Supplementary information

Materials and methods: 454-pyrosequencing and noise removal

Tag-pyrosequencing was done with Roche 454 Titanium platform following manufacturer protocols (454 Life Science). Amplification of the hypervariable regions V1-V3 was done using Primers 28F (5'-GAGTTTGATCNTGGCTCAG) and 519R (5'-GTNTTACNGCGGCKGCTG). Approximately 400 bp long tags were obtained. PCR and subsequent sequencing are described in Dowd *et al.* (2008).

The raw tag-sequences were processed using QIIME (Caporaso *et al*., 2010). Briefly, to reduce sequencing errors and their effects, the multiplexed reads were first trimmed, quality-filtered and assigned to the samples, surface or bottom. The filtering criteria included a perfect match to the sequence barcode and primer, at least 400 bp in length, an average quality score (phred) of 28 within sliding windows of 50bp. Additionally, denoiser was used to reduce the amount of erroneous sequences (Reeder & Knight, 2010). The sequences were then clustered into Operational Taxonomic Units (OTUs) based on the relatedness of the sequences (97% similarity) with UCLUST, version 1.1.579 (Edgar, 2010). A representative sequence from each OTU was selected as the first cluster seed chosen by UCLUST. ChimeraSlayer implemented in Mothur (Schloss *et al*., 2011) use to check for chimeras. Then, taxonomy assignment was made with QIIME by searching the representative sequences of each OTU against the SILVA 16S/18S rDNA non-redundant reference dataset (SSU Ref 108 NR) (Quast *et al*., 2013) using the Basic Local Alignment Search Tool (BLAST) and an e-value of 0.03. Chimera, chloroplast, eukarya and archaea sequences were removed from the output fasta file that was used for building a table with the OTU abundance of each sample and the taxonomic assignments for each OTU.

Materials and methods: Isolation of bacterial cultures

Isolates were obtained on board by plating 100 µl of undiluted and 10x diluted sea-water from the surface sample, in triplicates, onto modified Zobell agar plates (i.e. 5 g peptone, 1 g yeast extract and 15 g agar in 1 l of 0.2 µm filtered 75% sea water). Agar plates were incubated at *in situ* temperature (~20 °C), in the dark, for 14 days. 326 bacterial colonies were selected and the cultures were subsequently purified by re-isolation three times in a month. Next, isolates were grown at 20 ºC on the same liquid medium and stored at -80 ºC with 25% (v/v) glycerol.

Materials and methods: Bacterial isolates PCRs

PCR, using Taq polymerase (Boehringer-Mannheim), of the Internal Transcribed Spacer (ITS) was done using primers ITS-F (5'-GTCGTAACAAGGTAGCCGTA) and ITS-R (5'-GCCAAGGCATCCACC) and the following thermal conditions: 94ºC for 2 min, then 32 cycles of 94 ºC for 15 sec, 55 ºC for 30 sec, 72 ºC for 3 min, followed by one cycle of 72 ºC for 4 min and 4 ºC on hold.

PCR of the 16S rRNA gene of the 148 chosen by their different ITS pattern were then amplified using bacterial 16S rRNA gene primers 27F (5'-AGAGTTTGATCMTGGCTCAG) and 1492R (5'-GTTTACCTTGTTACGACTT). The thermal conditions were as follows: 94 ºC for 5 min, then 30 cycles of 94 ºC for 1 min, 55 ºC for 1 min, 72 ºC for 2 min, followed by one cycle of 72 ºC for 10 min and 4 ºC on hold.

Materials and methods: Simulating the Required Sequencing Effort (RSE)

For each of 80 ensemble members, we simulated a random sequence of 10N individual species labels, where N is the present sequencing effort, by: 1) sampling a set of parameter values from the posterior distribution, 2) sampling relative abundances (proportions in the water sample) from the taxon abundance distribution given the parameter values, 3) sampling species counts (from hypothetical sequencing) using the multinomial distribution given the proportions and the total number of individuals 10N, and 4) converting the species counts into a randomly-ordered sequence of individual labels. The simulated RSE was then identified as the individual (tag) index for which the number of species observed earlier in the sequence first exceeded 90% of the simulated total richness (S).

