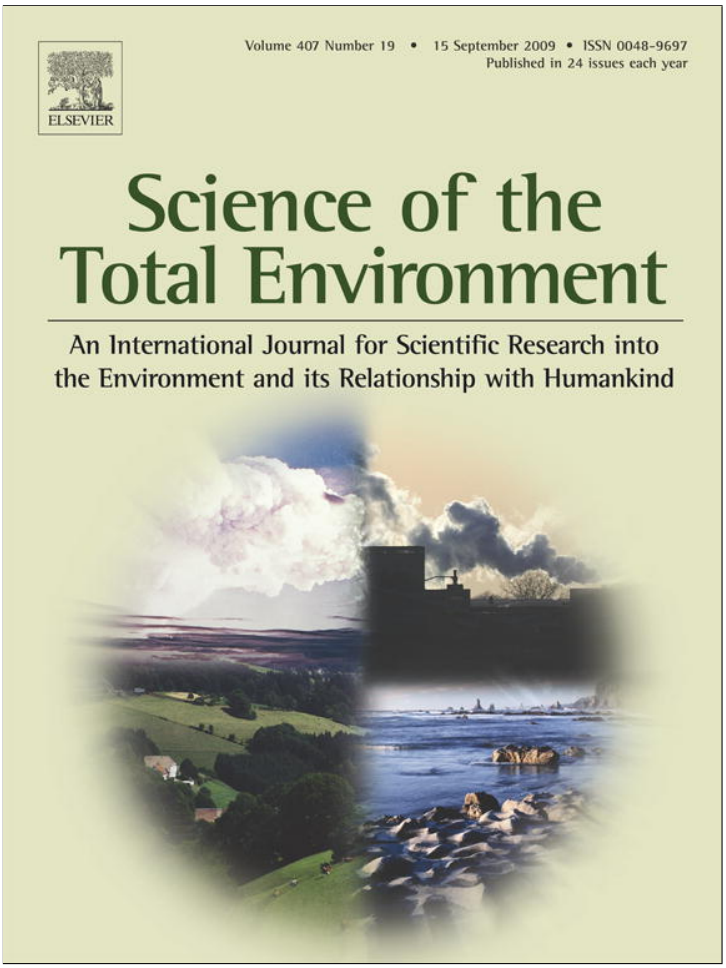

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Extensive bacterial diversity indicates the potential operation of a dynamic micro-ecology within domestic rainwater storage systems

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ABSTRACT

The concept that domestic rainwater storage tanks may host sustainable microbial ecosystems has not previously been addressed. The bacterial diversity, cultivated from more than 80 samples from 22 tanks at various locations across eastern Australia, is presented here as prima facie evidence for the potential operation of a functional micro-ecology within rainwater storage systems. Cultivated isolates were found to comprise members of four major bacterial divisions; Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes, including more than 200 species from 80 different genera. The pattern of abundance distribution was typical of that observed in most natural communities, comprising a small number of abundant taxa and a multitude of rare taxa, while the specific composition resembled that previously described in a number of natural aquatic systems. Although Proteobacteria from α, β and γ sub-classes were dominant, a set of core taxa comprising representative genera from all four phyla could be identified. Coliform and other species specifically associated with faecal material comprised <15% of the species identified, and represented <1.5% of total average abundance. The composition of the cultivated populations and scope of diversity present, suggested that rainwater tanks may support functional ecosystems comprising complex communities of environmental bacteria, which may have beneficial implications for the quality of harvested rainwater.

1. Introduction

Recent research has highlighted the potential capacity of widespread rainwater harvesting in cities to buffer the expected impacts of climate change on water supply, and enhance future water security for urban populations (Coombes and Barry, 2008). Additional benefits from extensive domestic harvesting of rainwater may also be expected via reductions in the energy demand and greenhouse gas emissions associated with the treatment and distribution of water from centralised supplies (Coombes, 2007). Despite the potential benefits however, uncertainty over water quality threatens to limit the utilisation of domestically harvested rainwater in cities.

Two factors contribute to this uncertainty. The first being the apparently frequent non-compliance of roof harvested rainwater with drinking water standards based on the presence of indicator organisms (Gould 1999; Lye 2002) — albeit that the actual health risks associated with non-compliant roof water are yet to be adequately demonstrated. The other, a generally limited knowledge of the processes at work within a rainwater storage tank, and their impact on end-product quality. Previous microbiological studies of roof-fed rainwater tanks have focussed largely on the potential presence of clinically significant species that may enter the tank via the faecal deposits of birds and other animals on the roof catchment surface. Data and knowledge of roof and tank-water microbiology has consequently been confined to total (heterotrophic) plate counts, faecal indicator (e.g. coliform) counts and the occurrence of specific pathogens (Gould, 1999; Uba and Aghogho, 2000; Savill et al., 2001; Simmons et al., 2001; Lye, 2002). Little recognition has thus far been given to non-faecal contamination, non-pathogenic species, specific composition of the heterotrophic count, or to the concept of the rainwater tank as a discrete and functional ecosystem.

Coombes et al. (2000) reported improvements in both the bacterial (based on coliform counts) and chemical quality of roof harvested rainwater with passage through the collection system, ascribing them to the operation of a 'treatment train' of naturally occurring processes. While simple physical processes such as settling are no doubt involved, resident populations of bacteria may also have a role to play via nutrient removal, bioremediation and competitive exclusion. This proposition alludes to the operation of dynamic micro-ecosystems within rainwater tanks. Given that ecosystem stability is widely considered to be a function of community biodiversity (Naeem et al., 1994; Tilman, 1996; Finlay et al., 1997), a measure of the viable microbial diversity present would appear to be a requisite starting point for assessing their likely existence in rainwater storage systems.

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1.1. Heterotrophic counts and potential diversity

A number of studies of water samples from rainwater harvesting systems have reported heterotrophic plate counts several orders of magnitude greater than the corresponding coliform indicator counts (Lye, 1987; Yaziz et al., 1989; Crabtree et al., 1996). While non-coliform heterotrophs may also be associated with faecal material, the findings of Evans et al. (2006) suggested that atmospheric deposition of airborne micro-organisms may be a significant contributor to the bacterial profile of roof run-off. Although the precise composition of these airborne populations was not established, the finding implied a significant presence of bacterial species of non-faecal origin.

