REVIEW ARTICLE

The microbial dimension of submarine groundwater discharge: current challenges and future directions

Clara Ruiz-González1,*,†, Valentí Rodellas2,‡ and Jordi Garcia-Orellana2,3,#

1Institut de Ciències del Mar (ICM-CSIC), Passeig Marítim de la Barceloneta, 37-49, E08003 Barcelona, Spain, 2Institut de Ciència i Tecnologia Ambientals (ICTA-UAB), Universitat Autònoma de Barcelona, E08193 Bellaterra, Spain and 3Departament de Física, Universitat Autònoma de Barcelona, E08193 Bellaterra, Spain

*Corresponding author: Institut de Ciències del Mar (ICM-CSIC), Paseo Marítimo de la Barceloneta, 37-49, E08003 Barcelona, Spain. Tel: 34-93-2309500, Ext. 6047; Fax: 34-93-2309555; E-mail: clararg@icm.csic.es

One sentence summary: The authors review the available literature on the microbial aspects of submarine groundwater discharge, from the freshwater aquifers to the coastal ocean, and identify current challenges and future directions to foster knowledge on microbial ecology at the land–ocean interface.

Editor: Prof. Corina Brussaard

1Clara Ruiz-González, http://orcid.org/0000-0003-3568-4943
2Valentí Rodellas, http://orcid.org/0000-0002-5896-9987
3Jordi Garcia-Orellana, http://orcid.org/0000-0002-0543-2641

ABSTRACT

Despite the relevance of submarine groundwater discharge (SGD) for ocean biogeochemistry, the microbial dimension of SGD remains poorly understood. SGD can influence marine microbial communities through supplying chemical compounds and microorganisms, and in turn, microbes at the land–ocean transition zone determine the chemistry of the groundwater reaching the ocean. However, compared with inland groundwater, little is known about microbial communities in coastal aquifers. Here, we review the state of the art of the microbial dimension of SGD, with emphasis on prokaryotes, and identify current challenges and future directions. Main challenges include improving the diversity description of groundwater microbiota, characterized by ultrasmall, inactive and novel taxa, and by high ratios of sediment-attached versus free-living cells. Studies should explore microbial dynamics and their role in chemical cycles in coastal aquifers, the bidirectional dispersal of groundwater and seawater microorganisms, and marine bacterioplankton responses to SGD. This will require not only combining sequencing methods, visualization and linking taxonomy to activity but also considering the entire groundwater–marine continuum. Interactions between traditionally independent disciplines (e.g. hydrogeology, microbial ecology) are needed to frame the study of terrestrial and aquatic microorganisms beyond the limits of their presumed habitats, and to foster our understanding of SGD processes and their influence in coastal biogeochemical cycles.

Keywords: aquatic prokaryotic communities; submarine groundwater discharge; coastal aquifers; subterranean estuaries; microbial diversity and ecology; ultrasmall prokaryotes

INTRODUCTION

Aquifers contain a significant portion of the Earth’s freshwater (~23 x 10^6 km^3; Gleeson et al. 2016) and a large fraction of the global microbial diversity (Magnabosco et al. 2018), yet they have been much less studied than surface aquatic ecosystems. Like in deep soils, deep marine sediments or the dark bathypelagic ocean (Acinas et al. 2019; Brewer et al. 2019; Wörner et al. 2019), the absence of photosynthesis-derived...
labile organic carbon has forced groundwater organisms (mostly prokaryotic microorganisms—Bacteria and Archaea) to develop diverse strategies to live or persist (Griebler and Lueders 2009). For example, heterotrophic groundwater microorganisms can use ancient organic carbon from rocks (Nowak et al. 2017), allochthonous organic carbon such as plant-derived material (Taubert et al. 2016; Kumar et al. 2017, 2018; Probst et al. 2017, 2018). Groundwater microorganisms, however, are largely under-represented in global diversity catalogs, as for example less than 2% of the public 16S ribosomal RNA (rRNA) gene sequences (i.e. the most common taxonomic marker for identifying prokaryotic taxa, Appendix 1) were calculated to derive from groundwater organisms (Schloss et al. 2016). The magnitude of this knowledge gap has become evident in recent years: 47 previously unknown phyla were found in a single aquifer (Anantharaman et al. 2016), highlighting a tremendous potential for taxonomic and metabolic discovery in subsurface ecosystems.

Among aquifers, those located in coastal areas are essential freshwater sources for humanity given that half of the global population is concentrated along the oceans’ shoreline (Small and Nichols 2003; Barragán and de Andrés 2015; Michael et al. 2017). Coastal aquifers are also vulnerable ecosystems because the intense anthropogenic pressure is causing pollution and salinization of coastal groundwater resources worldwide (Ferguson and Gleeson 2012; Werner et al. 2013). Their direct connection to the ocean through permeable sediments or rocks (Fig. 1) allows the discharge of groundwater, a process known as submarine groundwater discharge (SGD; Box 1), but also the entrance of seawater. This often results in an area of active mixing within the aquifer, referred to as ‘subterranean estuary’ (Moore 1999; Duque, Michael and Wilson 2020; Box 1). Globally, SGD fluxes (∼10^14 m^3 yr^-1) have been estimated to be 3–4 times greater than the riverine water fluxes (Kwon et al. 2014), representing a very important source of dissolved nutrients and other chemical compounds (e.g. metals, carbon, pollutants, greenhouse gases) to the coastal ocean (Moore 2010; Rodellas et al. 2015; Cho et al. 2018; Mayfield et al. 2021). SGD can comprise meteoric groundwater originating through aquifer recharge (fresh groundwater), seawater infiltrated into coastal aquifers and sediments (saline groundwater), or a mixture of both (brackish groundwater) (Moore 2010; Fig. 1). At the global scale, SGD is mainly composed of saline groundwater, with fresh groundwater representing only <1% of total SGD but sometimes being the dominant component at a more local scale (Luijendijk, Gleeson and Moosdorf 2020). In general, most SGD to the coastal ocean occurs through slow and diffusive seeps through permeable sediments (Fig. 1A), but in systems with fractured rocks or preferential flow paths (e.g. karstic carbonate or volcanic systems) groundwater can also be transferred to the coastal ocean through point-sourced seeps or submarine springs (Tovar-Sánchez et al. 2014; Fig. 1B).

**BOX 1.**

**Terms and definitions**

**Coastal aquifers:** Coastal aquifers are frequently defined as permeable geological units in the subsurface that can transmit significant flows of water and that are connected with the sea (Duque et al. 2020). Coastal aquifers consist of unconsolidated surficial deposits (created by alluvial, lacustrine, deltaic, glacial or eolian processes; e.g. Fig. 1A), permeable rocks (e.g. basalts, sandstones or limestones) or heavily fractured systems (e.g. karstified carbonate rocks or volcanic systems; Fig. 1B), where cavities and pores between mineral grains provide open spaces for water storage and transmission (Freeze and Cherry 1979).

**Subterranean estuary:** The term subterranean estuary was initially introduced to emphasize the importance of biogeochemical reactions occurring within coastal aquifers, where meteoric groundwater mixes with the seawater that has entered into the coastal aquifer (Moore, 1999). The subterranean estuary is now considered as the part of the coastal aquifer that actively interacts with the ocean, including thus the area of fresh groundwater that is connected to the sea (i.e. groundwater that could discharge to the ocean), the mixing zone between meteoric and marine groundwater, and the permeable sediments filled with completely saline groundwater that may exchange with overlying marine waters (Duque et al. 2020).

**Submarine groundwater discharge (SGD):** Groundwater is often considered as ‘any water in the ground’, regardless of its composition or origin, and thus it is synonymous with porewater (Burnett et al. 2003). Groundwater can thus consist of water of any salinity, ranging from freshwater from recharged aquifers on land to seawater that has invaded the subterranean estuary. Based on this concept, SGD is commonly defined as ‘the flow of water through continental and insular margins from the seabed to the coastal ocean, regardless of fluid composition or driving force’ (Burnett et al. 2003; Moore 2010; Taniguchi et al. 2019). SGD incorporates thus water flows with different characteristics and is driven by different forces, including discharge of fresh groundwater driven by the terrestrial hydraulic gradient, exchange of seawater driven by the movement of the freshwater–saltwater interface, or circulation of seawater driven by tidal inundation or wave run-up, among others (Santos et al. 2012; Robinson et al. 2018).

Coastal aquifers and SGD are of special interest from a microbiological perspective. First, coastal aquifers are located at the land–ocean interface, a transition zone with steep physicochemical gradients (e.g. freshwater–seawater, oxic–anoxic) that promote the establishment of spatially heterogeneous microbial communities (Héry et al. 2014; Beck et al. 2017; Seibert et al. 2020). Second, given that groundwater microbes control the cycling of nutrients, carbon and trace elements in aquifers (Hunter, Wang and Van Cappellen 1998; Griebler and Lueders 2009; Flynn et al. 2013; Griebler and Avramov 2015; Meckenstock et al. 2015; Hoffman et al. 2020), microbial activity in subterranean estuaries will determine the chemical composition of the groundwater reaching the ocean, but little is known regarding the metabolic functions of microorganisms in coastal aquifers (Santoro 2010; Seibert
et al. 2020). Finally, despite the relevance of bacterioplankton communities for marine food webs and biogeochemical processes (e.g. Whitman, Coleman and Wiebe 1998; Gasol et al. 2008), only a few studies have addressed marine bacterial taxonomic and functional responses to SGD (see references in Lecher and Mackey 2018). Improving our understanding on the microbial players along the entire aquatic continuum from freshwater aquifers to the coastal ocean is essential to gain insight into the complex processes underlying coastal groundwater biogeochemistry, the potential variations in these processes due to global change, and their influence on marine ecosystems.

The aim of this review is to provide an overview of the current knowledge on microbial ecology along the land–ocean transition zone connected by SGD. We first identify current challenges for the study of coastal groundwater microbiota based on recent discoveries in inland aquifers, which have been much more intensely studied than coastal groundwater systems (see the section ‘Challenges for assessing microbial diversity and their function in the coastal subsurface: lessons learned from inland aquifer research’). Second, we review the available literature on microbial diversity and ecology along subterranean estuaries and the adjacent coast influenced by SGD (see the section ‘Microbial ecology at the terrestrial–marine interface connected by groundwater’). Finally, we provide future research directions and strategies that may foster our understanding of the microbial dimension underlying SGD processes (see the section ‘Future directions and research avenues’).

**CHALLENGES FOR ASSESSING MICROBIAL DIVERSITY AND THEIR FUNCTION IN THE COASTAL SUBSURFACE: LESSONS LEARNED FROM INLAND AQUIFER RESEARCH**

Despite the growing interest in inland groundwater microbiology in the last decades, coastal aquifers have received comparably little attention. A simple search in the ISI Web of Science database looking for articles published between 2000 and 2020 containing ‘groundwater AND microbial’ in the title retrieves 369 studies that show a clear increasing trend over time (Fig. 2). Conversely, only 16 articles are recovered if we include terms such as ‘submarine’, ‘seawater’, ‘subterranean estuar’ or ‘coastal aquifer’, highlighting a wide gap of knowledge on the marine–groundwater interface from a microbial perspective. This growing body of inland groundwater research provides, nonetheless, a solid basis for anticipating the main challenges that the study of the microbiology in subterranean estuaries might face. Based on this knowledge, in this section we identify four main challenges related to groundwater microbial idiosyncrasies that future investigations should take into account for an accurate understanding of microbial ecology at the land–ocean interface.

Additional challenges related to sampling the subsurface or SGD sources are not discussed in this review. For example, aquifers need to be accessed through wells, piezometers, boreholes, cores or excavations. This not only makes the collection of groundwater and aquifer solids technically and economically challenging (Lehman 2007), but also precludes an accurate characterization of the geologic, physicochemical and biological 3D heterogeneity of subsurface ecosystems (Robinson et al. 2018; Smith et al. 2018, Folch et al. 2020). Similarly, SGD is a much more diffuse and heterogeneous source of water to the ocean than riverine discharge, which translates into many uncertainties associated with the magnitude and spatiotemporal variability of this process (Burnett et al. 2006). Current and future efforts to improve the characterization of aquifers and groundwater seeps to the ocean will be instrumental for developing the research lines identified in this review.

**Novel groundwater microbial taxa can be missed by current sequencing technologies**

The study of groundwater microorganisms has evolved progressively together with the development of taxon identification techniques (see Griebler and Lueders 2009 for an excellent review on groundwater prokaryotic diversity in inland aquifers since the earliest investigations). A compilation of studies examining microbial communities in coastal aquifers (Table S1, Supporting Information) evidences a clear technical shift moving from approaches such as clone libraries, quantitative polymerase chain reaction (qPCR) or fingerprinting of taxonomic or functional marker genes, to high-throughput sequencing (HTS) technologies such as 454 or Illumina (Logares et al. 2012; see Appendices 1 and 2 for definitions of microbiology concepts and methodologies). Currently, most studies on coastal aquifer microbiota have been based on the high-throughput amplicon sequencing of the 16S rRNA gene (Table S1, Supporting Information), which allows a much more detailed characterization of microbial communities than traditional approaches such as...
clique may be limited for the description of groundwater microbiota because it relies on the PCR amplification of particular genes (e.g. the 16S rRNA gene) through the use of primers, which are designed based on the sequences available in public databases. Therefore, diversity recovery through primer-based approaches can be biased against taxa that are less represented in public sequence catalogs, such as those from aquifers (and coastal aquifers in particular). In addition, primer pairs targeting different regions of the 16S rRNA gene can be biased against different taxa (Klindworth et al. 2013), preventing the direct comparison between studies using different primer pairs. Table S1 (Supporting Information) actually highlights a lack of consensus on primer usage, since up to 13 different 16S rRNA gene primer pairs have been used among the 25 studies describing groundwater prokaryotic diversity in coastal aquifers (Tables S1 and S2, Supporting Information). These primer pairs differ in their coverage of some of the main phyla detected in groundwater (Fig. 3A). Moreover, most fail to capture novel groundwater bacterial lineages discovered by studies applying primer-free approaches (Fig. 3A–C), such as the superphylum Candidatus (Ca.) Patescibacteria (also known as candidate phyla radiation, CPR), which includes a high diversity of small uncultivated bacterial groups (Brown et al. 2015; Luef et al. 2015; Anantharaman et al. 2016; Castelle et al. 2018; He et al. 2021; Tian et al. 2020; see the section ‘Ultrasmall prokaryotic groups are abundant in groundwater ecosystems’), or archaeal members of the DPANN radiation (Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota, Nanohaloarchaeae), also characterized by small cell sizes and genomes (Ludington et al. 2017; Castelle et al. 2018; He et al. 2021). The discovery of these groups has been possible through techniques such as metagenomics and metatranscriptomics that are not biased by the performance of primers (Appendix 2), but so far none of the microbial studies in subterranean estuaries have applied these techniques (Table S1, Supporting Information). Hence, despite the global distribution of the investigations on coastal aquifers conducted so far (Fig. 4), the unknown accuracy of the existing primers to recover groundwater diversity, together with the lack of consensus on which primers to use, precludes generalizations and presumably renders a biased view of groundwater microbial inhabitants in coastal areas.