Discussion: Simulation tests on the number of isolates retrieved in sequences

To simulate the number of isolates retrieved in sequences, we simulated 3000 sets of species counts using the method described above for RSE calculations, but with the total number of tags fixed at the present sequencing effort N. For each set of simulated sequencing counts, 38 species were selected at random without replacement from the list of all S counts (including zeros), and the number of these with non-zero counts was recorded to give the simulated number retrieved by sequencing r_s. The simulation p-value for the actual number of species retrieved by sequencing r was then taken as $(1 + \#(r_s \leq r))/3001$ following Davison & Hinkley (1997).

<u>Discussion: Simulation/bootstrap tests on the counts of isolates retrieved in sequences</u>

To simulate the counts of isolates retrieved in sequences, we again simulated 3000 sets of species counts as described above, and this time randomly selected without replacement either 9 or 14 species from the list of non-zero counts for each simulation. The mean, median, and maximum counts from this subset were recorded for each simulation, and p-values were calculated as described above assuming lower-than-random count statistics as an alternative hypothesis. For the bottom count statistics (14 species), we also performed the test assuming higher-than-random count statistics t on the alternative, hence calculating p-values as $(1 + \#(t_s \geq t))/3001$.

These tests were also repeated using a bootstrap method, thus avoiding the need to assume a parametric distribution. To do this, a vector of 9 or 14 species counts was randomly resampled *with* replacement from the observed species count vector. This was repeated over 9999 bootstraps and bootstrap p-values were calculated as $(1 + \#(t_s \leq t))/10000$ or $(1 + \#(t_s \geq t))/10000$, again following Davison & Hinkley (1997).

References:

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

Davison A, Hinkley D. (1997). Bootstrap methods and their applications. Cambridge University Press: New York.

Dowd SE, Callaway TR, Wolcott RD, Sun Y, McKeehan T, Hagevoort RG, *et al.* (2008). Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). *BMC Microbiol* **8**:125.

Edgar RC. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**:2460–2461.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, *et al.* (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* **41**:590–596.

Reeder J, Knight R. (2010). Rapidly denoising pyrosequencing amplicon reads by exploiting rank-abundance distributions. *Nat Methods* **7**:668-669.

Schloss PD, Gevers D, Westcott SL. (2011). Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. Gilbert, JA (ed). *PLoS One* **6**:e27310.

Figure legends

Figure S1. Goodness-of-fit of the best-approximating Sichel distribution to surface (A) and bottom (B) data sets. Observed and predicted count frequencies (numbers of OTUs with a given sample abundance) are plotted against tag counts (sample abundances) on a log-log scale. Goodness-of-fit is illustrated by the closeness of the predicted frequencies (posterior means, solid lines) to the observed frequencies (dots) as well as by the narrowness of the 95% prediction intervals (dashed lines) while still containing most of the data. The comparison is restricted to rare counts in the range 1–100 because these are likely the most important for estimating total richness and required sequencing effort, and because the computation of stable frequency prediction intervals for higher counts would require too many simulations (the intervals shown used 3 000). The distributions were however fitted to the full range of observed count frequencies ($f_{1–178569}$ and $f_{1–45414}$ for surface and bottom samples respectively).

Table captions

Table S1. Four different compound Poisson distributions were fitted to the surface and bottom data: the Poisson log-normal, the Poisson inverse Gaussian, the Poisson log-student, and the Poisson generalized inverse Gaussian (Sichel) distribution. As a robustness check we reran the Sichel fit for the surface sample excluding the counts of the most abundant species which, for this sample, was more than 3 times as abundant as the second most abundant species (see Surface*). The relative goodness-of-fit is assessed using Akaike's Information Criterion (AICc = -2×max(log likelihood) + $2p + 2p(p+1)/(n-p-1)$, where p is the number of fitted parameters and n is the number of data; Hurvich and

