REVIEW

Invertebrate traits, diversity and the vulnerability of groundwater ecosystems

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Abstract

1. Groundwater comprises the largest freshwater ecosystem on the planet. It has a distinct regime of extreme, yet stable environmental conditions that have favoured the development of similar morphological and functional traits in the resident invertebrate fauna (stygofauna).

2. The analysis of community traits is increasingly used as an alternative to taxonomy-based assessments of biodiversity, especially for monitoring ecosystem status and linking the functions of organisms to ecological processes, yet it has been rarely applied to stygofauna and groundwater ecosystems.

3. In this paper, we review the variation in functional traits among the invertebrate fauna of this important ecosystem. We focus on the stygofauna and processes of alluvium and fractured rock aquifers that are typified by small voids and fissures that constrain the habitats and environmental conditions.

4. As a first step, we compare trait variability between groundwater and surface water invertebrate communities and then examine the significance of the ranges of these traits to the vulnerability of the ecosystem to change.

5. Fifteen potentially useful functional traits are recognised. Eight of these have narrower ranges (i.e. exhibit fewer states, or attributes, of a particular trait) in groundwater than they do in surface water. Two traits have wider ranges.

6. Our synthesis suggests that the relative stability of groundwater environments has led to low trait variability. The low biomass and low reproductive rate of stygofauna suggest that recovery potential following disturbance is likely to be low.

7. For the purposes of both improved understanding and effective management, further work is needed to document additional functional traits and their states in groundwater fauna, enabling a better understanding of the relationship between response and effect traits in these ecosystems.

1 | INTRODUCTION

Groundwaters make up the most extensive freshwater ecosystem on the planet. They contain a suite of unique taxa, and a distinct regime of environmental conditions typified by extreme (e.g. total darkness, low energy) but relatively stable (e.g. low water flow, small thermal range) environmental conditions (Gibert et al., 1994). Given their low energy, groundwater environments typically support low biomass and diversity of taxa. However, isolation and fragmentation have given rise to a high degree of short-range endemism (Harvey et al., 2002), with typically few species in any one location, but many species across locations (Dumnicka et al., 2020). The pressures of the groundwater environment have led to a range of convergent and divergent evolutionary processes within and among groundwater invertebrate species. The pressure for food and resources has resulted in the diversification of niches in some fauna (e.g. Ercoli et al., 2019; Fišer et al., 2019), while the darkness, low energy and stable conditions have also led to convergent evolution, where species from divergent phylogenetic groups having similar morphological, physiological and behavioural traits, creating low inter-species variability across many biological attributes, most notably, the loss of pigmentation and vision.

In their seminal paper, McGill et al. (2006) stated that for traits to be useful in community ecology, they must vary more between taxa than within taxa. Yet, perhaps more importantly, there is a growing body of evidence showing that variability in inter- and intraspecific traits leads to greater stability of the ecosystem (Díaz et al., 2013; Wright et al., 2016) and, conversely, low trait variability leads to low productivity, ecosystem functioning and stability (Hodapp et al., 2016). While low variability in traits is not a problem in its own right, particularly in long-term stable environments such as groundwaters (and indeed maybe a response to that stability), reduced trait variability may increase vulnerability and decrease the resilience of the community to changes beyond the norm, such as those due to anthropogenic disturbance. This is particularly pertinent in the case of groundwater ecosystems, where over-pumping and reductions in recharge are diminishing these systems at a global scale (De Graaf et al., 2019; Jasechko & Perrone, 2021).

Trait-based analyses are increasingly used in ecology to provide an alternative to taxonomy-based assessments of biodiversity and for linking the functions of organisms to processes at the

> **Glossary**
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> Effect trait: An attribute of an organism that relates to its capacity to affect ecosystem properties or deliver ecosystem functions.
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> Epigean: Surface dwelling.
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> Hyporheic zone: The sediment and porous space beneath and alongside a stream bed that forms an ecotone between the stream and adjoining groundwater habitats.
>
> Response trait: An attribute of an organism that relates to its capacity to colonise or thrive in an environment and persist in the face of disturbance or environmental change.
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> State: An attribute of a trait depicting its category, level or kind. Elsewhere also referred to as 'modality'.
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> Stygobite: Obligate groundwater inhabiting species.
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> Stygofauna: General term for groundwater-dwelling organisms.
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> Stygophile: Essentially surface-dwelling (epigean) species with incipient adaptation to the groundwater life and able to maintain permanent subterranean populations.
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> Stygoxene: Species only occurring sporadically in groundwater habitats and unable to establish permanent subterranean populations.

ecosystem level (Hevia et al., 2017; Loreau & de Mazancourt, 2013; Menezes et al., 2010). In such approaches, biological attributes of taxa are quantified to describe and compare species and community responses to natural and human-impacted conditions. Trait-based approaches have the advantage of being able to indicate the mechanisms of biotic responses to environmental change, allow consistent descriptors or metrics across broad spatial scales that transcend taxonomic descriptions and distributions, and have less seasonal and interannual variability compared with traditional taxonomy-based community metrics (van den Brink et al., 2011). With groundwater resources and ecosystems under immense pressure globally (Mammola, Cardoso, et al., 2019; Mammola, Piano, et al., 2019), traits may provide a sensitive and informative means to better understand these systems and assess ecological risk and change (Culp et al., 2011; Di Lorenzo, Murolo, et al., 2019).

