AUTHOR(S): DELGADO-BAQUERIZO, M., GIARAMIDA, L., REICH, P.B., KHACHANE, A.N., HAMONTS, K., EDWARDS, C., LAWTON, L.A. and SINGH, B.K.

TITLE: Lack of functional redundancy in the relationship between microbial diversity and ecosystem functioning.

YEAR: 2016

Publisher citation: DELGADO-BAQUERIZO, M., GIARAMIDA, L., REICH, P.B., KHACHANE, A.N., HAMONTS, K., EDWARDS, C., LAWTON, L.A. and SINGH, B.K. 2016. Lack of functional redundancy in the relationship between microbial diversity and ecosystem functioning. Journal of ecology [online], 104(4), pages 936-946. Available from: https://dx.doi.org/10.1111/1365-2745.12585

OpenAIR citation: DELGADO-BAQUERIZO, M., GIARAMIDA, L., REICH, P.B., KHACHANE, A.N., HAMONTS, K., EDWARDS, C., LAWTON, L.A. and SINGH, B.K. 2016. Lack of functional redundancy in the relationship between microbial diversity and ecosystem functioning. Journal of ecology, 104 (4), pages 936-946. Held on OpenAIR [online]. Available from: https://openair.rgu.ac.uk

Lack of functional redundancy in the relationship between microbial diversity and ecosystem functioning.

Manuel Delgado-Baquerizo*[1,†]; Luca Giaramida[1,2,3,4†]; Peter B. Reich[1,5]; Amit N. Khachane[1], Kelly Hamonts[1], Christine Edwards[2]; Linda Lawton[2]; Brajesh K. Singh*[1,6].

[1]Hawkesbury Institute for the Environment, Western Sydney University, Penrith NSW 2751, Australia.

[2]IDEAS Research Institute, Robert Gordon University, Riverside East, Garthdee Road, Aberdeen, AB10 7GJ, UK.

[3]The James Hutton Institute, Craigiebuckler, Aberdeen AB15 8QH, UK.

[4]Assumption University, Bangkapi, Bangkok, 10240, Thailand

[5]Department of Forest Resources, University of Minnesota, St. Paul, MN 55108, USA.

[6]Global Centre for Land-Based Innovation, Western Sydney University, Penrith South DC, NSW 2751, Australia.

[†] Equal author contribution

*Correspondence authors. E-mail: M.DelgadoBaquerizo@westernsydney.edu.au or B.Singh@westernsydney.edu.au

Summary

1. Biodiversity is declining worldwide with detrimental effects on ecosystems. However, we lack a quantitative understanding of the shape of the relationship between microbial biodiversity and ecosystem function (BEF). This limits our understanding of how microbial diversity depletion can impact key functions for human well-being, including pollutant detoxification.

2. Three independent microcosm experiments were conducted to evaluate the direction (i.e. positive, negative or null) and the shape of the relationships between bacterial diversity and both broad (i.e. microbial respiration) and specialized (i.e. toxin degradation) functions in five Australian and two UK freshwater ecosystems using next-generation sequencing platforms.

3. Reduced bacterial diversity, even after accounting for biomass, caused a decrease in broad (i.e. cumulative microbial respiration) and specialized (biodegradation of two important toxins) functions in all cases. Unlike the positive but decelerating BEF relationship observed most frequently in plants and animals, most evaluated functional measurements were related to bacterial diversity in a non-redundant fashion (e.g. exponentially and/or linearly).

4. *Synthesis.* Our results suggest that there is a lack of functional redundancy in the relationship between bacterial diversity and ecosystem functioning; thus the consequences of declining microbial diversity on ecosystem functioning and human welfare have likely been considerably underestimated.

Key-words: Bacteria; Broad functions; Ecosystem services; Freshwater ecosystems; Microbial richness; Pyrosequencing; Respiration; Specialized functions; Toxin degradation.

Introduction

A large body of literature has provided evidence that losses in biodiversity will negatively impact ecosystem functions and services provided to humanity in both terrestrial and aquatic ecosystems (Tilman *et al.* 1997; Cardinale *et al.* 2011; Isbell *et al.* 2011; Maestre *et al.* 2012). Most of the studies conducted with plants and animals support a consensus view that the relationship between biodiversity and ecosystem functioning (i.e. BEF relationship) follows a positive but saturating shape indicating functional redundancy (Ehrlich & Ehrlich, 1981; Cardinale *et al.* 2011). A growing number of studies also suggest that microbial diversity enhances ecosystem functioning (Downing & Leibold 2002; Horner-Devine *et al.* 2003; Bell *et al.* 2005; Ptacnik *et al.* 2008; Langenheder *et al.* 2010; Peter *et al.* 2011a; Venail and Vives 2013). Unless there is a substantial functional redundancy in microbial communities (Allison and Martiny 2008), any loss in microbial diversity would likely alter the capacity of microbes to support ecosystem functions. However, none of the previous studies have explicitly examined the shape of the microbial BEF relationship, leaving a wide gap of knowledge that needs to be addressed (Bardgett and van der Putten 2014). Global environmental drivers such as climate change, land use intensification and nitrogen enrichment are impacting microbial diversity in both terrestrial and aquatic ecosystems (Gans *et al.* 2005; Wall *et al.* 2010; Cardinale *et al.* 2012; Singh *et al.* 2014). In order to evaluate the global consequences of shifting microbial diversity on ecosystem functioning, it is critical that we determine the shape of the microbial BEF relationship. However, we lack both the theoretical framework and solid empirical data to understand the shape of the microbial BEF relationship. This hampers our capacity to include microbial processes in ecosystem and earth system simulation models, as well as in conservation and management policy decision making (Singh *et al.* 2010; Bardgett & van der Putten 2014).

Given that bacterial communities play key roles in ecosystem functioning but are considered by far the most abundant and diverse living forms on Earth (Whitman *et al.* 1998; Singh et al. 2009), it is usually presumed that the microbial BEF relationship will show a high functional redundancy (Allison

and Martiny 2008). Previous studies suggested a wide range of shapes (mainly saturating or linear) for the relationships between bacterial diversity and both broad and specialized ecosystem functions (Bell *et al.* 2005; Ptacnik *et al.* 2008; Peter *et al.* 2011a; Ylla *et al.* 2013). However, none of these studies have statistically and simultaneously tested for the shape of the microbial BEF relationship by comparing multiple functions; such as Logarithmic, Michaelis-Menten [M-M], Linear, Power and Exponential (Cardinale *et al.* 2011; Reich *et al.* 2012). Each of these models implies a different ecological interpretation. For example, a linear BEF relationship suggests that each species has a proportional effect on ecosystem functionality with no functional redundancy. The exponential relationship suggests that a small decrease in species richness can have a high negative impact on ecosystem functionality (also no functional redundancy). On the other hand, the logarithmic relationship decelerates without saturating, suggesting that the initial loss of species has an impact, but is minimal due to the redundancy on ecosystem functioning (functional redundancy; Yachi & Loreau 1999). Similarly, the M-M relationship saturates, which suggests that some species are completely functionally redundant, and thus initial loss of diversity will not decrease functionality. Finally, the power function (fitted as in Reich *et al.* 2012) can fit multiple shapes and may represent either functional or no functional redundancy depending on each particular case. The lack of a quantitative understanding of the shape of these relationships limits our capacity to accurately predict the consequences of bacterial diversity reductions on critical functions and services for humanity; which include pollutant detoxification, primary production and climate regulation (e.g. CO_2 exchange).

Both broad (widely distributed across living organisms, e.g. decomposition) and specialized (conducted by particular groups of organisms, e.g. detoxification) functions are known to control key ecosystem processes such as respiration (aggregate CO_2 fluxes) and toxin degradation, which are critical for human well-being and development. Despite this fact, our current knowledge on how microbial diversity relates to these two types of ecosystem functions are limited (Schimel *et al.* 2005). It has been posited that broad functions may follow a different shape (i.e. saturating relationship; Yachi

& Loreau 1999) than specialized functions (i.e. immediate catastrophe; Cardinale *et al.* 2011). The rationale for this hypothesis is that initial loss of diversity may modestly influence broad functioning due to considerable redundancy among taxa in overall metabolic processes, but can collapse specialized functions, which are linked to extremely narrow microbial groups (Schimel *et al.* 2005). Determining the shape of the relationship between microbial diversity and both broad and specialized functioning is critical to understand the impact that future losses of microbial diversity may have on ecosystem functioning and human well-being.

Here, we conducted three independent microcosm experiments to evaluate the direction (i.e. positive, negative or null) and the shape of the relationships between bacterial diversity for both broad (i.e. microbial respiration) and specialized (i.e. toxin degradation) functions in five Australian and two UK freshwater ecosystems. Freshwater ecosystems are of paramount importance for human well-being since they provide water to Earth's 7 billion people for agriculture, industry, recreation and municipal use (Sala *et al.* 2000; MEA 2005; Cardinale *et al.* 2012). In these ecosystems, microbial communities play an important role maintaining key processes such as freshwater purification (e.g. breakdown of pollutants). We hypothesize that any loss in bacterial diversity will promote at least a proportional depletion (no functional redundancy) in both broad and specialized functions. We propose this idea because even broad ecosystems functions such as microbial respiration rely on more complex processes, including organic matter degradation (Schimel *et al.* 2005), which involve large and diverse groups of specialized functions (e.g. lignin degradation; Horwath 2007; Ruiz-Dueñas & Martínez, 2009). To properly interpret any pattern in the microbial BEF relationship, we need to consider the effects of microbial biomass, microbial composition, and other methodological issues. Moreover, if our hypothesis is valid, the shape of the microbial BEF relationship should arguably be similar across widely different systems (e.g. rivers, creeks and lakes) with different environmental status (e.g. pristine and polluted).

Materials and methods

Study sites

This study was based in two UK (Scotland) and five Australian (New South Wales) freshwater ecosystems. The five Australian sites belong to two independent microcosm studies including two (Australia.1) and three (Australia.2) sites, respectively. The two UK sites: Loch Freuchie (LF, 56°31'6.93"N, 3°51'4.27"W) and Loch Rescobie (LR, 56°39'18.53"N, 2°47'43.00"W), represent pristine and polluted lakes, respectively (SEPA 2010a; 2010b). The two Australia.1 sites: Hawkesbury River (HR, 33°33'22.06"S, 151°14'21.36"E) and Farmers Creek (FC, 33°28'27.61"S, 150°7'59.61"E), both represent polluted/high nutrient rivers belonging to the Hawkesbury-Nepean river system in New South Wales. Finally, the three Australia.2 sites: Parramatta River (PAR, 33°48'13.49"S, 150°59'56.44"E), Richmond Lagoon (RLA, 33°35'35.99"S, 150°44'31.38"E) and Wheeney Creek (WC, 33°25'32.49"S, 150°48'52.36"E) correspond to a polluted, and a pristine lake and a creek. We would like to highlight that the studies conducted in UK, Australia.1 and Australia.2 are independent from each other explaining the modest differences between experimental and sampling designs.

Water sampling

Three water samples (top 10cm) were randomly collected on August 2011 and February 2013 in the UK and Australia.1 sites, respectively. In addition, one water sample was collected in May 2015 at each location for the Australia.2 sites. In the case of Australia.1 and UK samples, the three water samples collected from each site were used to generate the three replicates. Water was sampled in 1L glass bottles wrapped in aluminum foil to minimize the input of light. One part of the water was directly stored (non-sterile water used for the microbial inoculums), while the other part was filter-sterilised via Stericup filters (0.22 µm, Millipore) and autoclaved at 121 °C for 20 minutes (sterile water). Water was then stored at 4°C ready to be used for the next steps.

Experimental design: dilution-to-extinction approach.

To develop bacterial diversity gradients, without using cultures, a dilution-to-extinction approach was

150 employed (Peter *et al.* 2011a). Here, we used two different versions of this approach including
151 replicated diversity levels (Australia.1 and UK sites) and non-replicated diversity gradient (Australia.2
152 sites) microcosms. The main reason to include these two approaches is that while replicated
153 approaches allow us to statistically test for significant differences among dilution (i.e. diversity) levels
154 (e.g. ANOVA), a wider range of dilutions, instead of 3 replicates, should enable a better description of
155 the shape of the function. By using both replicated level and regression gradient approaches, we aim to
156 provide robust scientific rigor to our findings. To create a gradient in bacterial diversity, a dilution
157 series of the inoculum was prepared by serial transfer of the inoculum into sterile medium (1:10).
158 These dilutions were conducted in a laminar flow hood to avoid contamination. In all cases (replicated
159 and non-replicated approaches), microcosms were constructed using the original sterilised water as
160 substrate, so we did not expect initial differences in substrate concentrations. In all cases, microcosms
161 were constituted in a final volume of 225mL.
162 *Replicated studies (Australia.1 and UK):* For the UK sites, dilutions of 10^{-1}, 10^{-4} and 10^{-7} were used,
163 while for the Australia.1 sites, dilutions of 1x (undiluted), 10^{-1}, 10^{-2} and 10^{-4} dilutions were used. Three
164 microcosms for each water dilution level were established, rendering a total of 42 microcosms (9 and
165 12 microcosms for each of the sites in the UK and Australia.1, respectively). We selected these
166 dilutions to cover a wide range of microbial diversity. Remaining dilutions not used throughout this
167 experiment were discarded due to time and space constraints. Sterile controls were included to ensure a
168 lack of contamination in our microcosms. For these samples, we used a replicated approach to
169 statistically test for biomass influence on the relationship between microbial diversity and functionality
170 by using ANCOVA analyses (see details below).
171 *Gradient approach (Australia.2)*: For the three Australia.2 sites, we established a continuous gradient
172 (non-replicated design) from $10^{-0.18}$ to 10^{-7} ($10^{-0.18}$, $10^{-0.48}$, $10^{-0.78}$, 10^{-1}, $10^{-1.18}$, $10^{-1.48}$, $10^{-1.78}$, 10^{-2}, 10^{-}
173 $^{2.48}$, $10^{-2.78}$, 10^{-3}, $10^{-3.48}$, 10^{-4}, $10^{-4.48}$, 10^{-5}, $10^{-5.48}$, 10^{-6}, $10^{-6.48}$, 10^{-7}). A total of 57 microcosms (19 for
174 each location) were established.

All microcosms (Australia.1, UK and Australia.2) were incubated at 20 °C shaking at 70 rpm in dark conditions during the biomass recovery stage (see below).

Biomass recovery stage.

To separate effects of bacterial diversity from effects of abundance, we allowed bacterial biomass to recover (i.e. to achieve roughly similar levels of microbial biomass among microcosms for a particular location; Fig. S1) before starting (time zero) our measurements of microbial functions. Moreover, we measured the biomass across treatments, and statistically accounted for biomass in our analyses (see details below). We used quantitative PCR (qPCR) to quantify microbial abundance in our microcosms. This method has been reported to provide similar results to direct cell counting using microscopy (Al-Tebrineh *et al.* 2010; Perez-Osorio *et al.* 2010; Castillo *et al.* 2006; Ammann *et al.* 2012; Furukawa *et al.* 2012). DNA extraction was carried out using the PowerWater DNA isolation kit (MoBio Laboratories Inc.; Appendix S1). 16S rRNA qPCR was carried out using a modified protocol of Fierer *et al.* (2005) and using a Rotor Gene-3000 (Corbett Research, Cambridge, UK; see Appendix S1 for complete protocol).

For UK and Australia.1 sites (replicated studies), biomass recovery was tested by means of bacterial 16S rRNA qPCR every three days starting from the moment when dilutions were made (till biomass recovery). Biomass recovery along the dilution (diversity) range was achieved within 3 days for LF, HR and FC, and within 6 days for LR (Fig. S1). Contrary to this, and based on previous experience, biomass from the Australia.2 sites (gradient approach), were directly tested one week after the time zero to ensure biomass recovery. We would like to highlight that we allowed biomass to recover so that all dilutions from each location started with a similar biomass (not amongst locations; Fig. S1).

Assessment of microbial diversity

Similar to microbial biomass, microbial richness and composition were measured immediately before starting (time zero) our measurements of microbial functions; first using T-RFLP (a rapid method that

provided results within 48 hours, Appendix S1 for methodological details) and then by next-generation sequencing.

In the case of the UK and Australia.1 sites, we used 454 pyrosequencing (454 life-sciences); however, because this technology was no longer available and supported by the sole provider (The Roche Ltd), we used Illumina Miseq (Illumina Inc.), a similar but advanced next generation sequencing approach, for Australia.2 sites (see Appendix S1). Regrettably, we failed to sequence 1/9 (LR), 2/9 (LF), 3/19 (PAR and WC) and 5/19 (RLA) of the water samples in the microcosms from UK and Australia.2 sites. Consequently, these samples were not used in further analyses. We used species richness (i.e. number of OTUs at 97 % similarity from 454 or Illumina sequencing) as a proxy of diversity for simplicity, but also because this approach was more commonly used in BEF literature (Gotelli & Colwell 2001). The data were rarefied to ensure even sampling depth between samples (see Appendix S1). Bioinformatic analyses were completed independently for each of our experiments (Australia.1, UK and Australia.2). In all cases, bacterial richness was highly related to the Shannon and Simpson diversity indexes calculated from next generation sequencing techniques (Table S1).

Broad functions

We first measured a broad ecosystem function (i.e. cumulative microbial respiration) in UK, Australia.1 and Australia.2 sites. We selected microbial respiration because this general function is widely distributed among different groups of microorganisms and it is considered as a good proxy of total biological activity (Campbell *et al.* 2003; Schimel & Weintraub, 2003). The day immediately after biomass recovery (the day in which we achieved roughly similar levels of microbial biomass among microcosms for a particular location), we transferred 40 mL of water from the original microcosms to 125 mL serum bottles under sterile conditions. Water samples were incubated at 20 ºC with continuous shaking at 70 rpm to ensure oxygenation. Respiration was measured using an infrared gas analyser (IRGA) every 2-3 days in each water microcosm for 13 and 18 days in the Australian (Australia.1 and Australia.2) and UK sites, respectively (See Appendix S1 for details). We then

calculated the total cumulative microbial respiration in our microcosms from these individual measurements. Finally, for the Australia.2 sites, we also measured the dissolved organic carbon (DOC) at the end of the experiment using a TOC analyzer (Shimatzu, Japan).