Tsai, 1989; Burnham and Anderson, 2002) and the deviance information criterion (DIC = -2×posterior mean(log likelihood) + p; Spiegelhalter *et al*., 2002; Quince *et al*., 2008). For the robustness check the selection criteria are placed in square parentheses since these cannot be compared to other rows. We also show the total species richness estimates from maximum likelihood ($\hat{S}_{ML}$) as well as the posterior median ($\hat{S}_{50\%}$) and the 95% credible bounds ($\hat{S}_{2.5\%}$ and $\hat{S}_{97.5\%}$) from the Bayesian MCMC method (Quince *et al*. 2008).

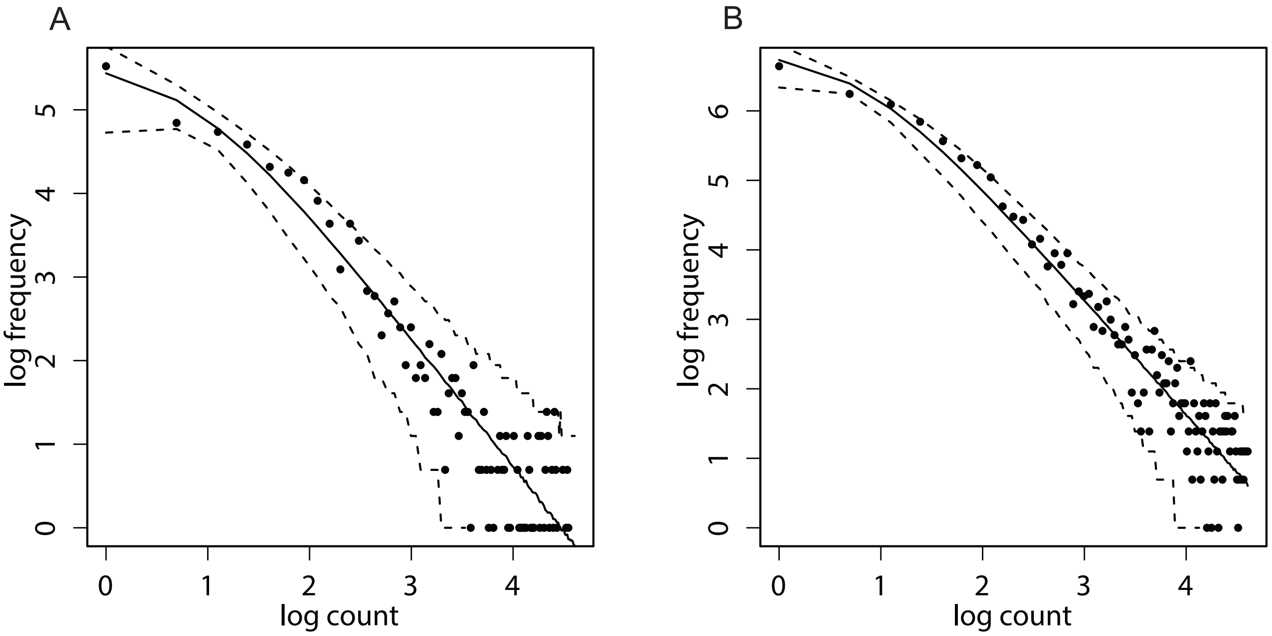
Reference: Hurvich CM and Tsai C-L (1989). Regression and time series model selection in small samples. *Biometrika* **76**: 297-307.