The use of traits to characterise biological assemblages has transformed how ecologists view and compare freshwater systems (e.g. Poff, 1997; Townsend & Hildrew, 1994), providing a lens through which to predict the functioning and stability of ecosystems (e.g. De Castro-Català et al., 2020; Wright et al., 2016), and assess ecological change. This is particularly true where changes become evident in the functional rather than taxonomic structure (Gagic et al., 2015; Loreau, 2010; Martini et al., 2021; Meyer & Kröncke, 2019; Winemiller et al., 2015). Such approaches have been applied to micro-organisms (Šimek et al., 2017), algae (Hoadley et al., 2021), land plants (Kattge et al., 2011, 2020), invertebrates (Worischka et al., 2015), fish (Mérigoux et al., 2001) and amphibians (Lourenço-de-Moraes et al., 2020), and across the spectrum of freshwater ecosystems, including rivers (e.g. Erős et al., 2009; Hoeinghaus et al., 2007; Várbíró et al., 2020), hyporheic zones (Descloux et al., 2014; Di Lorenzo, Fiasca, et al., 2021), lakes (Hébert et al., 2016) and wetlands (Van Bodegom et al., 2006). Except for a small number of recent papers, such as those by Di Lorenzo, Murolo, et al. (2019), who compared functional traits of groundwater invertebrate fauna (stygofauna) in response to nitrate contamination in a porous aquifer, and those by Borko et al. (2021) and Fišer et al. (2019), who analysed morphological traits to test niche differentiation in subterranean amphipods, there has been little analysis of biological traits of groundwater organisms.

The aim of this paper is to explore whether a trait-based approach can be applied to groundwater ecosystems. We address this by first discussing how groundwater geomorphology, climate, water chemistry and biological interactions shape the functional trait diversity of groundwater invertebrate assemblages. We subsequently review the paradigm that groundwater ecosystems have a narrow range of trait states (i.e. attributes of a trait depicting its category, level or kind, also referred to as 'modalities'; see Schmera et al., 2015) by comparing groundwater invertebrate assemblages with those of adjoining hyporheic and surface freshwater systems. We then consider the significance of trait ranges of groundwater taxa for the vulnerability of the ecosystem to change. Our focus is on fractured and granular porous aquifers, where void spaces are typically small and constrain biota and groundwater flow, and on sections of those aquifers where depth to groundwater is many metres below surface, so that the aquifer is not closely connected to surface processes (see below). We concentrate on stygofauna but recognise that microbes are critically important to the functioning of the ecosystem and may be equally amenable to a trait-based approach for groundwater systems (Madin et al., 2020).

2 | ENVIRONMENTAL FACTORS SHAPING TRAIT DIVERSITY IN GROUNDWATERS

Groundwater ecosystems exist in a continuum with adjoining terrestrial and aquatic ecosystems (Brunke & Gonser, 1997), sharing the attributes of those systems, including water and biota, near those ecosystem boundaries (Datry et al., 2005; Iannella et al., 2020). Remote from the boundaries, groundwater ecosystems are typified by relatively stable environmental conditions.

As there is no light in groundwaters, there is an absence of photosynthetic organisms and a general reliance on surface infiltration as a source of carbon and oxygen (Schmidt & Hahn, 2012). Consequently, carbon and oxygen concentrations in groundwaters are naturally lower than in surface waters. For example, dissolved organic matter concentrations in pristine groundwaters are typically 0.2–2 mg/L (Thurman, 1985), with the median global dissolved organic carbon concentration in groundwater being 1.2 mg/L (McDonough et al., 2020) compared to 5.7 mg/L in lake waters (Sobek et al., 2007). Even when present in higher concentrations, much of the organic matter in groundwaters may be non-labile (Hofmann et al., 2020), leading to nutrient limitation. Dissolved oxygen concentrations in groundwater may vary from <0.3 to >3.0 mg/L (Malard & Hervant, 1999), and decrease with distance from exchange zones with surface aquatic and terrestrial systems.

Groundwater ecosystems are profoundly influenced by the geology and geological history of the aquifer, as well as climatic conditions, water chemistry and biological interactions (Figure 1). The geological matrix of an aquifer provides a large thermal mass and buffers the ecosystem from daily and seasonal temperature cycles. As such, subsurface temperatures are typically close to the annual mean surface temperature, varying only 1–2°C over a year, with little or no seasonality (Taylor & Stefan, 2009). Geological factors (e.g. lithology, stratigraphy) also determine the size of voids, water chemistry and the hydraulic characteristics of the aquifer and its ecosystem. Such voids may be large in karst systems, allowing high flows and open water habitat, but in fractured rock and especially alluvial aquifers, void spaces are small (μm-mm range, Schmidt et al., 2017), which limits aquifer flows and creates a fine, tortuous habitat matrix. The result is a diversity of hydrological and habitat conditions ranging from habitats similar to those in surface aquatic systems, albeit in total darkness (e.g. cave streams), to more restrictive environments where pore size determines both biotic distribution and hydrodynamics. These environmental pressures have resulted in the evolution of a unique array of biota comprising microbes, invertebrate and, occasionally, vertebrate stygofauna (Humphreys, 2006).

Obligate groundwater species (stygobites) originated from surface-water ancestors, in both ancient and recent times. Some surface-water species entered groundwater due to their intrinsic capacity for dispersal (Rouch & Danielopol, 1987), whereas others were trapped underground during past marine transgression–regression events (Notenboom, 1991; Stock, 1980), or entered groundwater in response to climate variation at the surface (Barr Jr & Holsinger, 1985). Irrespective of their origins, these taxa possess a suite of traits, or plasticity in relevant traits, which have enabled them to persist underground (Danielopol & Rouch, 1991), with numerous processes having shaped their morphological and physiological adaptations (Figure 1).