Specialized functions

We measured two specialized functions (i.e. ability to degrade microcystin-LR and Triclosan) in the two Australia.1 freshwater ecosystems. Degradation of Microcystin-LR (MC-LR) and Triclosan was selected because these compounds are highly toxic, widely distributed and commonly used as proxies of natural and artificial toxins, respectively (Edwards & Lawton, 2009; Lee *et al.* 2012). In parallel, we transferred 40 mL from the original microcosms to 125 mL serum bottles. Then, MC-LR (0.5mg L^{-1}) and Triclosan (10µg L^{-1}; Bhargava & Leonard, 1996) were added aseptically to the water microcosms. Finally, we calculated the degradation rate constant k (after 16 days) for both MC-LR and Triclosan in our microcosms as explained in detail in Appendix S1. In brief, samples were incubated at room temperature (20 ºC) with continuous shaking at 70 rpm to ensure oxygenation in the dark for 16 days. Serum bottles were opened in sterile conditions every two days to allow oxygenation, taking 500 µL sub-samples for analysis every 4 days. Regarding MC-LR microcosms, sub-samples were analyzed by HPLC (Edwards *et al.* 2008). Quantification of Triclosan was achieved using a commercial kit (Abraxis kits, PA, USA). We then calculated the degradation rate constant k for both MC-LR and Triclosan in our microcosms using a first order kinetic curve as per the FOCUS software tool (http://focus.jrc.ec.europa.eu/dk/).

Statistical analyses I: testing the success of our experimental design: dilution-to-extinction approach.

The dilution-to-extinction approach used here mimics the response of natural communities, species and populations to environmental fluctuations where the rarest species are also more prone to extinction. In this regard, the dilution-to-extinction approach is considered as realistic as possible, and thus is an accepted method to quantify the effects of reductions in microbial diversity on ecosystem functionality (Peter *et al.* 2011a). To test whether we successfully achieved a diversity gradient from the dilution-to-

extinction approach, we first performed a one-way ANOVA to check for differences in the bacterial diversity between dilution levels for the Australia.1 and UK sites (replicated design). Further post-hoc analyses (i.e. Tukey) indicated that bacterial diversity consistently decreased with increasing dilution, even at comparable biomass (Figs. S2-3). In this sense, significant differences were observed in bacterial diversity across various dilution levels at all study sites ($P<0.01$; Figs. S2-3). In addition, we conducted correlation (Pearson's) analyses between dilution level and bacterial diversity in Australia.1/UK (replicated approach) and Australia.2 (non replicated approach) sites. Bacterial diversity was negatively related to dilution level in all cases ($P<0.05$).

Statistical analyses II: Comparing shapes of biodiversity and ecosystem functionality relationships

We first evaluated the direction (i.e. positive, negative or null) and shape of the relationship between bacterial diversity and a broad ecosystem function (i.e. cumulative microbial respiration) in the Australian (Australia.1 and Australia.2) and UK sites. Then, we assessed how bacterial diversity related to two specialized functions (biodegradation of Microcystin-LR and Triclosan) in the two Australia.1 freshwater ecosystems. To identify the best shape describing the relationship between bacterial diversity and functioning, we fitted five different functions that involve different biological interpretations (Logarithmic, M-M, Linear, Power and Exponential; Cardinale *et al.* 2011; Reich *et al.* 2012). Essentially these five functions are included in two groups of ecological shapes for the microbial BEF relationship: functional redundancy (logarithm and M-M) and no functional redundancy (linear or exponential). The power function can fit multiple shapes and may represent either functional or no functional redundancy depending on each particular case. We selected the best model fits by following Akaike Information Criteria (AICc; Burnham & Anderson, 2002). AICc is a corrected version of AIC that is highly recommended when dealing with small samples size as is our case (Burnham & Anderson, 2002). The lower the AICc index the better the model. Here, we consider a $\Delta AICc > 2$ threshold (Burnham & Anderson 2002; Burnham *et al.* 2011) to differentiate between substantially different models. In some cases, we were able to identify a single best function shaping

the microbial BEF relationship. This happened when a particular function (e.g. exponential) was much better (ΔAICc > 2) than the rest of the explored models (Logarithmic, M-M, Linear and Power). In other cases, we were only able to differentiate among groups of functions (functional redundancy vs. no functional redundancy), but unable to identify the best function describing the shape of the microbial BEF relationship. For example, if the best model linked to no redundant models (linear and exponential and/or no redundant-power) was better (ΔAICc > 2) than redundant models (logarithm and M-M and/or redundant power), but similar to other functions within the same category (linear and exponential and/or no redundant-power), we viewed this as providing evidence for a lack of redundancy in the relationship between bacterial diversity and ecosystem functioning. However, if the best redundant model was better (ΔAICc > 2) than no-redundant models, but similar to other functions within the same category (logarithm and M-M and/or redundant power), this indicated a redundancy in the microbial BEF relationship. Once we identified the best group of models, we then used the most representative function of this group (i.e. the one with the lowest ΔAICc value) in the main text. All functions and AICc indexes were fitted using Sigmaplot (London, UK).

Statistical analyses III: Evaluating the effect of microbial biomass on our analyses

We conducted ANCOVA and partial correlation to quantify the effect of biomass on the function being measured, and then partitioned that away from the diversity effect- hence the diversity effect tests whether diversity matters even at a standardized biomass. It is important to clarify that by conducting these analyses, we do not mean to express that biomass is not important for functionality. Contrary to this, because of the huge importance of biomass on ecosystem functioning we have allowed biomass to recover to roughly similar levels among microcosms before measuring functions and for statistical analyses.

Because differences in biomass can influence microbial diversity-functions relationships, the differences detected in biomass at the beginning of the measurements (Fig S1) (i.e. even after our best effort at biomass recovery) might have influenced the results. To assess this, for the Australia.1/UK

sites (replicated design), we statistically tested for any effect of these biomass differences among treatments by conducting a two-way ANCOVA (analyses of covariance) for each of the broad (microbial respiration) and specialized (MC-LR and Triclosan) functions, including site and dilution gradient as fixed factors and bacterial biomass based on qPCR (at the beginning of the experiment but after recovery; Fig S1) as a co-variable. Similar analyses were conducted to confirm the effect of bacterial diversity on the degradation of both MC-LR and Triclosan in the Australia.1 freshwater ecosystems. Besides ANCOVA analyses for replicated design, we conducted partial correlation to confirm the relationship between microbial diversity and ecosystem functionality after controlling for biomass (as measured with qPCR). ANCOVA, ANOVA and partial correlation analyses were performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA).

Statistical analyses IV and V: considering other methodological issues on our analyses

Further analyses were conducted to consider the effects of microbial composition on our analyses. We first used Random Forests analyses (Breiman, 2001) to explore whether the microbial diversity effect on ecosystem functionality (broad and specialized) remained important after considering main bacterial groups in our analyses (Appendix S2). Random forest is a novel machine-learning algorithm that extends standard classification and regression tree (CART) methods by creating a collection of classification trees with binary divisions (Breiman, 2001; Delgado-Baquerizo *et al.* 2015). Unlike traditional CART analyzes, the fit of each tree is assessed using randomly selected cases (1/3 of the data), which are withheld during its construction (out-of-bag or OOB cases). The importance of each predictor variable is determined by evaluating the decrease in prediction accuracy (i.e. increase in the mean square error between observations and OOB predictions) when the data for that predictor are randomly permuted. This decrease is averaged over all trees to produce the final measure of importance (Breiman, 2001; Delgado-Baquerizo *et al.* 2015). Here, we used Random Forest modeling to simultaneously evaluate the relative importance of microbial richness and composition (originally main bacterial phyla and axes from 2D nMDS analyses at the OTU level) on ecosystem functioning

(Appendix S2).

We further evaluated the effect of other methodological issues (e.g. filtering type, CO_2 fluxes measurement and shift in microbial richness and composition during incubation period) on our analyses (Appendix S3). As explained above the water used for microcosm preparation was filtered (0.22µm), with the exception of undiluted samples (1x microcosms in Australia.1 sites). Thus, it may be argued that filtering can be a bias explaining the differences among microcosms in Australia.1 sites (Note that this issue does not affect UK and Australia.2 sites which did not include 1x microcosms). Moreover, it could be argued that the responses in microbial respiration (i.e. broad functioning) that we reported here may be the consequence of a non-linear CO_2 release from water; and thus that CO_2 could be taken up by the water and not released into the headspace unless there is a lot of CO_2 being produced. Finally, both microbial richness and community composition might have changed during the incubation period during which ecosystem functions were measured, thereby biasing responses in ecosystem functioning. We evaluated the effects of filtering type, CO_2 fluxes measurement and shift in microbial richness and composition during incubation period on our analyses in Appendix S3.

Results

Bacterial diversity showed a positive relationship with both broad and specialized functions in freshwater ecosystems (Figs 1-3 and Figs S4-7). This was true in all studied ecosystems and for 100% of the measured functions (11 of 11). We were able to successfully identify a best group of models (functional vs. no functional redundancy) shaping the microbial BEF relationship in 7 of 11 cases (Table S2). In 6 of 7 cases, the best models supported a lack of functional redundancy in the microbial BEF relationship (linear and/or exponential and/or no redundant-power shapes; Figs 1-3; Table S2); indicating a major loss of function with the initial loss of diversity (Fig 4; Table S2). In particular, we have 1 of 7 cases of exponential/no redundant-power shapes (i.e. broad functioning in PAR; Fig. 2b; Table S2), 2 of 7 cases of linear/exponential/no redundant-power shapes (i.e. specialized functioning in

FC; Fig. 3a.1 and b.1; Table S2) and 3 of 7 exponential shapes (i.e. broad functioning in LF and HR and specialized functioning in HR; Figs. 1b and c and 2b.1; Table S2). Thus, most of the identified shapes for the microbial BEF relationship showed no functional redundancy patterns for broad (3 of 4 cases; HR, LF and PAR; Figs. 1 and 2) and specialized (3 of 3 cases; FC for MC-LR and FC and HR for triclosan degradation; Fig. 3) functioning, respectively. The only evidence of any redundancy whatsoever was Loch Rescobie in the UK where we found a near-linear but only very slightly saturating Michaelis-Menten relationship between bacterial diversity and cumulative microbial respiration (Fig 1d; Fig. 4). In the other 4 of 11 cases (i.e. broad functioning in FC, WC and RLA and specialized functioning in HR), we were not able to differentiate the best shape of the microbial BEF relationship among different groups of models including functional redundancy and no-functional redundancy (ΔAICc < 2; Table S2).

There were significant but minor differences in biomass among dilution treatments after the biomass recovery phases, at the start of the experiment (time zero; Fig S1), however proportionally much smaller than the differences in diversity among dilution treatments (Fig S2). To address this potential confounding factor in the present study, we tested for differences among treatments and for the effects of both biomass and diversity in the statistical analyses. In these ANCOVA analyses, we found significant differences between dilution treatments for all the measured functions ($P \leq 0.001$), but no significant main effects from bacterial biomass ($P > 0.275$) were observed on these functions (Table S3). This indicates that the differences in Figs 1-3 arise from differences in diversity, not in bacterial biomass, supporting the robustness of our results. Similarly, partial correlation analyses provide evidence that the positive relationship between microbial diversity and ecosystem functionality is maintained after controlling for biomass (Table S4).

As expected, we found some differences in bacterial composition across dilution levels (Appendix S2). To account for these differences and test whether diversity is still important compared to composition, we used Random Forest analyses. This approach provides insights on the relative

375 importance a several group of predictors (bacterial composition and diversity) on a particular response
376 variable (functions). Our Random Forest analyses (Figs S8-9) indicated that changes in microbial
377 diversity were more important (MC-LR degradation and microbial respiration) or similarly as
378 important (e.g. Triclosan degradation) for ecosystem functioning as changes in microbial communities.
379 Finally, further analyses provided evidence that key methodological details such as filtering
380 type, CO_2 fluxes measurements and shift in microbial composition during incubation period are not
381 influencing our results (Appendix S3).
382
383 **Discussion**
384 Our experimental results unequivocally show microbial diversity enhances function (11 of 11 cases)
385 and provide evidence that there is often a lack of functional redundancy in the relationship between
386 microbial diversity and broad and specialized ecosystem functioning. In particular, 6 of 11 of
387 regressions followed linear, exponential and/or no redundant-power shapes, while only 1 was slightly
388 decelerating (and the others four could not be distinguished in this regard). These observations are
389 consistent with previous reports of positive effects on microbial diversity on selected functions
390 (Downing & Leibold 2002; Horner-Devine *et al.* 2003; Bell *et al.* 2005; Ptacnik *et al.* 2008; Peter *et al.*
391 2011a; Ylla *et al.* 2013), that however, did not explicitly check for the shape of the microbial BEF
392 relationship.
393 Strikingly, while most of the classic studies assessing the links between changing biodiversity
394 and ecosystem functioning have observed a positive but decelerating relationship between BEF in
395 plant and animals (Tilman *et al.* 1997; Cardinale *et al.* 2011), here we show that the shape of this
396 relationship does not follow generally the same pattern in bacterial communities. The differences in the
397 shape of the BEF relationship may be related to the particular manner in which these groups of
398 organisms obtain their resources (Begon *et al.* 2006). For example, all plants acquire C, water and
399 nutrients in the same general manner and do not require pre-processing by other plants prior to doing

so (in fact, such "pre-processing" largely represents resource competition among all species in the community). In other words, in plant ecosystems, if one species disappears, in general, others will acquire the unused resources in its place – thus moving the system only very slightly down the decelerating functional redundancy curve (Cardinale *et al.* 2011). On the contrary, the resource consumption structure of freshwater bacterial communities, linked to key processes such as decomposition, is distinct because resources for some species only become available once other species have degraded and consumed a part of that resource. For example, the observed abrupt reduction in cumulative microbial respiration (aggregated process as defined in Schimel et al. 2005) with decreasing bacterial diversity may be the consequence of a decrease in the microbial community capacity to break down complex and recalcitrant polymers into simpler and more labile monomers (organic matter degradation) which are rapidly consumed and largely respired (i.e. complementary hypothesis; Loreau and Hector 2001; Schimel *et al.* 2005; Horwath 2007). Organic matter degradation usually involves many different specialized functions (e.g. lignin degradation), and is known to require the cooperation of large and diverse groups of micro-organisms (Horwath 2007). In this regard, any depletion in bacterial diversity may limit aggregated processes such as microbial respiration (Schimel *et al.* 2005) to the most labile C sources, negatively impacting upon this ecosystem function. Supporting this notion, in Australia.2 microcosms, where we measured dissolved organic carbon (DOC) at the end of the experiment, we found a negative relationship between microbial diversity and DOC in all three studied sites (WC: Pearson's r = -0.65; P = 0.003; PAR: Pearson's r = -0.47; P = 0.064; RLA: Pearson's r = -0.58; P = 0.032). This reduction in DOC was negatively related to microbial respiration (WC: Pearson's r = -0.76; P = 0.001; PAR: Pearson's r = -0.53; P = 0.038; RLA: Pearson's r = -0.45; P = 0.10); and suggests that a reduction in ecosystem functioning (e.g. microbial respiration) linked to losses in microbial diversity may impact upon important processes such as organic matter decomposition, promoting the accumulation of DOC in water with low levels of microbial diversity.

Consistent with the results observed for broad functions (at least in aggregated processes such as microbial respiration), we found a lack of functional redundancy in all three of the cases in which we identified the shape of the microbial BEF in specialized functions such as MC-LR and Triclosan degradation linked to bacterial diversity depletion. On the contrary to broad functions such as organic matter decomposition, which involve a large group of diverse microorganisms, specialized functions such as MC-LR and Triclosan degradation are carried out by small groups of microorganisms (Edwards & Lawton, 2009; Lee *et al.* 2012). Losses in the diversity of these particular groups of microorganisms, in parallel to the overall bacterial diversity depletion, may collapse the different metabolic routes and steps that allow the degradation of these toxins, explaining the observed exponential decrease in MC-LR and Triclosan degradation. These results can have important implications for freshwater ecosystems and human well-being. Both natural (i.e. MC-LR) and artificial (i.e. Triclosan) toxins such as used in this study are known to have negative effects on human health, vegetation growth, and animal and plant metabolism (Edwards & Lawton, 2009; Lee *et al.* 2012). A decrease of the natural capacity of ecosystems to remove nutrients and break down pollutants will increase the cost of water treatments as well as the percentage of people exposed to unclean water (Vörösmarty *et al.* 2010; Cardinale *et al.* 2012).

