Supplementary Figures and Tables for "Interaction capacity underpins community diversity"

Masayuki Ushio

Contents:

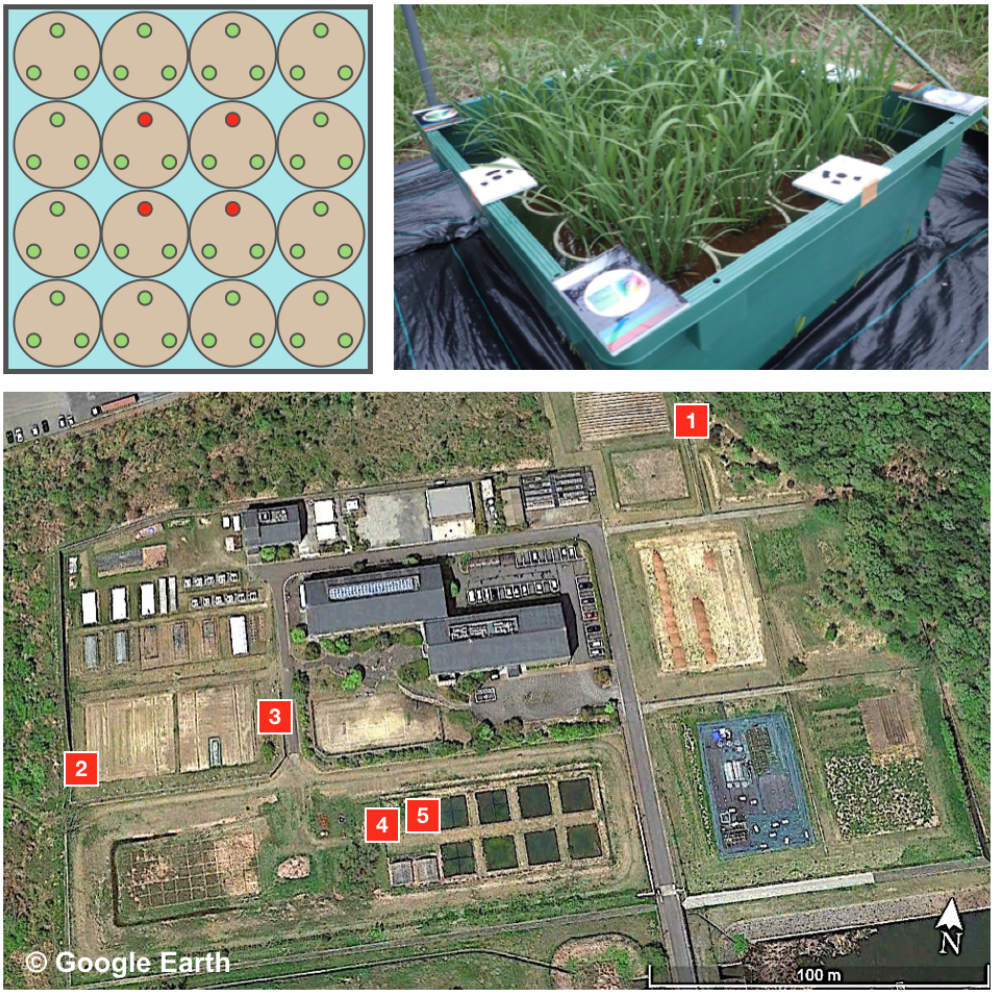

Figure S1 | Experimental rice plots established in Center for Ecological Research, Kyoto Univeristy (34° 58′18″ N, 135° 57′33″ E) in 2017. An image of the experimental rice plot (90 cm × 90 cm; upper right). Three rice individuals were grown in each Wagner pot (upper left). Growth rates of four individuals at the center of each plot were monitored for agricultural purposes. The growth data are being analyzed for different purposes and thus are not shown in this study. Five experimental rice plots were established in an experimental field in Center for Ecological Research, Kyoto University (bottom). Red squares indicate the locations of the rice plots.