Table S2. (A) Semiparametric functional fits to surface sample collector's curve data and corresponding estimates of total species richness. A set of 12 convex, saturating functions were fitted to the rarefied species accumulation curve, sampled at intervals of 1 000 tags (hence 502 data points), using the nonlinear least squares function "nls" in R to estimate the parameters a, b etc. The absolute quality of the fits was measured using the generalized R2 values (defined for nonlinear fit as 1 - RSS/SSM, where RSS is the residual sum of squares and SSM is the sum of squares of the sample mean). The best-approximating model was selected as that which minimized Akaike's Information Criterion (AICc, in this case the Power Michaelis Menten (2) function was selected). The selected model was then used to estimate the total sample richness S as the asymptotic value of the function at x = Inf (final column shows the estimates for all candidate functions). Required sequencing effort (not shown) was predicted by inverting the selected function for x such that the value of the function was 0.9 times the estimated sample richness. Note that for certain 3 and 4 -parameter functions the R2 values are extremely high and differ only in the fourth or fifth decimal places (R2>0.999) yet the estimated richnesses can differ substantially (cf. Power Michaelis Menten (2) vs. Weibull Cumulative). For such functions, the AICc values also tend to differ by relatively large amounts, such that a model averaging strategy based on AIC weights would differ little from simply choosing the lowest-AICc model (Burnham and Anderson, 2002), and any assessment of model selection uncertainty based on AIC-weights is unlikely to predict the level of selection uncertainty observed in simulations (see Table S3). This latter is likely the result of the neglected error correlation in the functional fits.

Table S2. (B) Semiparametric functional fits to bottom sample collector's curve data and corresponding estimates of total species richness. A set of 12 convex, saturating functions were fitted to the rarefied species accumulation curve, sampled at intervals of 1 000 tags (hence 576 data points), using the nonlinear least squares function "nls" in R to estimate the parameters a, b etc. The absolute quality of the fits was measured using the generalized R2 values (defined for nonlinear fit as 1 - RSS/SSM, where RSS is the residual sum of squares and SSM is the sum of squares of the sample mean). The best-approximating model was selected as that which minimized Akaike's Information Criterion (AICc, in this case the Power Michaelis Menten (2) + offset function was selected). The selected model was then used to estimate the total sample richness S as the asymptotic value of the function at x = Inf (final column shows the estimates for all candidate functions). Required sequencing effort (not shown) was predicted by inverting the selected function for x such that the value of the function was 0.9 times the estimated sample richness. Note that for certain 3 and 4 -parameter functions the R2 values are extremely high and differ only in the fourth or fifth decimal places (R2>0.999) yet the estimated richnesses can differ substantially (cf. Power Michaelis Menten (2) vs. Weibull Cumulative). For such functions, the AICc values also tend to differ by relatively large amounts, such that a model averaging strategy based on AIC weights would differ little from simply choosing the lowest-AICc model (Burnham and Anderson, 2002), and any assessment of model selection uncertainty based on AIC-weights is unlikely to predict the level of selection uncertainty observed in simulations (see Table S3). This latter is likely the result of the neglected error correlation in the functional fits.

Table S3. Simulation-based tests of estimator performance, considering estimates of both the total species richness (S) and the required sequencing effort (RSE) i.e. number of tags required to observe a given fraction of the total richness in a new sample (e.g. 0.7S means 70% of the total richness). For each of four parametric distributions (Poisson log-normal, Poisson log-student, Poisson inverse-Gaussian, and Sichel) an ensemble of 80 sets of community abundances were randomly sampled from the parametric distribution; species data were then simulated by sampling from multinomial distributions with probabilities defined by the community abundances for each ensemble member. Distribution parameter values, including the total species richness, were also varied between ensemble members by sampling from the posterior distributions fitted to the observed data. Estimator performance is summarized by the %BIAS (ensemble average of estimate minus true value) and %RMSE (root-mean-square error), normalizing by the ensemble mean of the true value in both cases. Non-parametric species richness estimators included the Chao1 lower bound estimate (Chao, 1984), the coverage-based estimator for highly heterogeneous communities (ACE-1; Chao & Lee, 1992; Chao *et al*., 2000) and