Notably, the low redundancy of the microbial BEF relationship in our short-term study seems to match with the reported long-term responses of the shape of the plant BEF relationship (Duffy, 2009; Reich *et al.* 2012). Recent studies suggest that although the shape of the plant BEF relationship is strongly saturating during the first years in BEF experiments, it became much less saturating over time (Duffy, 2009; Reich *et al.* 2012). In this regard, the recent BEF literature suggests that because of the common short time-scale employed in most terrestrial BEF experiments (≈2 years), classic studies conducted with macro-organisms may have underestimated the importance of biodiversity on ecosystem functioning; as the relationship becomes less saturating and more linear with time (Reich *et al.* 2012; Mora *et al.* 2014). This pattern of similarity of long-term plant BEF responses and short-term

450 microbial BEF responses could be related to the different life characteristics (e.g. growth and
451 reproduction rates) of these groups of organisms and thus the different time needed for biotic
452 interactions to develop. For example, microbes have a much more rapid life cycle (i.e. hours to days)
453 than macro-organisms (i.e. months to decades; Schmidt *et al.* 2007). This fact may promote a fast
454 establishment of positive effects from microbial diversity on ecosystem functioning, such as those
455 linked to both horizontal (i.e. symbiosis, competition and mutualism; Reiss 2009) and vertical (i.e.
456 multi-trophic food web interactions; Duffy et al. 2007) species interactions; whereas such interactions
457 can take much longer to develop in plant and animal communities. Thus, our results support the notion
458 that because of their fast growth and life cycle (e.g. up to 1×10^7 faster than animals; Schmidt *et al.*
459 2007), the full spectrum of biotic interactions fueled by bacterial diversity can play out quickly, and
460 bacterial communities in natural systems likely have little to no redundancy. In this respect, our study
461 provides a strong experimental framework to test for ecological questions related to community
462 interaction and succession that would take much longer to find an answer if using an experimental
463 approach involving longer life cycle organisms such as plants or animals (Mora *et al.* 2014).
464 It is important to note that the dilution-to-extinction approach used here is well-known to affect both
465 diversity and biomass, making results derived from this approach potentially difficult to interpret, if
466 steps to account for their co-variance are not taken (as was done in this study). The potential difficulty
467 can arise as both higher biomass and higher diversity could result in higher rates of functional
468 processes. In previous studies, this potential confounding of biomass and diversity was not accounted
469 for (Peter *et al.* 2011a; Ylla *et al.* 2013). Here, we allowed biomass to recover at all dilution levels, and
470 used ANCOVA and partial correlation analyses which indicated that the differences in Figs 1-3 arise
471 from differences in diversity, not in bacterial biomass. They therefore provide support for our
472 interpretation of the magnitude and shape of the relationship between bacterial diversity and broad and
473 specialized ecosystem functioning. Overall, the large reduction in functioning linked to bacterial
474 diversity losses, observed in all of the studied systems and functions, suggests that all bacterial species

475 are required to maintain both broad and specialized functions in freshwater ecosystems (i.e. no
476 redundancy). In addition to accounting for biomass, we explored the effect of microbial composition
477 on the reported microbial BEF relationships. Our Random Forest results (Figs S8-9) support the
478 finding that, in general, changes in microbial richness seem to be more important for ecosystem
479 functioning (microbial respiration, MC-LR and Triclosan degradation) than changes in microbial
480 composition. Even so, the relative abundance of particular groups of microbes such as *Proteobacteria*
481 and *Actinobacteria* were also important predictors of broad and specialized functioning in our study
482 (Figs. S9-10), highlighting the importance of bacterial composition as a driver of ecosystem
483 functioning (selection/sampling effect; Loreau and Hector 2001). Both *Proteobacteria* and
484 *Actinobacteria* are highly functional microbial communities which possess an impressive array of
485 genes allowing the breakdown of different organic components (Trivedi *et al.* 2013).
486 In conclusion, our findings provide direct evidence that similar to macro-organisms (plants and
487 animals), declining microbial diversity has direct, adverse consequences for important ecosystem
488 broad (aggregated) and specialized functions and the services they provide. However, unlike the
489 classical positive but decelerating relationship between ecosystem functions and macro-organism
490 species richness, we most often found a lack of functional redundancy in the relationship between
491 freshwater bacterial diversity with both broad and specialized ecosystem functions (exponential and
492 linear and/or no functional-power functions). These results suggest that a loss of even a small number
493 of bacterial species can have a strongly negative impact on overall ecosystem functioning and services,
494 adversely affecting both freshwater ecosystems and human welfare.
495

496 **Acknowledgments**

497 We thank Fernando T. Maestre (Universidad Rey Juan Carlos, Spain) and Nico Eisenhauer (University
498 of Leipzig, Germany) for helpful comments on previous versions of the manuscript, and all the
499 technicians and colleagues that helped with the field surveys and laboratory analyses. In addition, we

thank Jasmine Grinyer for revising the English of this manuscript. This research is supported by the ARC project (DP13010484). The authors declare no competing financial interests.

Data accessibility

Data from this study are available in Appendix S4.

References

Allison, S.D. & Martiny, J.B.H. (2008). Resistance, resilience, and redundancy in microbial communities. *Proc Natl Acad Sci USA* 105, 11512-11519.

Al-Tebrineh, J., Mihali, T.K., Pomati, F. & Neilan, B.A. (2010). Detection of saxitoxin-producing cyanobacteria and Anabaena circinalis in environmental water blooms by quantitative PCR. *Appl Environ Microbiol*. 76: 7836-42.

Ammann, T.W., Bostanci, N., Belibasakis, G.N. & Thurnheer, T. (2013). Validation of a quantitative real-time PCR assay and comparison with fluorescence microscopy and selective agar plate counting for species-specific quantification of an in vitro subgingival biofilm model. *J Periodontal Res.* 48, 517-26.

Bardgett, R.D. & van der Putten, W.H. (2014). Belowground biodiversity and ecosystem functioning. *Nature* 515, 505–511.

Bhargava, H.N & Leonard, P.A. (1996). Triclosan, Applications and Safety. *Am. J. Infect. Control* 24, 209-218.

Begon, M., Townsend, C.R. & Harper, J.L., (2006). *Ecology From Individuals to Ecosystems* (Blackwell Publishing, MA, USA, ed.4).

Bell, T., Newman, J.A., Silverman, B.W., Turner, S.L. & Lilley, A.K., (2005). The contribution of

species richness and composition to bacterial services. *Nature* 436, 1157-1160.

524 Breiman, L. (2001) *Machine Learning* 45, 5 (2001).

525 Burnham, K.P. & Anderson, D.R. (2002). Model Selection Multimodel Inference A Practical
526 Information-Theoretic Approach second edition (Springer, NY, USA).

527 Burnham, K. P., Anderson, D.R. & Huyvaert. K.P. (2011). AIC model selection and multimodel
528 inference in behavioral ecology: some background, observations, and comparisons. *Behav.*
529 *Ecol. Sociobiol.* 65, 23-35.

530 Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S. & Potts, J.M. (2003). A Rapid
531 Microtiter Plate Method To Measure Carbon Dioxide Evolved from Carbon Substrate
532 Amendments so as To Determine the Physiological Profiles of Soil Microbial Communities by
533 Using Whole Soil. *Appl Environ. Microbiol.* 69, 3593-1599.

534 Cardinale, B.J. *et al.* (2011). The Functional Role Of Producer Diversity In Ecosystems. *Am J Bot* 98,
535 572-592.

536 Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail,. P., Narwani, A., Mace,
537 G.M., Tilman, D., Wardle, D.A., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B.,
538 Larigauderie, A., Srivastava, D.S. & Naeem, S. (2012). Biodiversity loss and its impact on
539 humanity. *Nature* 486, 59-67.

540 Castillo, M., Martín-Orúe, S.M., Manzanilla, E.G., Badiola, I., Martín, M. & Gasa, J. (2006).
541 Quantification of total bacteria, enterobacteria and lactobacilli populations in pig digesta by
542 real-time PCR. *Vet. Microbiol.* 114, 165-70.

543 Delgado-Baquerizo, M., Gallardo, A., Covelo, F., Prado-Comesaña, A., Ochoa, V., Maestre, F.T.,
544 2015. Differences in thallus chemistry are related to species-specific effects of biocrust-forming
545 lichens on soil nutrients and microbial communities. Functional Ecology, 29, 1087–1098.

546 Downing, A.L. & M. Leibold, (2002). Ecosystem consequences of species richness and composition in
547 pond food webs. *Nature* 416, 837-41.

548 Duffy, J.E., B.J. Cardinale, K.E. France, P.B. McIntyre, E. Thébault, & Loreau. M. (2007). The

functional role of biodiversity in food webs: Incorporating trophic complexity. Ecol. Lett. 10, 522-538.

Duffy, J.E. (2009). Why biodiversity is important to the functioning of real-world ecosystems. *Front Ecol. Environ.* 7, 437-444.

Edwards, C. & Lawton, L. (2009). *Advances in Applied Microbiology* (Academic Press., London, UK).

Ehrlich, P.R. & Ehrlich, A.H., (1981). *Extinction, The causes and consequences of the disappearance of species* (Random House, New York, USA).

Fierer, N., Jackson, J.A., Vilgalys, A. & Jackson, R.B., (2005). Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Appl Environ Microbiol* 71, 4117-4120.

Furukawa, K., Noda, N., Tsuneda, S., Saito, T., Itayama, T. & Inamori Y. (2006). Highly sensitive real-time PCR assay for quantification of toxic cyanobacteria based on microcystin synthetase A gene. *J Biosci Bioeng.* 102, 90-6.

Gans, J., Wolinsky, M., Dunbar, J. (2005). Computational improvements reveal great bacterial diversity and high metal toxicity in soil. *Science* 309, 1387–1390.

Gotelli, N.J. & Colwell, R.K. (2001). Quantifying biodiversity, procedures and pitfalls in the measurement and comparison of species richness *Ecol Lett* 4, 379-391.

Horner-Devine, M.C., Leibold, M.A., Smith, V. & Bohannan, B.J.M.. (2003). Bacterial diversity patterns along a gradient of primary productivity. *Ecol. Lett.*, 6, 613-622.

Horwath, W. (2007). *Soil Microbiology and Biochemistry* (Springer, NY, USA).

Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W.S., Reich, P.B., *et al.* (2011). High plant diversity is needed to maintain ecosystem services. *Nature* 477, 199-202.

Lee, D.G., Zhao, F., Rezenom, Y.H., Russell, D.H. & Chua, K.H. (2012). Biodegradation of triclosan by a wastewater microorganism. *Water Research* 46, 4226-4234.

573 Loreau, M., Hector, A. (2001). Partitioning selection and complementarity in biodiversity experiments.
574 *Nature* 412, 72-76.
575 Maestre, F.T., Quero, J.L., Gotelli, N.J., Escudero, A., Ochoa, V., Delgado-Baquerizo, M. *et al.*
576 (2012). Plant Species Richness and Ecosystem Multifunctionality in Global Drylands. *Science*
577 335, 214-218.
578 Millennium Ecosystem Assessment (2005) *Ecosystems and Human Well-Being, Biodiversity Synthesis*
579 (World Resources Institute, Washington, DC).
580 Mora, C., Danovaro, R., & Loreau, M. (2014). Alternative hypotheses to explain why biodiversity-
581 ecosystem functioning relationships are concave-up in some natural ecosystems but concave-
582 down in manipulative experiments. Scientific Reports, *Nature*, 4, 5427.
583 Pérez-Osorio, A.C., Williamson, K.S. & Franklin, M.J. (2010) Heterogeneous rpoS and rhlR mRNA
584 levels and 16S rRNA/rDNA (rRNA gene) ratios within Pseudomonas aeruginosa biofilms,
585 sampled by laser capture microdissection. *J Bacteriol.* 192, 2991-3000.
586 Peter, H., Beier, S., Bertilsson, S., Lindström, E.S., Langenheder, S. & Tranvik, L.J. (2011a).
587 Function-specific response to depletion of microbial diversity. *ISME J* 5, 351-361.
588 Peter. H., P., Ylla, I., Gudasz, C., Romaní, A.R., Sabater, S. & Tranvik, L.J. (2011b).
589 Multifunctionality and Diversity in Bacterial Biofilms. *Plos ONE* 6, e23225.
590 Ptacnik, R., Solimini, A. G., Andersen, T., Tamminen, T., Brettum, P., Lepistö, L., Willen, E. &
591 Rekolainen, S. (2008). Diversity predicts stability and resource use efficiency in natural
592 phytoplankton communities. *Proc Natl Acad Sci USA 105*, 5134–5138.
593 doi:10.1073/pnas.0708328105.
594 Reich, P.B., Tilman, D., Isbell, F., Mueller, K., Hobbie, S.E., Flynn, D.F.B., Eisenhauer, N. (2012).
595 Impacts of Biodiversity Loss Escalate Through Time as Redundancy Fades. *Science* 336, 589-
596 592.
597 Reiss, J., Bridle, J.R., Montoya, J.M. & Woodward, G., (2009). Emerging horizons in biodiversity and

598 ecosystem functioning research. *Trends Ecol Evol* 24, 505-514.
599 Ruiz-Dueñas, F.J. & Martínez, A.T., (2009). Microbial degradation of lignin, how a bulky recalcitrant
600 polymer is efficiently recycled in nature and how we can take advantage of this. *Microb*
601 *Biotechnol* 2, 164–177.
602 Sala, O.E. (2000). Global Biodiversity Scenarios for the Year 2100. *Science* 287, 1770-1774.
603 Schimel, J.P. & Weintraub, M.N., (2003). The implications of exoenzyme activity on microbial carbon
604 and nitrogen limitation in soil, a theoretical model. *Soil Biol Biochem* 35, 549-563.
605 Schimel, J.P., Bennett, J. & Fierer, N. (2005). *Biological diversity and function in soils* (Cambridge
606 University Press, Cambridge, UK).
607 Schmidt, S.K., Nemergut, D.R., Cleveland, C.C., Reed, S.C. & Weintraub, M.N. (2007).
608 Biogeochemical Consequences of Rapid Microbial Turnover and Seasonal Succession in Soil.
609 *Ecology* 88, 1379-1385
610 SEPA (2010a). Water body information sheet for water body 100226 (Loch Rescobie) available via,
611 http,//www.sepa.org.uk/water/river_basin_planning/waterbody_data_sheets.aspx. Last Accessed
612 21/01/2013.
613 SEPA (2010b). Water body information sheet for water body 100242 (Loch Freuchie) available via,
614 http,//www.sepa.org.uk/water/river_basin_planning/waterbody_data_sheets.aspx. Last
615 Accessed 21/01/2013.
616 Singh, B.K., Campbell, C., Sorensen, S.J., Zhou, J., (2009) 'Soil genomics is the way forward', *Nature*
617 *Rev Microbiol* 7, 756-757.
618 Singh, B.K., Bardgett, R.D., Smith, P. & Reay, D.S., (2010). Microorganisms and climate change,
619 terrestrial feedbacks and mitigation options *Nature Rev Microbiol* 8, 779-790.
620 Singh, B.K., Quince C, Macdonald, C.A., Khachane, A., Thomas, N., Al-Soud, W.A., Sørensen, S.J.,
621 He, Z., White, D., Sinclair, A., Crooks, B., Zhou, J. & Campbell, C.D. (2014). Loss of

622 microbial diversity in soils is coincident with reductions in some specialized functions.
623 *Environm. Microbiol.* 16, 2408-2420.
624 Tilman, D., Lehman, D. & Thompson, K. (1997). Plant diversity and ecosystem productivity,
625 Theoretical considerations. *Proc Natl Acad Sci USA* 94, 1857.
626 Trivedi, P., Anderson, I.C., Singh, B.K. (2013), Microbial modulators of soil carbon storage:
627 integrating genomic and metabolic knowledge for global prediction. *Trends in Microbiol.* 21,
628 641–651.
629 Venail P. & Vives M.J. (2013). Phylogenetic distance and species richness interactively affect the
630 productivity of bacterial communities. *Ecology* 94, 2529-2536.
631 Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A. & Green, P. (2010).
632 Global threats to human water security and river biodiversity. *Nature* 467, 555-561.
633 Wall, D.H., Bardgett, R.D. & Kelly, E.F.. (2010). Biodiversity in the dark. *Nature Geosci*.. 3, 297-
634 298.
635 Whitman W.B., Coleman, D.C. & Wiebe, W.J. (1998). Prokaryotes, The unseen majority *Proc. Natl.*
636 *Acad. Sci USA*, 95, 6578-6583.
637 Yachi, S. & Loreau, M., (1999). Biodiversity and ecosystem productivity in a fluctuafing environment,
638 the insurance hypothesis. *Proc Natl Acad Sci USA* 96, 1493-1506.
639 Ylla, I., Peter, H., Romaní, A.M. & Tranvik, L.J.I. (2013). Different diversity-functioning relationship
640 in lake and stream bacterial communities. *FEMS Microbiol. Ecol.*, 85, 95-103.
641
642
643
644
645

647 **Figure legends**

648 **Figure 1.** Relationship between bacterial richness (number of OTUs) obtained via 454 pyrosequencing
649 and broad ecosystem functioning (cumulative microbial respiration over approximately two weeks) in
650 both Australian: a) Farmers Creek and b) Hawkesbury river and UK: c) Loch Freuchie and d) Loch
651 Rescobie microcosms (n = 3 replications at each initial dilution level). The solid lines represent the
652 best model. The solid lines represent fitted regressions for the best model. The long dashed lines
653 represent fitted regressions for alternative models to the best model. The short dashed lines represent
654 fitted linear regression and indicate that we failed to identify the best shape for this microbial BEF
655 relationship. See Table S2 for AICc values.

656 **Figure 2.** Relationship between bacterial richness (number of OTUs) obtained via Illumina Miseq and
657 broad ecosystem functioning (cumulative microbial respiration over approximately two weeks) in
658 Australia.2 sites. The solid lines represent fitted regressions for the best model. The long dashed lines
659 represent fitted regressions for alternative models to the best model. The short dashed lines represent
660 fitted linear regression and indicate that we failed to identify the best shape for this microbial BEF
661 relationship. See Table S2 for AICc values.

662 **Figure 3.** Relationship between bacterial richness (number of OTUs) obtained via pyrosequencing and
663 the degradation rate constant k for both specialized functions: Mycrocystin-LR (a) and Triclosan (b)
664 biodegradation (proportion of toxin degraded day^{-1}) in two Australia.1 freshwater ecosystems: Farmers
665 Creek (1) and Hakwesbury river (2; n = 3). The solid lines represent fitted regressions for the best
666 model. The long dashed lines represent fitted regressions for alternative models to the best model. The
667 short dashed lines represent fitted linear regression and indicate that we failed to identify the best shape
668 for this microbial BEF relationship. See Table S2 for AICc values.

669 **Figure 4.** Summary results on the shape of the microbial BEF relationship. Following our results, there
670 is a lack of functional redundancy in the relationship between microbial diversity with ecosystem
671 functioning which range from i) immediate catastrophe: even small losses in species richness can lead

672 to a large decline in ecosystem functioning to ii) proportional loss: each species has a proportional
673 effect on the ecosystems functioning. In this graph, we only represent those cases in which we
674 successfully differentiate between functional and no functional redundancy (7 of 11 cases). We failed
675 to identify the best shape for the microbial BEF relationship in the other 4 of 11 cases.

676

677 **Supporting Information**

678 Additional Supporting Information may be found in the online version of this article:

679 **Appendix S1.** Supplementary methods.

680 **Appendix S2.** Statistical analyses IV: Evaluating the effects of microbial composition on our analyses.

681 **Appendix S3.** Statistical analyses V. Evaluating the effects of filtering style, CO_2 fluxes measurements
682 and shifts in microbial composition and diversity during incubation period on our analyses.

683 **Figure S1.** Bacterial biomass over time at different dilution levels (1, 10^{-1}, 10^{-2} and 10^{-4} for Australia.1
684 and 10^{-1}, 10^{-4} and 10^{-7} for UK) estimated for: a) Farmers Creek, b) Hawkesbury river, c) Loch Freuchie
685 and d) Loch Rescobie. The different labels (1x, 10^{-1}, 10^{-2}, 10^{-4} and 10^{-7}) represent the exponent of each
686 dilution level. Significant differences arising from one-way ANOVA analyses for each of the time with
687 time as a fixed factor are as follows: ns = $p > 0.05$, * $p < 0.05$ and ** and $p < 0.01$. Error bars indicate
688 standard error (n=3). Arrows indicate the beginning of the experiment.

689 **Figure S2.** Bacterial richness (number of OTUs) estimated via pyrosequencing for the different
690 dilution levels (1, 10^{-1}, 10^{-2} and 10^{-4} for Australia.1 and 10^{-1}, 10^{-4} and 10^{-7} for UK) at the beginning of
691 the experiment for: a) Farmers Creek, b) Hawkesbury river, c) Loch Freuchie and d) Loch Rescobie.
692 Error bars indicate standard error (n=3). The different labels (1, 10^{-1}, 10^{-2}, 10^{-4} and 10^{-7}) representthe
693 exponent of each dilution level. Lower case letters represent the groups created by ANOVA post-hoc
694 tests which compare bacterial richness among dilution levels.

