bore distribution is depicted with the respective number of taxa (α-diversity) and species subniche positions per each sampling event are also displayed. Red dots represent the suborigins. E, realized environmental space; K, subset realized environmental space. Refer to Table 3 for the stygofaunal codes and to Karasiewicz et al. (2017) for further details about the indexes.

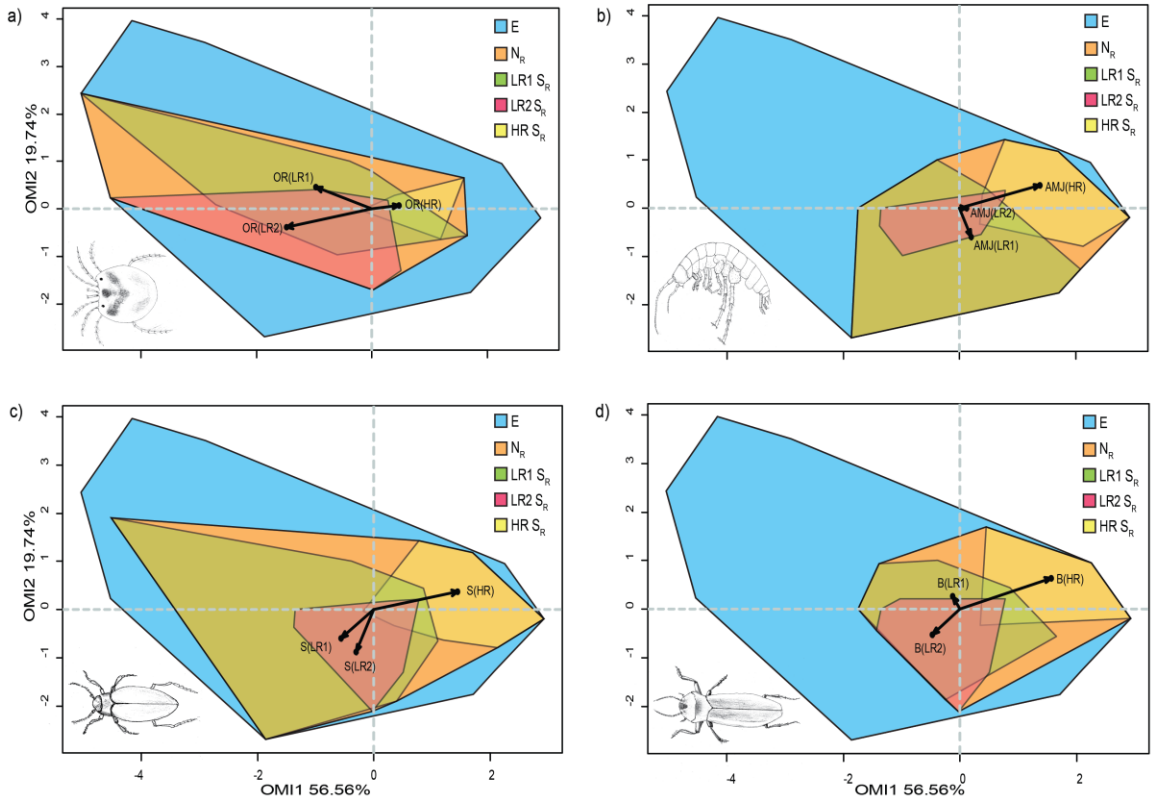

FIGURE 6 Species realized subniches positions of the statistically significant taxa for the OMI and WitOMI analyses: a) water mites (OR), b) amphipods juveniles (AMJ), c) *P. microsturtensis* (S) and d) *P. macrosturtensis* (B). The arrows represent the marginality vectors from the average habitat conditions. E, Environmental space; N_R realized niche; S_R, realized subniche. Refer to Karasiewicz et al. (2017) for further details about the ecological niche indexes.