**Ultrasmall prokaryotic groups are abundant in groundwater ecosystems**

Another peculiarity of groundwater microbial communities is their ability to shelter minuscule prokaryotes (Brown et al. 2015; Luef et al. 2015; Proctor et al. 2018; Herrmann et al. 2019) that may not be captured by current sampling strategies. Prokaryote biomass for DNA extraction or microscopic visualization is often collected onto 0.2-μm-pore-size filters (Table S1, Supporting Information) and these cells can be smaller than this pore size (Nakai 2020). The few groundwater studies (all conducted in inland aquifers) that used sequential filtration of water through 0.2- and 0.1-μm-pore-size filters unveiled that the fraction of small cells passing through the 0.2-μm filters is often dominated by members of the superphylum Ca. Patescibacteria (Miyoshi, Iwatsuki and Naganuma 2005; Herrmann et al. 2019; He et al. 2021) or by the small Archaea belonging to the DPANN radiation (Castelle et al. 2018; He et al. 2021). The reconstruction of individual genomes from metagenomes (MAGs) or from individually sorted cells (SAGs; Appendix 2) has yielded insight into the metabolic potential of these uncultivated groundwater groups, unveiling unusually reduced genomes and gaps in core metabolic potential that are typical of symbionts (Brown et al. 2015; Luef et al. 2015; Youssef et al. 2015; Anantharaman et al. 2016; Castelle et al. 2018; Probst et al. 2018; He et al. 2021). Members of Ca. Patescibacteria have been shown to be active in groundwater (Tauber et al. 2019) and seem to prefer a planktonic lifestyle (Lazar et al. 2019), although their detection in larger planktonic size fractions (e.g. >2 μm) has been interpreted as evidence of epipsymbiosis (i.e. surface attachment to other microorganisms; He et al. 2021). A recent study, however, claims that Ca. Patescibacteria and DPANN genomic features may not be indicative of symbiotic lifestyles but rather of an ancestral absence of electron transport chains, and thus that they rely on fermentative metabolisms (Beam et al. 2020).
Figure 3. Differential diversity coverage among the 16S rRNA gene primer pairs used in studies on subterranean estuaries (STE). The primers shown include those used in the studies included in Tables S1 and S2 (Supporting Information) to characterize prokaryotic diversity, which represent primers targeting only Archaea, only Bacteria or both (indicated by an asterisk). The different panels represent the diversity coverage of some of (A) the main bacterial phyla found in groundwaters, (B) the different phyla within the superphylum Ca. Patescibacteria (underlined in panel A) and (C) the main archaeal phyla known to be present in groundwater. The diversity coverage was tested in silico using the online tool TestPrime (Klindworth et al. 2013), by comparing the sequences captured by each primer pair against the SILVA taxonomic database, allowing for zero mismatches (i.e. no differences between the compared sequences). Primer sequences are indicated in Table S2 (Supporting Information).
any case, despite their apparent importance in the subsurface, the relevance of these ultrasmall prokaryotes in coastal aquifers remains unknown due to the previously mentioned primer limitations for capturing these taxonomic groups (Fig. 3), and the lack of studies exploring the diversity within the ultrasmall planktonic fraction (<0.2 μm) in subterranean estuaries (Table S1, Supporting Information; see the section ‘Microbial abundance, diversity and environmental drivers along subterranean estuaries’).

Miniaturized inactive or dormant taxa may be abundant in groundwater

Prokaryotes can also miniaturize in response to stress or starvation conditions (Velimirov 2001) becoming inactive or dormant (i.e. in a reversible state of low metabolic activity; Lennon and Jones 2011). Due to the typical scarcity of freshly produced carbon in subsurface ecosystems (Hofmann and Griebler 2018), groundwater microbes have probably evolved diverse strategies to persist in resting or dormant states until more favorable conditions occur. Given that an important fraction of the cells in aquifers are believed to be inactive (Griebler and Lueders 2009), it is possible that a fraction of the ultrasmall cell pool represents miniaturized dormant taxa rather than obligate ultramicrobacteria (sensu Nakai 2020). In support of this, the exposure of bacterial groundwater isolates to the oligotrophic conditions typical of pristine aquifers caused a significant cell size reduction (Herrmann et al. 2019). Also, we have observed that the incubation of 0.2-μm prefiltered groundwater from a coastal Mediterranean aquifer results in pronounced increases in the abundance, cell size and heterotrophic production of the bacteria passing through the 0.2-μm filter (Fig. 5), indicating that some cells had been miniaturized in situ (and were presumably dormant) likely due to the prevailing carbon scarcity.

Such miniaturized cells could comprise a microbial ‘seed bank’ (i.e. a reservoir of dormant bacteria that can reanimate upon changes in conditions; Lennon and Jones 2011). In general, though, little is known about the relevance and extent of dormancy in groundwater systems. A recent experimental study suggested that bacteria in oligotrophic aquifers can be locally co-limited by organic carbon and phosphorous, but that they can reanimate and reach high bacterial abundances when amended with different organic carbon sources and nutrients (Hofmann and Griebler 2018), or with soil dissolved organic matter (DOM) richer in labile compounds (Hofmann et al. 2020). Studies in deep marine sediments, where microbes face increasing starvation with depth, have shown that dormant endospore-forming cells can outnumber actively growing cells (Wörner et al. 2019) and that most microorganisms appear to be surviving instead of growing (Bradley, Amend and LaRowe 2019). Thus, rather than growth, maintenance mechanisms might be key to thrive in such oligotrophic habitats. Finally, environmental stresses such as salinity changes also appear to trigger dormancy in bacteria (Sachidanandham and Yew-Hoong Gin 2009; Aanderud et al. 2016), so this mechanism might allow repeated activation and inactivation of prokaryotes exposed to changing conditions caused by the mixing of seawater and groundwater in coastal aquifers.

Groundwater microbial communities might also include ‘allochthonous’ microorganisms transported from external sources that are hydraulically connected to the aquifer (e.g. soils, rivers, lakes; Hubalek et al. 2016; Graham et al. 2017; Meier et al. 2017; Herrmann et al. 2019; Fillinger et al. 2021). Although some of these transported microorganisms may comprise a traveling groundwater ‘seed bank’, being able to colonize different habitats within groundwater ecosystems (such as sediments or rock surfaces; Griebler et al. 2002; Griebler, Malard and Lefébure 2014; Fillinger et al. 2019b; Herrmann et al. 2019; see the section...
Groundwater biomass and activity is concentrated on aquifer solids

Compared with surface aquatic ecosystems, aquifers tend to have much higher solid–water ratios and slower flow velocities (usually on the order of millimeters to meters per day), which allows for an intense interaction between groundwater and solids that influences groundwater chemistry and surface characteristics of the geological matrix (Moore 2010; Tovar-Sánchez et al. 2014). This physical structure of aquifers challenges the study and the sampling of aquifer microbiota, as prokaryotes may not occur only as free-living planktonic cells but also attached onto solid aquifer surfaces as individual cells or aggregates (Smith et al. 2018). Actually, most groundwater microbial biomass and activity seems to be concentrated on solid surfaces (Griebler et al. 2002; Smith et al. 2018), often showing a highly patchy distribution at the microscale due to the microniches than can be established within pores and cavities (Goldscheider, Hunkeler and Rossi 2006; Schmidt, Cuthbert and Schwientek 2017; Smith et al. 2018).

Not many studies have compared the free-living and the attached microbial groundwater components. In inland aquifers, these investigations have shown that microbial communities adhered to karst sediments display higher activities than the suspended ones, likely due to a better access to organic carbon and nutrients (Wilhardt et al. 2009). The ratio of attached versus free-living bacteria was shown to increase pronouncedly as oligotrophic conditions increased, ranging from ~50:1 in a contaminated site to >1500:1 in pristine groundwater (Griebler et al. 2002). Attached microbial communities differ notably from the planktonic ones in their taxonomic composition, as has been shown by the direct sampling of microorganisms from aquifer rocks and groundwater (Herrmann et al. 2017; Meier et al. 2017; Lazar et al. 2019), or by incubation of sterilized sediments within wells (Zhou, Kellermann and Griebler 2012; Flynn et al. 2013) and in experimental microcosms or mesocosms (Longnecker and Kujawinski 2013; Fillinger et al. 2019b). Results from these studies show that groundwater transports taxa able to colonize sediments (Fillinger et al. 2019b) and that attached communities are more stable over time than planktonic microorganisms (Zhou, Kellermann and Griebler 2012), but more responsive to the presence of bacterial grazers (Longnecker and Kujawinski 2013).

No study on coastal aquifers has directly compared groundwater and sediment microbial communities (Table S1,
Supporting Information). However, prokaryotes living on sand grains and in saline porewater were found to differ at fine taxonomic levels (Gobet et al. 2012), and communities from two planktonic size fractions (0.2 and 0.45 μm, which may include small suspended particles) differed taxonomically in a subterranean estuary (Chen et al. 2019). Hence, targeting only the planktonic or the sediment-attached compartment may lead to a rather incomplete view of microbial ecology in inland or coastal groundwater. Given the relevance of hydrologic boundaries and flow paths for the assembly and functioning of the biofilm communities in inland shallow aquifers (Smith et al. 2018), it is likely that subterranean estuaries are also characterized by a highly complex spatiotemporal structuring of the sediment-attached microbial component.

**MICROBIAL ECOLOGY AT THE TERRESTRIAL–MARINE INTERFACE CONNECTED BY GROUNDWATER**

Environmental transitions or ecotones are relevant landscape components. Due to the mixing of resources and communities, these sites are often hotspots of biogeochemical activity, where abrupt physicochemical changes or gradients determine large variations in microbial community composition and activity (e.g. McClain et al. 2003; Stegen et al. 2016). Subterranean estuaries (Box 1), which are ubiquitous along coastlines, represent ideal systems to study microbial adaptations to the abrupt environmental transitions created by the mixing of groundwater and seawater, but they have been largely understudied compared with surface waters, estuaries or inland aquifers. In this section, we review the current knowledge on microbial communities from coastal aquifers based on the available studies (Table S1, Supporting Information; Fig. 4), highlighting what we have learned about the microbial diversity, drivers and functioning of these unexplored ecosystems. We also provide an overview of the few studies exploring the effects of SGD on coastal bacterial-plankton communities and the potential ecological implications (Table S3, Supporting Information; Fig. 4).

**Microbial abundance, diversity and environmental drivers along subterranean estuaries**

Despite the recent rise in the number of microbial studies focusing on aquifers connected to the sea (Table S1, Supporting Information; Fig. 4), little is known regarding the abundance, diversity and the roles of the microorganisms inhabiting these systems. Only a few studies have quantified prokaryotic cell abundances or bulk heterotrophic production in subterranean estuaries (Table S1, Supporting Information), even though this is something that is routinely done in microbial investigations on surface waters. Applying epifluorescence microscopy to porewater samples in a sandy beach aquifer, Santoro et al. (2008) reported up to 1.4 × 10⁶ cells mL⁻¹. Higher maximum abundances were observed in a coastal alluvial aquifer that is well connected to the surface, where large seasonal variations in cell numbers (range 2 × 10⁵ to >6 × 10⁷ cells mL⁻¹) were related to changes in temperature and nutrient concentrations (Velasco Ayuso et al. 2009a,b). These values fall all in the upper range of those typically found in pristine inland groundwater (10⁵–10⁶ cells mL⁻¹; Griebler and Lueders 2009), but the scarcity of data prevents any accurate comparison. In sediments, up to 2.6 × 10⁶ cells cm⁻² were measured (Beck et al. 2017), implying that likewise in inland aquifers, coastal sediments harbor higher prokaryotic biomass than ground- or porewater (see the section ‘Groundwater biomass and activity is concentrated on aquifer solids’).

In terms of microbial community composition, the recent application of high-throughput sequencing technologies (Table S1, Supporting Information) has shown that subterranean estuaries hide heterogeneous and diverse prokaryotic communities varying pronouncedly at small vertical and horizontal scales (e.g. Héry et al. 2014; Unno et al. 2015; Ye et al. 2016; Beck et al. 2017; Adyasari et al. 2019; Chen et al. 2019a; Hong et al. 2019; Adyasari et al. 2020; Chen et al. 2020b; Sola, Vargas-García and Vallejos 2020). Such studies have generally reported a dominance of the bacterial phyla Proteobacteria (mostly Gamma- and Alphaproteobacteria), Bacteroidetes and/or Actinobacteria, with other phyla like Planctomycetes, Chloroflexi or the endospore-forming Firmicutes being locally important depending on the environmental conditions (Héry et al. 2014; McAllister et al. 2015; Unno et al. 2015; Ye et al. 2016; Sang et al. 2018, 2019; Chen et al. 2019a; Hong et al. 2019; Zhang et al. 2021). Taxa belonging to the ultrasmall Ca. Fatesicibacteria have rarely been observed, often comprising <2% of the 16S rRNA sequences (Héry et al. 2014; Unno et al. 2015; Ye et al. 2016; Chen et al. 2019a), but locally reaching up to 10% (Adyasari et al. 2020). These abundances might be underestimates due to the previously mentioned primer biases for capturing these novel groups (see the section ‘Novel groundwater microbial taxa can be missed by current sequencing technologies’; Fig. 3) and/or to the loss of ultrasmall cells during 0.2 μm filtration (Table S1, Supporting Information; see the section ‘Ultrasmall prokaryotic groups are abundant in groundwater ecosystems’).