Following colonisation of the subterranean realm, evolution, as driven by the groundwater environment, has created a highly adapted fauna, which were likely pre-adapted to cope with low food

FIGURE 1 Overview of the organisational levels of response and effect traits in groundwater invertebrates (stygofauna) that are important in groundwater and their main influences from, and on, the environment.
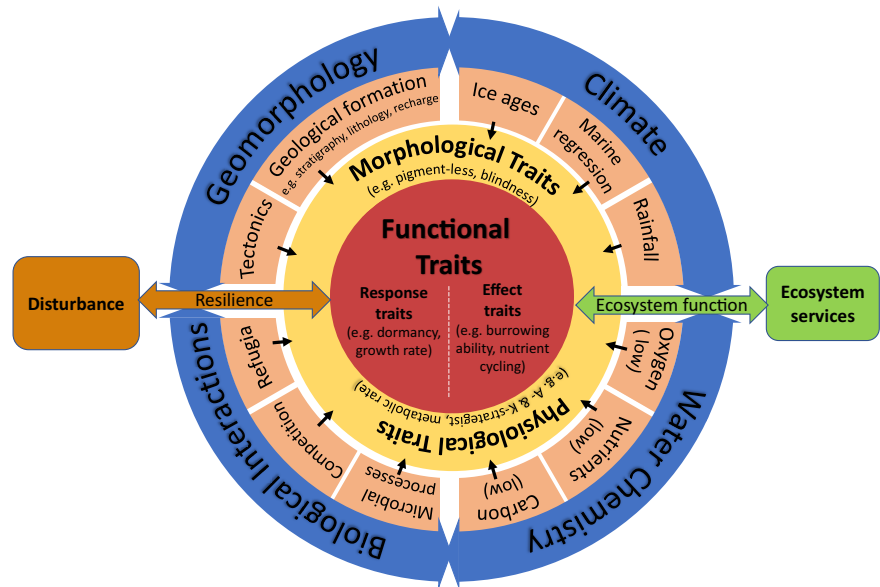
availability and total darkness (Holsinger, 1993). Stygofauna from multiple taxonomic lineages have evolved convergent morphological and physiological traits (Figure 1). The competitive advantage conferred by these traits means that undisturbed groundwaters are typically dominated by taxa with some or all of these traits (Korbel & Hose, 2011; Stein et al., 2010). While convergent morphologies and physiologies have been recognised across a range of stygofauna (e.g. Christiansen, 2012; Humphreys, 2006; Villani et al., 1999), the range of traits associated with those convergences relative to surface ecosystems has not been previously quantified.

Importantly, the environmental conditions in aquifers (e.g. low dissolved oxygen and nutrients) are at, or close to, the physiological limits of some organisms. Away from exchange zones, groundwaters are generally stable and buffered from the large fluctuations in water chemistry, temperature and flow that occur in surface waters, except, perhaps, during large recharge events (e.g. Datry et al., 2005; Reiss et al., 2019). As such, groundwater ecosystems are subject to strong, but typically stable, selective pressures that are common across aquifers of different geologies (Figure 1). Given this, it might be expected that these conditions reduce niche differentiation and thus diversity, and select for species with similar trait values (Cornwell & Ackerly, 2009; Keddy, 1992; Maire et al., 2012 but see Francois et al., 2016).

2.1 | Groundwater biodiversity and biomass

Groundwater ecosystems are typified by low diversity in any location (α diversity), but high diversity across locations (β diversity) (Hahn & Fuchs, 2009; Malard et al., 2009; Stoch & Galassi, 2010). For example, Hancock and Boulton (2008) identified up to 10 taxa per well, but up to 35 taxa per aquifer in samples from four alluvial aquifers in eastern Australia. The authors also found that taxa were all limited to a single aquifer, with one-quarter of all taxa appearing

to have highly localised distributions within the aquifer (Hancock & Boulton, 2008). By comparison, Göthe et al. (2014) reported a range of 18–55 macroinvertebrate taxa at sites within headwater streams, and 42–64 taxa within catchments, which reflects similar trends in rivers elsewhere (Clarke et al., 2008). Such patterns mean that: (a) there are fewer individuals and species to undertake ecosystem functions in groundwater, thus increasing the relative importance of species abundances to the overall ecosystem functioning; and (b) there is little functional redundancy (Gibert & Deharveng, 2002), despite there being fewer functional trait states for taxa to occupy (and thus greater likelihood of overlap). However, cryptic species that may co-occur in groundwaters could potentially contribute to functional redundancy.

While the traits of the taxa dictate the particular ecosystem services that are provided, the magnitude of services provided is dependent on the abundance/biomass of those taxa in the environment (Gaston et al., 2018). For groundwaters, biomass is typically low (e.g. Griebler & Lueders, 2009; Humphreys, 2006), and heterogeneous. Microbial densities in groundwaters are typically several orders of magnitude lower and cell size often smaller than in surface waters (Griebler & Lueders, 2009; van Driezum et al., 2018; Whitman et al., 1998). The invertebrate biomass of aquifers is even less than that of microbes; for example, Marxsen et al. (2021) estimated between 0 and 3800 µg invertebrates/L (median ~ 300 µg/L) in groundwater, which was typically only 1% of the prokaryote biomass. Elsewhere, Di Lorenzo et al. (2020) estimated that the total mass of carbon in stygofauna in a porous aquifer was only 43 ng/km^2, of which 27% was from non-stygobitic species.

What do this low abundance and low trait variability mean for ecosystem vulnerability? Does the loss of a small biomass of organisms that provide ecosystem services affect the state of the ecosystem? The relatively little information on metazoan biomass in groundwaters particularly limits the ability to predict ecosystem

changes to disturbance (Suding et al., 2008) because the extent to which a species contributes to ecosystem services depends strongly on its local abundance (Díaz et al., 2013). Understanding the biomass and abundance of stygofauna and its distribution within an aquifer is thus critical to advancing knowledge of these ecosystems.

3 | DO STYGOFAUNA HAVE NARROW RANGES OF TRAIT STATES IN COMPARISON TO SURFACE WATER FAUNA?