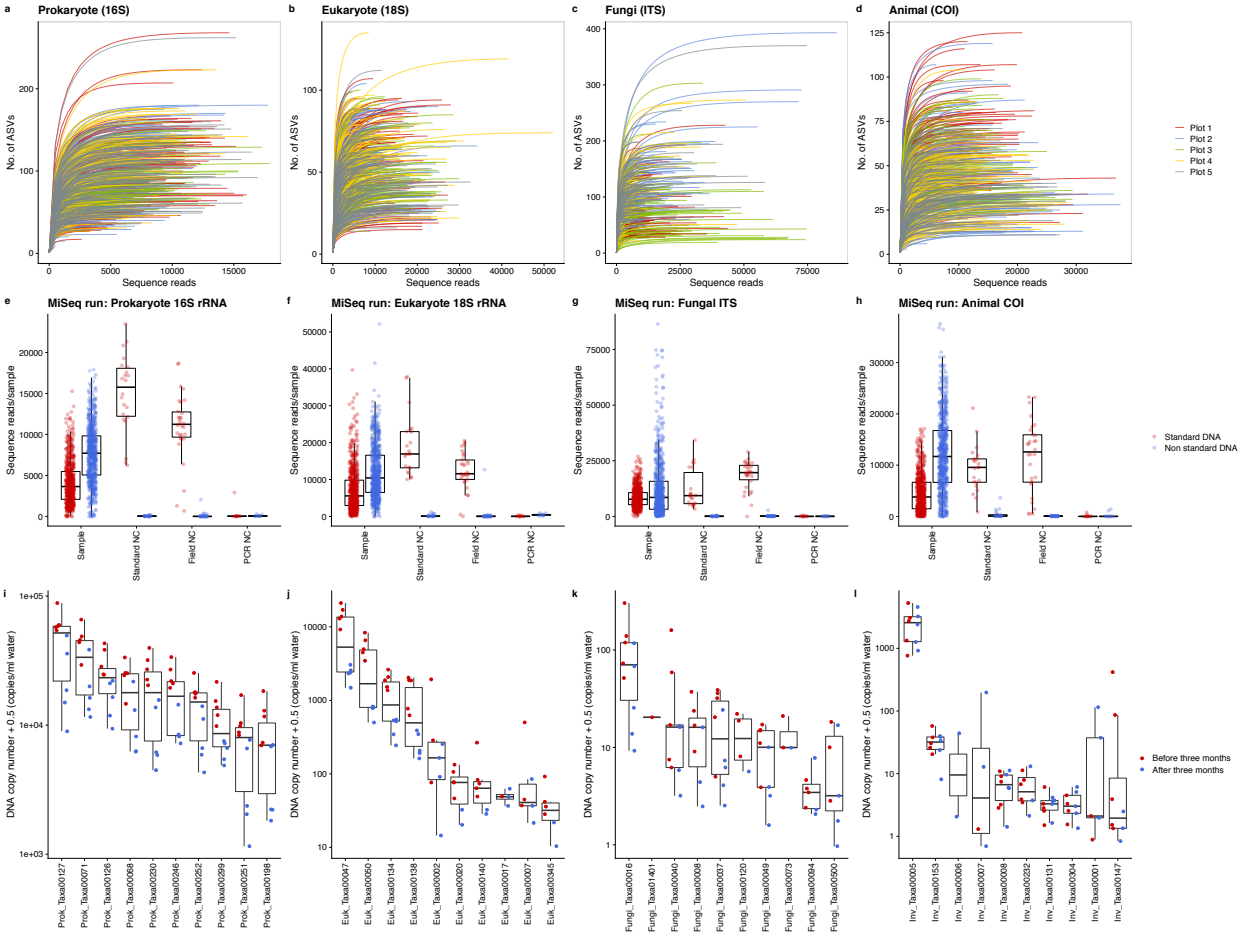

Figure S2 | Rarefaction curves and positive and negative controls for DNA analyses. Rarefaction curve (**a-d**) and the results of analysis of negative (**e-h**) and positive (**i-l**) controls. Most of the rarefaction curves reached a plateau, suggesting that the sequencing captured most diversity of the target taxa (**a-d**). Investigations of negative control samples suggested that no serious contaminations occurred during sample transportation, eDNA extraction or library preparation (Almost no non-standard DNAs were detected from Standard NC, Field NC or PCR NC; **e-h**). No dramatic reductions in DNA copy numbers of the top 10 dominant taxa were observed in samples from which DNAs were extracted before the storage duration exceeded 3 months (red points in **i-l**; note that DNAs of all samples analyzed in the time series analysis were extracted within 1-2 months after sampling). Together, these results suggest that the water sampling and DNA analysis in the present study enabled reliable illustrations of the dynamics of ecological communities in the rice plots. Detailed descriptions of the negative and positive control samples are provided in the Supplementary Information.