Archaea are generally less abundant than bacteria, but they harbor functional groups such as methanogens, ammonia oxidizers or nitrifiers that may play key roles in aquifer biogeochemistry (Santoro et al. 2008; Davis and Garey 2018; Adyasari et al. 2020; see the section ‘Microbial processes within subterranean estuaries’). Archaea often comprise <1% of total prokaryotic sequences in the planktonic compartment (Unno et al. 2015; Adyasari et al. 2019) but they can reach higher abundances (5% up to 50%) under specific conditions in both groundwater (Sang et al. 2018; Adyasari et al. 2020) or sediments (Misseri et al. 2014; Hong et al. 2019). Euryarchaeota, Crenarchaeota, Thaumarchaeota and Ca. Woesearchaeota are generally the dominant archaeal phyla (Rogers and Casciotti 2010; Missimer et al. 2014; Unno et al. 2015; Davis and Garey 2018; Sang et al. 2018; Hong et al. 2019; Adyasari et al. 2020) but phyla such as Ca. Batharchaeota and Ca. Parvarchaeota (the latter belonging to the DPANN radiation) have also been observed (Chen et al. 2019b; Hong et al. 2019).

Temperature, salinity, pH, dissolved oxygen and nutrient concentrations, redox conditions and DOM quality have commonly been identified as potential environmental drivers of the observed compositional variations in prokaryotic communities from coastal aquifers (McAllister et al. 2015; Beck et al. 2017; Davis and Garey 2018; Jiao et al. 2018; Adyasari et al. 2019, 2020; Chen et al. 2019a; Hong et al. 2019; Jiang et al. 2020; Sola, Vargas-García and Vallejos 2020). Hydrological conditions and connectivity with surrounding ecosystems, although less commonly studied, also appear as relevant drivers of the distribution of communities in these transition zones. For example, changes in microbial abundances, activity or composition have been reported in coastal aquifers driven by dynamic changes in environmental gradients due to tides or waves (Santoro et al. 2008; McAllister et al. 2015), seasonal changes in groundwater level (Menning et al. 2018; Chen et al. 2020a) or varying degrees of connectivity with the surface or coastal seawater (Velasco Ayuso et al. 2009a,b, 2010, 2011; Adyasari et al. 2019).
The reported changes in microbial community structure cannot always be predicted from the mixing of freshwater and seawater, since the specific conditions in the mixing zone promote the establishment of unique and diverse populations of taxa that are rare elsewhere (Héry et al. 2014; Hong et al. 2019). This is in accordance with experimental studies showing that the mixing of freshwater and marine bacterial assemblages can promote the growth of initially rare taxa (Shen, Jürgens and Beier 2018; Rocca et al. 2020). As the activation of rare taxa can impact relevant ecosystem processes (Sjöstedt et al. 2012; Aanderud et al. 2015; Stegen et al. 2016), such hotspots of microbial specialists along subterranean estuaries could have strong implications in aquifer biogeochemistry. However, most of the microbial investigations in subterranean estuaries have been restricted to a single sampling time or have low spatial resolution (Table S1, Supporting Information), so we lack a comprehensive understanding of the hydrologic and environmental drivers of these communities over time and space.

**Microbial processes within subterranean estuaries**

Microbial activity in subterranean estuaries is mainly fueled by the degradation of organic matter or the oxidation of inorganic compounds supplied by fresh groundwater and/or infiltrated seawater (Seibert et al. 2020; Fig. 6). For heterotrophic microorganisms, the principal pathways of organic matter degradation vary depending on the availability of electron acceptors, and include aerobic respiration, denitrification, manganese reduction, iron reduction, sulfate reduction and methanogenesis (Canfield and Thamdrup 2009). These pathways usually occur in a predictable sequence along a groundwater flow path, which results in freshwater aquifers usually having a characteristic spatial zonation of redox conditions and active microbial populations (Hunter, Wang and Van Cappellen 1998). In coastal aquifers, this metabolic zonation may be much more dynamic given that the availability of organic matter, oxygen and other electron acceptors and donors can change over time due to the dynamic mixing of fresh and marine waters and the complex interaction of terrestrial and marine forces (Slomp and Van Cappellen 2004; McAllister et al. 2015; Robinson et al. 2018; Fig. 6).

Hence, once the available oxygen supplied by fresh groundwater or seawater is consumed by aerobic microorganisms, other electron acceptors that are differentially delivered by fresh- or marine water will be used for anaerobic respiration by denitrifiers, manganese- and iron-reducers, and finally by sulfate reducers and methanogens (McAllister et al. 2015; Beck et al. 2017; Hong et al. 2019; Montiel et al. 2019). In turn, the reduced products of these reactions can be oxidized by other microorganisms through processes such as anammox (anaerobic ammonium oxidation), nitrification, and iron, sulfur or methane oxidation, which are often coupled to CO₂ fixation by chemolithoautotrophs (Santoro, Boehm and Francis 2006; Santoro et al. 2008; McAllister et al. 2015; Fig. 6). Although accumulating evidence from inland aquifers suggests that chemolithoautotrophy can be more important than previously believed in subsurface ecosystems (Alfreider, Schimer and Vogt 2012; Kellermann et al. 2012; Herrmann et al. 2015; Hutchins et al. 2016; Jewell et al. 2016; Brandovits et al. 2017), the relative contribution of autotrophic metabolisms to total carbon cycling is currently unknown in subterranean estuaries.

The physicochemical conditions imposed by the mixing between fresh groundwater and marine seawater in the subterranean estuary lead to a dynamic and heterogeneous distribution of functional capacities and metabolic rates. Salinity, for example, is known to impose a strong filter for microbial communities, as large changes in microbial community composition are always found along salinity gradients (Lozupone and Knight 2005). In a comprehensive review on the microbial nitrogen cycling in subterranean estuaries, Santoro (2010) suggested that increases in salinity may favor metabolisms such as DNRA, sulfate reduction and carbon remineralization while decreasing denitrification, nitrification and methanogenesis. Both anammox and denitrification potential rates were shown to decrease with increasing salinity in low permeable intertidal sediments (Jiao et al. 2018). Also, higher rates of methanogenesis within subterranean estuaries have been observed in fresher or brackish portions than in sulfate-rich saltwater sites (Brandovits et al. 2018; Pain, Martin and Young 2019), given that methanogens may be outcompeted for chemical substrates by sulfate-reducers (Whiticar 1999).

Besides salinity, many other factors such as redox conditions can differentially affect the distribution of microbial functional groups. Slomp and Van Cappellen (2004) classified subterranean estuaries based on the redox state of the fresh and saline groundwater that mix in the coastal zone, which has been proposed as a useful framework for predicting biogeochemical cycles in different subterranean estuaries (Santoro 2010). However, reality is extremely much more complex because there can be overlap in redox zones (Canfield and Thamdrup 2009) and a large spatiotemporal heterogeneity in this metabolic zonation at the microscale; for instance, discrete microenvironments and microbial communities can be established within interstitial pores (Schmidt, Cuthbert and Schwientek 2017; Smith et al. 2018), and respiration processes can be spatially restricted to the fringes of contaminant plumes due to depletion of electron acceptors in the plume core (Mechenstok et al. 2015; Pilloni et al. 2019). In any case, in order to be able to predict the distribution of different microbial groups and their functions, we need to gain insight into their environmental tolerances, preferences and metabolic capacities.

**Dynamics of microbial functional groups in coastal aquifers**

The recent application of approaches such as metagenomics and metatranscriptomics, or stable isotope probing (SIP), has yielded much insight into the metabolic potential of groundwater microbial communities from inland aquifers (see the section ‘Improving the description of microbial diversity and function in coastal aquifers’). On the contrary, most recent studies on subterranean estuaries have exclusively focused on the taxonomic composition of communities (Table S1, Supporting Information), which does not provide information on their metabolisms or activity rates. Some of these studies have attempted to predict microbial function based on taxonomic identity (Table S1, Supporting Information), unveiling a high diversity and highly heterogeneous distributions of potential microbial metabolisms (Davis and Garey 2018; Hong et al. 2019; Adyasari et al. 2020) and highlighting the importance of freshwater–saltwater transition zones as potential hotspots of functional diversity (Santoro, Boehm and Francis 2006; Hong et al. 2019). Although these metabolic predictions may be inaccurate or biased because they depend on the sequenced genomes available (Sun, Jones and Fodor 2020), the identification of particular taxa with known metabolisms might provide useful clues into the processes that could be operating in a given area (Montiel et al. 2019).
Other studies in subterranean estuaries have directly tracked functional microbial groups using primer-based approaches such as clone libraries or quantitative PCR (qPCR). These have reported pronounced spatial and temporal variations in the abundance or diversity of genes such as the ammonia monooxygenase gene (amoA) involved in the aerobic oxidation of ammonia (Santoro et al. 2008; Rogers and Casciotti 2010; Beck et al. 2017), nitrate reductase encoding genes (nirK and nirS) involved in denitrification (Santoro, Boehm and Francis 2006; Jiao et al. 2018) or genes involved in sulfate reduction (aprA) (Beck et al. 2017) (Fig. 6; Table 1). Metabolic biomarkers such as ladderane phospholipids or specific 16S rRNA gene primers have also been used to quantify anammox bacteria in subterranean estuaries (Sáenz et al. 2012; Jiao et al. 2018) as well as respiratory quinone composition for distinguishing certain metabolisms such as aerobic methanotrophy (Brankovits et al. 2017).

Despite this accumulating knowledge, our understanding of the links between the taxonomic composition of a community and its biochemical outcome is still limited, in part because microbial studies in coastal aquifers have rarely coupled microbial information with actual metabolic rates (Table S1, Supporting Information). For example, the different responses of ammonia-oxidizing bacteria or ammonia-oxidizing archaea to salinity resulted in large shifts in the ratio between the two along a subterranean estuary (Santoro et al. 2008), but the implications of this taxonomic shift for the in situ nitrification rates remain unknown. Using stable nitrogen tracer (15NO3−) assays, decreases in anammox and denitrification rates were observed with increasing salinity, but these were not accompanied by decreases in the estimated abundances of the responsible microorganisms (Jiao et al. 2018). Once again, PCR primer limitations, presence of inactive taxa or different activity rates between taxonomically different groups may be among the reasons explaining this lack of coupling between microbial identity and function. In addition, there may be functional redundancy among groundwater taxa, which implies that a given process could be maintained even if the main microbial players are replaced (Pilloni et al. 2019). Given that sequencing...
Table 1. Major metabolic pathways and some of the relevant marker genes targeted in groundwater studies.

| Metabolic pathway                | Gene            | Protein                                                   | Groundwater studies |
|----------------------------------|-----------------|-----------------------------------------------------------|---------------------|
| **Aerobic respiration**          | cyoA            | Cytochrome bo(3) ubiquinol oxidase subunit 2               | a                   |
|                                  | cydA            | Cytochrome bd-l ubiquinol oxidase subunit 1                | a                   |
|                                  | ccoN            | Cytochrome c oxidase                                       | a,b                 |
|                                  | coxA            | Cytochrome c oxidase subunit I                            | a                   |
| **Autotrophic carbon fixation**  | cbbL            | Ribulose bisphosphate carboxylase large chain              | b,c,f               |
|                                  | cbbM            | Ribulose bisphosphate carboxylase                         | c,f                 |
|                                  | cdhA            | Acetyl-CoA decarboxylase/synthase complex subunit α        | b                   |
|                                  | cdhB            | Acetyl-CoA decarboxylase/synthase complex subunit ε        | b                   |
| **Nitrification**                | amoA            | Ammonium monoxygenase subunit A                           | a,d,e,i,j,k          |
|                                  | nxrA            | Nitrite oxidoreductase subunit α                           | a,i                 |
| **Anammox**                      | hzsA            | Hydrazine synthase subunit A                              | b                   |
|                                  | hzsB            | Hydrazine synthase subunit B                              | b                   |
|                                  | hzsC            | Hydrazine synthase subunit C                              | b                   |
|                                  | hzoA            | Hydrazine oxidoreductase A                                 | b                   |
|                                  | hzoB            | Hydrazine oxidoreductase B                                 | b                   |
| **Denitrification**              | nirK            | Copper-containing nitrite reductase                        | a,b,h,i,k            |
|                                  | nirS            | Nitrite reductase                                          | a,b,h,i             |
|                                  | nosZ            | Nitrous-oxide reductase                                    | a,b,i,k             |
|                                  | napA            | Periplasmic nitrate reductase                              | a,b,i               |
|                                  | napB            | Periplasmic nitrate reductase, electron transfer subunit   | a,b,i,k             |
|                                  | narG            | Respiratory nitrate reductase 1 α chain                    | a,b,i               |
|                                  | narH            | Respiratory nitrate reductase 1 β chain                    | a,b,i               |
|                                  | narI            | Respiratory nitrate reductase 1 γ chain                    | b,i                 |
| **DNRA**                         | nrfA            | Nitrite reductase (formate dependent)                      | i,k                 |
|                                  | nrfB            | Nitrite reductase (NADH-dependent) large subunit           | i                   |
|                                  | nrfD            | Nitrite reductase (NADH-dependent) small subunit           | i                   |
| **Nitrogen fixation**            | nifD            | Nitrogenase molybdenum-iron protein α chain                | a,i                 |
|                                  | nifH            | Nitrogenase iron protein                                   | a,i,k               |
|                                  | nifK            | Nitrogenase molybdenum-iron protein β chain                | a,i                 |
| **Iron oxidation**               | mtoA            | Decaheme c-type cytochrome                                | b                   |
|                                  | mtoB            | Decaheme-associated outer membrane protein                | b                   |
| **Iron/manganese oxidation/reduction** | mtrB       | Decaheme-associated outer membrane protein                | a                   |
| **Sulfur oxidation/reduction**   | aprA            | Dissimilatory adenosine-5′-phosphosulfate reductase        | a,b,e               |
|                                  | dgrA            | Sulfite reductase α subunit                                | a,b,k,l             |
|                                  | dgrB            | Sulfite reductase β subunit                                | a,b                 |
| **Methanotrophy (aerobic)**      | pmoA            | Membrane-bound particulate methane monoxygenase            | g,m                 |
|                                  | mmoX            | Soluble methane monoxygenase                              | g,k                 |
| **Methanotrophy (anaerobic)**    | mcrA            | Methyl-coenzyme M reductase α subunit                      | k,l,m               |

Letters indicate examples of groundwater studies where the functional marker genes shown in Fig. 6 have been identified (or predicted from taxonomy as in ‘i’, ‘k’ and ‘l’), plus some genes indicative of aerobic respiration and autotrophic carbon fixation. Studies performed in subterranean aquifers are indicated in bold (see also Table S1, Supporting Information), but examples of inland aquifer investigations where other marker genes have been targeted are also included. Anammox (anaerobic ammonium oxidation), DNRA (dissimilatory nitrate reduction to ammonium), RuBiCo (ribulose-1,5-bisphosphate carboxylase/oxygenase). (a) Lavy et al. 2019; (b) Jewell et al. 2016; (c) Herrmann et al. 2015; (d) Santoro et al. 2008; (e) Beck et al. 2017; (f) Kellerman et al. 2012; (g) Shao et al. 2019; (h) Santoro et al. 2006; (i) Adyasari et al. 2020; (j) Rogers and Casciotti 2010; (k) Hong et al. 2019; (l) Sang et al. 2018; (m) Vigoren et al. 2017.