The coliform group of bacteria includes members of at least fifteen different genera, all of which belong to the Enterobacteriaceae family. In turn, the Enterobacteriaceae are just one of more than 160 families comprising heterotrophic bacteria (Garrity et al., 2004), defined here as those requiring organic carbon for growth. Thus the composition of a heterotrophic count is potentially very diverse, extending well beyond the comparatively narrow taxonomic range of a coliform count.

1.2. Apparent limitations on diversity

Nonetheless, the closed nature of modern rainwater storage tank systems appears to place certain limitations on the range of organisms that may exist within. By virtue of purpose and design, a rainwater tank is a rather strictly confined and relatively discrete habitat, having only limited continuity with the surrounding natural environment. Theoretically, inputs to the habitat would be intermittent, occurring only via the roof catchment during a rainfall event. The elevated position of the catchment surface renders it detached from the soil environment and beyond the reach of surface waters. In many cases the roof is isolated from the nearest vegetation and is often only accessible to birds and insects, which are generally restricted from entry to the tank by the presence of mesh screens. The tank is enclosed and devoid of light so that photosynthetic bacteria and algae cannot proliferate. Potential sources for bacterial contamination of rainwater tanks appear therefore to be limited to those animals that may frequent the roof environment and airborne debris atmospherically deposited on the catchment surface.

From a microbial perspective the roof catchment can be considered a harsh environment. In many cases, depending on the prevailing climate and specific site characteristics, the surface would periodically experience searing temperatures, offer little or no protection from solar UV radiation, and be essentially devoid of moisture and nutrients. Aside from those capable of producing spores, these are conditions that few bacterial species would be expected to survive for more than a brief period. Of course, bacteria delivered to the roof catchment within the faecal deposits of animals may be afforded protection from UV radiation and desiccation for a short time at least. Outside of these scenarios, only those arriving with or shortly before the advent of rainfall might be expected to survive and enter the tank. Those that do enter would be faced with the task of competing and proliferating in an oligotrophic environment. Under such conditions, one might expect the presence of more resilient and better adapted environmental species to facilitate the competitive exclusion of enteric organisms.

The objective of the current study was to investigate the potential scope of bacterial diversity to be found in rainwater tanks and describe the phylogenetic distribution of readily cultivatable organisms present. The underlying rationale was that the existence of diverse resident communities would be pre-requisite to the operation of functional ecosystems within rainwater tanks. Two basic questions were addressed: do rainwater tanks harbour a wide array of readily cultivated bacterial species? Is the apparent diversity and distribution of taxa consistent with the existence of dynamic but relatively stable microbial ecosystems within rainwater tanks?

2. Materials and methods

2.1. Sample collection

All samples were cold water samples collected from unfiltered tap outlets. At 7 of the sites the tanks were not connected for internal household applications, and samples at these sites were collected from outside taps. At all other sites samples were collected from kitchen tap outlets. In all cases faucets were opened sufficiently to produce a steady flow and allowed to run to waste for a minimum of 10 s prior to collection of the sample. All samples were collected in sterile 500 mL screw cap containers, immediately chilled, and analysed within 24 h.

2.2. Recovery, enumeration and identification of organisms

The analytical approach was designed to facilitate recovery of a broad range of mesophilic heterotrophic bacteria. Several combinations of non-selective growth media and incubations were employed, allowing recovery of both aerobic and anaerobic heterotrophs. Although many bacteria are fastidious, or resistant to cultivation on artificial media, it was considered that the adopted protocols would facilitate the recovery of a significant proportion of the species present and allow them to be reliably differentiated, identified and quantified.

2.2.1. Growth media

For aerobic incubations, samples were plated onto both nutrient agar (NA) and low nutrient R2 agar (R2A) (Oxoid Aust. Ltd.). NA was chosen as a complex, high nutrient, general growth medium for cultivation of a wide range of heterotrophic bacteria. R2A was included to facilitate recovery of stressed organisms and environmental species that do not readily respond to cultivation on complex high nutrient media. For anaerobic analysis, samples were plated onto Columbia horse blood agar (HBA), a popular medium for cultivation of anaerobic bacteria from faecal material and other biological samples.

2.2.2. Plating of samples

In all cases 1.0 mL sample aliquots were transferred aseptically (following thorough mixing) to standard 89 mm Petri dishes containing growth media as described, and dispersed by spread plate technique using sterile plastic spreaders. The large number of samples and range of media employed, precluded routine serial dilution of all samples. Samples suspected of being more heavily contaminated, based on appearance or prior knowledge, were serially diluted (10^{-1} to 10^{-3}) with autoclaved milli-Q water and plated accordingly. All undiluted samples were plated in duplicate on each of the growth media. Unused sample portions were maintained in refrigerated storage for dilution and re-testing where overgrowth of undiluted plates occurred. A negative control (dilution water only) was included whenever samples were diluted.

2.2.3. Incubations

In order to recover organisms with optimal growth temperatures across the mesophilic temperature range, 2 aliquots from each sample were plated onto separate NA plates, one for incubation at 37 °C and the other at 25 °C. All 37 °C NA plates were incubated for 24 h. The 25 °C NA plates were incubated for 24–48 h, depending on the extent and rate of colony growth observed following initial inspection at 24 h. To facilitate description and differentiation of colony types, light to moderately populated plates with tiny colonies were allowed to incubate for the entire 48 h. Incubation of heavily populated plates was terminated inside 48 h to ensure that accurate quantification was not hindered by confluent colony growth. R2A plates were incubated at 25 °C for 48–96 h. Because many bacteria, especially stressed organisms, exhibit a slow growth response on R2A, growth was assessed after 48 h and where appropriate incubation was continued for a further 24–48 h. For

anaerobic incubations plates were housed in an airtight jar containing an 'Anaerogen®' sachet (Oxoid Australia Ltd.) for oxygen removal. An 'Anaerotest®' indicator strip (Merck, Germany) was included in each jar to confirm that anaerobic conditions had been maintained throughout the incubation. All anaerobic incubations were conducted for a duration of 48 h.