the bias-corrected Chao estimate iChao (Chiu *et al*., 2014). The ACE-1 estimator was tested using two values of the cut-off count k to define "rare" species: the default value k = 10 and a larger value k = 100 as recommended by Chao & Shen (2012) for microbial communities (note, the estimated CV of the "rare" species was < 0.8 for k = 10 but > 0.8 for k = 100, where 0.8 is a threshold above which Chao & Shen (2012) recommend ACE-1 in preference to ACE). RSE was estimated for each nonparametric estimator by inverted the expression in Table 1 of Chao *et al*. (2014) and substituting the corresponding estimates of the zero-count frequency f0 = (S - S_{obs}) (using ACE-1 this is identical to the method proposed in Chao & Shen (2012) based on Shen *et al*. (2003) except for a negligible bias correction). Similar results (not shown) were obtained by substituting into equation (12) in Chao *et al*. (2009) (see also Colwell *et al*., 2012, equation 11). A semi-parametric AICc-selected estimator SP (AICc) was constructed by fitting 12 different functions to the collector's curves (rarefied species richness vs. sampling effort) and choosing the function with the lowest Akaike's Information Criterion (AICc). Total richness was then estimated as the asymptotic value of the selected function (see Table S2), and RSE was estimated by inverting the selected function for sampling effort given the required fraction of asymptotic richness. Nonparametric estimates were calculated using the R package SPECIES (Wang, 2011) and semiparametric functions were fitted using the nonlinear least squares function "nls" in R (R Core Team, 2013).



Supplementary Figure 1
Crespo et al.

Table S1.

Distribution	No. fitted parameters p	Sample	min(-log lik)	AICc	DIC	$\hat{S}_{\text{max. lik.}}$
Log-normal	3	Surface	869.4	1744.8	1744.8	2449
Log-student	4	Surface	840.6	1689.1	1689.3	1869
Inverse Gaussian	3	Surface	836.9	1679.8	1679.9	1644
Sichel	4	Surface	834.7	1677.4	1677.3	1618
Sichel	4	Surface*	[821.3]	[1650.7]	[1651]	1619
Log-normal	3	Bottom	1276.9	2559.8	2559.8	6843
Log-student	4	Bottom	1198.0	2404.1	2404.1	5850
Inverse Gaussian	3	Bottom	1230.0	2466.0	2466.0	5352
Sichel	4	Bottom	1176.9	2361.8	2362.1	5118

$\hat{S}_{\text{posterior mean}}$	$\hat{S}_{50\%}$	$\hat{S}_{2.5\%}$	$\hat{S}_{97.5\%}$
2501	2488	2238	2819
1897	1891	1797	2027
1644	1643	1594	1702
1615	1614	1568	1669
1616	1615	1568	1671
6856	6850	6544	7199
5867	5863	5701	6055
5353	5352	5250	5463
5109	5108	5027	5196

Table S2.

A

Function	Formula (x = #tags-1)	Number of Parameters	R^2	AICc
Michaelis Menten	$(ax)/(b+x)+1$	2	0.98414	4976
Negative Exponential	$a(1-\exp(-bx))+1$	2	0.93681	5670
Power Michaelis Menten (1)	$ax^c/(b+x^c)+1$	3	0.99977	2856
Power Michaelis Menten (2)	$ax^c/(b+x)^c+1$	3	0.99995	2086
Power Negative Exponential	$a(1-\exp(-bx))^c+1$	3	0.99947	3274
Weibull Cumulative	$a(1-\exp(-bx)^c)$	3	0.99992	2323
Michaelis Menten + offset	$(ax)/(b+x)+1+c$	3	0.99680	4174
Negative Exponential + offset	$a(1-\exp(-bx))+1+c$	3	0.98639	4901
Power Michaelis Menten (1) + offset	$ax^c/(b+x^c)+1+d$	4	0.99988	2545
Power Michaelis Menten (2) + offset	$ax^c/(b+x)^c+1+d$	4	0.99995	2088
Power Negative Exponential	a(1-exp(- bx))^c+1+d	4	0.99957	3169
Weibull Cumulative + offset	$a(1-\exp(-bx)^c)+d$	4	0.99992	2316