695 **Figure S3.** Relationship between the dilution to extinction gradient and bacterial diversity for
696 Australia.2 sites. Solid lines indicate either exponential or linear fits.

Figure S4. Cumulative microbial respiration for the different dilution levels (1, 10^{-1}, 10^{-2} and 10^{-4} for Australia.1 and 10^{-1}, 10^{-4} and 10^{-7} for UK) for: a) Farmers Creek, b) Hawkesbury river, c) Loch Freuchie and d) Loch Rescobie. The different labels (1, 10^{-1}, 10^{-2}, 10^{-4} and 10^{-7}) representthe exponent of each dilution level. Error bars indicate standard errors (n=3). Lower case letters represent the groups created by ANOVA post-hoc tests which compare bacterial richness among dilution levels.

Figure S5. Changes in microbial respiration with time during the functionality assay for a) Farmers Creek, b) Hawkesbury river, c) Loch Freuchie and d) Loch Rescobie. The different labels (1, 10^{-1}, 10^{-2}, 10^{-4} and 10^{-7}) representthe exponent of each dilution level. Error bars indicate standard error s (n=3). Differences among dilution treatments and time for microbial respiration of each studied site were evaluated using two-way ANOVAs, with dilution treatment as a fixed factor and repeated measures of time.

Figure S6. Scatter plots illustrating the dependency between cumulative microbial respiration and the dilution to extinction gradient for Australia.2 sites.

Figure S7. Mycrocystin-LR (a) and Triclosan (b) biodegradation rate constant k (proportion of toxin degraded day^{-1}) for the different dilution levels (1, 10^{-1}, 10^{-2} and 10^{-4} for Australia.1 and 10^{-1}, 10^{-4} and 10^{-7} for UK) for: 1) Farmers Creek and 2) Hawkesbury river. The different labels (1, 10^{-1}, 10^{-2} and 10^{-4}) representthe exponent of each dilution level. Error bars indicate standard errors (n=3). Lower case letters represent the groups created by ANOVA post-hoc tests which compare bacterial richness among dilution levels.

Figure S8. nMDS graph exploring the effect of the different dilution levels on bacterial composition at a OTUs level for Australia.1, UK and Australia.2 sites.

Figure S9. Random Forest mean predictor importance (% of increase of mean square error) of microbial richness and composition (i.e. relative abundance of main groups of microorganisms or main axes from a nMDS including information at a OTUs level) as drivers of cumulative microbial respiration in this study for the Australia.1, UK and Australia.2 sites. This accuracy importance

722 measure was computed for each tree and averaged over the forest (5000 trees). Significance levels are
723 as follows: *$P < 0.05$ and ** $P < 0.01$.
724 **Figure S10.** Random Forest mean predictor importance (% of increase of mean square error) of
725 microbial richness and composition (i.e. relative abundance of main groups of microorganisms or main
726 axes from a nMDS including information at a OTUs level) as drivers of MC-LR and Triclosan
727 degradation in this study for the Australia.1 sites. This accuracy importance measure was computed for
728 each tree and averaged over the forest (5000 trees). Significance levels are as follows: *$P < 0.05$ and
729 ** $P < 0.01$.
730 **Figure S11.** Calibration curve for microcosm CO_2 emissions. A known amount of CO_2 was added to
731 40ml of water in a 125ml serum bottle. Bottles were shaken for 10 min and then the concentration of
732 CO_2 was measured using an IRGA PP systems WMA2 (Amesbury, MA, USA).
733 **Table S1.** Correlation (Spearman's ρ) between bacterial diversity (bacterial richness obtained via 454
734 pyrosequencing for Australia.1 and UK and Illumina Miseq for Australia.2) and Shannon and Simpson
735 diversity (obtained via 454 pyrosequencing for Australia.1 and UK and Miseq Illumina for
736 Australia.2). FC = Farmers Creek; HR = Hawkesbury River; LF = Loch Freuchie; LR = Loch
737 Rescobie; WC = Wheeney Creek; PAR = Parramatta River; RLA = Richmond Lagoon.
738 **Table S2.** Model fit statistics and AICc index for the different functions describing the relationship
739 between bacterial species richness based on 454 pyrosequencing / Illumina Miseq (X) and ecosystems
740 functions (cumulative microbial respiration, Microcystin-LR and Triclosan; Y). AICc measures the
741 relative goodness of fit of a given model; the lower its value, the more likely it is that this model is
742 correct. Two models models with $\Delta AICc > 2$ are substantially different. The selected models are in
743 bold. FC = Farmers Creek; HR = Hawkesbury River; LF = Loch Freuchie; LR = Loch Rescobie; WC =
744 Wheeney Creek; PAR = Parramatta River; RLA = Richmond Lagoon. The power function can fit
745 multiple shapes and may represent either functional or no functional redundancy depending on each
746 particular case.

747 **Table S3.** Summary results of the two-way ANCOVA analyses carried out with the ecosystem
748 functions (cumulative microbial respiration, Microcystin-LR and Triclosan) as dependent variables and
749 the site (FC and HR for Australia and LF and LR for UK) and dilution (1, 10^{-1}, 10^{-2} and 10^{-4} for
750 Australia and 10^{-1}, 10^{-4} and 10^{-7} for UK) as fixed factors. Bacterial biomass was always included as a
751 co-variable. df = degrees of freedom. *P* values below 0.05 are in bold. FC = Farmers Creek; HR =
752 Hawkesbury River; LF = Loch Freuchie; LR = Loch Rescobie.

753 **Table S4.** Partial correlation (Pearson's r) between bacterial richness (obtained via 454
754 pyrosequencing for Australia.1 and Uk and Illumina Miseq for Australia.2) and ecosystem functions
755 controlling for biomass. FC = Farmers Creek; HR = Hawkesbury River; LF = Loch Freuchie; LR =
756 Loch Rescobie; WC = Wheeney Creek; PAR = Parramatta River; RLA = Richmond Lagoon.

757

758

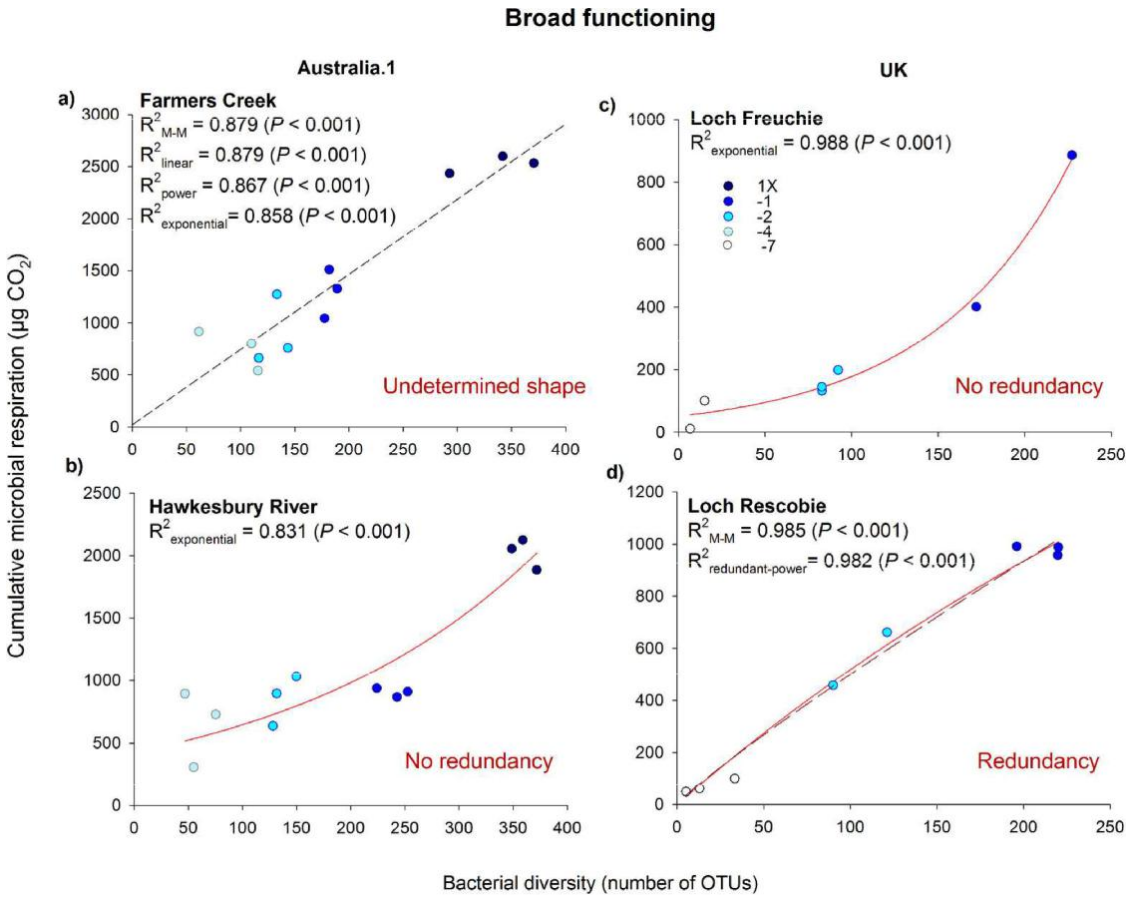


Figure 1. Relationship between bacterial richness (number of OTUs) obtained via 454 pyrosequencing and broad ecosystem functioning (cumulative microbial respiration over approximately two weeks) in both Australian: a) Farmers Creek and b) Hawkesbury river and UK: c) Loch Freuchie and d) Loch Rescobie microcosms (n = 3 replications at each initial dilution level). The solid lines represent the best model. The solid lines represent fitted regressions for the best model. The long dashed lines represent fitted regressions for alternative models to the best model. The short dashed lines represent fitted linear regression and indicate that we failed to identify the best shape for this microbial BEF relationship. See Table S2 for AICc values.

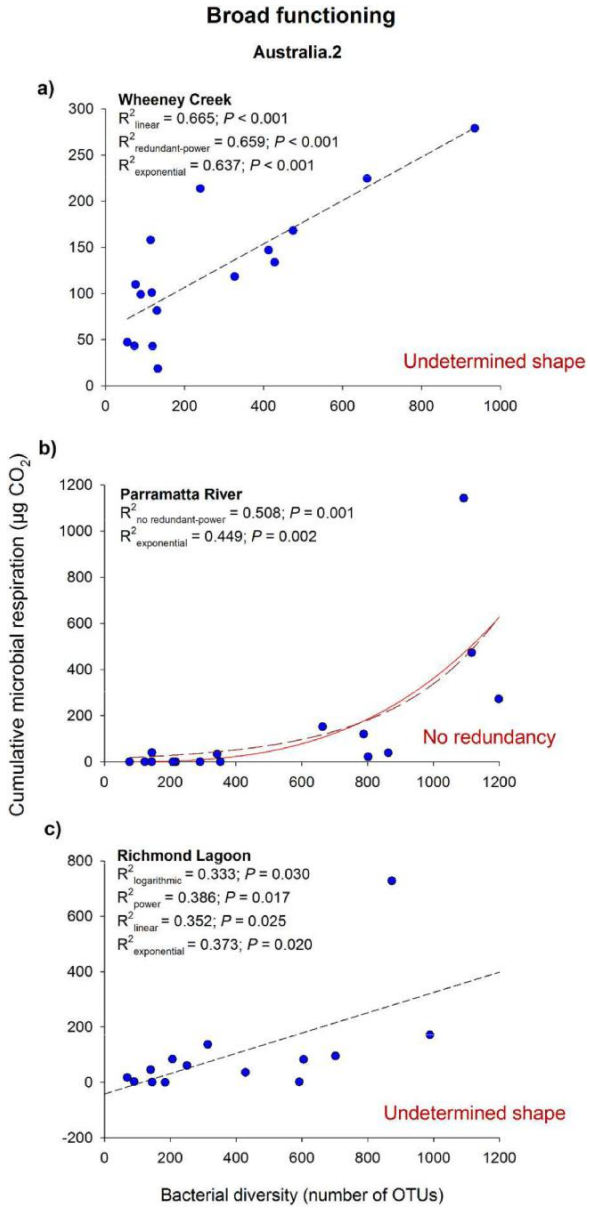


Figure 2. Relationship between bacterial richness (number of OTUs) obtained via Illumina Miseq and broad ecosystem functioning (cumulative microbial respiration over approximately two weeks) in Australia.2 sites.

The solid lines represent fitted regressions for the best model. The long dashed lines represent fitted regressions for alternative models to the best model. The short dashed lines represent fitted linear regression and indicate that we failed to identify the best shape for this microbial BEF relationship. See Table S2 for AICc values.

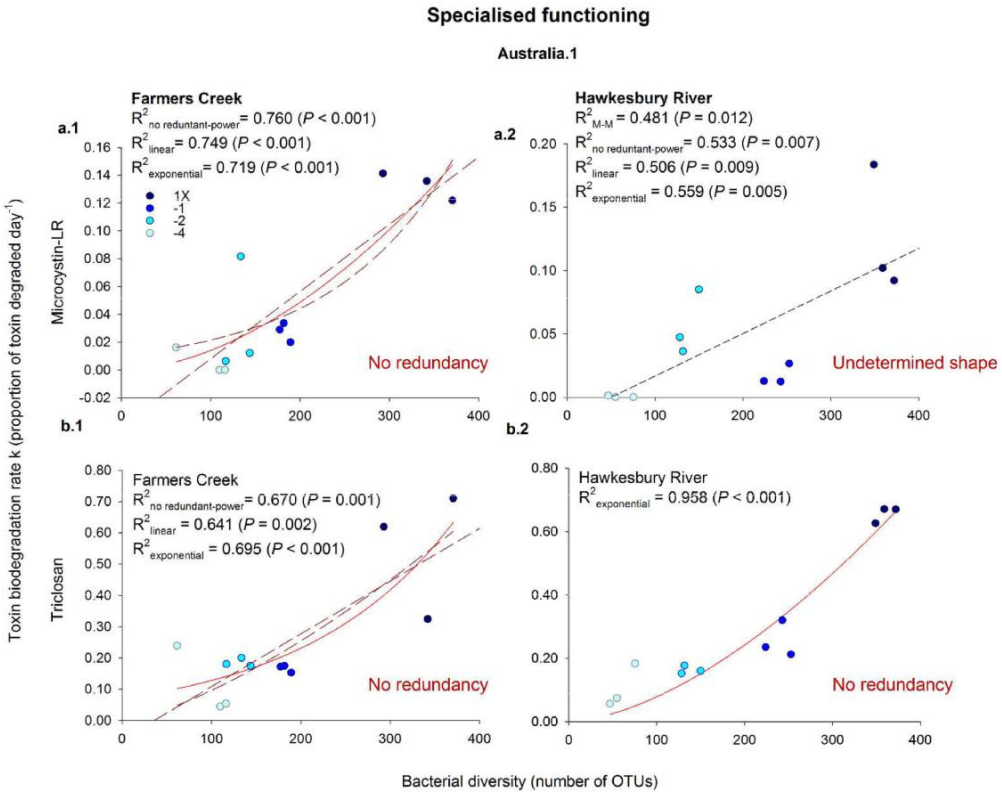


Figure 3. Relationship between bacterial richness (number of OTUs) obtained via pyrosequencing and the degradation rate constant k for both specialized functions: Mycrocystin-LR (a) and Triclosan (b) biodegradation (proportion of toxin degraded day-1) in two Australia.1 freshwater ecosystems: Farmers Creek (1) and Hakwesbury river (2; n = 3). The solid lines represent fitted regressions for the best model. The long dashed lines represent fitted regressions for alternative models to the best model. The short dashed lines represent fitted linear regression and indicate that we failed to identify the best shape for this microbial BEF relationship. See Table S2 for AICc values.

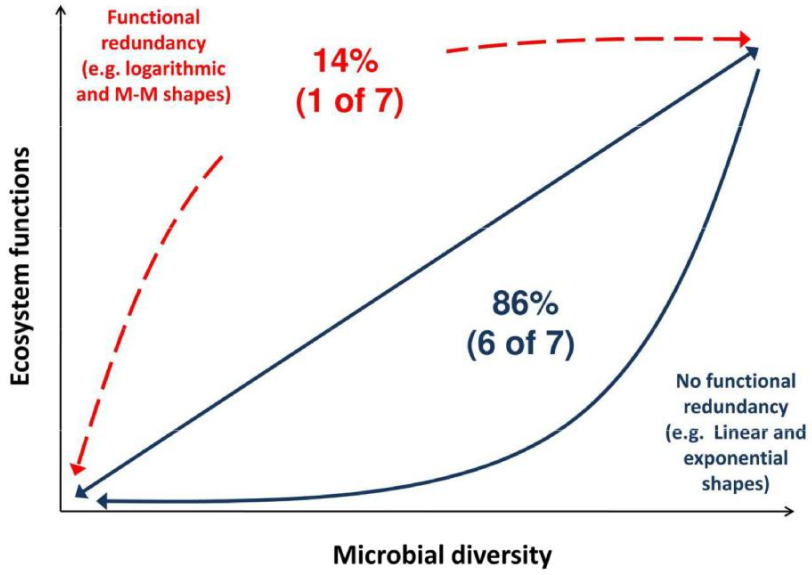


Figure 4. Summary results on the shape of the microbial BEF relationship. Following our results, there is a lack of functional redundancy in the relationship between microbial diversity with ecosystem functioning which range from i) immediate catastrophe: even small losses in species richness can lead to a large decline in ecosystem functioning to ii) proportional loss: each species has a proportional effect on the ecosystems functioning. In this graph, we only represent those cases in which we successfully differentiate between functional and no functional redundancy (7 of 11 cases). We failed to identify the best shape for the microbial BEF relationship in the other 4 of 11 cases.

Supplementary information

Appendix S1. Supplementary methods.

DNA extraction. 25 mL of water from each bottle were filtered (0.2 µm, Stericup filter unit, Millipore) and the filters were stored in sealed sterile Petri dishes at -20 ºC until DNA extraction. DNA extraction were carried out using the PowerWater® DNA Isolation Kit (Mobio, Carlsbad, USA) following the manufacturers protocol, except that the DNA was eluted in 50 µL and not in 100 µL as suggested by the manufacturer.