2.2.4. Differentiation of colonies for enumeration and identification

Isolates recovered from each sample were initially differentiated by careful examination of colony morphology using a dissecting microscope. Colony types were differentiated on the basis of size, form (shape), elevation, margin, texture/surface appearance, colour and any other distinctive features not defined by the above criteria. Colonies that could not be conclusively placed with others of similar description were treated as separate types. Counts were adjusted if further analysis revealed them to be the same organism (note that counts were performed by manual spotting, and those within the range of 30–300 colonies per plate were considered valid). To distinguish obligate anaerobes from facultative organisms, all isolates selected from anaerobic plates were tested for aero-tolerance by sub-plating and subsequent aerobic incubation. For each colony type a single representative colony was selected for sub-culturing to provide a pure culture for further analysis.

2.2.5. Identification of isolates via DNA sequence analysis

2.2.5.1. DNA extraction. Extraction of DNA from the various bacterial cultures was conducted using the QIAmp® DNA Mini Kit (Qiagen Australia P/L) following the manufacturer's protocol. All procedures were carried out in a laminar flow cabinet using sterile instruments and reagents.

2.2.5.2. PCR amplification of extracted DNA. Extracted DNA was amplified by PCR using the *Taq* DNA polymerase enzyme (Fermentas, #EP0072) and the universal primers, POmod (5′AGAGTTTGATCMTGG) and PC3mod (5′GGACTAMAGGGTATCTAAT), which amplify a 789 bp region of the gene encoding the 16S ribosomal RNA sub-unit. PCR amplifications were performed in 200 µL tubes containing a 50 µL reaction mixture comprising the following reagents: 10× *Taq* buffer [100 mM Tris–HCl (pH 8.8 at 25 °C), 500 mM KCl, 0.8% Nonidet P40] (5 µL), 25 mM $MgCl_2$ (5 µL), 20 mM dNTP's (2 µL), 25 µM primers (1 µL), 5U/µL *Taq* polymerase (0.25 µL), 0.5% diethyl pyro-carbonate (DEPC) water (31.75 µL) and 5 µL of template (sample) DNA at variable concentration. Positive and negative controls were included with each batch of samples analysed. Amplifications were conducted using a 'DYAD™ DNA Engine' programmable bench-top thermal cycler. The thermal cycling programme included initial denaturation of template DNA at 94.5 °C for 3 min, followed by 42 cycles of denaturation at 94.5 °C for 1 min; annealing of primers at 46 °C for 30 s; and strand replication/elongation at 72 °C for 30 s, terminating with a 5 min elongation step at 72 °C on the final cycle.

2.2.6. Purification and sequencing of PCR products

PCR products were prepared for sequencing by removal of PCR reagents using the QIAmp® Quick Purification Kit (Qiagen Australia P/L), according to the manufacturer's protocol. Big Dye Terminator Cycle Sequencing technology (Applied Biosystems) was used for all DNA sequencing reactions with sequencing fragments separated and collected on an ABI377 automated DNA sequencer.

2.2.7. Analysis of sequence data

The identity of cultivated organisms was obtained by comparison of the purified DNA sequence data to 'GenBank' sequence database entries, using the BLAST 2.2 program (http://www.ncbi.nlm.nih.gov/blast/). Positive identity was assigned if the nearest database match exceeded 98% homology with the sample sequence.

3. Results

3.1. Scope of the survey

A total of 83 samples collected from roof-fed rainwater storage tanks at twenty two sites distributed from Melbourne to Brisbane along the east coast of Australia, were analysed over a 2 year period. These geographically disparate sites encompassed a range of urban, suburban, peri-urban and rural locations and included a varied cross-section of local climatic conditions and individual site characteristics. These factors, coupled with an extended sampling period to prevent potential seasonal bias in the data, provided a pilot sample set that may be considered generally representative of rainwater tanks in eastern Australia.

3.2. Taxonomic summary of the cultivated diversity

The bacterial composition of these samples was found to be taxonomically diverse, with 202 different species identified among the recovered isolates (refer Supporting Information, Table S1). Based on current phylogenetic classification of prokaryotes (Garrity et al., 2004), the species recovered comprised representatives of 80 different genera spanning 38 families, 17 separate orders, 8 classes and 4 major phyla of the domain Bacteria (Fig. 1).

Proteobacteria and Firmicutes accounted for more than 90% of the species identified (and >80% of the genera represented), the remainder being members of the Actinobacteria and Bacteroidetes phyla. With the exception of the *Clostridium* and *Desulfotomaculum* species, all Firmicutes identified belonged to the class 'Bacilli', while the Proteobacteria comprised members of eleven separate orders from the α, β and γ sub-classes (Fig. 1).

The Enterobacteriaceae (γ-Proteobacteria) were the most diversely represented family with 14 member genera identified, including 10 coliform groups. Diverse representation was also found among families of the β-Proteobacteria including 7 genera from the Comomonadaceae, and 5 each from the Oxalobacteriaceae and Nesseriaceae families. Almost half of the 80 genera recorded were represented by a single species only, although a relatively large number of constituent species were identified among several widely distributed genera including the *Pseudomonas* group of γ-Proteobacteria (17 species), and four groups of Firmicutes, *Clostridium* (12 species), *Bacillus* (24 species), *Staphylococcus* (9 species) and *Enterococcus* (7 species) (Supporting Information, Table S1).

3.3. Distribution of taxa

3.3.1. Frequency of detection

The occurrence of phylogenetic groups was considered in terms of the average proportion of samples in which they were detected at each site. Indicative of their large species representation, Proteobacteria (detected in 94% of samples) and Firmicutes (70% of samples) were the most commonly encountered phyla (Fig. 2A). Actinobacteria and Bacteroidetes were represented in 27% and 24% of samples respectively.

Although subdivided among 11 separate orders, the prevalence of Proteobacteria can be largely attributed to the occurrence of 3 main groups, the Burkholderiales order of β-class (75% of samples), and the Pseudomonadales (64%) and Enterobacteriales (39%) of the γ-class (Fig. 2C). Firmicutes were confined to 3 orders, the Clostridiales, Bacillales and Lactobacillales, of which the Bacillales (51% of samples) were most prominent, although Clostridiales were also frequently detected (37% of samples).