Microbial control of SGD-derived chemical fluxes

Ultimately, microbial activity, together with other geochemical reactions occurring in the subterranean estuary (e.g. desorption, dissolution, precipitation), will determine the chemical composition of the groundwater flowing through coastal aquifers into the ocean (Moore 1999; Slomp and Van Cappellen 2004; Seibert et al. 2020; Fig. 6). Microbial activity in coastal sediments was shown to reduce significantly anthropogenic nitrate through denitrification and DNRA, acting as a natural filter, whereas techniques have replaced, rather than complemented, the use of traditional bulk activity assays such as estimates of active biomass, heterotrophic production or exoenzymatic activity (Table S1, Supporting Information), the gain in taxonomic resolution has been accompanied by a notable loss of information on the actual functioning of communities and its regulation. As noted by Smith et al. (2018), merging traditional activity measurements with novel sequencing technologies may provide the needed multifaceted view of these underexplored communities.
organic matter mineralization resulted in large amounts of ammonia and dissolved organic nitrogen to the ocean (Montiel et al. 2019). Other studies have reported that processes such as methanogenesis and methanotrophy are important sources and sinks of methane in subterranean estuaries, largely determining SGD methane fluxes to the ocean (Schutte et al. 2016; Brankovits et al. 2018; Pain, Martin and Young 2019), and that the transport of particulate marine matter through the seepage zone can fuel microbial dissolved organic carbon production, resulting in a net release of SGD-driven DOM (Jiang et al. 2020). The different seasonal patterns shown by denitrification, DNRA or anammox in a sandy subterranean estuary further illustrate that the multiple microbial processes involved in the cycling of a given element (e.g. nitrogen) may be differentially regulated (Wong et al. 2020). Consequently, only a deep understanding of these processes and their natural controls will enable an accurate understanding or prediction of the potential changes in SGD-derived fluxes upon variations in environmental conditions.

**Influence of SGD on marine microbial communities**

Since the awareness of the global relevance of SGD in coastal biogeochemical processes, multiple investigations have explored the effects of SGD on marine biota, including macro- and microorganisms (see Lecher and Mackey 2018 for a review). Microbial-related SGD studies have focused mostly on the responses of phytoplankton communities because of their key role as marine primary producers, and have shown that the nutrient inputs or salinity changes associated with SGD often result in variations in the abundances and composition of planktonic communities, promoting the growth of certain groups and sometimes causing eutrophication or harmful algal blooms (Lecher and Mackey 2018; Adolf et al. 2019; Taniguchi et al. 2019; Chen et al. 2020c). Despite the relevance of marine bacteria for the functioning of coastal biogeochemistry (Gasol et al. 2008), much less is known about marine bacterioplankton taxonomic and functional responses to SGD, as the few available studies have focused mostly on exploring changes in bulk abundances or activity of bacteria (Table S3, Supporting Information; Fig. 4). Photosynthetic unicellular cyanobacteria such as Synechococcus and Prochlorococcus are responsible for ~25% of the primary production globally (Kirchman 2012) and can be important primary producers also in coastal waters (Agawin et al. 2003; Scanlan et al. 2009). Experimental addition of groundwater to marine communities resulted in fast abundance increases of Synechococcus in the Mediterranean Sea (Garcés, Basterretxea and Tovar-Sánchez 2011), but not in other coastal settings (California and Hawaii) where other phytoplankton groups were favored (Chamberlain et al. 2014; Lecher et al. 2015). These findings agree with experiments reporting variable responses of coastal Synechococcus to different nutrient additions (Lekunberri et al. 2012), and highlight that microbial responses to SGD will depend largely on the chemical composition of groundwater and specific local and biotic conditions.

On the other hand, heterotrophic bacterioplankton largely determine the flow and fate of carbon in the ocean. Community-level processes such as bacterial respiration or biomass production thus have implications at the ecosystem level (del Giorgio and Williams 2005; Gasol et al. 2008), and appear to be affected by SGD (Carlson and Wiegner 2016). The recurrent observation that coastal bacterial respiration rates often exceed primary production indicates that bacteria rely on allochthonous (i.e. land-derived) dissolved organic carbon (Duarte, Agustí and Vaque 2004; Duarte and Prairie 2005), but to our knowledge no study has explored how much of this biologically used carbon derives from SGD.

Bacteria are also relevant competitors for inorganic nutrients, accounting for ~40% of total uptake of inorganic phosphorus, nitrogen and iron (Kirchman 2000). Bacterial ability to outcompete eukaryotic phytoplankton may vary depending on the type of inorganic nutrients and carbon added, and hence the chemical composition of the SGD may determine the biological fate of nutrients or carbon. For example, bacteria outcompeted diatoms in mesocosms with additions of phosphate and nitrate (with or without glucose), but if silicate was added, diatoms competed successfully with bacteria for the uptake of mineral nutrients (Havskum et al. 2003). In the study of García et al. (2011) only a moderate response in the total number of bacteria upon groundwater addition was reported after 3 days of incubation, probably caused by a better performance of phytoplankton upon the increased availability of silicate. However, marine bacteria are the fastest responders to nutrient inputs (e.g. Lekunberri et al. 2012), so SGD-driven effects on bacterial abundance or activity may be missed if not sampled at the adequate temporal resolution.

Bacterial consumption of DOM and inorganic nutrients depends on community composition because different bacterial taxa exhibit distinct metabolic capabilities and substrate preferences, and not all bacteria are equally active in carbon consumption, respiration or biomass production (Cottrell and Kirchman 2000; Alonso-Sáez and Gasol 2007; Ruiz-González et al. 2012; Sarmiento and Gasol 2012). In turn, bacterial community composition and activity are strongly shaped by environmental gradients and biological factors such as viral infection or bacterivory, with some bacterial groups being more susceptible to these mortality factors than others (Boavida and Wetzel 1998; Bouvier and del Giorgio 2007; Mojica and Brussaard 2014; Teira et al. 2019). Responses of heterotrophic bacteria to SGD may hence be either direct (if responding to nutrient or carbon inputs or changes in conditions such as salinity) but also indirect if SGD has an influence on other planktonic organisms such as phytoplankton, viruses or bacterial predators. As an example, large but transient increases in the abundance and activity of opportunistic marine bacterial groups such as Roseobacter were observed upon experimental groundwater addition, which peaked in the first 24 h and decreased quickly afterward likely due to intense predation (Maister 2018).

Depending on the type of discharge, microbial responses to SGD may differ. Continuous diffusive SGD (Fig. 1A) may create stable gradients of groundwater influence, leading to the establishment of a succession in microbial communities like those observed along surface estuarine plumes (Bouvier and del Giorgio 2002; Troussellier et al. 2002). Point-sourced SGD inputs (Fig. 1B) or discharge peaks following precipitation events (Kim et al. 2003; Mejías et al. 2012), conversely, might trigger localized or ephemeral pulses of intense growth of taxa that may disproportionately contribute to carbon cycling, e.g. by channeling large amounts of carbon toward higher trophic levels. However, groundwater is hardly ever included as a factor shaping bacterial communities in coastal waters, highlighting the lack of interaction between microbial oceanographers and terrestrial ecologists or hydrogeologists.

**SGD-driven transport of microbial diversity into the coastal ocean**

Given the high microbial diversity hidden in coastal aquifers and the magnitude of groundwater discharge at local and global scales, SGD may also influence coastal microbial communities
through the transport of groundwater prokaryotes. In freshwater ecosystems, the terrestrial–aquatic connectivity of microbial assemblages has emerged as an important driver of microbial community composition and biogeography at the landscape scale (Langenheder and Lindström 2019). For example, lake and river bacterial assemblages are strongly impacted by the dispersal of microorganisms from the surrounding terrestrial landscape or upstream water bodies, some of which seem able to grow and dominate the aquatic environment (Crump, Amaral-Zettler and Kling 2012; Ruiz-González, Niño-García and del Giorgio 2015a; Niño-García, Ruiz-González and del Giorgio 2016). This SGD-driven microbial connectivity has mostly been addressed with regard to the transport of terrestrial pathogenic or fecal indicator bacteria (FIB, i.e. enterococci and Escherichia coli) through coastal aquifers or sediments, which can have health implications (Yau et al. 2014; Vollberg et al. 2019). Although some studies concluded that SGD may not be a main source of FIB (Knee et al. 2008), others reported a clear link between FIB and the concentration of SGD tracers such as radium isotopes (Paytan, Boehm and Shellenbarger 2004), as well as the presence of FIB and human enteric viruses sourced by groundwater even 10 km offshore (Futch, Griffin and Lipp 2010). Interestingly, different FIB can be transported from different sources (e.g. E. coli from sand washing and enterococci from groundwater; Russell et al. 2013) and different types of sediments can differentially retain fecal indicator bacteria or viruses (de Sieyes et al. 2016). Hence, processes operating at the land–ocean transition zone may modify not only the chemical but also the microbial composition of SGD.

SGD discharging to the ocean is presumably also loaded with the diverse microbial communities naturally inhabiting the subterranean estuary or coastal sediments (see the section ‘Microbial abundance, diversity and environmental drivers along subterranean estuaries’). Several hydrogeological characteristics (e.g. groundwater velocity, type of aquifer and discharge, mechanisms driving the flow, degree of interaction between groundwater and seawater) may determine the identity of the aquifer microbes arriving to the ocean, as for example not all bacterial taxa are equally mobilized from intertidal sands by seawater (Boehm, Yamahara and Sassoubre 2014). To our knowledge, only two studies have suggested a potential role of SGD as conveyor of groundwater microorganisms from coastal aquifers into the marine environment: Lee et al. (2017) showed that both SGD flow and tidal fluctuation determined the periodic occurrence of freshwater-related bacteria in coastal bacterioplankton assemblages, and Menning et al. (2018) reported that changes in aquifer discharge controlled the microbial abundance and taxonomic richness in a spring-fed estuary.

It is still uncertain whether any of the groundwater or sediment prokaryotes transported via SGD can thrive in seawater, or whether marine taxa can survive within sandy sediments. However, there is experimental evidence that it is possible to retrieve living marine bacteria from sources such as freshwater, air or lake sediments (Comte et al. 2014; Langen-heder et al. 2016) and that mixing of fresh- and saline water promotes the growth of initially rare taxa (Shen, Jürgens and Beier 2018; Roccia et al. 2020). Also, active freshwater bacterial taxa were detected at the marine extreme of an estuarine plume (Troussellier et al. 2002). These findings suggest that coastal terrestrial and freshwater landscape components could represent reservoirs of microbial taxonomic and functional diversity for marine communities, with yet unknown implications for marine community structure and functioning. The fact that most coastal groundwater microbial research has been focused on single aquifers, rarely including the adjacent seawater and disregarding the land–ocean microbial connectivity (Table S1, Supporting Information), has precluded establishing microbial singularities or commonalities across coastal aquifers or determining the magnitude of microbial fluxes between the aquifers and the sea.

**FUTURE DIRECTIONS AND RESEARCH AVENUES**

Deepening our knowledge of the microbial dimension at the land–ocean interface will be beneficial from multiple points of view, ranging from the discovery of novel species and metabolisms, to achieving more accurate predictions of SGD fluxes and their consequences in marine biogeochemical cycles. Based on the needs of the field identified in the previous sections, here we propose five future research avenues and strategies that may help researchers advance toward a better understanding of the microbial dimension of SGD (Fig. 7).

**Improving the description of microbial diversity and function in coastal aquifers**

Given the high degree of taxonomic novelty found within single aquifers (Anantharaman et al. 2016), the study of the gene pools within both the active and the dormant fractions of communities from the undersampled coastal aquifers ensures the discovery of unimaginable living or surviving strategies, enriching current sequence catalogs (Fig. 7.1). PCR-free sequencing techniques like metagenomics, metatranscriptomics or single cell genomics (Appendix 2) have uncovered an enormous variety of novel taxa and metabolisms in inland aquifers that are not captured by current primer-based approaches (Jewell et al. 2016; Lau et al. 2016; Probst et al. 2017, 2018; Bell et al. 2018), so their application to coastal aquifers will offer key insight into the functional potential hidden in these systems. The ongoing accumulation of genomic information from inland aquifers will facilitate the application of these techniques to coastal aquifer microbiota by providing reference genomes and sequences. Moreover, metagenomic information may also help in the design of new PCR taxonomic or functional primers (e.g. Cornejo-Castillo 2017), which could be optimized for groundwater prokaryotic diversity. Applied to the ultrasmall fraction of groundwater communities (<0.2 μm), these approaches will shed light on the unknown role of the minute Ca. Patescibacteria or the DPANN Archaea in subterranean estuaries.