Multiple frameworks and typologies have been developed to classify and analyse biological traits. Suding et al. (2008) proposed a framework distinguishing between *response* and *effect* traits, which can be used to describe how a community responds to change, and how a changed community affects ecosystem functioning (Mensens et al., 2017). *Response trait*s are those that determine the capacity of an organism to colonise or thrive in an environment and persist in the face of disturbance or environmental change (Díaz et al., 2013; Suding et al., 2008), and relate directly to the resilience and resistance of an ecosystem (Figure 1). Response traits include reproduction, growth rate and dispersal that potentially support recovery or mitigate the impacts of changes in populations. *Effect traits* determine how an organism affects ecosystem properties or delivers ecosystem functions (such as bioturbation or nutrient cycling) (Díaz et al., 2013; Suding et al., 2008; Figure 1). By examining a selection of key response and effect traits, below we consider whether the ranges of trait states in groundwater invertebrates are truly narrower than those of surface water invertebrates.

We acknowledge that in the discussion below we identify some response and effects traits that relate directly to an ecological function (e.g. number of eggs, metabolic rate) while some traits are surrogate measures; furthermore, that individual traits are not independent, having often co-evolved in response to the same pressures. These problems are not restricted to groundwater ecosystems and invertebrates (e.g. Endler, 1995; Klug & Bonsall, 2019). However, the dearth of knowledge of the traits of groundwater invertebrates relative to other ecosystems highlights the pressing need to identify and quantify functions and traits, and to better understand the evolution of traits and the delivery of ecosystem functions.

3.1 | Response traits in stygofauna

In response to darkness, many subterranean species lack functional eyes and pigmentation, and have developed enhanced non-ocular sensory appendages or chemo-sensory structures to assist in swimming, mating and finding food (Christiansen, 2012; Galassi et al., 2009). The absence of eyes avoids the metabolic costs of eye development and maintenance, and the vulnerability that sensitive optical structures create (Moran et al., 2015). The vision states in subterranean fauna are limited (Friedrich, 2013), with blindness (anophthalmy) being the most common state (Table S1). Eye spots

are retained in some copepods and amphipods (microphthalmy) and groundwater insects typically lack the compound eyes of their surface relatives. Thus, there are more states for groundwater invertebrates compared to surface water invertebrates where well-developed eyes (macrophthalmy) are the norm (Table S1). Although anophthalmy and microphthalmy are widely shared traits, groundwater species differ in their response to light. For example, while most groundwater species are insensitive to light, others, such as some amphipods from the genus *Niphargus*, are photophobic. This feature helps *Niphargus* distinguish between surface and subterranean environments, avoiding surface habitats where UV rays may be dangerous for depigmented animals (Manenti & Barzaghi, 2021), and competition and predation may be more extensive (Fišer et al., 2016). Hence, we propose the additional states 'anophthalmy and photosensitive' and 'anophthalmy and non-photosensitive' (Table S1).

Most stygofauna are white (pigmentless), which avoids the unnecessary metabolic cost of pigment production, although some taxa (e.g. diving beetles) have residual pigmentation from their surface water origins (Langille et al., 2021), but lack the diversity of colours seen in surface freshwater and marine invertebrates. Body coloration provides protection from ultraviolet radiation, thermal resistance and visual communication to conspecifics and predators (De Bruyn & Gosselin, 2014), and thus seemingly offers little advantage in a dark world. Consequently, we expect decreased variation in this trait relative to surface water invertebrates. Trait classifications for colour are limited, and Spitz et al. (2014) propose a binary classification of 'cryptic' or 'conspicuous' for marine environments. This should be adapted for stygobites to include 'unpigmented', capturing the two states of this trait (cryptic and unpigmented) in groundwater fauna (Table S1).

The confines of the small void spaces have also constrained (or selected for) the shape of most larger groundwater organisms to be generally vermiform (worm-like), enabling them to negotiate the small voids within the aquifer matrix. Body shape is likely to vary more in caves than in small-void aquifer types, as multiple habitat types in caves should allow for more diverse morphologies (Trontelj et al., 2012), although there may be significant variation in interstitial species across some taxa (see Fišer et al., 2019). Descloux et al. (2018) suggested four states for body shape in aquatic invertebrates (streamlined, flattened, cylindrical and spherical), all of which are known for surface water and groundwater fauna (Table S1); there may be no difference in the trait states between surface water, hyporheic and groundwater invertebrates because all environments typically have some limited void spaces (Peralta-Maraver et al., 2018; Schmidt et al., 2017).

The physically confined aquifer environment limits access to open air and restricts respiratory options, thus we expect fewer states to be present in groundwater compared to surface water invertebrates. States relating to respiration in groundwater fauna are limited to gills and tegument (cutaneous) respiration. Spiracles are generally absent (Table S1), with plastrons being only present in a few stygobitic elmid beetle species (Hernando et al., 2001). Adult groundwater dytiscids (diving beetles) are unable to carry and replenish a sub-elytral air

store and lack the setal tracheal gills found in some small surface dytiscids (Kehl & Dettner, 2009). Consequently, they rely on less efficient cutaneous respiration and have a lower metabolic rate and are of small size due to the limited capacity for O_2 uptake (Jones et al., 2019). Organisms using gill and tegument forms of respiration are also more sensitive to chemical stressors than air breathers (Van den Berg et al., 2019).