3.3.2. Relative abundance

The relative abundance of phylogenetic groups was assessed at all taxonomic levels by determination of their average abundance per sample. The distribution was dominated by Proteobacteria at an

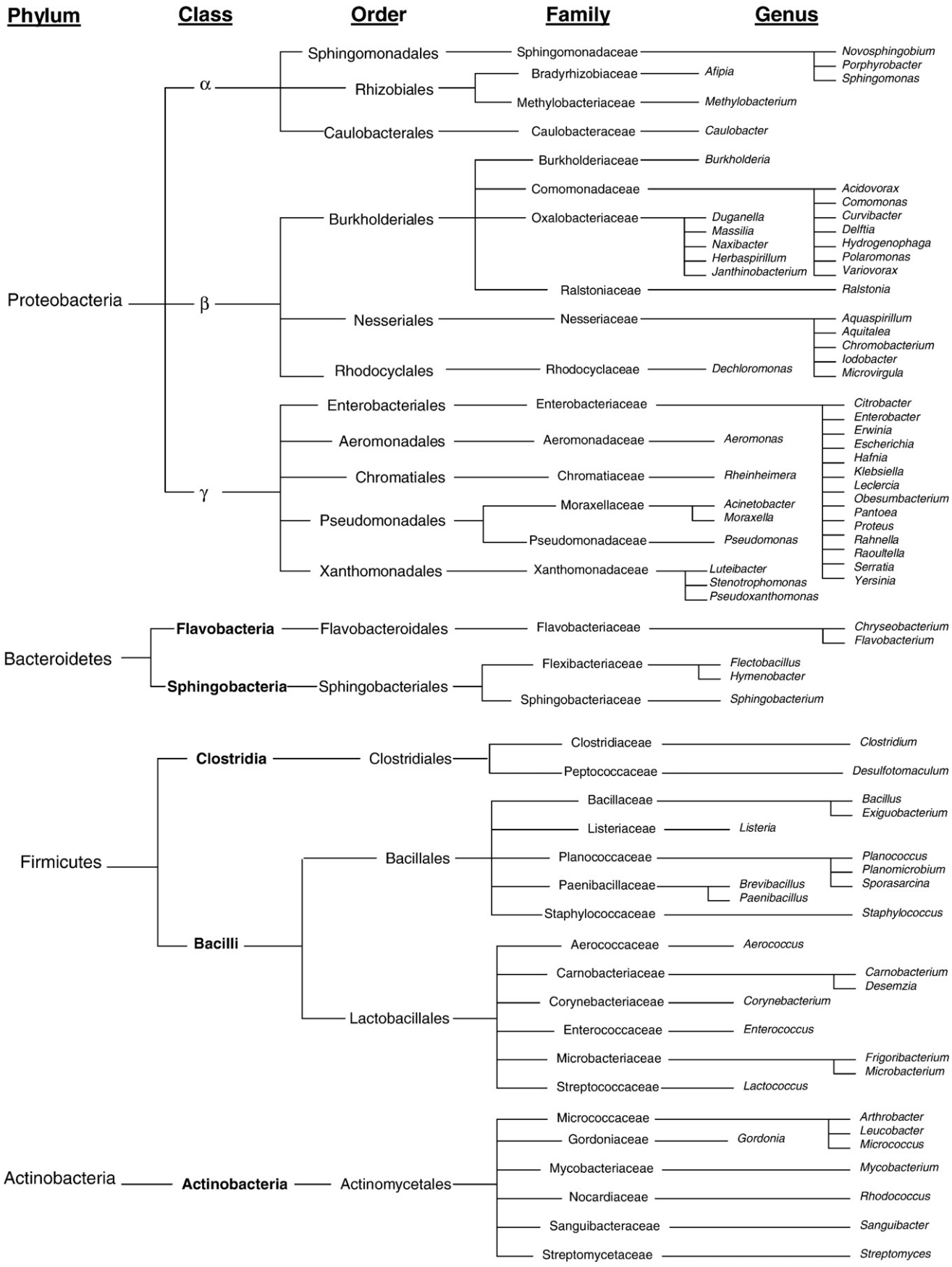


Fig. 1. Taxonomic tree indicating the phylogenetic distribution down to genus level, of all bacterial groups represented among isolates recovered from the 22 rainwater tanks.