Techniques that allow linking metabolic processes and activity rates to specific microbial groups offer ways to improve our understanding of the role of different prokaryotic groups in the cycling of substrates in coastal aquifers. For example, SIP of DNA can identify microorganisms involved in the use of different compounds (labeled with stable isotopes) through the selective recovery of heavy isotope-enriched DNA: The microbial taxa involved in autotrophic fixation of CO₂ (Lazar et al. 2017) or in the degradation of plant-derived polysaccharides (Taubert et al. 2019) were identified in inland aquifers applying SIP. Also, SIP coupled to Raman spectroscopy (Raman-SIP) unveiled the groundwater bacterial populations activated by different plant-derived compounds using heavy water (D₂O) as a tracer (Taubert et al. 2018) and demonstrated complete denitrification by a groundwater microbial community adding ¹⁵NO₃⁻ (Kumar et al. 2018).

The relative contribution of different microbial taxa to substrate uptake can also be elucidated at the single-cell level...
Figure 7. Future research directions to gain insight into the microbial dimension of SGD. Future studies should (A) improve the characterization of the taxonomic and functional diversity (including the ultrasmall, sediment-attached and previously unknown taxa) within subterranean estuaries; (B) elucidate the main environmental and hydrologic drivers of prokaryotic communities and their functions, considering the high spatiotemporal heterogeneity of these systems and features such as dormancy or dispersal of allochthonous taxa that may obscure links between community taxonomic composition (indicated by the color of prokaryotes), community functioning and external factors; (C) explore the role of biotic interactions (e.g. viruses, predators or symbiotic associations) and their implications for microbial community functioning; (D) incorporate knowledge on microbial processes and their drivers to constrain chemical SGD fluxes and their potential variations upon changes in conditions; and (E) gain insight into the responses of microbial communities to SGD, particularly bacterioplankton, focusing on the responses of specific microbial taxa, cascading effects and the dispersal of microorganisms from groundwater to the ocean. DOM, dissolved organic matter; DIC, dissolved inorganic carbon.

through techniques such as microautoradiography (Brock 1967) or BONCAT (bioorthogonal non-canonical amino acid tagging) (Hatzenpichler et al. 2014), which combined fluorescence in situ hybridization techniques (FISH or CARD-FISH; Appendix 2) allow the visual identification of different active prokaryotic taxa. Microautoradiography has widely been used to quantify the uptake of different radiolabeled compounds by bacterial groups in freshwater and marine systems (Pérez and Sommaruga 2006; Ruiz-González et al. 2012), but only rarely in groundwater (Wülhartitz et al. 2009; Kellermann et al. 2012). More recently developed, BONCAT is based on the fluorescent labeling of the newly synthesized proteins. Combined with CARD-FISH or flow cytometry (Sebastián and Gasol 2019), it allows visually identifying active cells within specific taxonomic groups, making it a
promising tool for understanding the metabolic status of microbial inhabitants in coastal aquifers (Maister, 2018).

The visualization of microorganisms and their activity is actually becoming increasingly needed in an era dominated by sequencing technologies, as it provides essential information on microbial processes or features that cannot be derived from sequencing data (Sebastián and Gasol 2019). For example, size-fractionated filtration for DNA sequencing does not allow unequivocally determining whether a given bacterial taxon is actually small, as filters may clog and retain small cells. Indeed, we have found cells identified as ultrasmall Ca. Parcubacteria (see the section ‘Ultrasmall prokaryotic groups are abundant in groundwater ecosystems’) onto the 0.2-µm filter in a coastal alluvial aquifer (Fig. 8A and B), which, to our knowledge, represents the first observation of ultrasmall cells in coastal saline groundwater. The comparison with the large Gammaproteobacteria detected in another subterranean estuary (Fig. 8C and D) highlights the relevance of visualization for understanding the contribution of the different groundwater microbial groups to total biomass, for learning about their lifestyles (free-living, attached), or for deciphering their contribution to community activity (Fig. 8E; Sebastián and Gasol 2019).

Finally, the sampling of prokaryotes attached to surfaces should also be implemented in parallel to water sampling to achieve the full picture of the groundwater microbiota (Smith et al. 2018). Whereas characterizing the taxonomic composition of microbial communities in unconsolidated sediments is relatively easy if the sediment can be accessed, estimating the microbial biomass and activity associated with these surfaces remains challenging (Smith et al. 2018). Recent protocols for the direct quantification of prokaryotic abundance in groundwater sandy sediments (Bayer et al. 2016) and the combination of traditional activity assays with new sequencing techniques performed at scales relevant for microbial processes (Smith et al. 2018) open the door for the simultaneous assessment of the two microbial compartments.

Expanding knowledge of chemical and hydrologic drivers of microbial communities in subterranean estuaries

Most current information on groundwater microbial diversity (in particular that from coastal aquifers) comes from snapshot observations in specific locations (Table S1, Supporting Information), so we lack a comprehensive view of the large-scale spatial patterns and the temporal dynamics of the microbial communities in subterranean estuaries. Comparative studies covering large environmental and spatial gradients are needed to gain insight into the most important drivers of groundwater microbial communities. Experimental manipulations to promote or detect increases in the abundances of rare or dormant taxa (Rajala and Bomberg 2017; Hofmann and Griebler 2018; Fig. 5), sequencing the RNA instead of the DNA to target the potentially active community (Aanderud et al. 2016; Wisnoski et al. 2020), quantification of relic DNA (Carini et al. 2016; Lennon et al. 2018), or approaches that allow linking taxonomy with actual microbial activity (see the section ‘Improving the description of microbial diversity and function in coastal aquifers’) offer ways to learn about the metabolic status and role of different microbial taxa at the land–ocean transition zone.

Flow cytometry may also be used to readily characterize the physiological status of cells along the land–ocean transition zones, such as through the nucleic acid double-staining protocol (Gre’gori et al. 2001) that allows distinguishing dead or damaged cells from healthy cells in planktonic samples, or the detection of actively respiring cells stained with 5-cyano-2,3-ditolyl tetrazolium chloride (Sieracki, Cucci and Nicinski 1999). Distinguishing high versus low nucleic acid content cells (RNA vs LNA) may help estimate variations in ultrasmall planktonic cells along environmental gradients (Proctor et al. 2018).

Considering the bidirectional dispersal of groundwater and marine species is also critical, as it may impact aquifer functioning incoherent vital taxa in both directions, or mask relevant patterns by delivering significant amounts of inactive allochthonous taxa. Microbial source tracking methods, which allow assigning the most plausible habitat to a given taxon in order to infer potential dispersal sources (Comte et al. 2017; Ortiz-Alvarez et al. 2019) could allow defining connectivity pathways within aquifers and tracking the abundances of potentially inactive allochthonous taxa (e.g. Fillinger et al. 2021). The quantification of endospores as done in deep marine sediments (Wörmer et al. 2019) or of genes involved in dormancy (Lennon and Jones 2011) may further help assess the extent of dormancy and the factors governing activity in these dynamic environmental transitions. Importantly, for any of these microbiological investigations to yield accurate insight, they must be accompanied by detailed hydrogeological assessments of the relevant characteristics of the system, including the physical forces driving the flow, the dynamic movement of the fresh–saline interface, or the groundwater residence time in the subterranean estuary.

Elucidating the role of biotic interactions as drivers of microbial communities in coastal aquifers

Besides environmental or hydrologic factors, biotic interactions are emerging as key drivers of food webs and biogeochemical cycles in ecosystems such as the ocean (Lima-Méndez et al. 2015; Wilkins et al. 2019), but little is known about the interactions between indigenous microorganisms in the groundwater environment (Fig. 7.3). Some studies in inland aquifers have suggested that planktonic prokaryotes are more strongly controlled by viral lysis than by bacterivores (Wilhartitz et al. 2013), and that viruses can influence carbon biogeochemistry and prokaryotic community structure (Pan et al. 2014). Predation by flagellates was shown to be intense and size
Figure 8. Visualization is essential to gain insight into the ecology of different groundwater microbial groups. Examples of microscopy pictures showing the presence of ultrasmall Ca. Parcubacteria (A, B) or large Gammaproteobacteria (C–E) in two Mediterranean coastal aquifers in Spain [a limestone-karstified coastal aquifer (García-Solsona et al. 2010) and an alluvial aquifer (Folch et al. 2020), respectively]. Microscopic images show: (A, C) all microbial cells stained with 4',6-diamidino-2-phenylindole (DAPI); specific bacterial groups hybridized with the CARD-FISH probes OD1-289 (Gong et al. 2014) for Parcubacteria clades (B), and Gamma42a (Manz et al. 1992) for Gammaproteobacteria (D). (E) Translationally active cells (detected with BONCAT, see the section ‘Improving the description of microbial diversity and function in coastal aquifers’). Scale bars are 20 μm. Note the large difference in size between both groups, with obvious implications for their contributions to total biomass and activity, information that cannot be derived from sequencing approaches. Gammaproteobacteria (D) seem to be mostly concentrated on particles, as well as the majority of active cells (E).
selective in a contaminated aquifer (Kinne et al. 1998) and predatory bacteria (i.e. bacteria that feed on other bacteria) were isolated from a coastal alluvial aquifer (Banning, Casciotti and Kujawinski 2010), yet the relevance of this mechanism in shaping prokaryotic communities is largely unknown. Interestingly, experimental exposure to protozoan grazers caused changes in sediment-associated bacterial communities and increased the retention of $^{13}$C-labeled organic carbon in groundwater sediments (Longnecker and Kujawinski 2013). This suggests that these biotic interactions may impact not only the microbial structure of communities but also biogeochemical processes in both the planktonic and the sediment-attached compartments.

Recently, some multi-approach studies have started to shed light into the complexity of trophic interactions within subsurface ecosystems. Combining isotopic composition of food resources and consumers with information on food web structure, predator species diversity and organic matter, Hutchins et al. (2016) unveiled that chemolithoautotrophic organic matter production supported complex macroinvertebrate food webs and their species diversity in a groundwater system. Similarly, a combination of geochemical, genomic and biomarker approaches revealed that carbon from methanotrophic bacteria supported a large fraction of the diet of cave-adapted shrimps in a tropical subterranean estuary (Brankovits et al. 2017), highlighting the relevance of chemolithoautotrophic metabolisms for sustaining life in these coastal systems.

Microscopy may also help elucidate biological interactions within coastal groundwater microbial assemblages. Cryotransmission electron microscopy (cryo-TEM) was essential to determine that ultrasmall cells in an inland aquifer were metabolically active rather than starved and dormant, as they appeared infected by viruses or physically interacting with other bacterial cells, further indicating a potential symbiotic lifestyle (Luef et al. 2015; He et al. 2021). Also, using genomic information to design FISH probes, Schwank et al. (2019) uncovered a host–symbiont association between two uncultivated archaeal phyla in a deep aquifer. Due to the small genome of many of the novel phyla discovered in groundwater, it has been hypothesized that many groundwater groups, including members within Ca. Patescibacteria, may have a symbiotic lifestyle (Nelson and Stegen 2015; Youssef et al. 2015, but see Beam et al. 2020). This, coupled to the genomic evidence that groundwater microbial community members are intimately linked through the exchange of chemical substrates (Anantharaman et al. 2016; Lau et al. 2016), and that most groundwater biomass is in the form of biofilms where cells are in close contact to one another (Smith et al. 2018), suggests that symbiotic relationships (either through obligate or facultative syntrophy, mutualism or commensalism) might be essential in sustaining the microbiome and biogeochemical cycling in groundwater ecosystems. The study of biotic interactions within subterranean estuaries is, to our knowledge, a largely unexplored but promising field of research.

**Incorporating microbial processes to constrain and better estimate SGD fluxes**

Integrating the microbial perspective into SGD studies should improve our capacity to constrain the magnitude of SGD-driven solute fluxes and their ecological implications (Fig. 7.4). Accurate estimates of solute fluxes supplied by SGD (at local, regional and global scales) require understanding the chemical transformations occurring to groundwater before it reaches the sea, including the chemical cycling controlled by microbial communities. Knowledge of microbial ecology in the subterranean estuary thus needs to be coupled with estimates of biogeochemical and hydrogeological processes to unravel the role of subterranean estuaries in controlling solute fluxes to the ocean; this is actually one of the major knowledge gaps in the SGD field highlighted by recent reviews (Robinson et al. 2018; Taniguchi et al. 2019).

Most knowledge about element cycling in subterranean estuaries has been achieved by piecing together physicochemical snapshots obtained from profiles along cross-shore transects, and by comparing nutrient concentrations in the fresh and marine endmembers (e.g. Gonneea et al. 2014; Reckhardt et al. 2017). While these evaluations provide key information on the behavior of chemicals in the subterranean estuary, they do not always allow identifying the controlling mechanisms or predicting the potential consequences due to changes in the environment (because different processes may yield similar chemical snapshots). For example, coastal aquifers are especially sensitive to salinization (i.e. seawater intrusion) due to both sea level rise and excessive withdrawal of water for human purposes (Ferguson and Gleeson 2012; Werner et al. 2013; Retter, Karwautz and Griebler 2021) and hence the salinity tolerances of different microbial taxa will control how a given process respond to the expected salinity changes. Actually, seawater intrusion has been shown to impact the distribution and activity of microbial communities in coastal peat deposits, resulting in increases in the release of greenhouse gases (CH$_4$, CO$_2$) driven by SGD (Kreuzburg et al. 2020). Salinity increases might also enhance the delivery of NH$_4^+$ from groundwater to the coast due to increased N mineralization and decreased nitrification (Santoro 2010). Investigations in anhialine systems (i.e. coastal sinkholes with a subterranean connection with the ocean; see Fig. 1B) might shed light on some of the processes and the drivers operating in coastal underground ecosystems; their higher accessibility may allow a coverage of the spatial heterogeneity in environmental gradients and/or microbial dynamics that is not feasible in most subterranean estuaries (e.g. Seymour et al. 2007; González et al. 2011) or the sampling and functional exploration of surface-attached microbial communities (Haas et al. 2018).