B

Function	Formula	Number of Parameters	R^2	AICc
Michaelis Menten	$(ax)/(b+x)+1$	2	0.98873	6899
Negative Exponential	$a(1-\exp(-bx))+1$	2	0.95179	7737
Power Michaelis Menten (1)	$ax^c/(b+x^c)+1$	3	0.99986	4380
Power Michaelis Menten (2)	$ax^c/(b+x)^c+1$	3	0.99999	2897
Power Negative Exponential	$a(1-\exp(-bx))^c+1$	3	0.99959	4996
Weibull Cumulative	$a(1-\exp(-bx)^c)$	3	0.99999	3062
Michaelis Menten + offset	$(ax)/(b+x)+1+c$	3	0.99758	6014
Negative Exponential + offset	$a(1-\exp(-bx))+1+c$	3	0.98893	6891
Power Michaelis Menten (1) + offset	$ax^c/(b+x^c)+1+d$	4	0.99992	4081
Power Michaelis Menten (2) + offset	$ax^c/(b+x)^c+1+d$	4	0.99999	2566
Power Negative Exponential	a(1-exp(- bx))^c+1+d	4	0.99974	4729
Weibull Cumulative + offset	$a(1-\exp(-bx)^c)+d$	4	0.99999	2924

$\hat{S}$
1520
1317
1927
1679
1459
1568
1590
1373
1864
1679
1467
1565

$\hat{S}$
4947
4224
6122
5425
4666
4981
5157
4397
5971
5435
4702
4996

Table S3.

Estimator	Sample	S(lognormal)		RSE(0.7S, lognormal)	
		%BIAS	%RMSE	%BIAS	%RMSE
Chao	Surface	-25.8	26.8	-86.2	116.1
ACE-1(k=10)	Surface	-24.5	25.4	-85.5	114.4
ACE-1(k=100)	Surface	0.9	4.3	-21.0	54.4
iChao	Surface	-23.4	24.5	-84.8	114.1
SP(AICc)	Surface	-1.0	4.5	-6.2	33.3
Chao	Bottom	-14.9	15.1	-70.1	71.9
ACE-1	Bottom	-14.2	14.4	-70.8	72.4
ACE-1(k=100)	Bottom	7.5	8.1	54.1	55.2
iChao	Bottom	-12.7	12.9	-71.1	72.5
SP(AICc)	Bottom	3.7	4.1	23.3	27.5

S(logstudent)		RSE(0.8S, logstudent)		S(inverse Gaussian)		RSE(0.9S, inv
%BIAS	%RMSE	%BIAS	%RMSE	%BIAS	%RMSE	%BIAS
-22.1	22.9	-76.2	90.1	-5.7	6.6	-36.6
-18.9	19.4	-71.3	84.0	-3.5	4.2	-26.9
50.2	60.0	95.2	113.4	40.6	45.9	283.0
-19.1	19.9	-72.1	85.9	-3.3	4.3	-25.9
-2.4	11.6	-2.8	70.3	1.4	6.8	81.6
-24.3	24.8	-73.9	81.3	-5.9	6.0	-33.5
-18.8	19.0	-66.2	72.6	-3.7	3.8	-23.8
93.8	101.5	167.0	178.3	43.5	44.3	306.2
-20.6	21.0	-69.0	76.4	-3.2	3.5	-21.6
3.6	10.0	31.3	60.8	1.3	7.1	51.6

erse Gaussian)	S(Sichel)		RSE(0.9S, Sichel)	
%RMSE	%BIAS	%RMSE	%BIAS	%RMSE
49.6	-6.3	7.8	-38.3	53.9
36.6	-3.7	4.9	-27.5	40.3
313.4	53.2	60.5	323.0	361.5
39.2	-3.6	5.5	-27.5	43.8
448.9	4.1	12.2	237.9	921.5
34.9	-7.4	7.9	-38.5	44.1
25.0	-3.6	3.9	-24.7	28.5
309.8	76.0	81.2	396.9	416.9
23.3	-4.2	4.8	-27.2	33.0
206.8	3.1	12.5	138.5	391.6