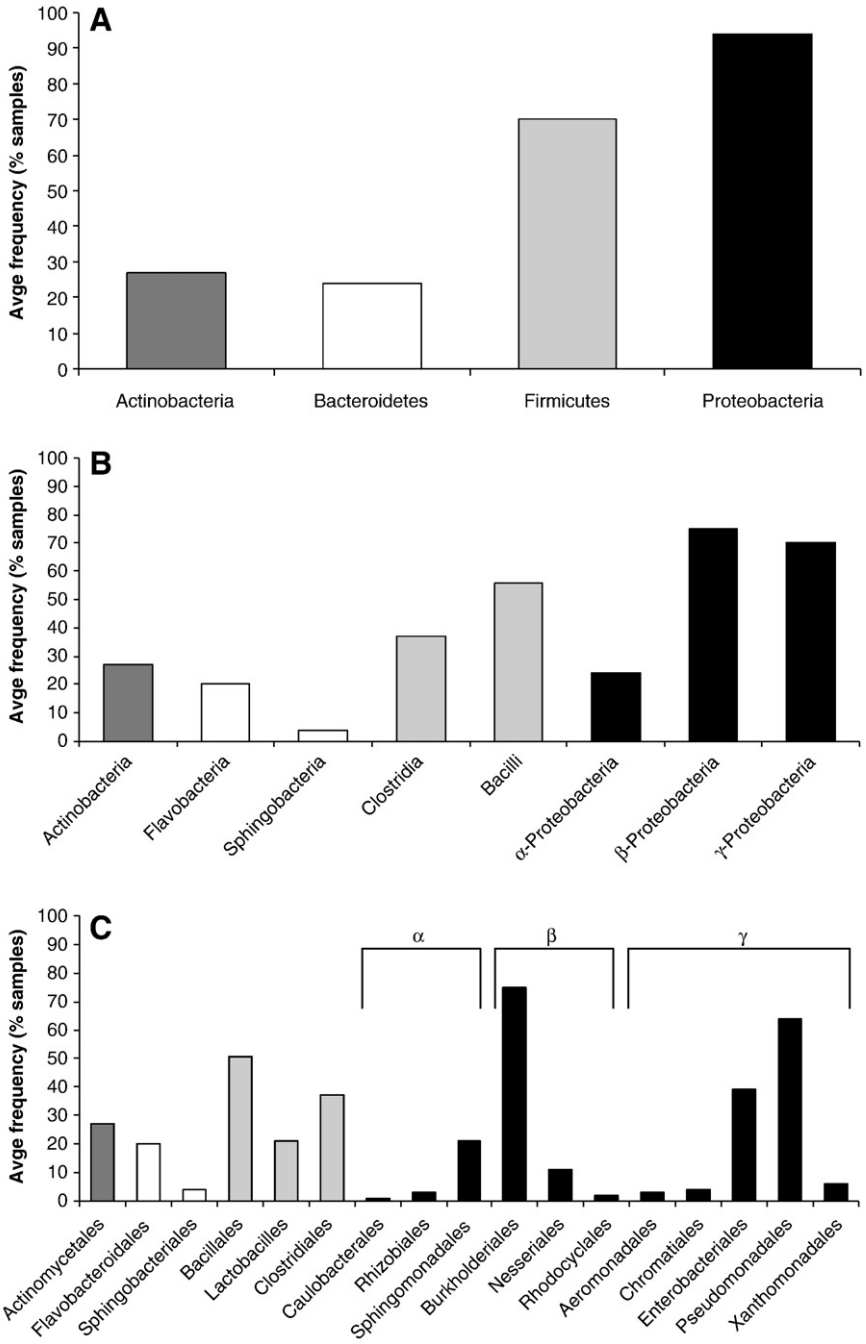


Fig. 2. Detection frequency (average % of samples per site) of represented phylogenetic groups at the level of (A) phylum, (B) class and (C) order.

average abundance >5000 cfu/mL, compared with counts of <100 cfu/mL for each of the other phyla (Fig. 3A). As with the frequency distribution, the Burkholderiales group were dominant among β-Proteobacteria in terms of abundance, while the Sphingomonadales (1873 cfu/mL) and Pseudomonadales (584 cfu/mL) groups were most prominent among α and γ classes (Fig. 3B and C). Despite frequent occurrence of the Enterobacteriales group and the large number of representative genera identified, this group was not found in high relative abundance (Fig. 3C), nor were any individual genera of the Enterobacteriaceae family (refer Fig. 1).

At genus level the abundance distribution was found to be characterised by a small number of relatively abundant taxa (~10% of genera) and a long tail of rare taxa comprising 90% of those identified (Fig. 4). Distribution probability plots indicated that the observed average abundances most closely approximated a log-normal pattern of distribution (Fig. 4 insert). Four of the most abundant groups belonged to the Comomonadaceae family of β-Proteobacteria including *Acidovorax* (1558 cfu/mL), *Hydrogenaphaga* (259 cfu/mL), *Polaromonas* (253 cfu/mL) and *Variovorax* (119 cfu/mL). Despite a general dominance of β-Proteobacteria, the *Sphingomonas* group of α sub-class were found to be most abundant in terms of maximum count (139,000 cfu/mL), average abundance (1823 cfu/mL), and frequency at high abundance (>1000 cfu/mL in 10% of samples). *Pseudomonas* species (496 cfu/mL) were most prominent among the γ-Proteobacteria.

As with abundance, the frequency distribution at genus level was skewed towards infrequent detection with almost 30% of genera detected once only across the sample set, although many of these groups may have been present in other samples at abundances too low for detection. Regression analysis (Spearman r) confirmed a positive correlation between average frequency and abundance scores ($r = 0.79$,