The mobility and dispersal of groundwater invertebrates are typically less than that of surface taxa (Galassi et al., 2009), hence we expect fewer states in groundwater than in surface water taxa (Table S1). Groundwater species tend to disperse slowly between aquifers and even within heterogeneous areas of the same aquifer (Galassi et al., 2017; Holsinger, 2005; Verovnik et al., 2004). A noteworthy exception is the blind shrimp, *Parisia unguis*, which has a range of at least 300 km throughout a karst aquifer in the Northern Territory, Australia (Oberprieler et al., 2021). However, the mobility of stygofauna is overall poorly known (Vadher et al., 2017). Smith et al. (2016) suggested that stygobitic Amphipoda could migrate between 17 and 35 km/year, while Di Lorenzo, Di Cicco, et al. (2019) measured the swimming speed and relative inactivity of the groundwater copepod *Diacyclops belgicus* (1.5 mm/s and 50% respectively), which translates to potential migration up to 24 km/year. Although such long-distance movements are theoretically possible, they are likely to be limited by barriers, food availability (long-term energetics) and the heterogeneous aquifer matrix. Three states of crawling rates (very low, low and high) for surface water fauna have been proposed by Brown and Milner (2012) (Table S1). Of these, high crawling rates (>100 cm/hr) have been observed in groundwaters (Smith et al., 2016; Stumpp & Hose, 2017). Low (10–100 cm/hr) and very low (<10 cm/hr) crawling rates may also be possible but are probably uncommon because attached or very slow-moving fauna (such as Cnidaria) that may move at these rates are rare in groundwaters.

The number of states for mobility in stygobites is slightly reduced relative to surface water systems. Usseglio-Polatera et al. (2000) identified eight states among benthic fauna (Table S1). Of these, five (excluding flyer and permanent attached) might readily describe stygobitic invertebrates (Table S1). Although surface swimmers and epibenthic burrowers may be unlikely in porous aquifer habitats, they may be present in cave waters (Pipan & Culver, 2007).

Unlike surface water taxa, and stygoxene and stygophile forms, in which flight of adult insect stages is possible, stygobites typically lack both active and passive aerial dispersal stages (Holsinger, 1993; Humphreys, 2006) and are constrained to aquatic dispersal states (Table S1). Within the dispersal states, active dispersal seems more likely in most porous aquifers given the typically low flow rates, although passive dispersal is possible in large voids where there are higher water flow velocities, enabling stygobites to drift, such as from karstic aquifers into springs (e.g. Di Lorenzo et al., 2018; Gibson et al., 2008; Hutchins et al., 2021). Overall, there are fewer states of dispersal among groundwater than surface water invertebrates.

Resource limitations, particularly low carbon, nutrient and oxygen concentrations in groundwaters, and the relative stability of those and other environmental conditions create a 'predictably unfavourable' habitat (Greenslade, 1983). This set of conditions selects for the conservation of adaptations ('A-selection') among species, rather than K- or r-selection (Greenslade, 1983). A-selection is correlated with strategies such parthenogenesis, poor migratory ability, long life histories, and low reproductive rates (Greenslade, 1983), even more so than with K-selected taxa. These traits contrast with those of r-selected species. It is likely that both A- and K-selected taxa are present in groundwater ecosystems.

The timing of, and cues for, reproduction among groundwater invertebrates remain poorly known but are likely linked to resource availability rather than seasonality (see Edler & Dodds, 1996; Reiss et al., 2019; Saccò et al., 2020). Accordingly, the trait states linked to reproductive timing and cycles proposed for surface taxa (see Dunscombe et al., 2018; Usseglio-Polatera et al., 2000) are not relevant for groundwater. Some reproductive techniques, such as terrestrial oviposition are not possible for stygobites, meaning the range of traits is less than for surface species (Table S1), although the reproductive strategies of groundwater invertebrates remain poorly known.

The brood sizes of stygofauna are often small and occupy fewer states (brood size categories) than related surface species (Table S1), probably to invest limited energy in fewer offspring than many. Predation pressure is low in groundwater, so it is not necessary to invest in the high numbers of offspring characteristic of r-selected taxa (see above). For example, around 50 individual offspring per brood are produced by stygobitic representatives of the isopod genus *Caecidotea* in contrast with more than 300 eggs in epigean species of this genus (Zigler & Cooper, 2011). Similarly, the stygobitic cyclopoid *Eucyclops graeteri* carries one to three eggs while the female of the epigean *Eucyclops serrulatus* carries two egg-sacs, each with 15–20 eggs (Dole-Olivier et al., 2000).

Metabolic rates of groundwater fauna are typically low as an evolutionary adaptation to survive chronically low and/or discontinuous food and oxygen supplies (Mezek et al., 2010; Wilhelm et al., 2006), and lower than those of related surface taxa across a range of temperatures (e.g. Di Lorenzo et al., 2015; Hervant et al., 1998; Issartel et al., 2005; but see Simčič & Sket, 2019). For example, during fasting, electron transport system activity and the respiration rate of the stygobitic amphipod *Niphargus stygius* did not change while significantly varying in the epigean relative *Gammarus fossarum* (Simčič et al., 2005). Low metabolic rate is linked to low activity (Colson-Proch et al., 2009; Gerhardt et al., 2020; Hervant et al., 1998, 2001) and reproduction (Carpenter, 2021), which are critical to ecosystem effects and responses. Groundwater invertebrates may also be better able to recover from hypoxia than surface species (Hervant et al., 1998). We propose three states of metabolic rates (lower than surface-water relatives, comparable to those of surface-water relatives, and higher than those of surface water relatives) (Table S1).