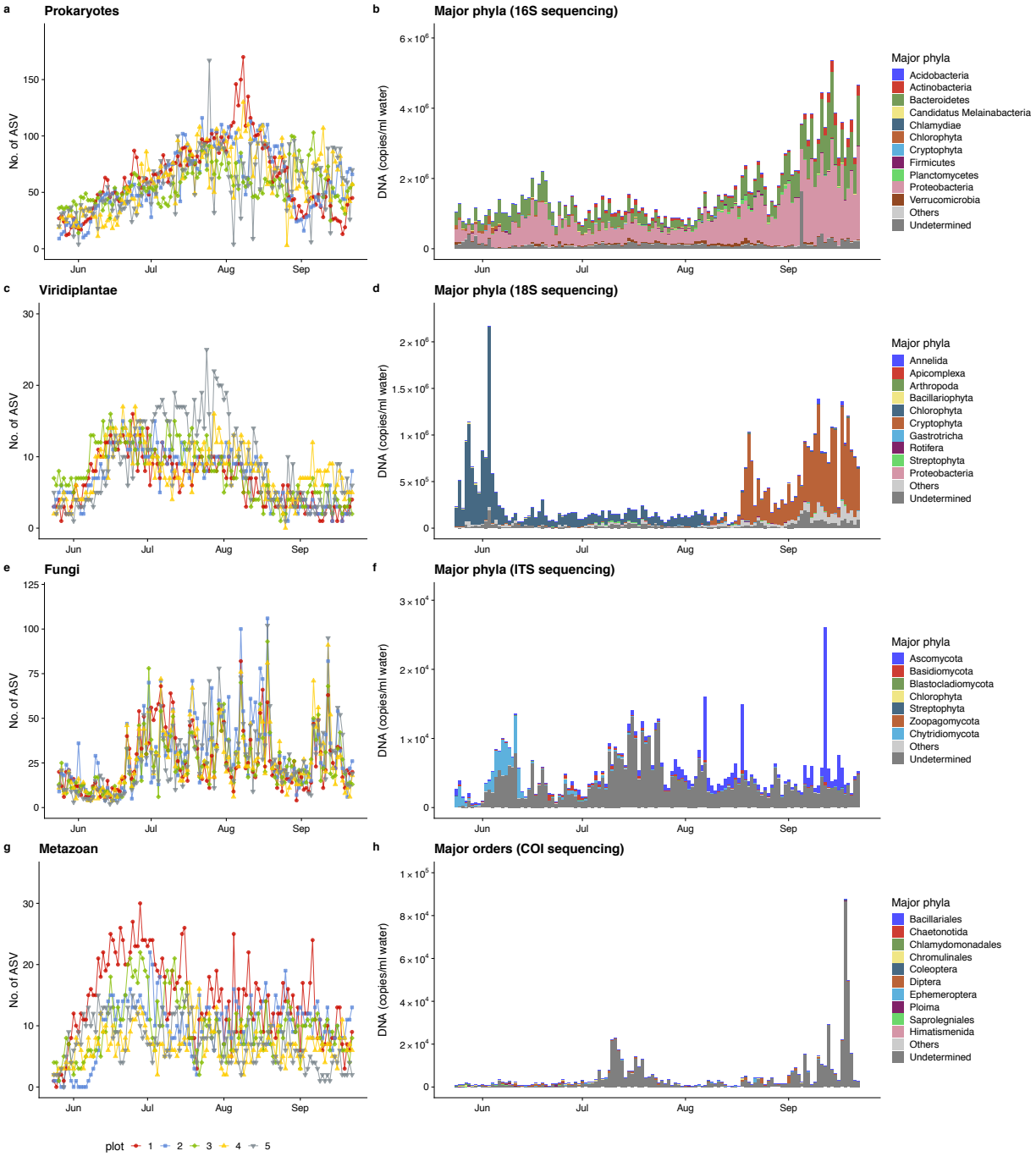

Figure S3 | Patterns in DNA time series of major taxonomic groups in the study. Temporal patterns of ASV diversity, major taxa and the DNA concentrations for **a-b** prokaryotes, **c-d** fungi, **e-f** metazoans, and **g-h** viridiplantae.