Quantitative PCR. qPCR assays were carried out on a Rotor Gene-3000 (Corbett Research, Cambridge, United Kingdom) in polypropylene thin-walled tubes. Each 25 µL reaction contained: 12.5 µL of GoTaq® qPCR Master Mix (Promega), 1 µL of bovine serum albumin (20 mg mL-1; Roche), 0.625 µL of primer EUB338 (20 µM, Seq: ACTCCTACGGGAGGCAGCAG) (Kolb *et al.* 2003), 0.625 µL of primer EUB518 (20 µM, Seq: ATTACCGCGGCTGCTGG) (Muyzer *et al.* 1993), 5.25 µL of nuclease-free water (Promega) and 5 µL of template. PCR conditions were: 5 min at 95°C, followed by 30 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 60 s and 83°C for 15 s. To produce an amplicon standard, a plasmid containing the target regions was constructed and used as the template for PCR. Amplified products were run on 2% agarose gel to confirm specificity. Standard curves were generated in duplicate via 10-fold dilutions of the quantified PCR amplicon. At least five non-zero standard concentrations per assay were included, with standard concentration ranging from 10^{-9} to 10^{-2} copies μL^{-1}. Melting curve analysis was carried out following each assay during the optimization stage of the assay to verify the specificity of the fluorescence signal, however once the assay gave optimal results (i.e. $R^2 \geq 0.99$ and efficiency at 100±5%) melting curve was removed to shorten the assay run time. Target copy numbers for each reaction were calculated assuming a product size of 200 bp from the standard curves, which in all assays gave optimal correlation coefficient and efficiency.

454 pyrosequencing analyses (Australia.1 and UK). Due to the low concentration of DNA in individual samples, 16S rRNA gene amplicons were used as the template DNA. Amplicons were produced using three sets of primer (bacteria 16S genes). The amplicons were then cleaned up using Wizard® SV Gel and PCR Clean-Up System (Promega) following manufacturer protocol. 454 Pyrosequencing of 16S rRNA gene was performed on a Roche Junior Titanium Series. A 466-bp fragment of 16S rRNA gene was amplified using the modified primers 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT; Caporaso *et al.* 2010). Data analyses, including assessment of main bacterial phyla abundance, were performed using the 'Quantitative Insights Into Microbial Ecology' (QIIME v 1.6.0) software package (Caporaso *et al.* 2010). Barcode, linker primer and reverse primer sequences were removed from the raw sequence

reads using the 'split_libraries.py' script while setting minimum sequence length of 200 and minimum quality score of 20. The 'Acacia' tool was used with default options to remove pyrosequencing noise (Bragg *et al.* 2012). Potential chimeras were removed using the UCHIME chimera detection utility of the USEARCH v6.0.307 tool (Edgar *et al.* 2011). Similar sequences were binned into OTUs using 'UCLUST' method (minimum pairwise identity of 97%). OTU abundance tables were constructed using QIIME. Taxonomy was assigned to OTUs using Greengenes database version 13_5 (DeSantis *et al.* 2006; McDonald *et al.* 2012). Alpha diversity metrics were calculated on the rarefied OTU table. We used data rarefied at 4672 sequences for UK and 1366 for Australia.1 sites to ensure even sampling depth between samples.

Illumina Miseq analyses (Australia.2). Samples were sequenced using the Illumina MiSeq platform and the same primers as used for 454 pyrosequencing sequencing. Low quality regions (Q < 20, 5 bp and 76 bp from forward and reverse reads, respectively) were trimmed from the 5' end of the sequences using SEQTK (https://github.com/lh3/seqtk) and the paired ends were joined using FLASH (Magoc & Salzberg 2011). Primers were removed from the resulting sequences using SEQTK and a further round of quality control was subsequently conducted using trimmomatic (Bolger *et al.* 2014), applying the sliding window: 4:20 option and setting the minimum sequence length at 200. The resulting sequences were subsequently screened in MOTHUR (Schloss *et al.* 2009) to discard sequences with ambiguous characters or more than 8 homopolymers. Operational Taxonomic Units (OTUs) were built at 97% sequence similarity using UPARSE (Edgar 2013). Singletons were discarded, as well as chimeric sequences identified by the UCHIME algorithm using the SILVA gold 16S rRNA gene reference database (Edgar *et al.* 2011). OTU abundance tables were constructed by running the usearch global command and uc2otutab.py script (http://www.drive5.com/). Taxonomy was assigned to OTUs in MOTHUR using the naïve Bayesian classifier (Wang *et al.* 2007) with a minimum bootstrap support of 60% and the Greengenes database version 13_5 (DeSantis *et al.* 2006; McDonald *et al.* 2012). The OTU abundance table was rarefied at 22118 sequences to ensure even sampling depth between samples. Alpha diversity metrics (bacterial richness and Shannon diversity) were calculated on the rarefied OTU table using MOTHUR (Schloss *et al.* 2009).

Terminal restriction fragment length polymorphism analyses (T-RFLP; All samples). Regarding T-RFLP analysis, amplicons for terminal restriction fragment analysis were produced using the bacterial 16S primer sets: 63F (CAGGCCTAACACATGCAAGTC) and 1087R (CTCGTTGCGGGACTTACCCC) primer sets (Lane, 1991). For PCR amplification of the bacteria 16S rRNA gene, the reaction mix (50 µL) consisted of: 1 x NH4 reaction buffer, 2 mM MgCl2, 400 µM of each deoxynucleoside triphosphate, and 2.5 U of Biotaq DNA polymerase (all reagents from

BIOLINE, UK), 20 µg bovine serum albumin (BSA, Roche Diagnostics, UK) and 5 µL of template DNA. Bacterial primers were used at a concentration of 200 nM. PCR reactions were performed with a DYAD DNA Engine Peltier thermal cycler (MJ Research, Waltham, MA). The cycle consisted of 5 min at 95°C, followed by 30 cycles of denaturing at 94°C for 30s, annealing at 55°C for 30s, elongation at 72°C for 1 min, and a last cycle of 10 minutes extension period at 72°C. PCR amplicons were purified using the Wizard® SV Gel and PCR clean up system (Promega) following the manufacturer instructions. Once the samples were purified using the commercial kit, the concentration and purity of DNA were measured using a NanoDrop™ 1000 spectrophotometer (Thermo Scientific). DNA concentration estimates were then used to normalize the amount of DNA at the restriction digestion step. The pools of bacterial DNA were digested at 37°C for 3 hours with the restriction enzyme HhaI (Promega) following manufactures guidelines in a 10 µL reaction. DNA fragment analysis was carried out on an ABI PRISM® 3130xl Genetic Analyzer (Applied Biosystems). After ensuring that the quality of the capillary electrophoresis run was satisfactory, relative abundance tables were obtained for statistical analysis that was carried out with GenStat (version 11.1, VSN). Before statistical analysis, only terminal fragments in the length range 30-500 base pairs were selected to comply with the range of the T-RFLP standard. Baseline was set up based on overall fluorescence noise of each run to exclude peaks resulting from technical artifacts. Also, peaks with relative abundance below 5% were removed from analysis and remaining peaks were combined when differing for less than one base pair.

Broad functions (All microcosms). We first measured a broad ecosystem function (i.e. cumulative microbial respiration) in UK, Australia.1 and Australia.2 sites. The day immediately after biomass recovery stage (day in which we achieved roughly similar levels of microbial biomass among microcosms for a particular location), we transferred 40 mL of water from the original microcosms to 125 mL serum bottles under sterile conditions. Water samples were incubated at 20 ºC with continuous shaking at 70 rpm to ensure oxygenation during 13 and 18 days for the Australian (Australia.1 and Australia.2) and UK sites, respectively. Bottles were wrapped with aluminium foil to ensure minimal exposure to light. Microbial respiration was measured using an infrared gas analyser (IRGA: EGM-4, PP systems for UK and WMA2, PP systems for Australia.1 and Australia.2 sites) in each of these serum bottles. Respiration was measured every 2-3 days in each water microcosm for 13 and 18 days in the Australian (Australia.1 and Australia.2) and UK sites, respectively. Gaseous samples (10 mL) from the headspace of each serum bottle were taken using syringes just before opening the bottle for atmospheric re-equilibration at each sampling time. The samples were injected into the IRGA to obtain the CO_2 concentration. Thus, each measurement represents the CO_2 accumulated in each microcosm

after 2-3 days. From one time step to the next, we calculated the difference in CO_2 concentration relative to ambient. Finally, we calculated the total cumulative microbial respiration in our microcosms from these individual measurements (expressed as µg CO_2).

Specialized functions (Australia.1). We measured two specialized functions (i.e. ability to degrade microcystin-LR and Triclosan) in the two Australia.1 freshwater ecosystems. In parallel, we transferred 40 mL from the original microcosms to 125 mL serum bottles. Then, MC-LR (0.5 mg L^{-1}) and Triclosan (10 µg L^{-1}) were added aseptically to the water microcosms. Bottles were wrapped with aluminium foil to ensure minimal exposure to light. Samples were incubated at room temperature (20ºC) and with continuous shaking at 70 rpm to ensure oxygenation in the dark for 16 days. Serum bottle were opened in sterile conditions every two days to allow oxygenation, taking aliquots (500 µL) for analysis every 4 days. Regarding MC-LR microcosms, aliquots were frozen, freeze-dried, reconstituted in 80% aqueous methanol and centrifuged at 15000 × g then the supernatant analyzed by HPLC (Edwards *et al.* 2008). HPLC eluents were milli-Q water-0.05% trifluoroacetic acid (TFA) (Fisher Scientific, Leicestershire, UK) and acetonitrile (Rathburn, Walkesburn, UK) 0.05% TFA, the latter being used as the ion pairing agent. The detector resolution was set at 1.2 µm and data were acquired in the wavelength range 200-400 µm. Separation was obtained with a Sunfire C18 column (2.1 mm i.d. x 150 mm long x 5 µm particle size) supplied by Waters corporation (Wilmslow, UK) kept at a temperature of 40 ºC. The instrument used was a Waters 2695 Separation Module with a Waters 2996 Photodiode Array Detector (Waters, Elstree, UK) at a flow rate of 0.3 mL min^{-1}. Finally, quantification of Triclosan was achieved using a commercial kit (Abraxis kits, PA, USA) that applies the principles of enzyme linked immunosorbent assay (ELISA) with quantitation range from 0.05 to 2.5 ppb. Prior to analysis, samples were diluted with the diluent provided by the manufacturer to meet the assay specifications. We then calculated the degradation rate constant k for both MC-LR and Triclosan in our microcosms using a first order kinetic curve as per the FOCUS software tool (http://focus.jrc.ec.europa.eu/dk/). The FOCUS tool requires the percentage of remaining compound in the flask at the different sampling time in order to calculate the degradation rate constant k (proportion of toxin degraded day^{-1}) and the half life (DT-50 in days; time required for 50% dissipation of the initial concentration). For degradation with first order kinetic, k and DT-50 are linked by the relation $DT\text{-}50 = \ln(2)\, k^{-1}$.

Appendix S2. Statistical analyses IV. Evaluating the effects of microbial composition on our analyses.

First, we obtained information on the relative abundance of main bacteria phyla for the Australia.1, UK and Australia.2 sites using next generation sequencing. Moreover, we used a non-metric

multidimensional ordination (nMDS) on the matrix of bacterial composition at a OTUs level to obtain a metric of community composition at the lowest taxonomic rank. The two-dimensional nMDS solution sufficed to represent the data. We conducted nMDS ordinations on previously normalized data (Z-score) with the PRIMER v6 statistical package for Windows (PRIMER-E Ltd., Plymouth Marine Laboratory, UK), using the Bray-Curtis similarity measure.

We then conducted a classification Random Forests analysis (RF; Breiman, 2001) to assess the relative importance of bacterial composition (i.e. relative abundance of main bacterial and phyla or main axes from a nMDS) and richness in controlling both broad and specialized functions, and to explore whether the microbial richness effect on ecosystem functionality was still important after considering main bacterial groups in our analyses. Random Forest is a novel machine-learning algorithm that extends standard classification and regression tree (CART) methods by creating a collection of classification trees with binary divisions (Wei *et al.* 2010). Unlike traditional CART analyses, the fit of each tree is assessed using randomly selected cases (1/3 of the data), which are withheld during its construction (out-of-bag or OOB cases). The importance of each predictor variable was determined by evaluating the decrease in prediction accuracy (i.e. increase in the mean square error between observations and OOB predictions) when the data for that predictor are randomly permuted. This decrease was averaged over all trees to produce the final measure of importance (Wei *et al.* 2010). This accuracy importance measure was computed for each tree and averaged over the forest (5000 trees). In RF, the different microbial variables (microbial diversity and percentage of abundance of the main groups of microorganisms) were included as predictor of the different functions in this study (response variables). We conducted these analyses independently for Australia.1, UK and Australia.2 sites because of the different next generation sequencing approaches (454 pyrosequencing vs. Illumina MIseq) and rarefaction levels used in this study. In addition, we merged those sites from Australia.1/UK (Farmers Creek, Hawkesbury River, Loch Freuchie and Loch Rescobie) and Australia.2 (Wheeney Creek, Parramatta River and Richmond Lagoon) to improve our number of samples. These analyses were conducted using the randomForest package (Liaw & Wiener, 2002) for the R statistical software, version 3.0.2 (http://cran.r-project.org/). The significances of the model and the cross-validated R^2 were assessed with 5000 permutations of the response variable using the A3 R package (Fortmann-Roe, 2013). Similarly, the significance of the importance measures of each predictor (here microbial variables) on the response variable (functions) was assessed by using the rfPermute package for R (Archer, 2013).

Proteobacteria was the dominant phylum of bacteria in all our sites ranging from 60 to 95%, followed by *Bacteroidetes* and *Actinobacteria* that ranged from 3 to 25% and from 1 to 9%,

respectively. As expected, we found some differences in composition across dilution levels. For example, relative abundance of *Proteobacteria* increased with dilution level in five of our seven sites (Spearman $\rho > 0.825$; $P < 0.05$), whereas relative abundance of other phyla such as *Actinobacteria* (5 of 7; Spearman $\rho < -0.732$; $P = 0.001$), *Bacteroidetes* (4 of 7; Spearman $\rho < -0.567$; $P = 0.022$) *and Gemmatimonadetes* (4 of 7; Spearman $\rho < -0.552$; $P = 0.026$) proportionally decreased with dilution level. To account for these differences and test whether diversity is still important compared to composition, we used Random Forest analyses. Our Random Forest analyses (Figs S8-10) indicated that, in general, changes in microbial diversity were more important (MC-LR degradation, Triclosan degradation and cumulative microbial respiration) for ecosystem functioning as changes in microbial community composition (relative abundance of main bacterial phyla and main axes from a nMDS).

Appendix S3. Statistical analyses V. Evaluating the effects of filtering style, CO_2 fluxes measurements and shifts in microbial composition and diversity during incubation period on our analyses.

Here, we conducted further analyses considering the effects of filtering style, CO_2 fluxes measurements and shifts in microbial composition and diversity during incubation period on our analyses. The main goal of the analyses conducted in this appendix was to explore whether any of these important factors could have influenced the reported relationship between microbial diversity and ecosystem functioning. Please, notice that because of the differences in terms of experimental design and measured variables across microcosm studies, we conducted these analyses for the microcosms for which this information was available (indicated in brackets).

Filtering style (Australia.2). Because the water used for microcosm preparation was filtered (0.22µm), with the exception of the undiluted samples (1x microcosms in Australia.1 sites), it may be argued that filtering can be a bias explaining the differences among microcosms. Thus, for a subset of our microcosms (Australia.2) we prepared parallel microcosms with (0.22µm; included in the main text) and without filtering (both of them autoclaved). We selected microcosms 1x, 10^{-1}, 10^{-3}, 10^{-5} and 10^{-7} to cover the whole dilution gradient. We measured cumulative respiration in both water-filtered and no filtered microcosms in exactly the same way as explained in the Method section in the main text. Then, we used Pearson correlations to explore whether similar respiration trends were found in both water-filtered and non filtered microcosms. Our results supported that filtered and non filtered microcosms provide exactly the same results for the three water systems included here (Wheeny Creek: Pearsons'r = 0.98; $P = 0.004$; Richmond Lagoon: Pearsons'r = 0. 98; $P < 0.001$; and Parramata River: Pearsons'r = 0.99; $P < 0.001$).

CO_2 fluxes measurements (Milli-Q water). It could be argued that the responses in microbial

respiration that we reported here may be the consequence of a non-linear CO_2 release from water; and thus that CO_2 could be taken up by the water and not released into the headspace unless there is a lot of CO_2 being produced. Such a process could produce the linear or accelerating pattern observed. To discard this issue we prepared 18 water microcosms (40 mL of water) in 125mL serum bottles using Milli-Q water and injected different CO_2 concentrations in water across these microcosms. We then incubated these bottles for 10 minutes at 150rpm and measured the concentration of CO_2 in the headspace with an infrared gas analyser (IRGA PP systems WMA2, Amesbury, MA, USA) as explained in the Material and Method section of this manuscript. Results indicate that CO_2 release follows a linear trend (Fig S10), discarding any bias in our posteriori analyses.

Shift in microbial richness and composition during incubation period (Australia.1 and UK). It could be argue that both microbial richness and community composition might have changed during the incubation period during which ecosystem functions were measured. To address this important point, we measured richness and composition at the end of the experiment (after 2 weeks incubation period) in two of our experiments (Australia.1 and UK) using 454 sequencing (UK) and T-RLFP (Australia.1), respectively. We then conducted further analyses to explore the relationship of microbial diversity and composition at the beginning and at the end of the experiment (linear regressions for microbial richness and mantel tests using Bray-Curtis similarity for microbial composition) to ensure that the reported patterns in richness and composition were maintained for each dilution during these two weeks incubation period. Note that in the case of 454 data, the matrix of composition was analysed at the OTU level.

In all cases (two sites from Australia.1 and two sites from UK), microbial richness at the beginning of the experiment was highly related to those at the end of the experiments (LF: Pearson's r = 0.84, $P = 0.010$; LR: Pearson's r = 0.95, $P < 0.001$; HR: Pearson's r = 0.75, $P = 0.019$; FC: Pearson's r = 0.58, $P = 0.10$). Similarly, microbial composition at the beginning of the experiment was highly related to the one at the end of the experiments (LF: Pearson's r = 0.94, $P < 0.001$; LR: Pearson's r = 0.75, $P < 0.001$; HR: Pearson's r = 0.56, $P < 0.001$; FC: Pearson's r = 0.26, $P = 0.050$). Thus, albeit differences along time are probable, these results support the main patterns in bacterial richness and composition are maintained with time.

References:

Archer, E., (2013). *Estimate permutation p-values for importance metrics*. R package version 1.5.2.