Metabolic rates of organisms typically vary with body mass but, unusually, some groundwater species deviate from this general rule. Metabolic scaling is commonly described by the power function $Y = aM^b$ (e.g. Kleiber, 1932) in which Y = metabolic rate, M = body

mass and *a* and *b* are constants. In both endo- and ectothermic organisms, from unicellular microbes to multicellular plants and animals, metabolic rates scale linearly with the body mass with a factor *b* in the range between 0.66 and 0.75 ('allometric scaling', e.g. Brown et al., 2004; Gillooly et al., 2001, 2002). The metabolic rates of the adults of the stygobitic amphipod *Gammarus acherondytes* and the stygobitic copepod *D. belgicus* do not vary with body size in contrast to most surface water invertebrates (Di Lorenzo et al., 2015; Wilhelm et al., 2006). A constant low metabolism which does not scale with the body size (called 'ametric' scaling, Di Lorenzo et al., 2015) may be a physiological adaptation to food-limited environments. This pattern may only apply to adult stygobites, since isometric scaling (i.e. where *b* is close to 1, indicating that oxygen consumption increases at the same rate as body mass) has been reported for the juveniles of *D. belgicus* (*b* = 1.01; Di Lorenzo et al., 2015). Isometric scaling during development is a response to rapid growth rates necessary to avoid predation and reduce juvenile mortality (e.g. Glazier, 2006). Shifts in the scaling of standard metabolic rates from near isometry in juveniles to allometry in adults have been observed in many animals and plants (Glazier et al., 2015). We propose three states of metabolic scaling (allometric, isometric and ametric) (Table S1), and we assume 'ametric scaling' to be limited to groundwater fauna.

The *A*- and *K*-life history strategies of long life span and low reproductive rates that are typical of stygofauna (in contrast to *r* strategies) have been associated with high ecotoxicological risk profiles (Daam et al., 2010; Posthuma & Van Straalen, 1993), and thus a high sensitivity to chemical disturbance. This may be exacerbated by the low mobility of stygofauna which makes them unable to avoid chemical stressors (e.g. Araújo et al., 2020; Di Cicco et al., 2021). The limited data on the chemical sensitivity of stygofauna (Castaño-Sánchez et al., 2020a) show that groundwater species are sensitive to chemicals, although it is not possible to state whether they are consistently more or less sensitive to chemicals than related taxa from other environments (Di Lorenzo, Di Marzio, et al., 2019; Hose, 2005). Von der Ohe and Liess (2004) provided an index of chemical sensitivity (S) for aquatic fauna by comparison to *Daphnia magna*. Such a dichotomous approach, that is, more (S > 1) or less (S < 1) sensitive than *Daphnia magna* may be applicable to groundwaters. Although the limited existing ecotoxicological data from stygobitic species (Castaño-Sánchez et al., 2020a) suggest that they are generally less sensitive than *D. magna* for the same chemicals, both states of this trait are likely (Hose, 2005, 2007, Table S1).

3.2 | Effect traits in stygofauna

The effect traits of stygofauna underpin key ecosystem services such as the maintenance of the physical environment through bioturbation (Hose & Stumpp, 2019) and trophic interactions (Saccò, Blyth, Humphreys, et al., 2019; Weitowitz et al., 2019) that serve to simultaneously promote microbial growth and control microbial clogging of the aquifer, and nutrient cycling (Griebler et al., 2019). Collectively, these services maintain or improve groundwater flow

and quality. The traits relating to these processes include body size and feeding strategies, which may have fewer states among stygofauna (Table S1). Groundwater ecosystems also provide other services, but many of these, such as bioremediation, are supplied by microbial taxa (Griebler et al., 2019; Griebler & Lueders, 2009).

The ability of fauna to bioturbate (Che & Dorgan, 2010), and their influence on the hydraulic properties of the aquifer, are directly related to their body size (Hose & Stumpp, 2019). However, not all stygobitic invertebrates are capable of burrowing, with many utilising existing voids in the matrix. For these non-burrowing taxa, there is frequently a relationship between the body size and habitat pore size (Dumnicka et al., 2020; Korbel et al., 2019). The minute voids present in unconsolidated sediments of alluvial aquifers are a spatial constraint for even small stygobitic cyclopoids and harpacticoids, which often have reduced body size, shortening of the swimming legs and a reduction in setation of the cephalic appendages relative to surface water species (Bruno et al., 2009; Galassi, 2001). In addition to the physical constraints of the environment, it is likely that oxygen and carbon availability limits animal size as observed for epigean animals (Harrison et al., 2010). Body size is modulated by environmental factors and, along with temperature, oxygen and food play a critical role in the evolution of animal size (e.g. Allen et al., 2006; Harrison et al., 2010). In groundwater, the constantly low oxygen level and food availability likely contribute to the relatively smaller size range of invertebrates in groundwater communities (consistently <40mm) compared to those in surface waters (Table S1).

Mediating microbial assemblages is a critical function of groundwater fauna (Griebler et al., 2019; Weitowitz et al., 2019), which is achieved through grazing and sediment ingestion. Many taxa are sediment swallowers, able to strip bacteria before excreting the sediment, which, in turn, may stimulate microbial activity (Mattison et al., 2005; Saccò, Blyth, Bateman, et al., 2019). The relative scarcity of carbon in groundwater ecosystems favours opportunists and omnivores able to utilise any available carbon source, rather than trophic specialists. Nevertheless, some taxa display a high degree of trophic specialisation (Premate et al., 2021) and associations with particular trophic pathways (e.g. Hermann et al., 2020). For example, the stygobitic isopods *Proasellus valdensis* and *P. cavaticus* exhibited a strong specialisation on sedimentary biofilm and most probably feed selectively on this food source (Francois et al., 2016), but this may not necessarily expand the range of traits among taxa within the ecosystem. Usseglio-Polatera et al. (2000) listed eight feeding habits for aquatic invertebrates, of which stygobites likely include seven: deposit feeder, shredder, scraper, filter feeder, piercer, predator and parasite. However, limited knowledge of the trophic ecology of stygofauna means that some categories, such as 'absorber', are currently unknown (Table S1). Despite coarse organic matter in the form of tree root material being available in shallow aquifers (and influencing stygofauna assemblages; see Jasinska et al., 1996; Korbel & Hose, 2015), specialist shredders are not known in alluvial aquifers, but may be present there and in caves where plant root material and allochthonous coarse organic matter are more common. Hence, based on current knowledge, the range of trophic traits of

groundwater invertebrates is likely narrower than that of surface water invertebrates.