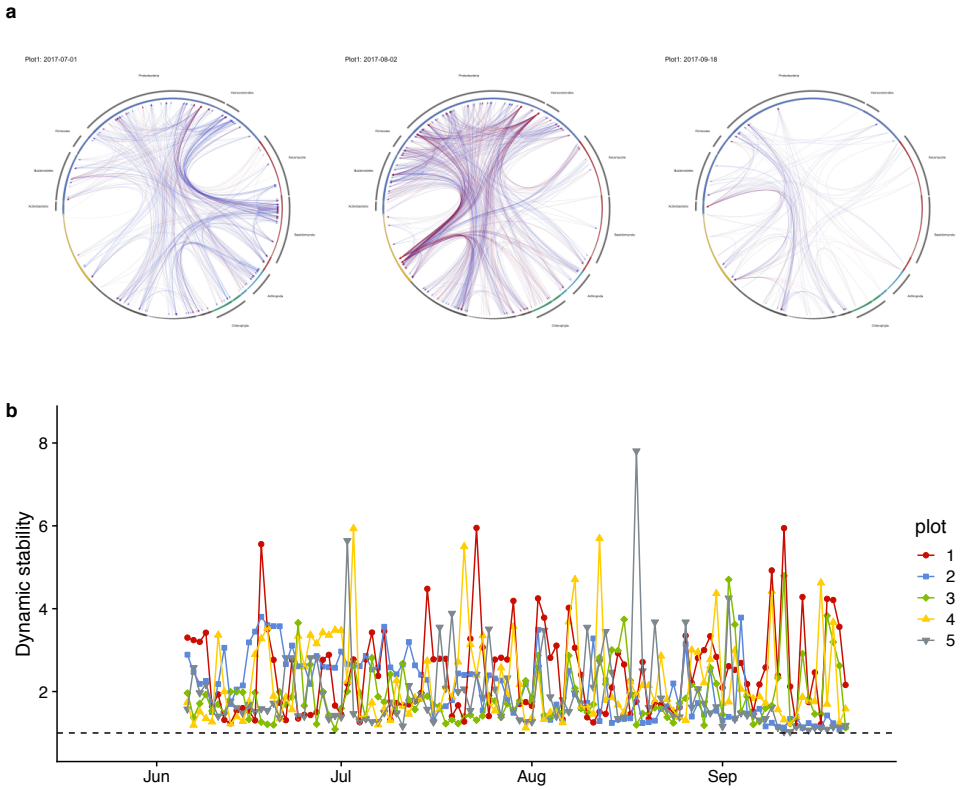

Figure S4 | Time-varying properties of the interaction network. a Changes in the network structures. Interaction networks in Plot 1 on 1 July, 2 August and 18 September 2017 are shown as examples of time-varying interaction networks. **b** Dynamic stability of the ecological community. Different colours, lines and symbols indicate different plots.