Bolger, A.M., Lohse, M. & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114-2120.

Bragg, L., Stone, G., Imelfort, M., Hugenholtz, P. & Tyson, G.W. (2012). Fast, accurate error-correction of amplicon pyrosequences using Acacia. *Nature Methods* 9, 425-426.

Breiman, L. (2001) *Machine Learning* 45, 5 (2001).

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N. *et al.* (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7, 335-336.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalev,i D., Hu, P., Andersen, G.L. (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental,Microbiology* 72, 5069-72.

Edgar, R., Haas, B.J., Clemente, J.C., Quince, C., Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27, 2194-2200.

Edgar, R.G. et al. (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* 10, 996-998.

Edwards, C.D., Fowler, G.N. & Lawton, L.A. (2008). Biodegradation of microcystins and nodularin in freshwaters. *Chemosphere* 73, 1315-1321.

Fortmann-Roe, S. (2013). *Accurate, Adaptable and Accessible Error Metrics for Predictive Models*. R package version 0.9.

Kolb, S., Knief, C., Stubner, S. & Conrdad, R. (2003). Quantitative detection of methanotrophs in soil by novel pmoA-targeted real-time PCR assays. *Applied and Environmental Microbiology* 69, 2423-2429.

Liaw, A. & Wiener, M. (2002). *Rnews*, 2/3, 18.

Magoc, T., Salzberg, S.L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27, 2957-2963.

McDonald, D., Price, M.N., Goodrich, J. et al. (2012). An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *The ISME Journal* 6, 610-618.

Muyzer, G., De Waal, E.C., Uitterlinden, A.G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, 59, 695-700.

Schloss, P.D. et al. (2009) Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities *Applied And Environmental Microbiology* 75, 7537-7541.

Lane, D.J. (1991). *Nucleic Acid Techniques in Bacterial Systematics* (John Wiley and Sons, NY, USA)

Wang, Q. et al. (2007) Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy.*Applied and Environmental Microbiology* 73, 5261-5267.

Wei, C-L., Rowe, G.T., Escobar-Briones, E., Boetius, A., Soltwedel, T., Caley, M.J., *et al.* (2010). Global Patterns and Predictions of Seafloor Biomass Using Random Forests *PLOS ONE* 5, e15323.

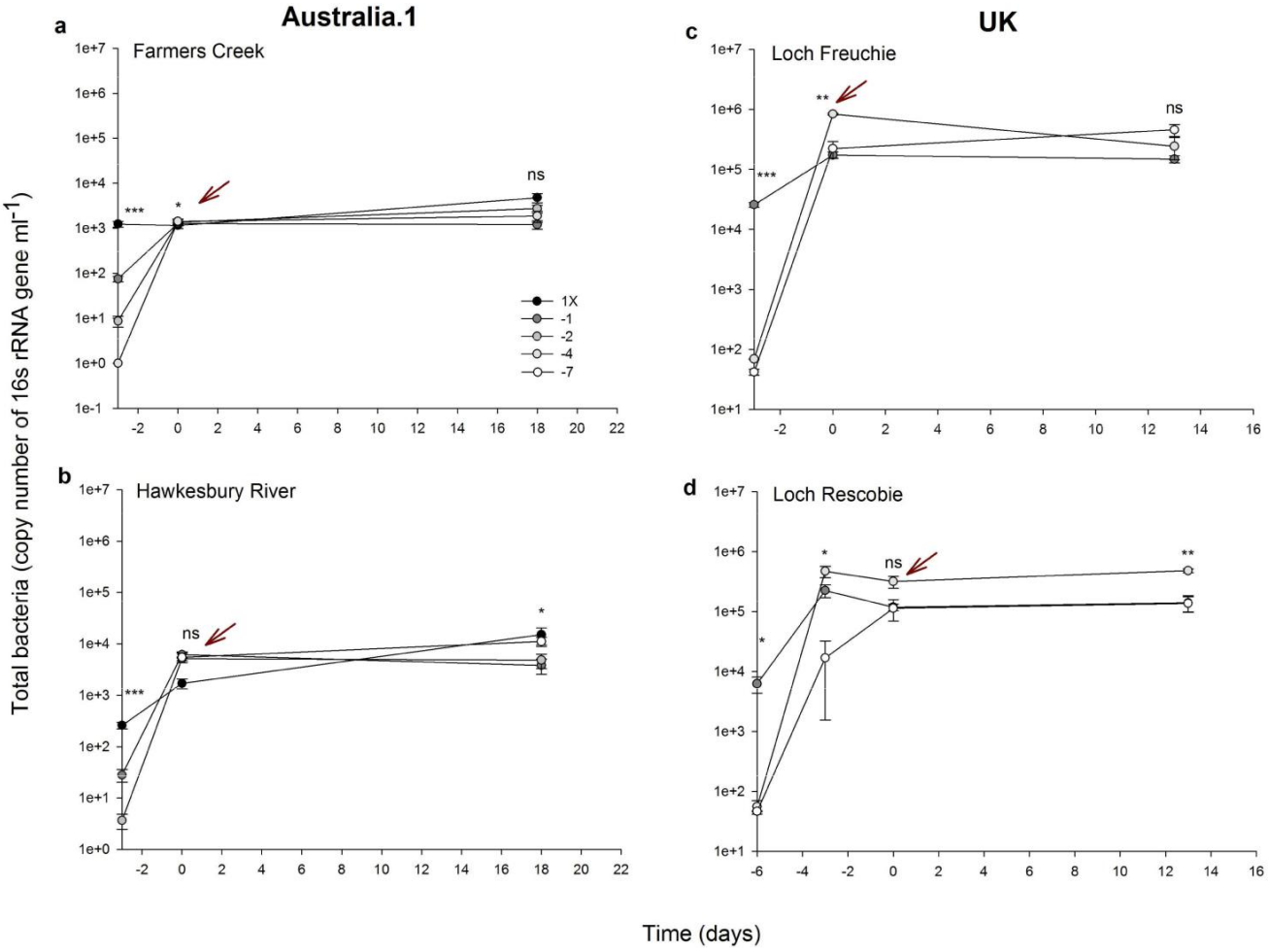


Figure S1. Bacterial biomass over time at different dilution levels (1, 10^{-1}, 10^{-2} and 10^{-4} for Australia.1 and 10^{-1}, 10^{-4} and 10^{-7} for UK) estimated for: a) Farmers Creek, b) Hawkesbury river, c) Loch Freuchie and d) Loch Rescobie. The different labels (1x, 10^{-1}, 10^{-2}, 10^{-4} and 10^{-7}) represent the exponent of each dilution level. Significant differences arising from one-way ANOVA analyses for each of the time with time as a fixed factor are as follows: ns = $p > 0.05$, * $p < 0.05$ and ** and $p < 0.01$. Error bars indicate standard error (n=3). Arrows indicate the beginning of the experiment.

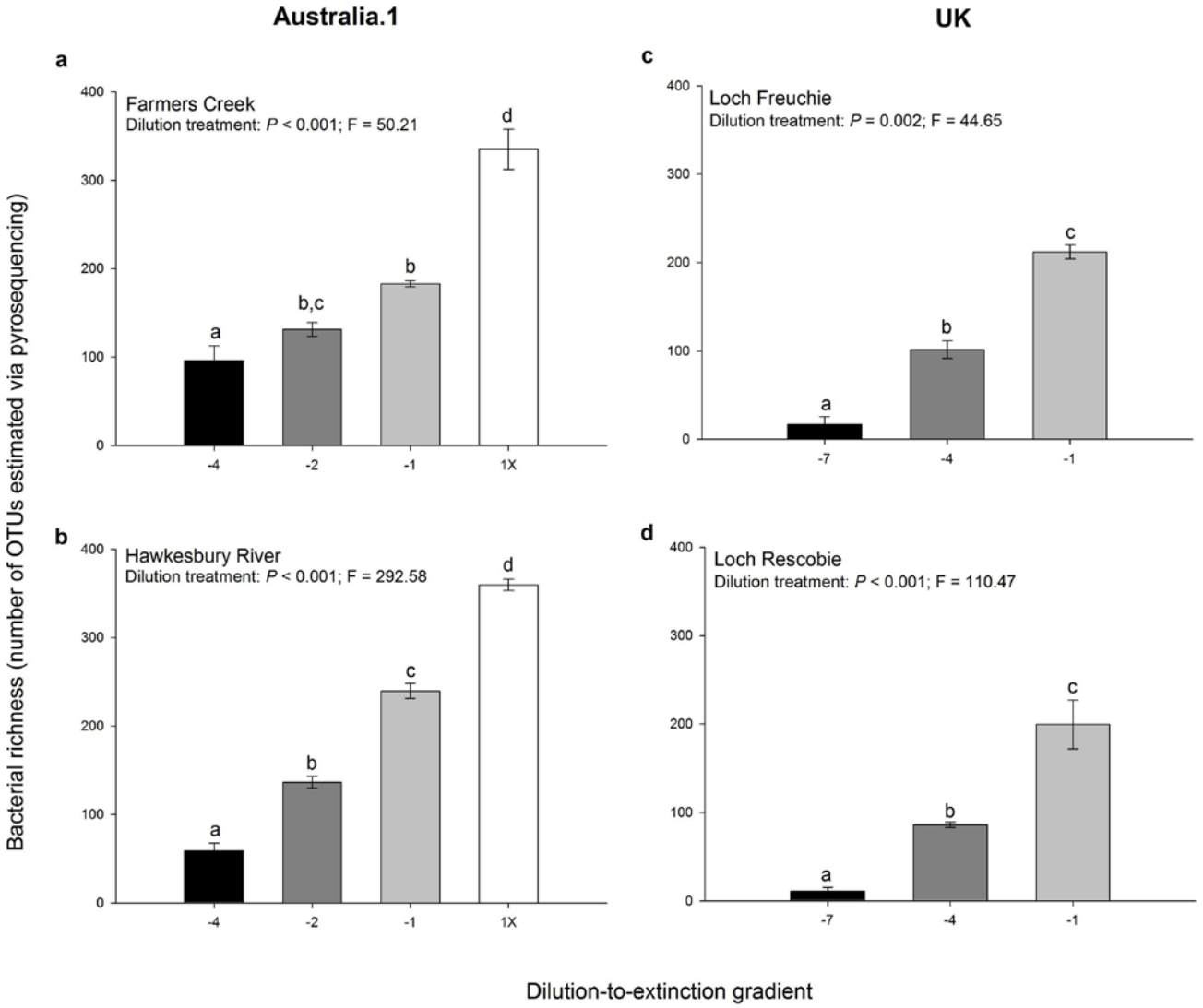


Figure S2. Bacterial richness (number of OTUs) estimated via pyrosequencing for the different dilution levels (1, 10^{-1}, 10^{-2} and 10^{-4} for Australia.1 and 10^{-1}, 10^{-4} and 10^{-7} for UK) at the beginning of the experiment for: a) Farmers Creek, b) Hawkesbury river, c) Loch Freuchie and d) Loch Rescobie. Error bars indicate standard error (n=3). The different labels (1, 10^{-1}, 10^{-2}, 10^{-4} and 10^{-7}) represent the exponent of each dilution level. Lower case letters represent the groups created by ANOVA post-hoc tests which compare bacterial richness among dilution levels.

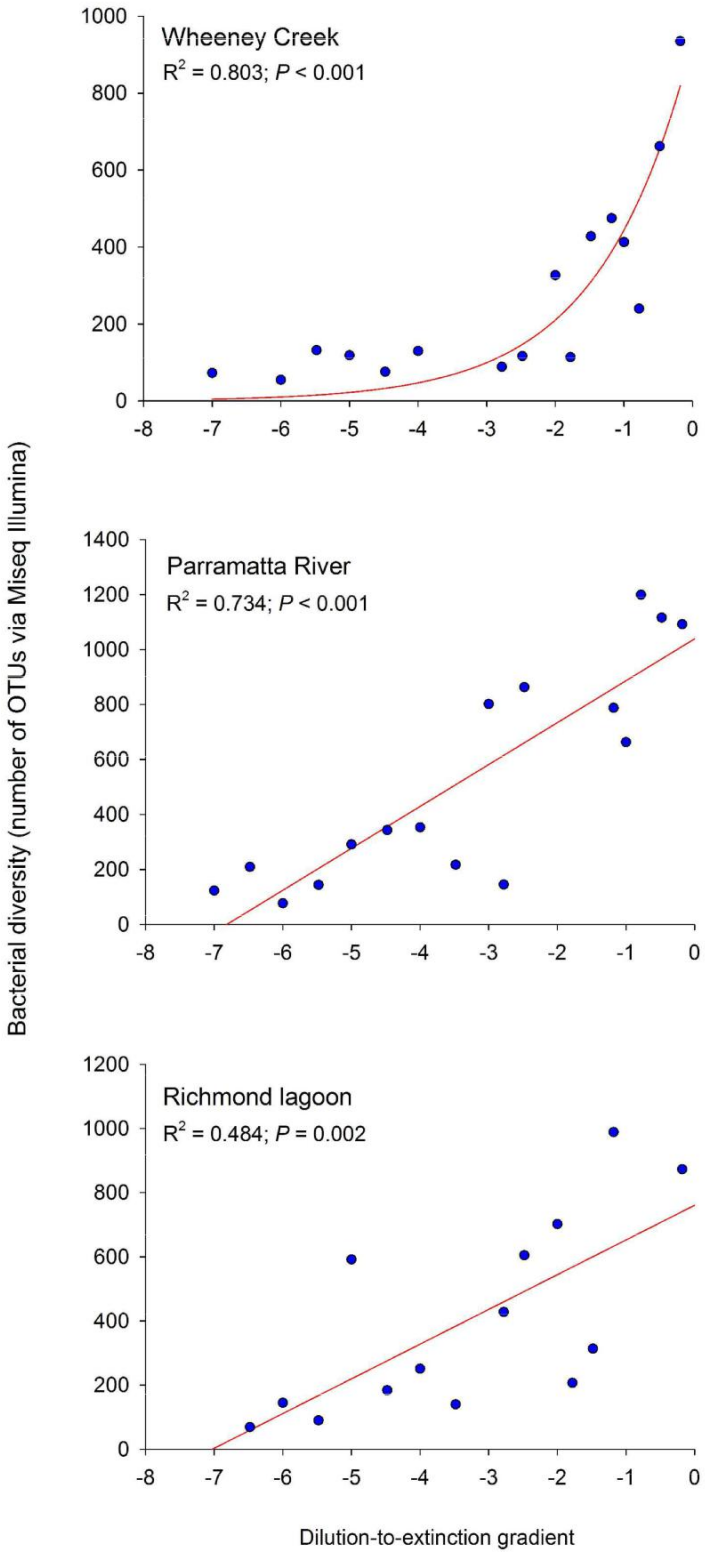


Figure S3. Relationship between the dilution to extinction gradient and bacterial diversity for Australia.2 sites. Solid lines indicate either exponential or linear fits.

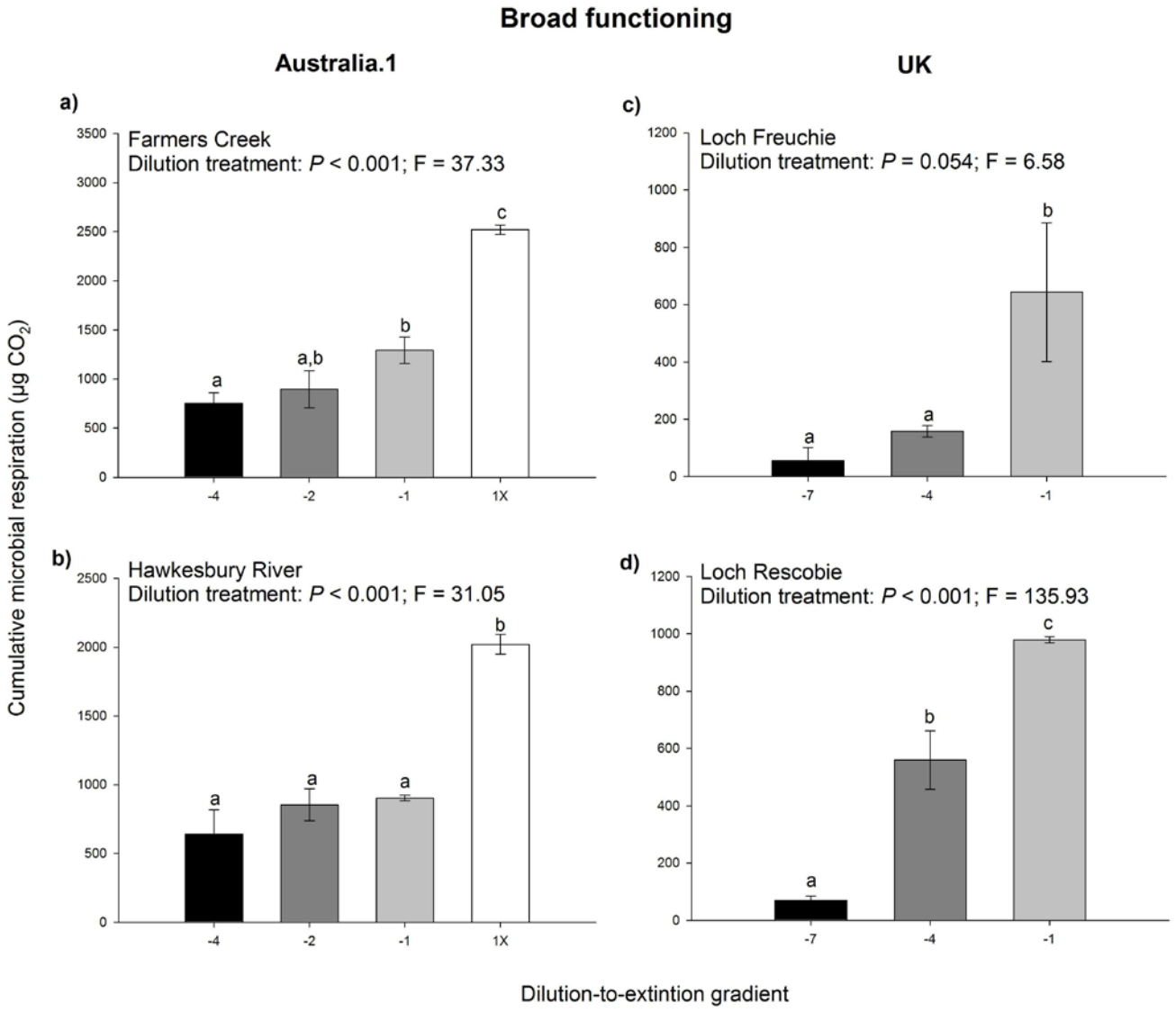


Figure S4. Cumulative microbial respiration for the different dilution levels (1, 10^{-1}, 10^{-2} and 10^{-4} for Australia.1 and 10^{-1}, 10^{-4} and 10^{-7} for UK) for: a) Farmers Creek, b) Hawkesbury river, c) Loch Freuchie and d) Loch Rescobie. The different labels (1, 10^{-1}, 10^{-2}, 10^{-4} and 10^{-7}) representthe exponent of each dilution level. Error bars indicate standard errors (n=3). Lower case letters represent the groups created by ANOVA post-hoc tests which compare bacterial richness among dilution levels.