Life spans of stygobites are longer than those of related surface taxa (Ginet & Decou, 1977; Strayer, 1994; Voituron et al., 2011). While evidence of longevity of stygofauna is piecemeal and anecdotal, there is some evidence to suggest that the life span of some stygobitic Crustacea exceeds several years (Galassi, 2001; Glatzel, 1990; Rouch, 1968; Venarsky et al., 2012; Table S1) compared to <1 year for many surface-water taxa (Descloux et al., 2014). Sarremejane, Cid, et al. (2020) proposed four states for life span (<1 week, ≥1 week–1 month, ≥1 month–1 year, ≥1 year). We expect stygofauna will fit only two of these states, adult life span ≥1 month–1 year and ≥1 year. Fauna that may depend on episodic events to stimulate breeding (Reiss et al., 2019; Saccò, Blyth, Bateman, et al., 2019) must have the capacity to survive for several years. Di Lorenzo, Fiasca, et al. (2021) and Di Lorenzo, Cifoni, et al. (2021) showed that adults of the stygobitic harpacticoid *Nitocrella achaiae* survive for a year in the laboratory, meaning that the overall life span (including juvenile stages) is likely to be much longer than 1 year. We thus propose additional states for groundwater fauna that reflect their multi-year life span (Table S1), but overall, there is likely to be a narrower range of life spans among groundwater than surface-water invertebrates.

4 | NARROW TRAIT RANGES—WHAT MIGHT THIS MEAN FOR GROUNDWATER ECOSYSTEM VULNERABILITY?

The above synthesis demonstrates that across a range of traits, including response and effect traits, the variability in states among groundwater invertebrates is different, and more frequently narrower, than in surface water systems. While not exhaustive, of the 15 traits listed in Table S1, 8 had fewer states for groundwater than surface water invertebrates (Figure 2). Specifically, 6 of the 12 response traits and 2 of the 3 effect traits had fewer states in groundwaters than are expected in surface waters. For vision and metabolic scaling, there were likely more trait states present in groundwater than surface water invertebrates (Figure 2). The consequences of the low trait and taxonomic diversity of groundwater invertebrates are discussed below.

Our discussion above suggests that groundwater invertebrates have narrow ranges for numerous response and effect traits, and biodiversity and biomass within any location are typically low, relative to other aquatic ecosystems. Low biomass and diversity alone can predispose an ecosystem to vulnerability and impact its capacity to recover (Mori et al., 2013). In groundwaters, where the evolutionary convergence of fauna translates to reduced variation in effect *and* response traits (i.e. correlated effect and response functions sensu Díaz et al., 2013), the ecosystem is particularly vulnerable to perturbation, with little capacity to resist or recover (Wright et al., 2016).

Low variability in response traits and low functional redundancy across a community mean that there exist few mechanisms for groundwater invertebrate communities to maintain their structure
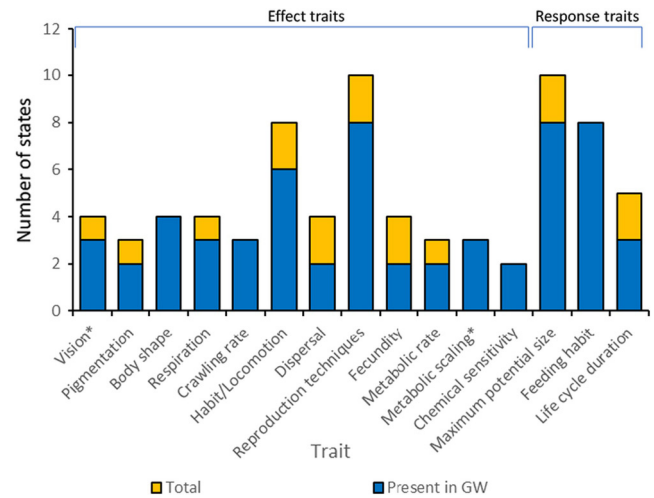

FIGURE 2 Number of trait states expected in groundwater invertebrates compared to the number of states for the same trait in total among groundwater, surface water and hyporheic invertebrates. * Vision and metabolic scaling have more trait states in groundwater than in surface waters. See Table S1 for details of states.

and function following disturbance, and that the potential impact of that disturbance to the community will be large (Castaño-Sánchez et al., 2020a; Di Lorenzo, Cifoni, et al., 2021). If variability in effect traits is low across a community, taxa that are able to persist through the often-long-lasting disturbances in groundwater may have only a narrow range of mechanisms to provide ecosystem services, and some services may no longer be provided as a result (Gitay et al., 1996). Therefore, the general observation that narrow trait ranges lead to a high degree of functional redundancy and support ecosystem resistance and resilience (Mori et al., 2013), is unlikely to apply in groundwater. The low diversity and biomass of organisms in groundwater ecosystems mean that there are relatively few taxa (and individuals) sharing suites of traits and trait states, and functional redundancy is low compared to more speciose systems. From a starting point of low abundance and biomass, even small changes, either positive or negative, may have relatively significant ecosystem effects. This emphasises the importance of understanding the links between effect and response traits, and their interactions with the abundance and distribution of taxa. Despite being critical for predicting ecosystem vulnerability, this is an area of research for which there is a dearth of empirical data for both groundwaters and aquatic ecosystems.