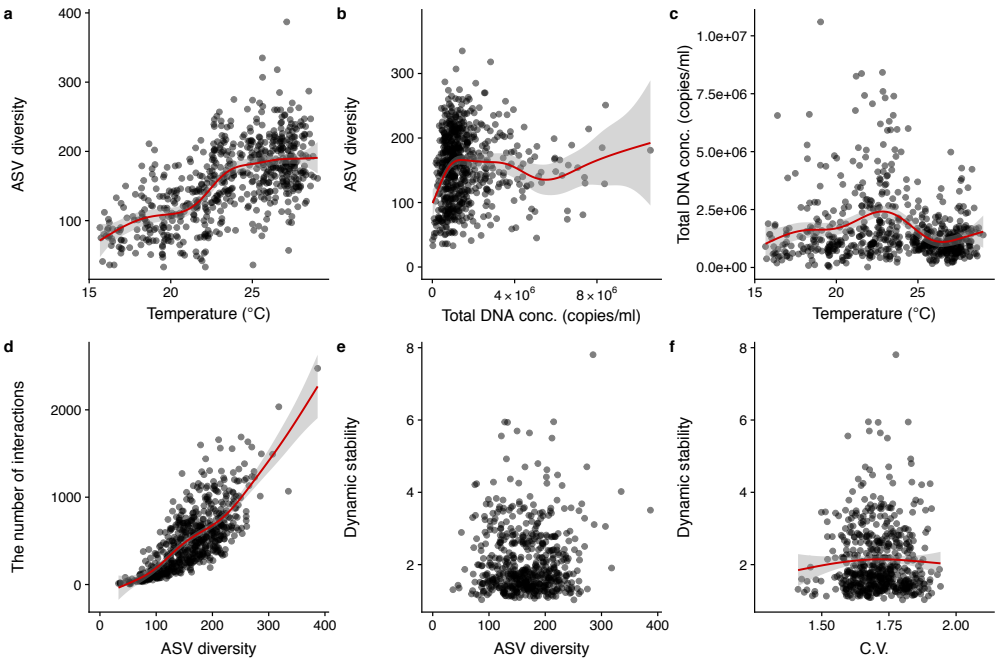

Figure S5 | Additional patterns in network properties. **a** Mean air temperature and ASV diversity, **b** total DNA concentrations and ASV diversity, **c** Mean air temperature and total DNA concentrations, **d** The number of interactions and ASV diversity. **e** ASV diversity and dynamic stability and **f** Dynamic stability and coefficients of variation of community dynamics. Red lines indiate significant nonlinear regressions by general additive model (GAM).

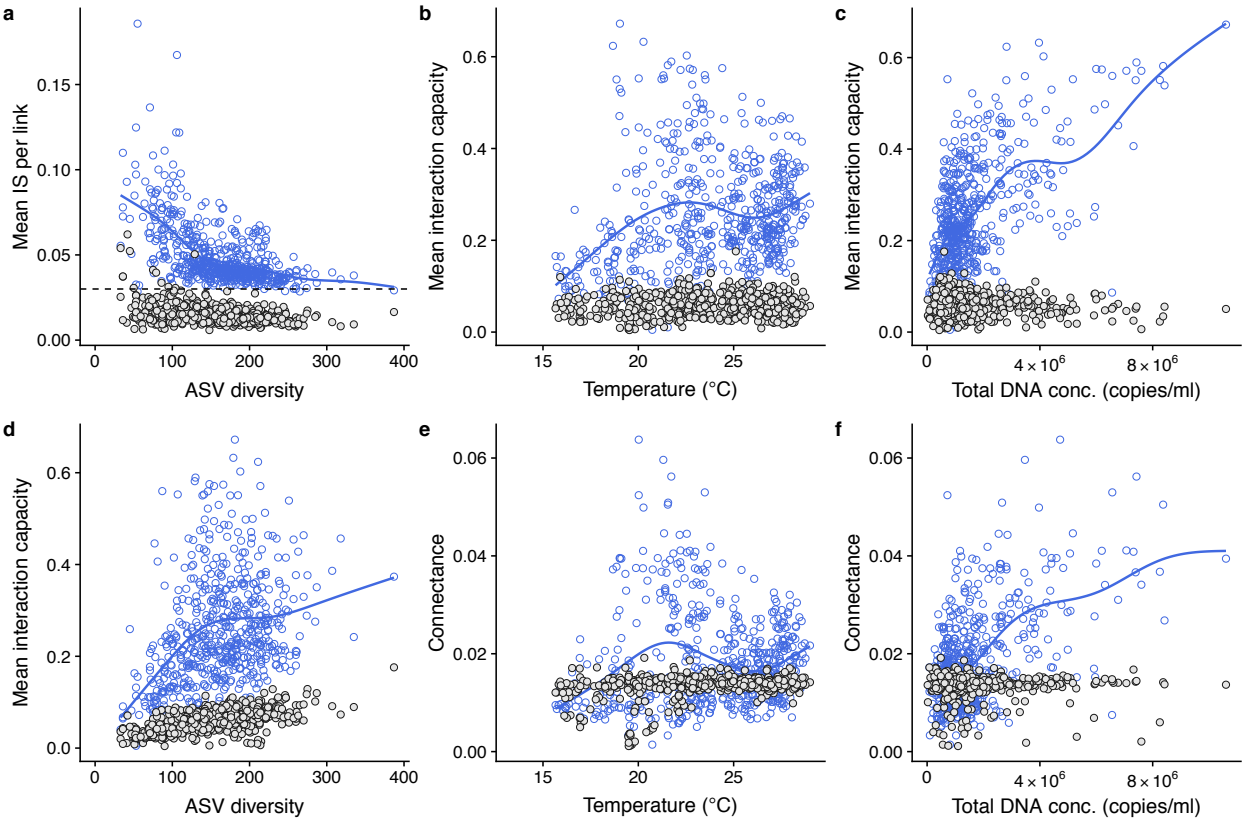

Figure S6 | Comparisons between observed network properties and those of randomly shuffled time series. **a** Mean interaction strength and ASV diversity, **b, c** Mean interaction capacity, mean air temperature and total DNA concentrations, and **d** mean interaction capacity and ASV diversity, and **e, f** connectance, mean air temperature and total DNA concentrations. Gray points indicate patterns generated by analyzing randomly shuffled time series. Blue points and line indicate patterns for the original time series. The observed patterns were not reproduced by the analyses of the randomly shuffled time series.