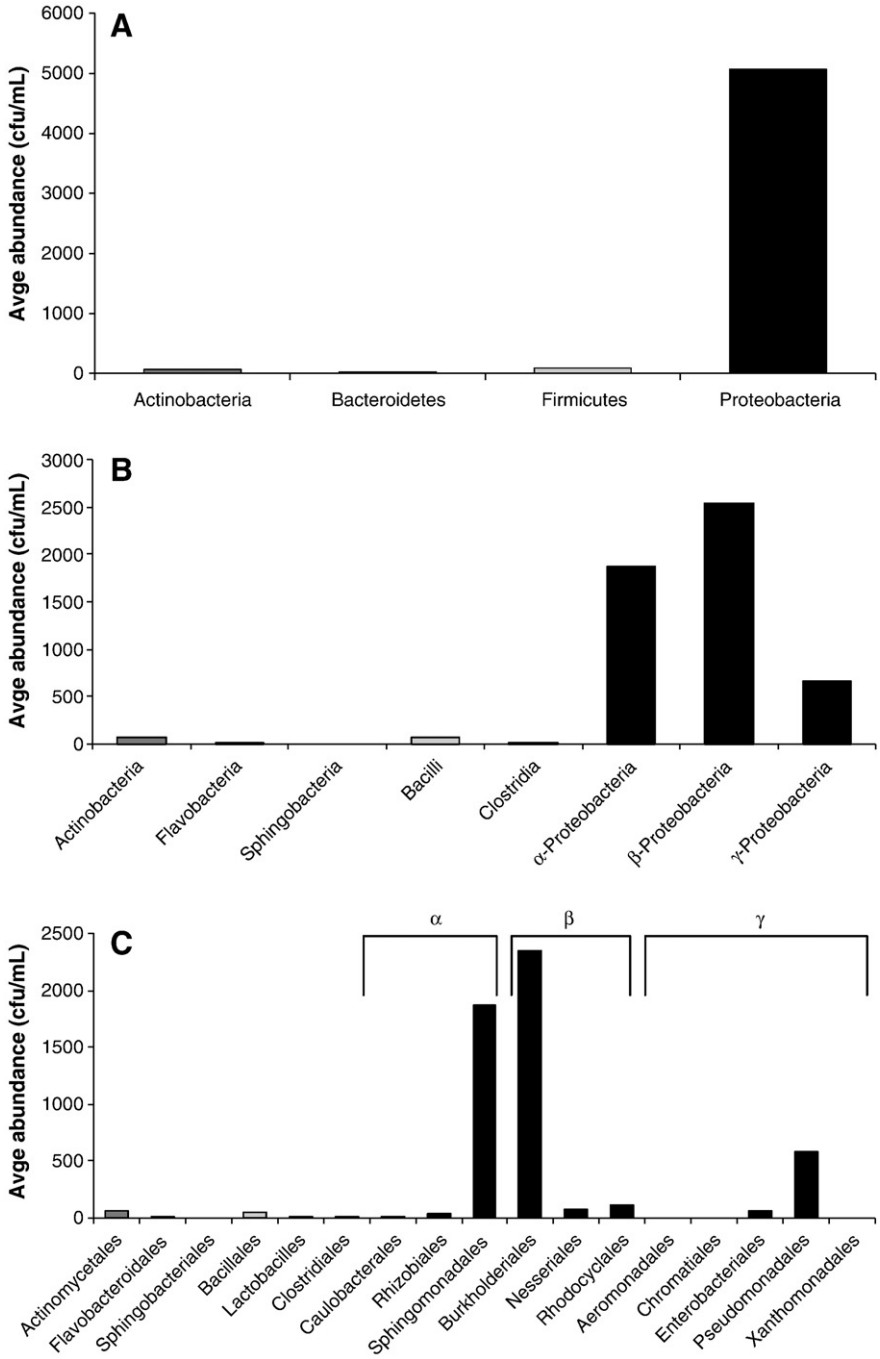


Fig. 3. Average abundance per sample (cfu mL^{-1}) of represented phylogenetic groups at the level of (A) phylum, (B) class and (C) order.

$p<0.01$), the pattern of which was clearly evident when detection frequencies were superimposed against the abundance distribution curve (Fig. 4).

The groups most frequently encountered included the 3 highly abundant Proteobacteria; *Sphingomonas* sp., *Acidovorax* sp., and *Pseudomonas* sp., along with the Firmicutes; *Bacillus* sp., *Clostridium* sp. and *Paenibacillus* sp. (refer Supporting Information Table S2). *Pseudomonas* species were dominant (58% of samples) with *P. putida*, *P. fluorescens* and *P. lanceolata* most prevalent among them. The relatively high occurrence of the *Bacillus* group (42% of samples) was primarily due to the prevalence of the *B. cereus/thuringiensis* phylotype (39% of samples) although 4 other *Bacillus* species (*B. pumilis*, *B. licheniformis*, *B. fusiformis*, and *B. subtilis*) were each detected in >10% of samples. Similarly, *Clostridium bifermentans* (22% of samples) was prominent among the *Clostridium* group (37% of samples). Other genera represented in the upper 10 percentile for occurrence included *Arthrobacter* (Actinobacteria), *Chryseobacterium* (Bacteroidetes) and *Duganella* (Proteobacteria) (Table S2, Supporting Information).

3.3.3. Key taxonomic features of the data

The key features of the taxonomic composition of these samples are most clearly illustrated by examination of the phylogenetic relationships of prominent groups (Fig. 5). While the set of frequently occurring taxa comprised <15% of all genera detected, the group was found to be diverse, including members of all 4 phyla, all but one class, a half of all orders, and more than a fifth of all families represented. Thus although the distribution of individual genera was skewed towards infrequent occurrence, frequent presence at detectable levels was observed across

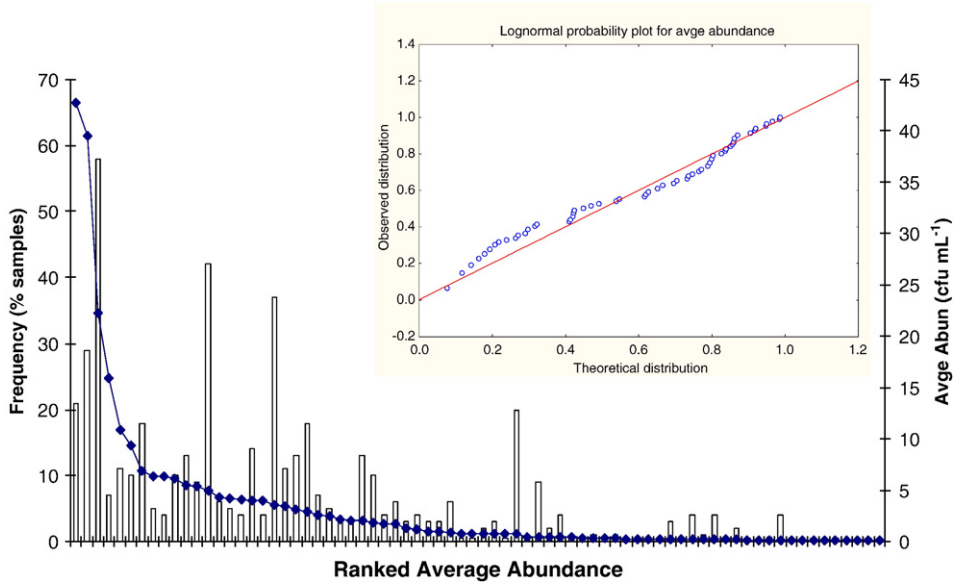


Fig. 4. Combined plot of abundance and occurrence for each bacterial genus represented. Line = ranked plot of the average abundance scores* for each genus. Columns = detection frequency for each group (average proportion of samples per site in which each was detected). Inset: log-normal probability plot of average abundance scores (note: in a perfect log-normal distribution all data points lie along the theoretical diagonal [line]). *The square root of the abundance scores have been plotted to reduce the scale and enhance visualisation of the overall pattern of distribution.

the full phylogenetic range of identified taxa, indicating a general preservation of diversity.

The abundance distribution was more phylogenetically discrete, with dominant taxa comprised exclusively of Proteobacteria, the majority belonging to the β sub-class. Despite wide representation and frequent detection of Enterobacteriaceae, no single genus from this family featured among the most abundant or most frequently detected groups.

In general, the cultivatable bacterial populations of the rainwater tanks in this study appeared to be dominated at higher taxonomic levels by Proteobacteria and Firmicutes although certain Actinobacteria and Bacteroidetes were relatively prominent. The Burkholderiales order of β-Proteobacteria was significant for both the diversity and prevalence of its member genera, especially among the Comomonadaceae family. At genus level five prominent groups could be readily identified including the widely distributed spore-forming Firmicutes, *Bacillus* and *Clostridium*, and 3 groups of Proteobacteria, *Sphingomonas* (α), *Acidovorax* (β) and *Pseudomonas* (γ), which were both persistent and abundant.