Meta-analyses on both terrestrial and aquatic ecosystems have reported that functional redundancy positively affects community stability and resilience to disturbance (Biggs et al., 2020). Although more research into the mechanism(s) underlying this relationship is needed, it is possible that the low variability in response traits of groundwater fauna relative to surface aquatic fauna implies a limited capacity to respond to change. However, having relatively few or a narrow range of states for a given trait is only problematic if the environmental change exceeds the community's range of tolerances. That is, a community in which all taxa have a high degree of tolerance

to change (but thus has low variability in the tolerance trait), may maintain ecosystem function despite significant disturbance (e.g. Boersma et al., 2014; Sarremejane, England, et al., 2020). As a hypothetical (and optimistic) example, limited data suggest stygofauna from alluvial and fractured rock aquifers can tolerate temperatures 3–5°C above background before showing an increase in mortality (Brielmann et al., 2011; Castaño-Sánchez et al., 2020b). If all taxa share this trait range, the direct effects of 'best case' temperature increases (1–2°C) forecasted under climate change in these systems may be limited (Castaño-Sánchez et al., 2020b; but see Avramov, Rock, et al., 2013 and Di Lorenzo & Galassi, 2017 for sublethal changes). However, the response to temperature increases among groundwater crustaceans, in particular, may be variable (Mermillod-Blondin et al., 2013), and they may evolve tolerance to thermal change over longer time-scales (see Delić et al., 2022; McInerney et al., 2014) potentially enabling them to survive temperature changes associated with climate change. Response traits to other stressors, such as sensitivity to xenobiotics, are more likely to be variable and, under the current knowledge of stygofauna physiology, are unpredictable (Avramov, Schmidt, & Griebler, 2013; Castaño-Sánchez et al., 2020a, 2020b; Di Lorenzo, Cifoni, et al., 2021; Hose, 2005, 2007). Importantly, the slow rates of groundwater movement and replenishment, and difficulties remediating contaminated groundwaters, mean that contaminant exposure may persist for longer in groundwater than in surface environments, putting stygofauna at particular risk (Hose, 2005). Predicting the ecological effects of change requires further knowledge of the magnitude of change and the range of values of related traits in the community, and their inter-relationship.

If low variability in response traits confers vulnerability, the net ecosystem function may be maintained if tolerant taxa are able to increase their functional output or increase in abundance following the disturbance (Hinz et al., 2021). However, the recruitment of stygofauna following disturbance is typically low and slow, due to inherently low reproductive rates and dispersal of fauna (Table S1), meaning that there is likely to be a relatively extended period where ecosystem function remains impaired (Galassi et al., 2014; Mammola et al., 2022; Mammola, Piano, et al., 2019). While there has been some evidence of biomass increases in some taxa following contamination of aquifers with readily degradable organic matter (e.g. Sinton, 1984), such changes are unlikely to be sufficient to compensate for the loss of biomass and function of other taxa, at least in the short term (given low reproduction and immigration rates). Furthermore, contamination with less degradable organic substances can eradicate stygobitic crustacean communities (e.g. Graening & Brown, 2003). Even if disturbance favours the introduction of surface taxa to the aquifer, it is uncertain whether those taxa can provide the same types and level of services provided by groundwater organisms and whether the natural stygofauna communities can be re-established. Further quantification of the roles of invertebrates to ecosystem services in both surface and groundwaters is urgently needed, as well as thresholds of invertebrate biomass (or abundance) needed to sustain ecosystem services.

5 | TRAITS IN GROUNDWATER MONITORING

Trait-based analyses are increasingly incorporated in environmental monitoring, where changes in the suites of traits held by the community, rather than changes in terms of the taxonomic structure, indicate environmental change (see Culp et al., 2011). Trait-based approaches are among a suite of taxonomy-free metrics that may be incorporated into biomonitoring (see Makiola et al., 2020). Such approaches have enormous potential for use in groundwater ecosystems where the taxonomy of invertebrates is often challenging, and cryptic and endemic species are common (e.g. Bradford et al., 2010; Harvey, 2002; Iannella et al., 2021; McInerney et al., 2014; Mokany et al., 2019). Trait-based approaches are also less subject to spatial and temporal nuances than taxonomy-based assessments (van den Brink et al., 2011) and may be more consistent in responses to stressor gradients (Pollard & Yuan, 2010). While there are attributes of traits incorporated into existing monitoring approaches (e.g. ratios of juveniles/adults and crustaceans/non-crustaceans; e.g. Di Lorenzo et al., 2015; Fattorini et al., 2017; Galassi et al., 2014; Korbel et al., 2011, 2017; Malard et al., 1996; Plenet et al., 1996; Stein et al., 2010) overall greater knowledge and quantification of the functional traits of stygofauna are needed before such approaches can become routine.

6 | CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Traits have great potential as a tool for better understanding groundwater ecosystems because they provide a framework to circumvent the taxonomic challenges of a (generally) poorly explored and described fauna (Ficetola et al., 2019) as well as providing a possible tool for risk assessment (Díaz et al., 2013). The provision of ecosystem services, which for groundwaters includes reliable drinking water supplies, is critically linked to the traits of the fauna. More work is needed to identify appropriate traits and their states in groundwater fauna. As in other ecosystem types, there is currently little understanding of the relationship between response and effect traits in groundwater, and this should be an area for further research (Suding et al., 2008) so that the nature and extent of changes to the ecosystem, and the delivery of ecosystem services following disturbance, can be better predicted. We have speculated that interspecific trait variability among groundwater invertebrates is low in most situations as a likely consequence of the intense selective pressures of the groundwater environment. Models of traits and ecosystem stability suggest that low trait variability makes groundwater ecosystems particularly vulnerable to change, with limited capacity for recovery, emphasising the recent and urgent calls for the improved recognition, conservation and management of groundwater ecosystems (Boulton, 2020; Fattorini et al., 2020; Iannella et al., 2021; Mammola, Cardoso, et al., 2019; Sánchez-Fernández et al., 2021; Wynne et al., 2021).

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Grant Hose, Kathryn Korbel and Anthony Chariton conceived the idea; Grant Hose led the workshop and writing of the manuscript. All authors contributed critically to the workshop and manuscript drafts and gave final approval for publication.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.