In turn, prospection of subsurface microbial genes and metabolisms might help counteract our impact on groundwater by means of microbiologically mediated management practices, like for example the degradation of pollutants (Posman, DeRito and Madsen 2017) or those enhancing nitrogen removal from aquifers (Ludington et al. 2017). Denitrification has long been considered as the main process removing nitrogen from coastal aquifers, but other less studied metabolisms performed by largely unknown microbial players, e.g. DNRA and anammox, seem to be important pathways of nitrogen loss in aquifers globally (Wang et al. 2020). In any case, it will not be possible to accurately interpret microbial data without a good knowledge of the system (geological setting, geochemistry, hydrology). Attempts to join different approaches such as nutrient mass-balances, stable isotopic signatures of water and sediments, information on DOM composition, redox conditions, water residence time and microbial communities have provided useful insight into the different sources of chemical elements, the complexity of subsurface biogeochemical transformations and resulting SGD-driven solute fluxes (Beck et al. 2017; Montiel et al. 2019). Therefore, only through multidisciplinary efforts we may achieve a holistic view of the biotic interactions and biogeochemical processes within coastal aquifers.
Deepening our understanding of SGD effects on marine food webs and biogeochemical cycles

Studies exploring the effects of SGD on the microbial components at the base of food webs (including bacterioplankton) will provide useful insight into the ecological and biogeochemical consequences of SGD in the marine environment, improving our understanding of the microbial regulation of nutrient, carbon and metal cycles in the coastal ocean (Fig. 7.5). As the element fluxes differ largely depending on the characteristics of the aquifer (e.g. natural and anthropogenic inputs into groundwater, geologic characteristics and type of discharge, water flow velocity; Tovar-Sánchez et al. 2014), an accurate understanding of the microbial responses to SGD necessarily requires detailed evaluations of the magnitude of SGD-driven chemical fluxes.

Coupling microbial dynamics with estimations of SGD-driven solute fluxes derived from chemical tracers (e.g. radium isotopes, radon, methane, silica; Dulaiova et al. 2010; Rodellas et al. 2017; Oehler et al. 2019) or the application of mixing models along salinity gradients (as in Stegen et al. 2016) may be useful to identify and quantify localized microbial responses to SGD. Experimentation could allow differentiating between direct SGD effects on microbial communities (through the acquisition of SGD solutes or changes in the environment), or indirect cascade effects such as bacterial responses to SGD-driven changes in phytoplankton production of DOM or grazer or viral activity. Isotopic fractionation may provide valuable insight on the transfer of nutrients or carbon from groundwater into different marine microbial compartments (e.g. primary producers; Andrisoa et al. 2019), allowing to estimate the fate of the delivered elements in coastal ecosystems. The degree of allochthony in aquatic bacteria (i.e. the contribution of terrestrial dissolved organic carbon to aquatic bacterial biomass) has been extensively studied in freshwater systems (e.g. Guillemette, McCaIlistier and del Giorgio 2016), yet to our knowledge this has never been explored in coastal bacterial communities influenced by SGD. Finally, microbial transformations controlling dissolved organic matter (DOM) quality within coastal aquifers (Chailou, Lemay-Borduas and Couturier 2016; Couturier, Nozais and Chailou 2016; Jiang et al. 2020) may in turn determine the taxonomic or functional responses of planktonic marine bacterioplankton influenced by SGD, given that DOM quality is a main driver of aquatic bacterioplankton structure and functioning (Landa et al. 2014; Ruiz-González et al. 2015b). Applying the above-mentioned techniques to link taxonomy with function (see the section ‘Improving the description of microbial diversity and function in coastal aquifers’) along natural groundwater gradients or upon experimental groundwater additions will help identify specific responses of different marine bacterial groups to SGD.

On the other hand, the influence of the dispersal of groundwater microbial communities on coastal ecosystems could be resolved by means of experimental assessments of the capacity of groundwater microorganisms to thrive in marine conditions (e.g. mixing water types; Maister 2018) or through transplant experiments (Shen, Jürgens and Beier 2018), or by tracking the presence of groundwater taxa in marine sites. Microbial source tracking methods (see the section ‘Expanding knowledge of chemical and hydrologic drivers of microbial communities in subterranean estuaries’) have been applied to identify the origin of fecal bacteria in the surf zone (Russell et al. 2013) and could be used to detect groundwater taxa in coastal areas (or vice versa) if simultaneously characterizing coastal aquifers and marine sites (Unno et al. 2015; Adyasarri et al. 2019). Specifically, the SGD-driven transport of taxa able to thrive in coastal seawater might represent a diversity pathway not previously considered, with potential implications for marine community structure and functioning. Finally, whether viruses, bacteria or protists delivered with coastal groundwater can directly interact with marine bacterioplankton communities remains completely unknown.

Groundwater taxa arriving to the sea may be used as microbial ‘indicators’ or tracers of groundwater inputs to the coastal zone. Recent studies have used microbial information to identify tidal or temporal variations of SGD (Lee et al. 2017), to distinguish groundwater recharge sources to shallow wells (Higgins et al. 2020), to uncover sporadic episodes of intense groundwater influence (Kim et al. 2020) or to detect contamination episodes, given that specific taxa can be used as biosensors even after a given pollutant has been fully degraded (Smith et al. 2015). Similarly, the presence of marine microbial taxa in coastal aquifers can indicate active land–ocean connectivity, and bacterial taxa within Oceanospirillales and Alteromonadaceae (Chen et al. 2019a) or within Rhodobacteraceae and Flavobacteriaceae (Unno et al. 2015) have been proposed as potential indicators of seawater intrusions in coastal aquifers. The identification of pathogens or fecal bacteria may further allow the detection of wastewater contamination in coastal aquifers or in the adjacent seawater (Boehm, Shellenbarger and Paytan 2004; Yau et al. 2014; Ahmed et al. 2019). As many of these ‘indicators’ may not survive in marine systems, their detection will refine our capacity to distinguish between allochthonous and truly marine taxa.

CONCLUDING REMARKS

The submarine discharge of groundwater to the coastal ocean constitutes a terrestrial and marine linkage of high relevance for the functioning of marine ecosystems, which is modulated to a large extent by the microbial processes occurring in the coastal aquifer. Despite the growing interest on groundwater microbiology in recent years, coastal aquifers have received little attention from microbial studies in comparison to surface waters. In turn, SGD research has been historically dominated by studies performed from either a ‘chemical oceanography’ or a ‘hydrological’ point of view, lacking a biological understanding of the underlying processes.

Here, we have identified some of the current challenges and future directions that should be tackled for a deeper understanding of the microbial dimension underlying SGD processes. These include improving the characterization of groundwater microbiota by overcoming current technical limitations and considering the specific characteristics of coastal aquifers (i.e. dynamic mixing of water, chemicals and communities). In turn, the evaluation of solute fluxes (e.g. nutrients, metals, carbon, contaminants) supplied by SGD needs to rely on an accurate understanding of the microbiologically mediated chemical cycling in the coastal aquifer, requiring an improved knowledge on their drivers and metabolic capacities and responses. Finally, coastal marine microbial ecology needs to incorporate the connectivity with the continent, and in particular this largely overlooked groundwater linkage, good knowledge of the hydrogeology and the chemical and microbial fluxes from the coast to the ocean will expand our understanding of the structuring and function of coastal planktonic communities, as well as the ecological consequences of SGD. Only by moving beyond the presumed limits of ecosystems and research fields (Xenopoulos et al. 2017; Kayler et al. 2019), we may obtain a comprehensive understanding of the interlinked physical and biogeochemical processes occurring throughout the terrestrial–marine continuum.
ACKNOWLEDGMENTS

Particular thanks to Marta Sebastián and Andrea G. Bravo for their useful and constructive comments on the manuscript, and to Josep M. Gasol, Olena Maister, Marc Diego-Feliu and Aaron Alorda-Kleinglass for their help during some of the first exploratory field surveys. We acknowledge support of the publication fee by the CSIC Open Access Publication Support Initiative through its Unit of Information Resources for Research (URICI).

SUPPLEMENTARY DATA

Supplementary data are available at FEMSRE online.

FUNDING

This work was funded by the Spanish Ministry of Science, Innovation and Universities (MICINN) through the GRAMMI project RTI2018-099740-J-I00 and partly by the projects MEDIS-TRAES II (CGL2016-77122-C2) and OPAL (PID2019-110311RB-C21). Additional financial support was provided by grants SPIP2020-02595 (Ministry for the Ecological Transition and the Demographic Challenge (MITECO)) and 2017SGR/156 (Generalitat de Catalunya), with funding from the Spanish government through the 'Severo Ochoa Centre of Excellence' accreditation (CEX2019-000928-S). VR acknowledges financial support from the Beatriz Program of the Catalan Government (2017-BP-00334). This study contributes to the work carried out by the MERS research group 2017 SGR 1588.

Competing Interests. The authors declare no competing financial interests.

REFERENCES

Aanderud ZT, Jones SE, Fierer N et al. Resuscitation of the rare biosphere contributes to pulses of ecosystem activity. Front Microbiol 2015;6:24.

Aanderud ZT, Vert JC, Lennon JT et al. Bacterial dormancy is more prevalent in freshwater than hypersaline lakes. Front Microbiol 2016;7:853.

Acinas SG, Sánchez P, Salazar G et al. Metabolic architecture of the deep ocean microbiome. bioRxiv 2019;635680.

Adolf JE, Burns J, Walker JK et al. Near shore distributions of phytoplankton and bacteria in relation to submarine groundwater discharge-fed fishponds, Kona coast, Hawai‘i, USA. Estuar Coast Shelf Sci 2019;219:341–53.

Adyasari D, Hasenrühr C, Montiel D et al. Microbial community composition across a coastal hydrological system affected by submarine groundwater discharge (SGD). PloS One 2020;15:e0235235.

Adyasari D, Hasenrühr C, Oehler T et al. Microbial community structure associated with submarine groundwater discharge in northern Java (Indonesia). Sci Total Environ 2019;689:590–601.

Agawin NSR, Duarte CM, Agusti S et al. Abundance, biomass and growth rates of Synechococcus sp. in a tropical coastal ecosystem (Philippines, South China Sea). Estuar Coast Shelf Sci 2003;56:493–502.

Ahmed W, Hamilton K, Toze S et al. A review on microbial contaminants in stormwater runoff and outfalls: potential health risks and mitigation strategies. Sci Total Environ 2019;692:1304–21.

Alfreider A, Schimer M, Vogt C. Diversity and expression of different forms of RubisCO genes in polluted groundwater under different redox conditions. FEMS Microbiol Ecol 2012;79:649–60.

Alonso-Sáez L, Gasol JM. Seasonal variations in the contributions of different bacterial groups to the uptake of low-molecular-weight compounds in northwestern Mediterranean coastal waters. Appl Environ Microbiol 2007;73:3528–35.

Anantharaman K, Brown CT, Hug LA et al. Thousands of microbial genomes shed light on interconnected biogeochemical processes in an aquifer system. Nat Commun 2016;7:13219.

Andrisoa A, Stiegelz TC, Rodellas V et al. Primary production in coastal lagoons supported by groundwater discharge and porewater fluxes inferred from nitrogen and carbon isotope signatures. Mar Chem 2019;210:48–60.

Banning EC, Casciotti KL, Kujawinski EB. Novel strains isolated from a coastal aquifer suggest a predilection for flavobacteria. FEMS Microbiol Ecol 2010;73:254–70.

Barragán JM, de Andrés M. Analysis and trends of the world’s coastal cities and agglomerations. Ocean Coast Manag 2015;114:11–20.

Bayer A, Drexel R, Weber N et al. Quantification of aquatic sediment prokaryotes: a multiple-steps optimization testing sands from pristine and contaminated aquifers. Limnologia 2016;56:6–13.

Beam JP, Bercraft ED, Brown JM et al. Ancestral absence of electron transport chains in Patescibacteria and DPANN. Front Microbiol 2020;11:1848.

Beck M, Reckhardt A, Amelberg J et al. The drivers of biogeochemistry in beach ecosystems: a cross-shore transect from the dunes to the low-water line. Mar Chem 2017;190:35–50.

Bell E, Lamminmäki T, Alneberg J et al. Biogeochemical cycling by a low-diversity microbial community in deep groundwater. Front Microbiol 2018;9:2129.

Boavida G, Wetzel RG. Inhibition of phosphatase activity by dissolved humic substances and hydrolytic reactivation by natural UV. Freshw Biol 1998;40:285–93.

Boehm AB, Shellenbarger GG, Paytan A. Groundwater discharge: potential association with fecal indicator bacteria in the surf zone. Environ Sci Technol 2004;38:3558–66.

Boehm AB, Yamahara KM, Sassoubre LM. Diversity and transport of microorganisms in intertidal sands of the California Coast. Appl Environ Microbiol 2014;80:3943–51.

Bouvier T, del Giorgio PA. Key role of selective viral-induced mortality in determining marine bacterial community composition. Limnol Oceanogr 2002;47:453–70.

Bouvier T, del Giorgio PA. Key role of selective viral-induced mortality in determining marine bacterial community composition. Limnol Oceanogr 2007;9:287–97.

Bradley JA, Amend JP, LaRowe DE. Survival of the fewest: microbial dormancy and maintenance in marine sediments through deep time. Geobiology 2019;17:43–59.

Brankovits D, Pohlman JW, Niemann H et al. Hydrologic controls of methane dynamics in karst subterranean estuaries. Glob Biogeochem Cycles 2018;32:1759–75.

Brankovits D, Pohlman JW, Niemann H et al. Methane- and dissolved organic carbon-fueled microbial loop supports a tropical subterranean estuary ecosystem. Nat Commun 2017;8:1835.

Brewer TE, Aronson EL, Arogyswamy K et al. Ecological and genomic attributes of novel bacterial taxa that thrive in sub-surface soil horizons. mBio 2019;10:e01318–9.
Brock TD. Bacterial growth rate in the sea—analysis by thymidine autoradiography. Science 1967;155:81–3.

Brown CT, Hug LA, Sharon TI et al. Unusual biology across a group comprising more than 15% of domain Bacteria. Nature 2015;523:208–11.

Burnett WC, Aggarwal PK, Aureli A et al. Quantifying submarine groundwater discharge in the coastal zone via multiple methods. Sci Total Environ 2006;367:498–543.

Burnett WC, Bokuniewicz M, Huettel WS et al. Groundwater and pore water inputs to the coastal zone. Biogeochemistry 2003;63:3–33.