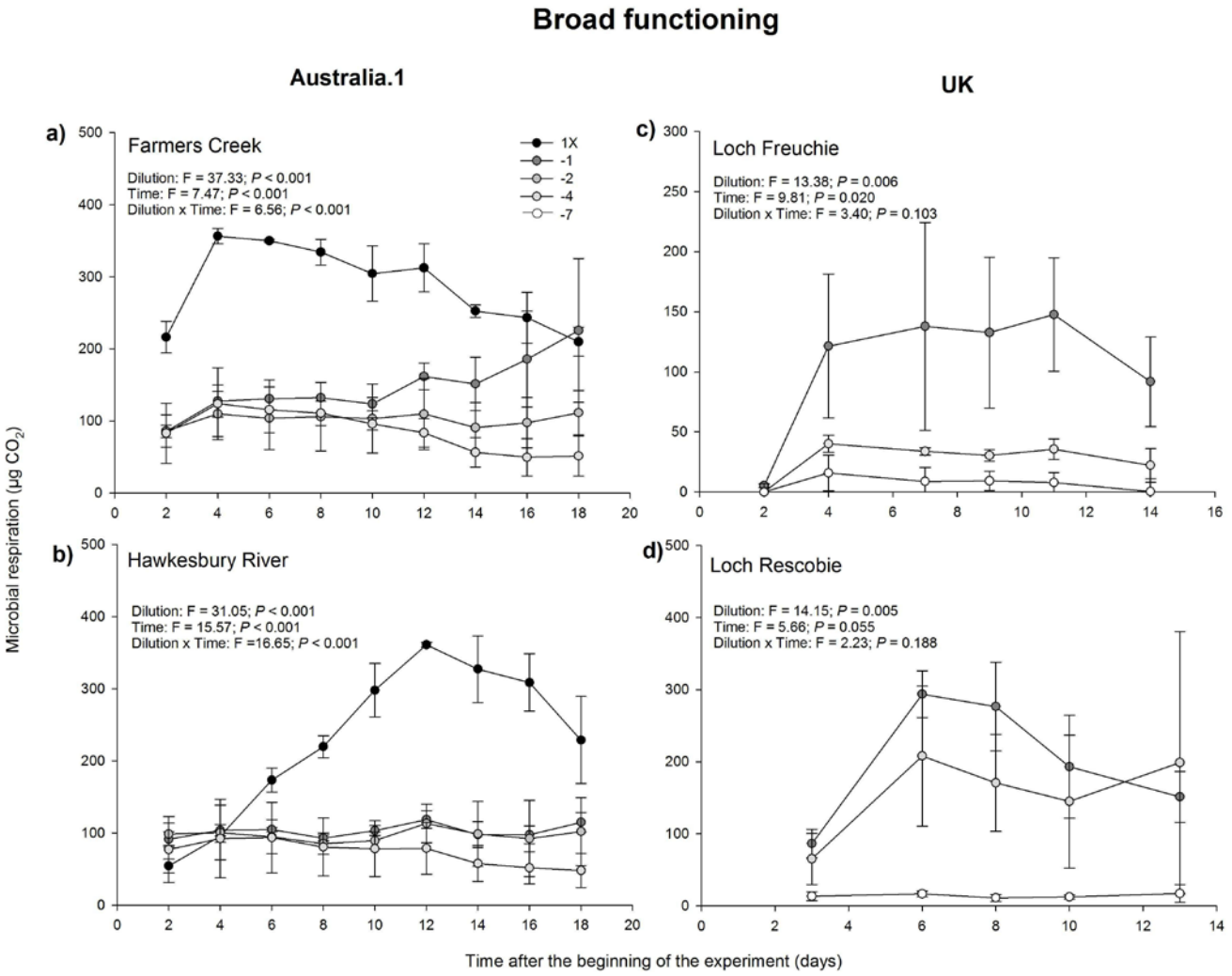


Figure S5. Changes in microbial respiration with time during the functionality assay for a) Farmers Creek, b) Hawkesbury river, c) Loch Freuchie and d) Loch Rescobie. The different labels (1, 10^{-1}, 10^{-2}, 10^{-4} and 10^{-7}) representthe exponent of each dilution level. Error bars indicate standard error s (n=3). Differences among dilution treatments and time for microbial respiration of each studied site were evaluated using two-way ANOVAs, with dilution treatment as a fixed factor and repeated measures of time.

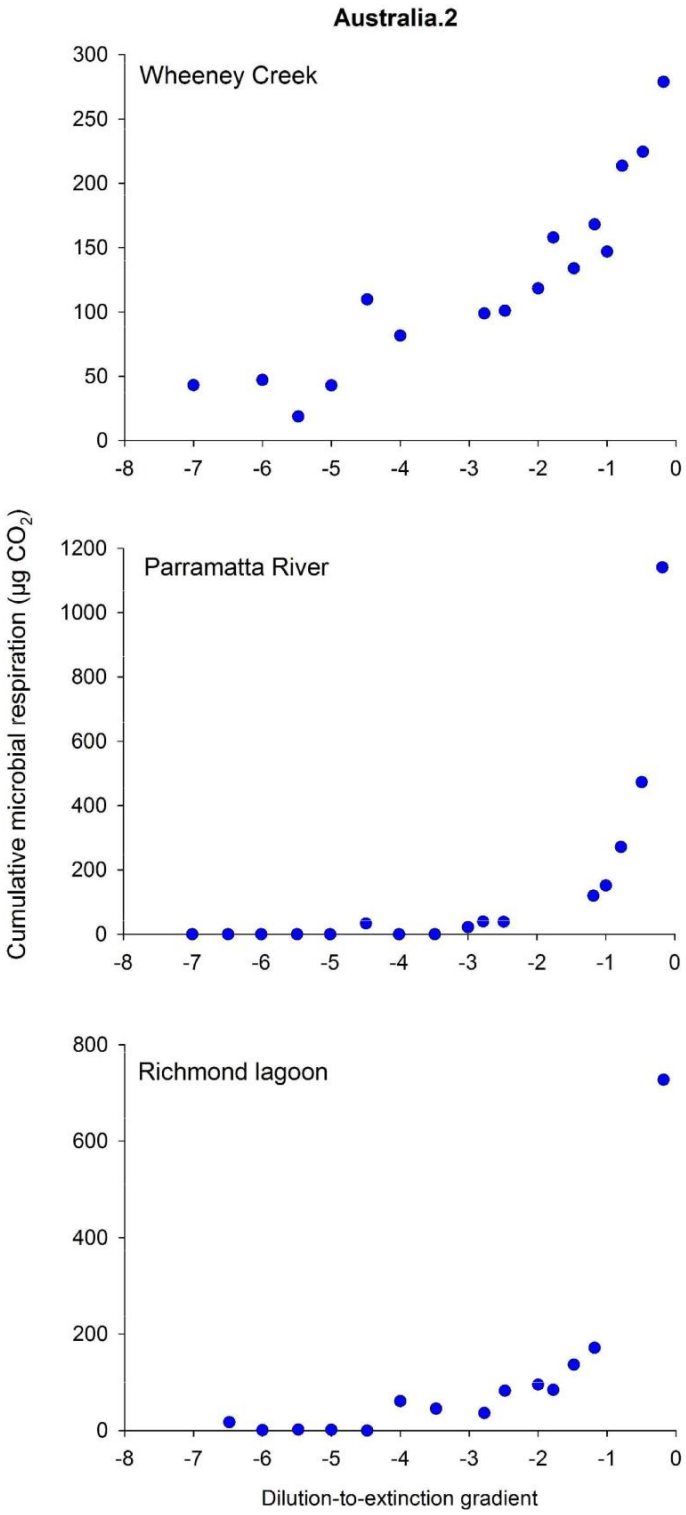


Figure S6. Scatter plots illustrating the dependency between cumulative microbial respiration and the dilution to extinction gradient for Australia.2 sites.

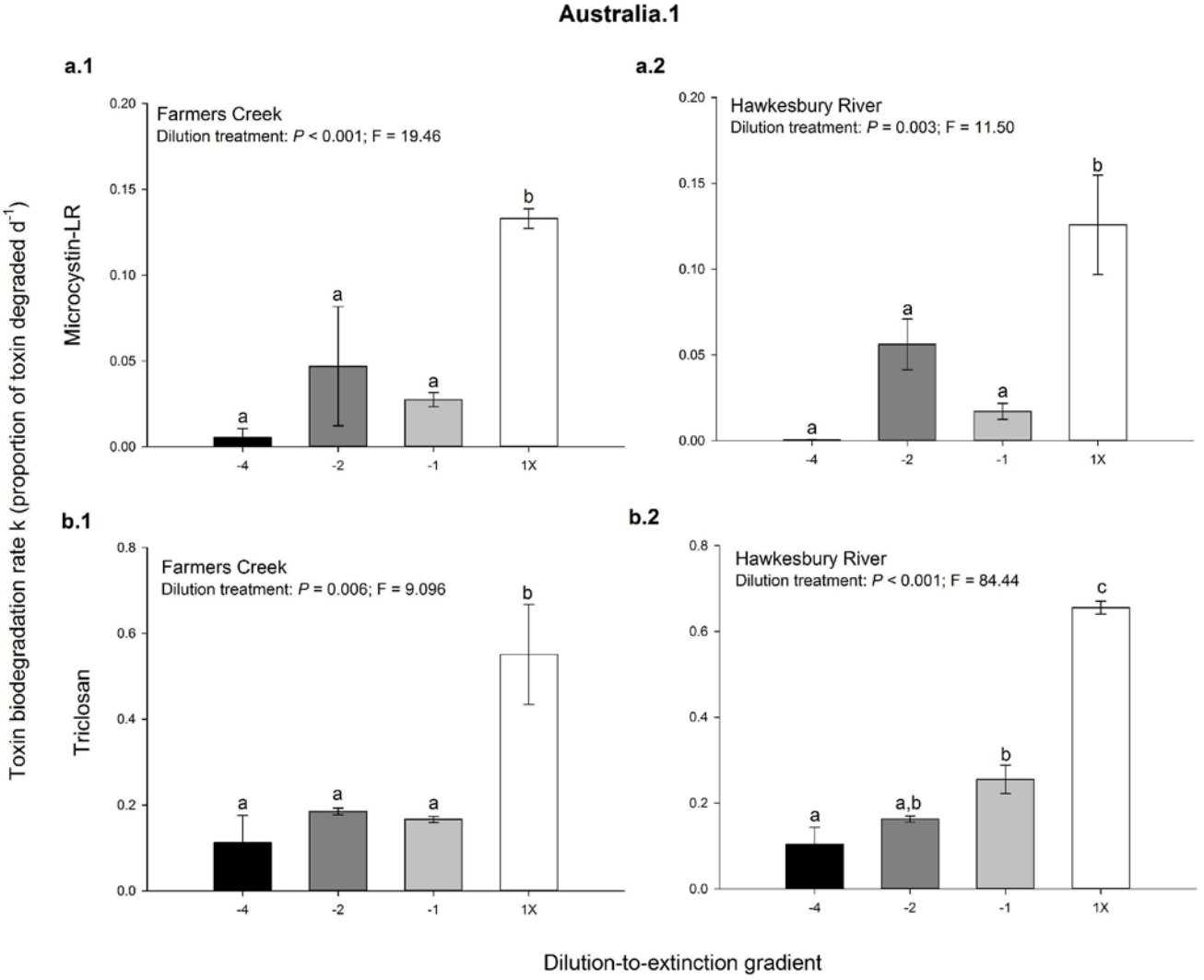


Figure S7. Mycrocystin-LR (a) and Triclosan (b) biodegradation rate constant k (proportion of toxin degraded day^{-1}) for the different dilution levels (1, 10^{-1}, 10^{-2} and 10^{-4} for Australia.1 and 10^{-1}, 10^{-4} and 10^{-7} for UK) for: 1) Farmers Creek and 2) Hawkesbury river. The different labels (1, 10^{-1}, 10^{-2} and 10^{-4}) representthe exponent of each dilution level. Error bars indicate standard errors (n=3). Lower case letters represent the groups created by ANOVA post-hoc tests which compare bacterial richness among dilution levels.

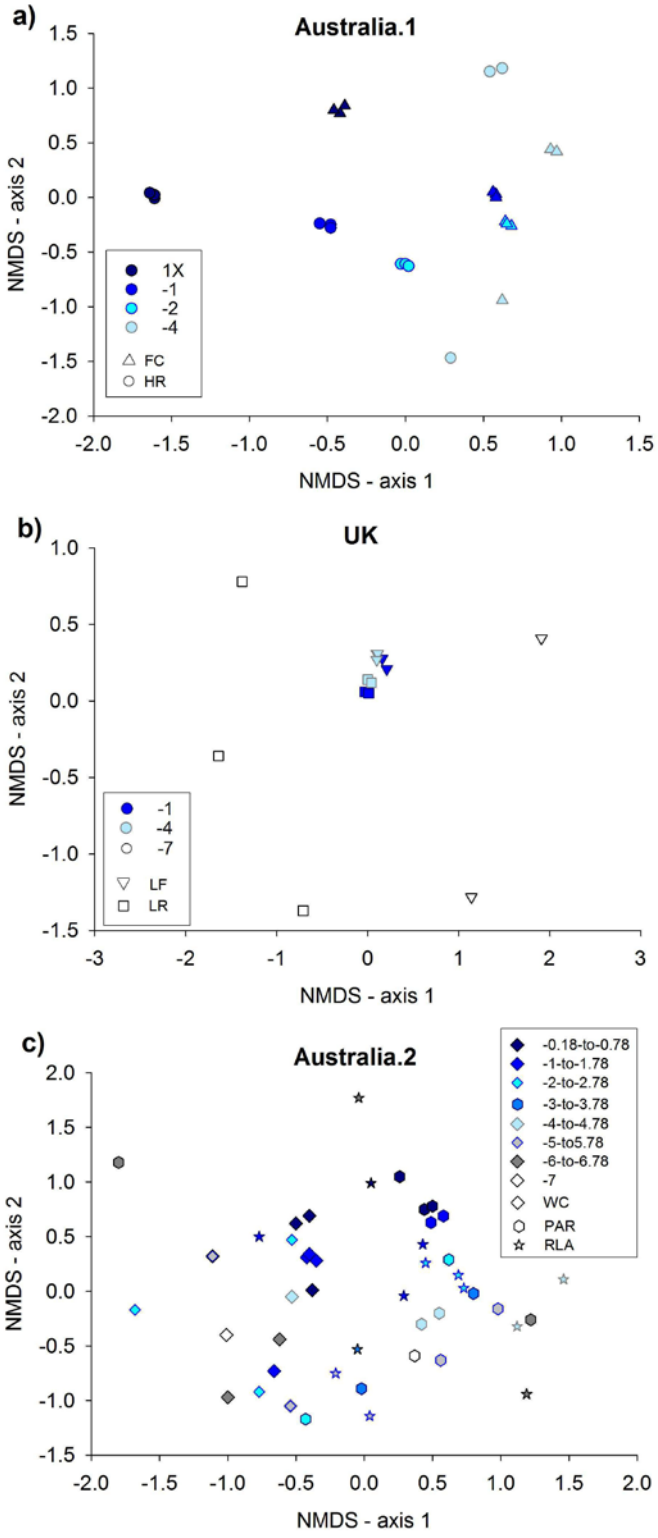


Figure S8. nMDS graph exploring the effect of the different dilution levels on bacterial composition at a OTUs level for Australia.1, UK and Australia.2 sites.

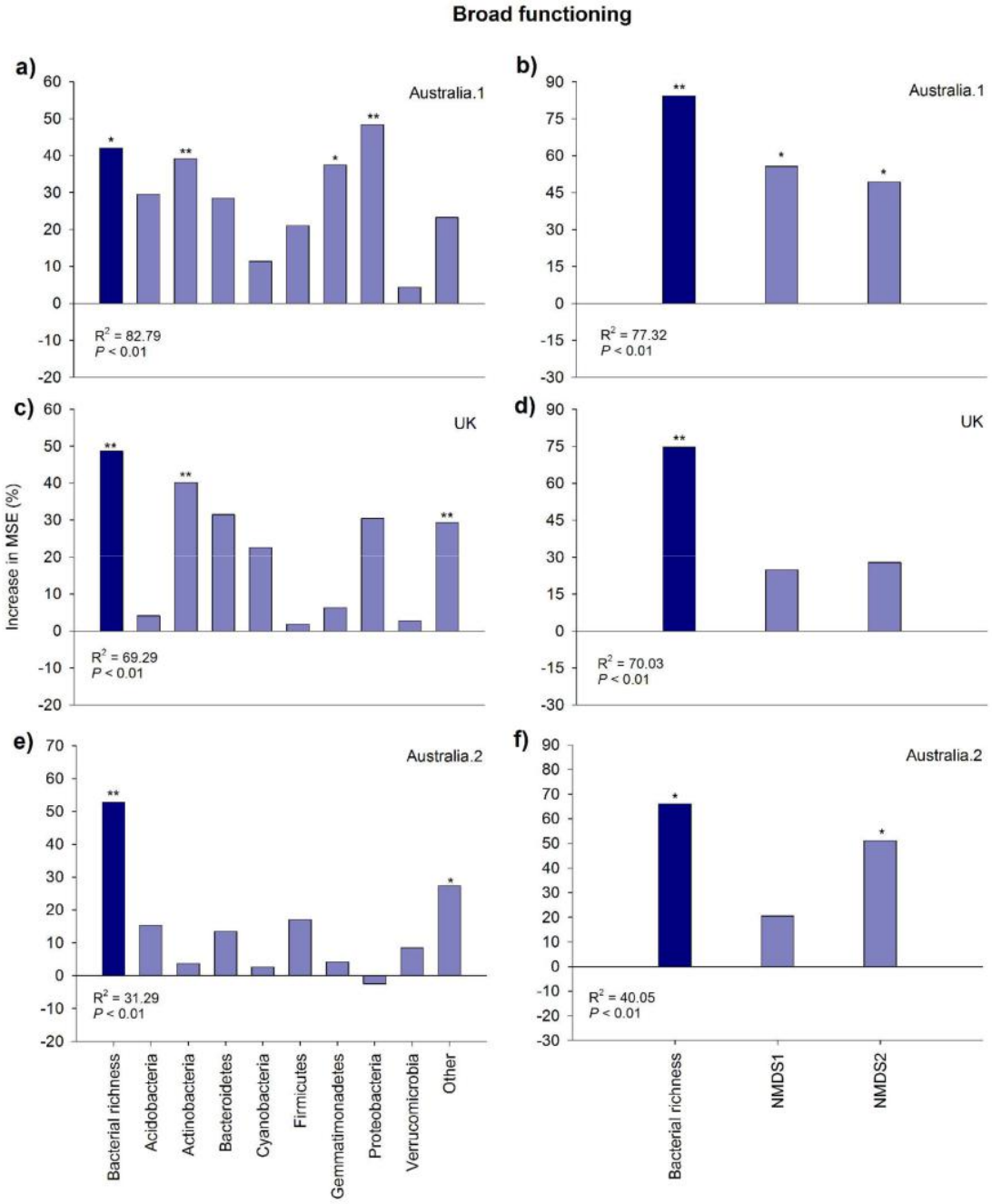


Figure S9. Random Forest mean predictor importance (% of increase of mean square error) of microbial richness and composition (i.e. relative abundance of main groups of microorganisms or main axes from a nMDS including information at a OTUs level) as drivers of cumulative microbial respiration in this study for the Australia.1, UK and Australia.2 sites. This accuracy importance measure was computed for each tree and averaged over the forest (5000 trees). Significance levels are as follows: *$P < 0.05$ and ** $P < 0.01$.