4. Discussion

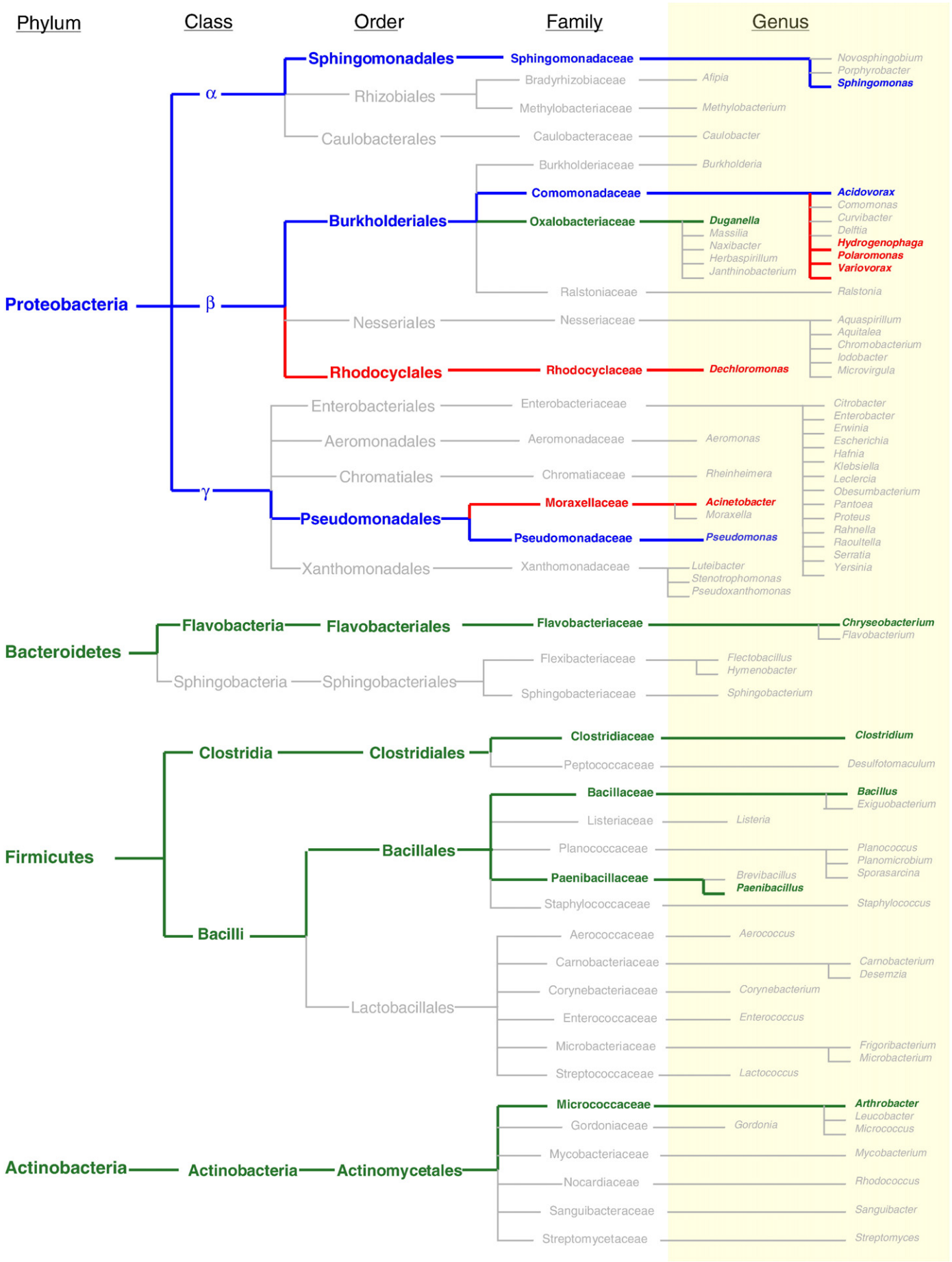
4.1. Comparison with other aquatic systems

In this study, more than 200 bacterial species of considerable phylogenetic diversity have been cultivated and identified from less than a litre of stored rainwater. Although almost certainly a conservative estimate, given that the readily cultivatable fraction may represent as little as 1% of total prokaryotic diversity (Dykhuizen, 1998; Fuhrman and Campbell, 1998), this figure is comparable to the cultivated diversity reported in studies of other aquatic systems (Hantula et al., 1996; Pinhassi et al., 1997; Agogue et al., 2005). The apparent log-normal pattern of abundance distribution, comprising a small number of abundant taxa and a long tail of rare taxa, is also consistent with that found in virtually all natural ecosystems (Dykhuizen, 1998; Magurran and Henderson, 2003; Pedros-Alio, 2006). Thus with regard to the widely accepted hypothesis that "diversity begets stability" (Finlay et al., 1997), evidence from this survey supports the thesis that rainwater tanks represent discrete and functional microbial ecosystems.

Despite general acknowledgement that the readily culturable fraction of overall prokaryotic diversity is small, some evidence suggests that it may in fact be highly representative of total diversity in many aquatic habitats. By examination of a substantial body of 16S rDNA libraries, Kemp and Aller (2004) assessed bacterial diversity in a range of environments and found that the majority of aquatic systems actually comprised <200 phylotypes. This would certainly be considered a minimum value, since many of the libraries were not exhaustive samples of diversity in the source community. However its comparability to the number of species identified in this study, suggests that the cultivatable portion of the total diversity present may indeed be substantial for many aquatic communities.