Canfield DE, Thamdrup B. Towards a consistent classification scheme for geochemical environments, or, why we wish the term ‘suboxic’ would go away. Geobiology 2009;7:385–92.

Carini P, Marsden PJ, Leff JW et al. Relic DNA is abundant in soil and obscured estimates of soil microbial diversity. Nat Microbiol 2016;2:16242.

Carlson K, Wiegener T. Effects of submarine groundwater discharge on bacterial growth efficiency in coastal Hawaiian waters. Aquat Microb Ecol 2016;77:167–81.

Castelle CJ, Brown CT, Anantharaman K et al. Biosynthetic capacity, metabolic variety and unusual biology in the CPR and DPANN radiations. Nat Rev Microbiol 2018;16:629–45.

Chaillou G, Lemay-Borduas F, Couturier M. Transport and transformation of groundwater-borne carbon discharging through a sandy beach to a coastal ocean. Can Water Resour J 2016;41:455–68.

Chamberlain SD, Kaplan KA, Modanu M et al. Biogeography of planktonic and benthic cyanobacteria in coastal waters of the Big Island, Hawai‘i. FEMS Microbiol Ecol 2014;89:80–8.

Chen L, Hu BX, Dai H et al. Characterizing microbial diversity and community composition of groundwater in a salt–freshwater transition zone. Sci Total Environ 2019a;678:574–84.

Chen L, Zhang J, Dai H et al. Comparison of the groundwater microbial community in a salt-freshwater mixing zone during the dry and wet seasons. J Environ Manage 2020a;271:110969.

Chen X, Cukrov N, Santos JR et al. Karstic submarine groundwater discharge into the Mediterranean: radon-based nutrient fluxes in an anchialine cave and a basin-wide upscaling. Geochim Cosmochim Acta 2020c;268:467–84.

Chen X, Ye Q, Du J et al. Bacterial and archaeal assemblages from two size fractions in submarine groundwater near an industrial zone. Water 2019b;11:1261.

Chen X, Ye Q, Sanders CJ et al. Bacterial-derived nutrient and carbon source-sink behaviors in a sandy beach subterranean estuary. Mar Pollut Bull 2020b;160:111570.

Cho H-M, Kwon KG, Moosdorff EY et al. Radon tracing nutrient inputs through submarine groundwater discharge in the global ocean. Sci Rep 2018;8:2439.

Comte J, Berga M, Severin I et al. Contribution of different bacterial dispersal sources to lakes: population and community effects in different seasons. Environ Microbiol 2017;19:2391–404.

Comte J, Lindström ES, Eller A et al. Can marine bacteria be recruited from freshwater sources and the air? ISME J 2014;8:2423–30.

Cornejo-Castillo FM (2017). Diversity, Ecology and Evolution of Marine Diazotrophic Microorganisms. Universidad Politécnica de Cataluña. Ph.D. Thesis, Dec 2017 (https://digital.csic.es/handle/10261/164042).

Cottrell MT, Kirchman DL. Natural assemblages of marine proteobacteria and members of the Cytophaga–Flavobacter cluster consuming low- and high-molecular-weight dissolved organic matter. Appl Environ Microbiol 2000;66:1692–7.

Couturier M, Nozais C, Chaillou G. Microbial subterranean estuaries as a source of fresh terrestrial dissolved organic matter to the coastal ocean. Mar Chem 2016;186:46–57.

Crump BC, Amaral-Zettler LA, Kling GW. Microbial diversity in arctic freshwaters is structured by inoculation of microbes from soils. ISME J 2012;6:1629–39.

Davis M, Garey J. Microbial function and hydrochemistry within a stratified anchialine sinkhole: a window into coastal aquifer interactions. Water 2018;10:972.

del Giorgio PA, Williams PF. The global significance of respiration in aquatic ecosystems: from single cells to the biosphere. In: del Giorgio PA, Williams PF (eds). Respiration in Aquatic Ecosystems. New York: Oxford University Press, 2005, 267–303.

de Sieyes NR, Russell TL, Brown KL et al. Transport of enterococci and F+ colipage through the saturated zone of the beach aquifer. J Water Health 2016;14:26–38.

Duarte CM, Agustí S, Vaque D. Controls on planktonic metabolism in the Bay of Blanes, northwestern Mediterranean littoral. Limnol Oceanogr 2004;49:2162–70.

Duarte CM, Prairie YT. Prevalence of heterotrophy and atmospheric CO2 emissions from aquatic ecosystems. Ecosystems 2005;8:862–70.

Dulaiova H, Camilli R, Henderson PB et al. Coupled radon, methane and nitrate sensors for large-scale assessment of groundwater discharge and non-point source pollution to coastal waters. J Environ Radioact 2010;101:553–63.

Duque C, Michael HA, Wilson AM. The subterranean estuary: technical term, simple analogy, or source of confusion? Water Resour Res 2020;56:e2019WR026554.

Ferguson G, Gleeson T. Vulnerability of coastal aquifers to groundwater use and climate change. Nat Clim Chang 2012;2:342–5.

Fillinger L, Hug K, Griebler C. Aquifer recharge viewed through the lens of microbial community ecology: initial disturbance response, and impacts of species sorting versus mass effects on microbial community assembly in groundwater during riverbank filtration. Water Res 2021;189:116631.

Fillinger L, Hug K, Griebler C. Selection imposed by local environmental conditions drives differences in microbial community composition across geographically distinct groundwater aquifers. FEMS Microbiol Ecol 2019a;95:ffz160.

Fillinger L, Zhou Y, Kellerman C et al. Non-random processes determine the colonization of groundwater sediments by microbial communities in a pristine porous aquifer. Environ Microbiol 2019b;21:327–42.

Flynn TM, Sanford RA, Ryu H et al. Functional microbial diversity explains groundwater chemistry in a pristine aquifer. BMC Microbiol 2013;13:146.

Folch A, del Val L, Luquot L et al. Combining Fiber Optic (FO-DTS), cross-hole ERT and time-lapse formation electrical conductivity to characterize and monitor a coastal aquifer. J Hydrol 2020;588:125050.

Freeze RA, Cherry JA. Groundwater. Englewood Cliffs, N.J.: Prentice-Hall, 1979.

Futch JC, Griffin DW, Lipp EK. Human enteric viruses in groundwater indicate offshore transport of human sewage to coral reefs of the Upper Florida Keys. Environ Microbiol 2010;12:964–74.
et al. Langenheder S, Lindström ES. Factors influencing aquatic and terrestrial bacterial communities across a hillslope–riparian transect shaped by proximity to the stream, groundwater table, and weathered bedrock. Ecol Evol 2019;9:6869–900.

Lazar CS, Lehmann R, Stoll W et al. The endolithic bacterial diversity of shallow bedrock ecosystems. Sci Total Environ 2019;679:35–44.

Lazar CS, Stoll W, Lehmann R et al. Archaeal diversity and CO2 fixers in carbonate–siliciclastic-rock groundwater ecosystems. Archaea 2017;2017:2136287.

Lecher AL, Mackey K, Kudela R et al. Nutrient loading through submarine groundwater discharge and phytoplankton growth in Monterey bay, CA. Environ Sci Technol 2015;49:6665–73.

Lecher AL, Mackey KRM. Synthesizing the effects of submarine groundwater discharge on marine biota. Hydrology 2018;5:60.

Lee E, Shin D, Hyun SP et al. Periodic change in coastal microbial community structure associated with submarine groundwater discharge and tidal fluctuation. Limnol Oceanogr 2017;62:437–51.

Lehman RM. Understanding of aquifer microbiology is tightly linked to sampling approaches. Geomicrobiol J 2007;24:331–41.

Lekunberri I, Lefort T, Romera-Castillo C et al. Relationship between induced phytoplankton blooms and the structure and dynamics of the free-living heterotrophic bacterial community. Mar Ecol Ser 2012;448:23–U58.

Lennon JT, Jones SE. Microbial seed banks: the ecological and evolutionary implications of dormancy. Nat Rev Microbiol 2011;119:119–30.

Lennon JT, Muscarella ME, Placella SA et al. How, when and where relic DNA affects microbial diversity. mBio 2018;9:e00637–18.

Lima-Médé an G, Faust K, Henry N et al. Determinants of community structure in the global plankton interactome. Science 2015;348:126073.

Logares R, Haverkamp THA, Kumar S et al. Environmental microbiology through the lens of high-throughput DNA sequencing: synopsis of current platforms and bioinformatics approaches. J Microbiol Methods 2012;91:106–13.

Longnecker K, Kujawinski EB. Using stable isotope probing to characterize differences between free-living and sediment-associated microorganisms in the subsurface. Geomicrobiol J 2013;30:362–70.

Lopezone CA, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 2005;71:8228–35.

Ludington WB, Seher TD, Applegate O et al. Assessing biosynthetic potential of agricultural groundwater through metagenomic sequencing: a diverse anammox community dominates nitrate-rich groundwater. PLoS One 2017;12:e0174930.

Luef B, Frischkorn KR, Wrighton KC et al. Diverse uncultivated ultra-small bacterial cells in groundwater. Nat Commun 2015;6:6372.

Luijendijk E, Gleeson T, Moosdorf N. Fresh groundwater discharge insignificant for the world’s oceans but important for coastal ecosystems. Nat Commun 2020;11:1260.

Magnabosco C, Lin L-H, Dong H et al. The biomass and biodiversity of the continental subsurface. Nat Geosci 2018;11:707–17.

Maister O. Effects of Submarine Groundwater Discharge on Coastal Bacterioplankton Communities from the NW Mediterranean. M.Sc.
Miyoshi T, Iwatsuki T, Naganuma T. Phylogenetic oligodeoxynucleotide probes for the major subclasses of Proteobacteria: problems and solutions. Syst Appl Microbiol 1992;15:593–600.

Mayfield KK, Eisenhauer A, Santiago Ramos DP et al. Groundwater discharge impacts marine isotope budgets of Li, Mg, Ca, Sr, and Ba. Nat Commun 2012;12:148.

McAllister SM, Barnett JM, Heiss JW et al. Dynamic hydrologic and biogeochemical processes drive microbially enhanced iron and sulfur cycling within the intertidal mixing zone of a beach aquifer. Limnol Oceanogr 2015;60:329–45.

McClain ME, Boyer EW, Dent CL et al. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 2003;6:301–12.

Meckenstock RU, Elsner M, Griebler C et al. Biodegradation: updating the concepts of control for microbial cleanup in contaminated aquifers. Environ Sci Technol 2015;49:7073–81.

Meier A, Singh MK, Kastner A et al. Microbial communities in carbonate rocks-from soil via groundwater to rocks. J Basic Microbiol 2017;57:752–61.

Mejías M, Ballesteros BJ, Antón-Pacheco C et al. Methodological study of submarine groundwater discharge from a karstic aquifer in the Western Mediterranean Sea. J Hydrol 2012;464:527–40.

Menning DM, Carraher-Stross WA, Graham ED et al. Aquifer discharge drives microbial community change in karst estuaries. Estuaries Coast 2018;41:430–43.

Michael HA, Post VEA, Wilson AM et al. Science, society, and the coastal groundwater squeeze. Sci Soc Coast Groundw Squeeze 2017;53:2610–7.

Missimer TM, Hoppe-Jones C, Jadoon KZ et al. Hydrogeology, water quality, and microbial assessment of a coastal alluvial aquifer in western Saudi Arabia: potential use of coastal wadi aquifers for desalination water supplies. Hydrogeol J 2014;22:1921–34.

Miyoshi T, Iwatsuki T, Naganuma T. Phylogenetic characterization of 16S rRNA gene clones from deep-groundwater microorganisms that pass through 0.2-micrometer-pore-size filters. Appl Environ Microbiol 2005;71:1084–8.

Mojica KDA, Brussaard CPD. Factors affecting virus dynamics and microbial host-virus interactions in marine environments. FEMS Microbiol Ecol 2014;89:495–515.

Montiel D, Lamore AF, Stewart C et al. Natural groundwater nutrient fluxes exceed anthropogenic inputs in an ecologically impacted estuary: lessons learned from Mobile Bay, Alabama 2019;145:1–33.

Moore WS. The effect of submarine groundwater discharge on the ocean. Ann Rev Mar Sci 2010;2:59–88.

Moore WS. The subterranean estuary: a reaction zone of ground water and sea water. Mar Chem 1999;65:111–25.

Nakai R. Size matters: ultra-small and filterable microorganisms in the environment. Microbes Environ 2020;35:n/a.

Nelson WC, Stegen JC. The reduced genomes of Parcubacteria (OD1) contain signatures of a symbiotic lifestyle. Front Microbiol 2015;6:713.

Niño-García JP, Ruiz-González C, del Giorgio PA. Landscape-scale spatial abundance distributions discriminate core from random components of boreal lake bacterioplankton. Ecol Lett 2016;19:1506–15.

Nowak ME, Schwab VF, Lazar CS et al. Carbon isotopes of dissolved inorganic carbon reflect utilization of different carbon sources by microbial communities in two limestone aquifer assemblages. Hydrol Earth Syst Sc 2017;21:4283–300.

Oehler T, Tamborski J, Rahman S et al. DSI as a tracer for submarine groundwater discharge. Front Mar Sci 2019;6:563.

Ortiz-Alvarez R, Cáiz J, Camarero I et al. Regional community assembly drivers and microbial environmental sources shaping bacterioplankton in an alpine lacustrine district (Pyrenees, Spain). Environ Microbiol 2019;22:297–309.

Pain AJ, Martin JB, Young CR. Sources and sinks of CO2 and CH4 in silicilastic subterranean estuaries. Limnol Oceanogr 2019;64:1500–14.

Pan D, Watson R, Wang D et al. Correlation between viral production and carbon mineralization under nitrate-reducing conditions in aquifer sediment. ISME J 2014;8:1691–703.

Paytan A, Boehm AB, Shellenbarger GG. Bacterial contamination and submarine groundwater discharge: a possible link. Environ Chem 2004;1:1–2.

Pilloni G, Bayer A, Ruth-Anneser B et al. Dynamics of hydrology and anaerobic hydrocarbon degrader communities in a tar-contaminated aquifer. Microorganisms 2019;7:46.