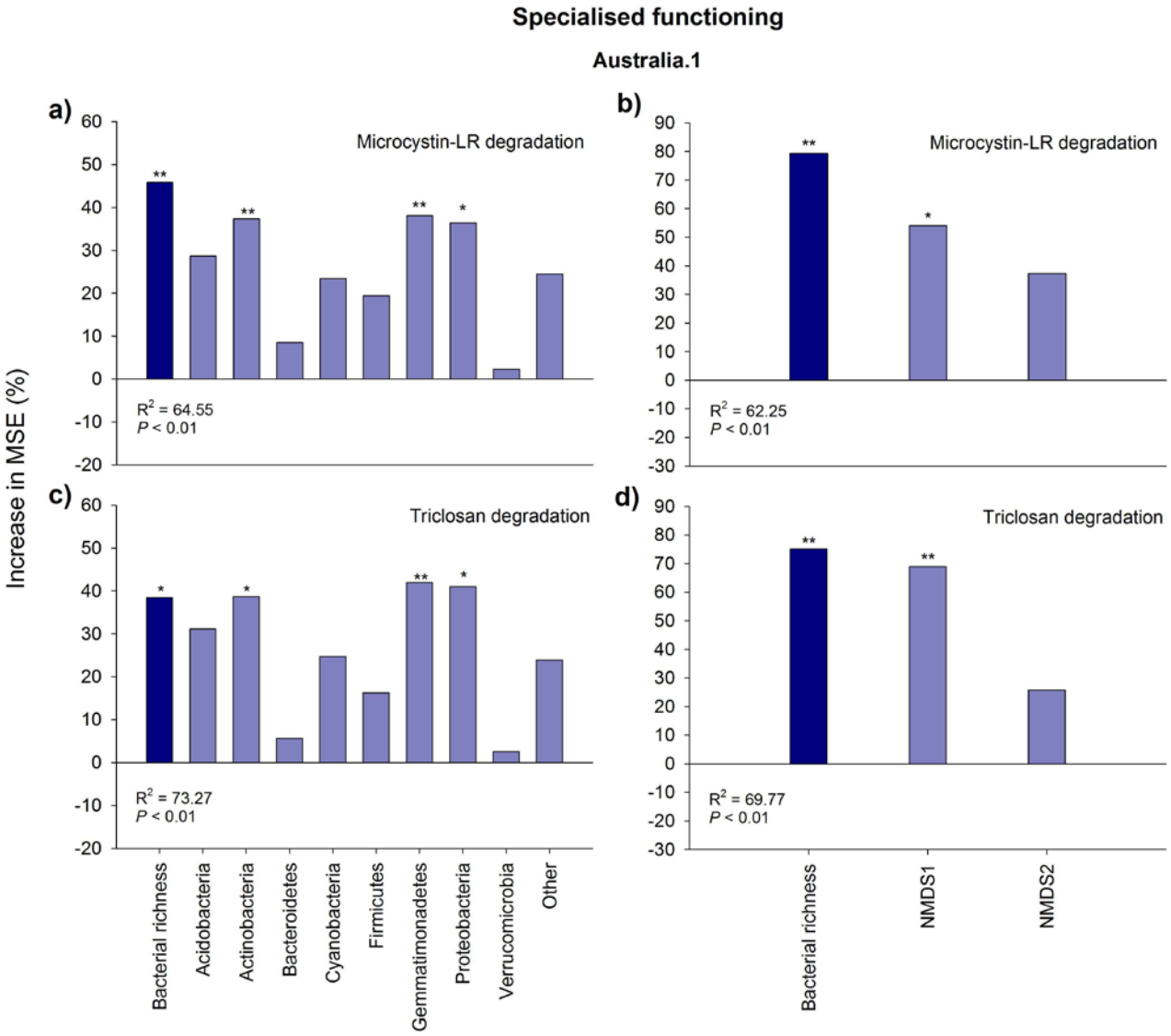


Figure S10. Random Forest mean predictor importance (% of increase of mean square error) of microbial richness and composition (i.e. relative abundance of main groups of microorganisms or main axes from a nMDS including information at a OTUs level) as drivers of MC-LR and Triclosan degradation in this study for the Australia.1 sites. This accuracy importance measure was computed for each tree and averaged over the forest (5000 trees). Significance levels are as follows: *$P < 0.05$ and ** $P < 0.01$.

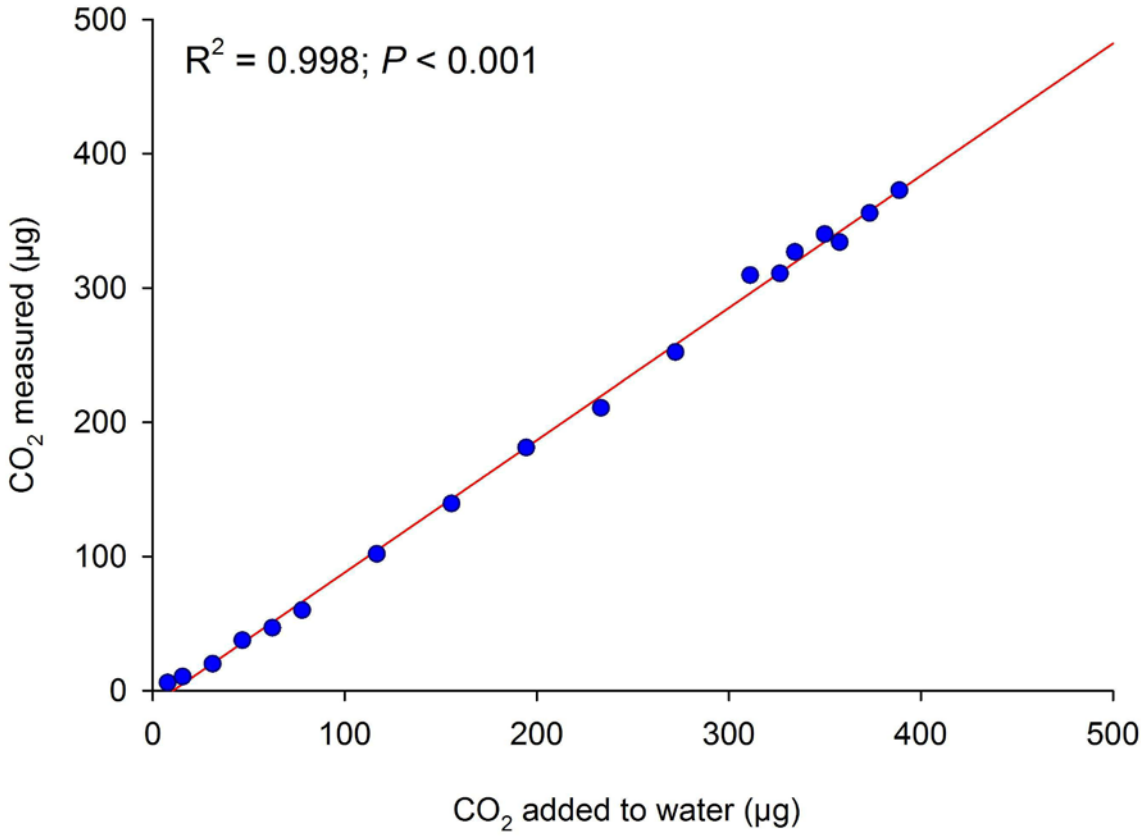


Figure S11. Calibration curve for microcosm CO_2 emissions. A known amount of CO_2 was added to 40ml of water in a 125ml serum bottle. Bottles were shaken for 10 min and then the concentration of CO_2 was measured using an IRGA PP systems WMA2 (Amesbury, MA, USA).

Table S1. Correlation (Spearman's ρ) between bacterial diversity (bacterial richness obtained via 454 pyrosequencing for Australia.1 and UK and Illumina Miseq for Australia.2) and Shannon and Simpson diversity (obtained via 454 pyrosequencing for Australia.1 and UK and Miseq Illumina for Australia.2). FC = Farmers Creek; HR = Hawkesbury River; LF = Loch Freuchie; LR = Loch Rescobie; WC = Wheeney Creek; PAR = Parramatta River; RLA = Richmond Lagoon.

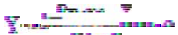
	Shannon		Simpson	
Site	**ρ**	***P***	**ρ**	***P***
FC	0.944	<0.001	0.867	<0.001
HR	0.993	<0.001	0.919	<0.001
LF	0.964	<0.001	1.000	<0.001
LR	0.976	<0.001	0.967	<0.001
WC	0.868	<0.001	0.802	<0.001
PAR	0.903	<0.001	0.857	<0.001
RLA	0.833	<0.001	0.874	<0.001

Table S2. Model fit statistics and AICc index for the different functions describing the relationship between bacterial species richness based on 454 pyrosequencing / Illumina Miseq (X) and ecosystems functions (cumulative microbial respiration, Microcystin-LR and Triclosan; Y). AICc measures the relative goodness of fit of a given model; the lower its value, the more likely it is that this model is correct. Two models models with ΔAICc > 2 are substantially different. FC = Farmers Creek; HR = Hawkesbury River; LF = Loch Freuchie; LR = Loch Rescobie; WC = Wheeney Creek; PAR = Parramatta River; RLA = Richmond Lagoon. The power function can fit multiple shapes and may represent either functional or no functional redundancy depending on each particular case.

Function	Site	Model	R^2	*P*	AICc	DeltaAICc	Selected Model(s)	Model group
Microbial respiration	**FC**	**Logarithmic** [1]	0.755	<0.001	150.556	8.553		
		[2] Michaelis-Menten	0.879	<0.001	142.024	0.021	✓	**Redundancy**
		Power [3]	0.867	<0.001	142.008	0.005	✓	**No redundancy**
		Linear [4]	0.879	<0.001	142.003	**0.000**	✓	**No redundancy**
		Exponential [5]	0.858	<0.001	142.856	0.853	✓	**No redundancy**
	HR	**Logarithmic** [1]	0.570	0.004	151.273	11.223		
		Michaelis-Menten [2]	0.696	<0.001	147.104	7.054		
		Power [3]	0.712	<0.001	146.455	6.405		
		Linear [4]	0.747	<0.001	144.891	4.841		
		Exponential [5]	0.831	<0.001	140.050	**0.000**	✓	**No redundancy**
	LF	**Logarithmic** [1]	0.510	0.071	87.981	25.952		
		Michaelis-Menten [2]	0.833	0.004	80.437	18.408		
		Power [3]	0.957	<0.001	70.8561	8.827		
		Linear [4]	0.856	0.002	79.381	17.352		
		Exponential [5]	0.988	<0.001	62.029	**0.000**	✓	**No redundancy**
	LR	**Logarithmic** [1]	0.855	0.001	92.642	18.245		
		Michaelis-Menten [2]	0.985	<0.001	74.397	**0.000**	✓	**Redundancy**
		Power [3]	0.982	<0.001	75.860	1.463	✓	**Redundancy**
		Linear [4]	0.979	<0.001	76.891	2.494		
		Exponential [5]	0.900	<0.001	89.699	15.302		
	WC	**Logarithmic** [1]	0.618	<0.001	128.676	2.116		
		Michaelis-Menten [2]	0.618	<0.001	128.689	2.129		
		Power [3]	0.659	<0.001	126.863	0.303	✓	**Redundancy**
		Linear [4]	0.665	<0.001	126.560	**0.000**	✓	**No redundancy**
		Exponential [5]	0.637	<0.001	127.839	1.279	✓	**No redundancy**
	PAR	**Logarithmic** [1]	0.310	0.025	183.116	5.414		
		Michaelis-Menten [2]	0.374	0.011	181.543	3.841		
		Power [3]	0.508	0.001	177.702	**0.000**	✓	**No redundancy**

		Linear[4]	0.389	0.005	180.068	2.366		
		Exponential[5]	0.449	0.002	178.394	0.692	✓	**No redundancy**
	RLA	**Logarithmic**[1]	0.333	0.030	148.144	1.159	✓	**Redundancy**
		Michaelis-Menten[2]	0.263	0.060	149.544	2.559		
		Power[3]	0.386	0.017	146.985	**0.000**	✓	**No redundancy**
		Linear[4]	0.352	0.025	147.738	0.753	✓	**No redundancy**
		Exponential[5]	0.373	0.020	147.288	0.303	✓	**No redundancy**
MC-LR	**FC**	**Logarithmic**[1]	0.637	0.002	-73.932	4.959		
		Michaelis-Menten[2]	0.629	0.002	-73.689	5.202		
		Power[3]	0.760	<0.001	-78.891	**0.000**	✓	**No redundancy**
		Linear[4]	0.749	<0.001	-78.385	0.506	✓	**No redundancy**
		Exponential[5]	0.719	<0.001	-77.006	1.885	✓	**No redundancy**
	HR	**Logarithmic**[1]	0.435	0.019	-68.094	2.982		
		Michaelis-Menten[2]	0.481	0.012	-69.124	1.952	✓	**Redundancy**
		Power[3]	0.533	0.007	-70.384	0.692	✓	**No redundancy**
		Linear[4]	0.506	0.009	-69.707	1.369	✓	**No redundancy**
		Exponential[5]	0.559	0.005	-71.076	**0.000**	✓	**No redundancy**
Triclosan	**FC**	**Logarithmic** [1]	0.479	0.012	-37.780	6.432		
		Michaelis-Menten[2]	0.622	0.002	-41.624	2.588		
		Power[3]	0.670	0.001	-43.240	0.972	✓	**No redundancy**
		Linear[4]	0.641	0.002	-42.241	1.971	✓	**No redundancy**
		Exponential[5]	0.695	<0.001	-44.212	**0.000**	✓	**No redundancy**
	HR	**Logarithmic**[1]	0.686	<0.001	-41.396	24.152		
		Michaelis-Menten[2]	0.842	<0.001	-49.687	15.861		
		Power[3]	0.910	<0.001	-56.444	9.104		
		Linear[4]	0.862	<0.001	-51.258	14.290		
		Exponential[5]	0.958	<0.001	-65.548	**0.000**	✓	**No redundancy**

[1] $Y = a + b \cdot \log(X)$

[2] 

[3] $Y = a \cdot X^{b}$

[4] $Y = a + b \cdot X$

[5] $Y = a \cdot e^{bX}$

Table S3. Summary results of the two-way ANCOVA analyses carried out with the ecosystem functions (cumulative microbial respiration, Microcystin-LR and Triclosan) as dependent variables and

the site (FC and HR for Australia and LF and LR for UK) and dilution (1, 10^{-1}, 10^{-2} and 10^{-4} for Australia and 10^{-1}, 10^{-4} and 10^{-7} for UK) as fixed factors. Bacterial biomass was always included as a co-variable. df = degrees of freedom. *P* values below 0.05 are in bold. FC = Farmers Creek; HR = Hawkesbury River; LF = Loch Freuchie; LR = Loch Rescobie.

Location	Function	Factor	df	F	*P*
Australia.1	**Microbial respiration**	**Biomass**	1	0.834	0.376
		Dilution	3	39.007	**<0.001**
		Site	1	0.254	0.621
		Dilution x Site	3	1.871	0.178
		Res	15		
UK		**Biomass**	1	0.125	0.733
		Dilution	2	36.734	**<0.001**
		Site	1	4.709	0.062
		Dilution x Site	2	2.531	0.141
		Res	8		
Australia.1	**Microcystin-LR**	**Biomass**	1	0.043	0.839
		Dilution	3	12.457	**<0.001**
		Site	1	0.033	0.859
		Dilution x Site	3	0.499	0.688
		Res	15		
	Triclosan	**Biomass**	1	1.28	0.275
		Dilution	3	16.03	**<0.001**
		Site	1	0.26	0.615
		Dilution x Site	3	1.24	0.330
		Res	15		

Table S4. Partial correlation (Pearson's r) between bacterial richness (obtained via 454 pyrosequencing for Australia.1 and Uk and Illumina Miseq for Australia.2) and ecosystem functions controlling for biomass. FC = Farmers Creek; HR = Hawkesbury River; LF = Loch Freuchie; LR = Loch Rescobie; WC = Wheeney Creek; PAR = Parramatta River; RLA = Richmond Lagoon.

Function	Site	Parameter	
	FC	**Pearson's r**	0.897
		P-value	<0.001
	HR	**Pearson's r**	0.861
		P-value	0.001
	LF	**Pearson's r**	0.938
		P-value	0.006
	LR	**Pearson's r**	0.994
		P-value	<0.001
	WC	**Pearson's r**	0.821
		P-value	<0.001
	PAR	**Pearson's r**	0.694
		P-value	0.004
	RLA	**Pearson's r**	0.564
		P-value	0.045
MC-LR	FC	**Pearson's r**	0.769
		P-value	0.006
	HR	**Pearson's r**	0.667
		P-value	0.025
Triclosan	FC	**Pearson's r**	0.644
		P-value	0.032
	HR	**Pearson's r**	0.928
		P-value	<0.001