Importantly, the range of taxa identified in these tanks and their relative distribution appears to be similar to that observed in many freshwater and marine systems, as determined by both cultivation and molecular techniques. In their comprehensive genomic study of seawater from an oligotrophic ocean site in the Sargasso Sea, Venter et al. (2004) found an abundance distribution dominated by Proteobacteria of α, β and γ sub-classes, as well as Firmicutes, Actinobacteria and Bacteroidetes in similar ratio to that found in the rainwater tanks of this study. All cultivated isolates identified by Agogue et al. (2005) in sea surface micro-layer samples from the Mediterranean, were also found to belong to the same four bacterial divisions. The latter also reported Proteobacteria to be consistently more abundant at their pristine sampling site, while Firmicutes and Actinobacteria were dominant at a polluted site. The predominance of Proteobacteria in rainwater tanks might be taken as an indication of the generally clean oligotrophic nature of tank water. At a finer taxonomic scale, many of the genera found to be prevalent in these tanks, including *Pseudomonas*,

Fig. 5. Taxonomic tree indicating the phylogenetic distribution of all bacterial groups represented among isolates recovered from 22 rainwater tanks. Highlighted groups comprise the upper 10 percentile for frequency of occurrence (green), and average abundance (red). Groups ranked among the upper 10 percentile for both frequency and abundance are highlighted in blue.



Sphingomonas, *Bacillus* and *Arthrobacter*, were also reported to be among the most abundant cellular isolates cultivated from seawater samples by Suzuki et al. (1997).

As in the rainwater tanks, a dominance of β-Proteobacteria has been a consistent finding in freshwater systems, particularly among free-living groups, while α and γ sub-classes appear to dominate among particle-attached groups (Crump et al., 1999; Glockner et al., 1999; Cottrell et al., 2005; Boucher et al., 2006). The amount of particulate matter entering a rainwater tank would be under the influence of such factors as the surrounding terrain, prevailing winds, rainfall frequency and catchment cleaning practices. Further investigation of the relative abundance of the sub-classes of Proteobacteria in relation to dissolved and suspended solids in different tanks may therefore prove useful in assessing the extent to which these factors influence the microbial composition of tank water.

The resemblance of the bacterial composition to that of other aquatic systems is striking given that the tank environment differs markedly in several fundamental respects. Isolation within an opaque, impervious vessel prevents unrestricted exchange of materials with the natural surrounds and essentially precludes photosynthetic organisms from the system. Nonetheless, the rainwater tank might be regarded as a 'mesocosm' or model aquatic system, from which the effects of light and the leaching of nutrients have largely been removed. In mesocosm studies of other aquatic systems (not light deprived), resident bacterial populations dominated by Proteobacteria have responded in a definable and systematic manner to manipulated changes in nutrient levels and the introduction of non-indigenous organisms (Hofle, 1992; Riemann et al., 2000). Such experimental manipulations would be akin to the influx of nutrients and organisms to a rainwater tank that occur during a rain event. By way of analogy, one might anticipate the response of rainwater tank communities to these intermittent inputs to be equally predictable.

Such responses would be driven largely by the 'core' taxa, which can be defined in any community as those that are dominant, persistent and primarily responsible for ecosystem function (Magurran and Henderson, 2003; Pedros-Alio, 2006). By simple observation of the data from this study, just five groups at genus level might be considered to comprise the core bacterial residents of rainwater tanks. These include the abundant (dominant) and frequently detected (persistent) *Pseudomonas*, *Sphingomonas* and *Acidovorax*, as well as the frequently occurring spore-forming Firmicutes, *Bacillus* and *Clostridium*. However, Magurran and Henderson (2003) have described a relationship between persistence and abundance that provides a statistical means of defining the range of core taxa within a community. Application of this approach to the rainwater data resulted in an expansion of this core group to include at least 6 genera additional to those identified above (marked with * in Table S2).

4.2. Implications for water quality

Although faecal deposition was considered a primary pathway by which bacteria might enter rainwater tanks, identified members of the Enterobacteriaceae family (which included ten coliform groups) were neither persistent nor abundant in these samples. Since the survivability of coliform groups on the catchment surface is unlikely to differ substantially from that of other non-sporing gram negative Proteobacteria (e.g.. *Pseudomonas*, *Sphingomonas*, *Acidovorax*), two possible explanations for their comparatively low occurrence and abundance seem likely. Either the incidence of faecal deposition on the roof catchments was low relative to contributions from other sources or pathways (refer Evans et al., 2006) or, organisms of faecal origin may simply be less tolerant of the oligotrophic tank conditions than non-enteric groups commonly found in other aquatic systems. The latter may result in their competitive exclusion, representing a means by which the operation of a resident ecosystem may facilitate maintenance of water quality in tanks.

Nutrient cycling and other metabolic activities of the resident communities may also have beneficial consequences for the chemical quality of tank water. Contamination of roof harvested rainwater with halogenated, aromatic and heavy metal pollutants has been identified as potentially problematic in urban settings (Forster, 1999; Hu and Balasubramanian, 2003; Deboudt et al., 2004) while contamination with pesticides may be of concern in rural environs (Van Dijk and Guicherit, 1999). Many of the bacterial groups frequently detected in this study including *Pseudomonas*, *Sphingomonas*, *Bacillus*, *Arthrobacter* and *Rhodococcus*, have demonstrated a capacity to degrade such compounds, or otherwise facilitate their removal from water, in other scenarios (Aislabie and Lloyd-Jones, 1995; Greene et al., 2000; Remoudaki et al., 2003; Salehizadeh and Shojaosadati, 2003).

Aside from the obvious benefits, such activity within rainwater tanks may carry implications with regard to the treatment of stored rainwater. Any potential bio-remedial capacity of resident populations would provide a case for their retention within the tank, rather than elimination via disinfection for example, especially in adequately maintained systems where pathogenic risk is considered minimal. At sites with higher risk of pathogenic load, and where the stored water is used for drinking, treatment may include post-tank measures such as UV disinfection or passage through a water heater (Spinks et al., 2006; Evans et al., 2008), rather than chemical disinfection of the tank itself.

The scope of bacterial diversity present, the general abundance distribution, and the resemblance of the composition to that of other aquatic systems, has indicated the likely existence of definable micro-ecosystems within rainwater tanks. The functional operation of a stable micro-ecology, dominated by well adapted core resident groups, may have beneficial implications with regard to the regulation and maintenance of both the microbial and chemical quality of roof harvested rainwater. System design, maintenance practices and recommendations regarding safe domestic utilisation of harvested rainwater are currently guided by a limited understanding of the relationship between roof catchment contamination, 'in-tank' processes and end-product quality. Investigation of bio-reactor processes, facilitated by diverse microbial communities, may provide valuable insight into this relationship.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.scitotenv.2009.06.009.

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