Posman KM, DeRito CM, Madsen EL. Benzene degradation by a Varioruca species within a coal tar-contaminated groundwater microbial community. Appl Environ Microbiol 2017;83:e02658–16.

Probst AJ, Castelle CJ, Singh A et al. Genomic resolution of a cold subsurface aquifer community provides metabolic insights for novel microbes adapted to high CO2 concentrations. Environ Microbiol 2019;17:459–74.

Probst AJ, Ladd B, Jarett JK et al. Differential depth distribution of microbial function and putative symbionts through sediment-hosted aquifers in the deep terrestrial subsurface. Nat Microbiol 2018;3:328–36.

Proctor CR, Besmer MD, Langenegger T et al. Phylogenetic clustering of small low nucleic acid-content bacteria across diverse freshwater ecosystems. ISME J 2018;12:1344–59.

Pérez MT, Sommaruga R. Differential effect of algal- and soil-derived dissolved organic matter on alpine lake bacterial community composition and activity. Limnol Oceanogr 2006;51:2527–37.

Rajala P, Bomberg M. Reactivation of deep subsurface microbial community in response to methane or methanol amendment. Front Microbiol 2017;8:431.

Reckhardt A, Beck M, Greskowiak J et al. Cycling of redox-sensitive elements in a sandy subterranean estuary of the southern North Sea. Mar Chem 2017;188:6–17.

Retter A, Karwautz C, Griebler C. Groundwater microbial communities in times of climate change. Curr Issues Mol Biol 2021;41:509–38.

Robinson CE, Xin P, Santos IR et al. Groundwater dynamics in subterranean estuaries of coastal unconfined aquifers: Controls on submarine groundwater discharge and chemical inputs to the ocean. Adv Water Resour 2018;115:315–31.

Rocca JD, Simonin M, Bernhardt ES et al. Rare microbial taxa emerge when communities collide: freshwater and marine microbiome responses to experimental mixing. Ecol 2020;101:e02956.

Rodellass V, García-Orellana J, Masqué P et al. Submarine groundwater discharge as a major source of nutrients to the Mediterranean Sea. Proc Natl Acad Sci USA 2015;112:3926–30.

Rodellas V, García-Orellana J, Trezzi G et al. Using the radium quartet to quantify submarine groundwater discharge and porewater exchange. Geochim Cosmochim Acta 2017;196:58–73.

Rogers DR, Casciotti KL. Abundance and diversity of archaeal ammonia oxidizers in a coastal groundwater system. Appl Environ Microbiol 2010;76:7938–48.
Ruiz-González C, Lefort T, Massana R et al. Diel changes in bulk and single-cell bacterial heterotrophic activity in winter surface waters of the northwestern Mediterranean Sea. Limnol Oceanogr 2012;57:29–42.

Ruiz-González C, Niño-García JP, del Giorgio PA. Terrestrial origin of bacterial communities in complex boreal freshwater networks. Ecol Lett 2015a;18:1198–206.

Ruiz-González C, Niño-García JP, Lapierre JF et al. The quality of organic matter shapes the functional biogeography of bacterioplankton across boreal freshwater ecosystems. Glob Ecol Biogeogr 2015b;11:1–11.

Russell TL, Sassoubre LM, Wang D et al. A coupled modeling and molecular biology approach to microbial source tracking at Cowell Beach, Santa Cruz, CA, United States. Environ Sci Technol 2013;47:10231–9.

Sachidanandham R, Yew-Hoong Gin K. A dormancy state in nonspore-forming bacteria. Appl Microbiol Biotechnol 2009;81:927–41.

Sang S, Dai H, Hu BX et al. The study of hydrogeochemical environments and microbial communities along a groundwater salinity gradient in the Pearl River Delta, China. Water 2019;11:804.

Sang S, Zhang X, Dai H et al. Diversity and predictive metabolic pathways of the prokaryotic microbial community along a groundwater salinity gradient of the Pearl River Delta, China. Sci Rep 2018;8:17317.

Santoro AE, Boehm AB, Francis CA. Denitrifier community composition along a nitrate and salinity gradient in a coastal aquifer. Appl Environ Microbiol 2006;72:2102–9.

Santoro AE, Francis CA, de Sieyes NR et al. Shifts in the relative abundance of ammonia-oxidizing bacteria and archaea across physicochemical gradients in a subterranean estuary. Environ Microbiol 2008;10:1068–79.

Santoro AE. Microbial nitrogen cycling at the saltwater-freshwater interface. Hydrog Geol J 2010;18:187–202.

Santos IR, Eyre BD, Huettem M. The driving forces of porewater and ground-water flow in permeable coastal sediments: a review. Estuaries Coast Shelf Sci 2012;98:1–15.

Sarmiento H, Gasol JM. Use of phytoplankton-derived dissolved organic carbon by different types of bacterioplankton. Environ Microbiol 2012;14:2348–60.

Scanlan DJ, Ostrowski M, Mazard S et al. Ecological genomics of marine picocyanobacteria. Microbiol Mol Biol Rev 2009;73:249–99.

Schloss PD, Girard RA, Martin T et al. Status of the Archaeal and Bacterial census: an update. mBio 2016;7:e00201–16.

Schmidt SI, Cuthbert MO, Schwientek M. Towards an integrated understanding of how micro scale processes shape groundwater ecosystem functions. Sci Total Environ 2017;592:215–27.

Schutte CA, Wilson AM, Evans T et al. Methanotrophy controls groundwater methane export from a barrier island. Geochim Cosmochim Acta 2016;179:242–56.

Schwank K, Bornemann TL V, Dombrowski N et al. An archaeal symbiont-host association from the deep terrestrial subsurface. ISME J 2019;13:2135–9.

Sebastián M, Gasol JM. Visualization is crucial for understanding microbial processes in the ocean. Philos Trans R Soc B 2019;374:20190083.

Seibert SL, Degenhardt J, Ahrens J et al. Investigating the land-sea transition zone. In: Jungblut S, Liebich V, Bode-Dalby M (eds). YUMARES 9 - The Oceans: Our Research, Our Future. Switzerland: Springer Nature, 2020, 225–42.

Seymour J, Humphreys W, Mitchell J. Stratification of the microbial community inhabiting an anoxicine sinkhole. Aquat Microb Ecol 2007;50:11–24.

Shao Y, Hatzinger PB, Streger SH et al. Evaluation of methanotrophic bacterial communities capable of biodegrading trichloroethene (TCE) in acidic aquifers. Biodegradation 2019;30:173–90.

Shen D, Jürgens K, Beier S. Experimental insights into the importance of ecologically dissimilar bacteria to community assembly along a salinity gradient. Environ Microbiol 2018;20:1170–84.

Sieracki ME, Cucci TL, Nicinski J. Flow cytometric analysis of 5-cyano-2,3-ditolyl tetrazolium chloride activity of marine bacterioplankton in dilution cultures. Appl Environ Microbiol 1999;65:2409–17.

Sjoestedt J, Koch-Schmidt P, Pontarp M et al. Recruitment of members from the rare biosphere of marine bacterioplankton communities after an environmental disturbance. Appl Environ Microbiol 2012;78:1361–9.

Slomp CP, Van Cappellen P. Nutrient inputs to the coastal ocean through submarine groundwater discharge: controls and potential impact. J Hydrol 2004;295:64–86.

Small C, Nichols RJ. A global analysis of human settlement in coastal zones. J Coast Res 2003;19:584–99.

Smith D, Azam F. A simple, economical method for measuring bacteria protein synthesis rates in seawater using 3H-leucine. Mar Microb Food Webs 1992;6:107–14.

Smith HJ, Zelaya AJ, De León KB et al. Impact of hydrologic boundaries on microbial planktonic and biofilm communities in shallow terrestrial subsurface environments. FEMS Microbiol Ecol 2018;94:1–16.

Smith MB, Rocha AM, Smillie CS et al. Natural bacterial communities serve as quantitative geochemical biosensors. mBio 2015;6:e00326–15.

Sola F, Vargas-García MC, Vallejos A. Interrelation prokaryotic community-aquifer in a carbonate coastal environment. Aquat Sci 2020;82:13.

Stegen JC, Fredrickson JK, Wilkins MJ et al. Groundwater-surface water mixing shifts ecological assembly processes and stimulates organic carbon turnover. Nat Commun 2016;7:11237.

Sun S, Jones RB, Fodor AA. Inference-based accuracy of metagenome prediction tools varies across sample types and functional categories. Microbiome 2020;8:46.

Sáenz JP, Hopmans EC, Rogers D et al. Distribution of anaerobic ammonia-oxidizing bacteria in a subterranean estuary. Mar Chem 2012;136:7–13.

Taniguchi M, Dulai H, Burnett KM et al. Submarine groundwater discharge: updates on its measurement techniques, geo-physical drivers, magnitudes, and effects. Front Environ Sci 2019;7:141.

Taubert M, Stockel S, Geesink P et al. Tracking active groundwater microbes with D2O labelling to understand their ecosystem function. Environ Microbiol 2018;20:369–84.

Taubert M, Stähly J, Kolb S et al. Divergent microbial communities in groundwater and overlying soils exhibit functional redundancy for plant-polysaccharide degradation. PLoS One 2019;14:e0212937.

Teira E, Logares R, Gutierrez-Barral A et al. Impact of grazing, resource availability and light on prokaryotic growth and diversity in the oligotrophic surface global ocean. Environ Microbiol 2019;21:1482–96.

Tian R, Ning D, He Z et al. Small and mighty: adaptation of superphylum Patescibacteria to groundwater environment drives their genome simplicity. Microbiome 2020;8:51.
Tovar-Sánchez A, Basterretxea G, Rodellas V et al. Contribution of groundwater discharge to the coastal dissolved nutrients and trace metal concentrations in Majorca Island: karstic vs detrital systems. Environ Sci Technol 2010;44:11819–27.

Troussellier M, Scharer H, Batailler N et al. Bacterial activity and genetic richness along an estuarine gradient (Rhone River plume, France). Aquat Microb Ecol 2002;28:13–24.

Unno T, Kim J, Kim Y et al. Influence of seawater intrusion on microbial communities in groundwater. Sci Total Environ 2015;532:337–43.

Velasco Ayuso S, Acebes P, López-Archilla AI et al. Environmental factors controlling the spatiotemporal distribution of microbial communities in a coastal, sandy aquifer system (Doñana, southwest Spain). Hydrogeol J 2009a;17:677–80.

Velasco Ayuso S, del Carmen Guerrero M, Montes C et al. Regulation and spatiotemporal patterns of extracellular enzyme activities in a coastal, sandy aquifer system (Doñana, SW Spain). Microb Ecol 2011;62:162–76.

Velasco Ayuso S, Guerrero MC, Montes C et al. Spatiotemporal distribution of microbial communities in a coastal, sandy aquifer system (Doñana, SW Spain). Geobiology 2009b;7:66–81.

Velasco Ayuso S, López-Archilla AI, Montes C et al. Microbial activities in a coastal, sandy aquifer system (Doñana Natural Protected Area, SW Spain). Geomicrobiol J 2010;27:409–23.

Velimirov B. Nanobacteria, ultramicrobacteria and starvation forms: a search for the smallest metabolizing bacterium. Microbes Environ 2001;16:67–77.

Vigneron A, Bishop A, Alsop EB et al. Microbial and isotopic evidence for methane cycling in hydrocarbon-containing groundwater from the Pennsylvania Region. Front Microbiol 2017;8:1–12.

Vollberg F, Walther M, Gärdes A et al. Modeling the potential of submarine groundwater discharge to facilitate growth of Vibrio cholerae bacteria. Hydrology 2019;6:39.

Wang S, Zhu G, Zhuang L et al. Anaerobic ammonium oxidation is a major N-sink in aquifer systems around the world. ISME J 2020;14:151–63.

Werner AD, Bakker M, Post VEA et al. Seawater intrusion processes, investigation and management: recent advances and future challenges. Adv Water Resour 2013;51:3–26.

Whiticar MJ. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. Chem Geol 1999;161:291–314.

Whitman WB, Coleman DC, Wiebe WJ. Prokaryotes: the unseen majority. Proc Natl Acad Sci USA 1998;95:6578–83.

Wilhartitz IC, Kirschnic AKT, Brussaard CPD et al. Dynamics of natural prokaryotes, viruses, and heterotrophic nanoflagellates in alpine karstic groundwater. Microbiology-open 2013;2:533–43.

Wilhartitz IC, Kirschnic AKT, Stadler H et al. Heterotrophic prokaryotic production in ultraoligotrophic alpine karst aquifers and ecological implications. FEMS Microbiol Ecol 2009;68:287–99.

Wilkins LGE, Leray M, O’Dea A et al. Host-associated microbiomes drive structure and function of marine ecosystems. PLoS Biol 2019;17:e3000533.

Witoski NI, Muscarella ME, Larsen ML et al. Metabolic insight into bacterial community assembly across ecosystem boundaries. Ecology 2020;101:e02968.

Wong WW, Applegate A, Poh SC et al. Biogeochemical attenuation of nitrate in a sandy subterranean estuary: insights from two stable isotope approaches. Limnol Oceanogr 2020;65:3098–113.

Wörner I, Hoshino T, Bowles MW et al. Microbial dormancy in the marine subsurface: global endospore abundance and response to burial. Sci Adv 2019;5:eava1024.

Xenopoulos MA, Downing JA, Kumar MD et al. Ecological and biogeochemical contrasts across the aquatic continuum. Limnol Oceanogr 2017;62:53–14.

Ye Q, Liu J, Du J et al. Bacterial diversity in submarine groundwater along the coasts of the Yellow Sea. Front Microbiol 2016;6:1519.

Youssef NH, Rinke C, Stepanauskas R et al. Insights into the metabolism, lifestyle and putative evolutionary history of the novel archaeal phylum ‘Diapherotrites’. ISME J 2015;9:447–60.

Zhang X, Qi L, Li W et al. Bacterial community variations with salinity in the saltwater-intruded estuarine aquifer. Sci Total Environ 2021;755:142423.

Zhou Y, Kellermann C, Griebler C. Spatio-temporal patterns of microbial communities in a hydrologically dynamic pristine aquifer. FEMS Microbiol Ecol 2012;81:230–42.