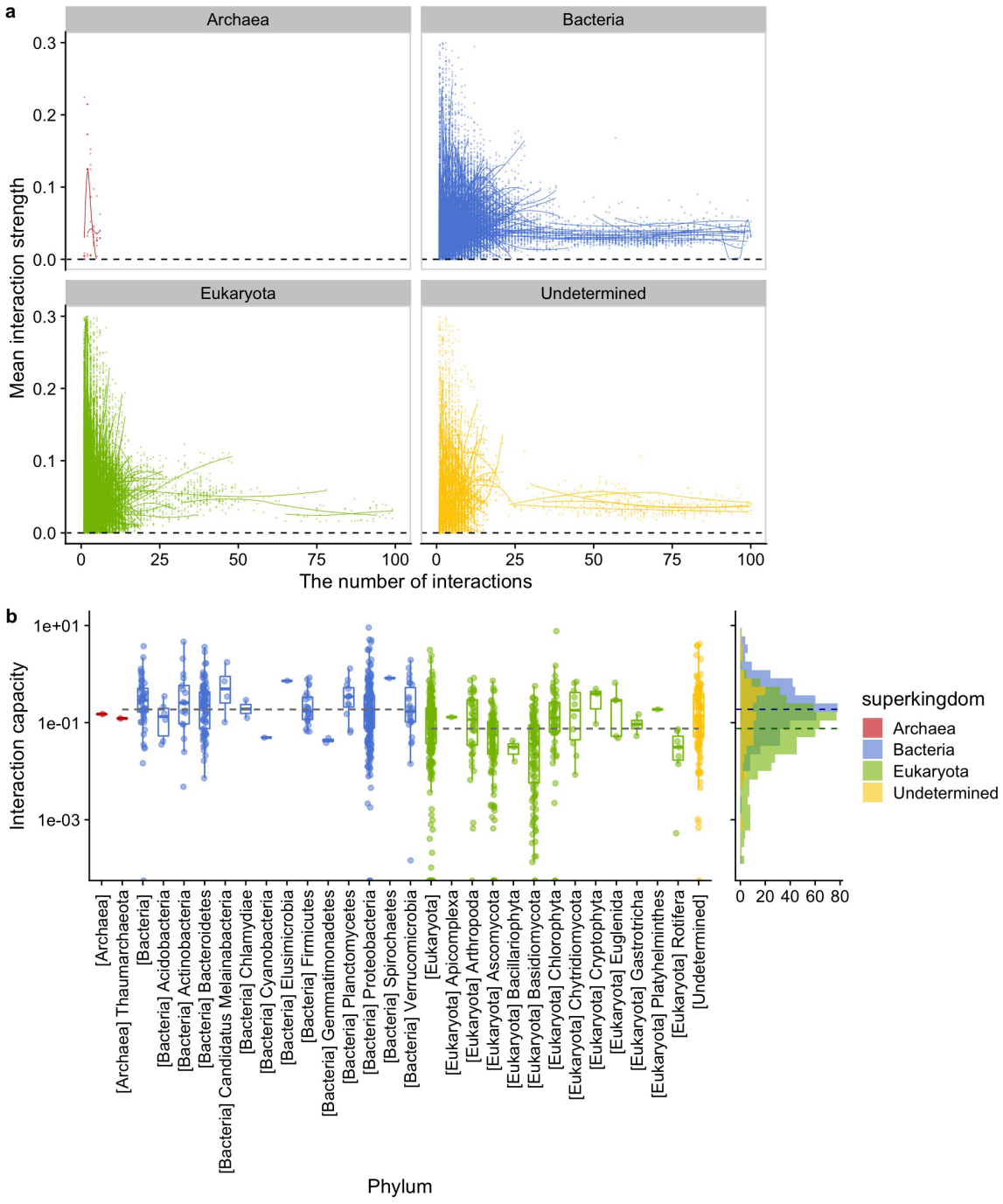

Figure S7 | Species-level patterns of interaction strength and capacity. a Relationships between the number of interactions and mean interaction strength per link. **b** Relationship between species-level interaction capacity and phylum . Bacterial taxa generally show a higher interaction capacity than eukaryotes, suggesting that the potental maximum diversity of Bacteria is higher than that of Eukaryota.

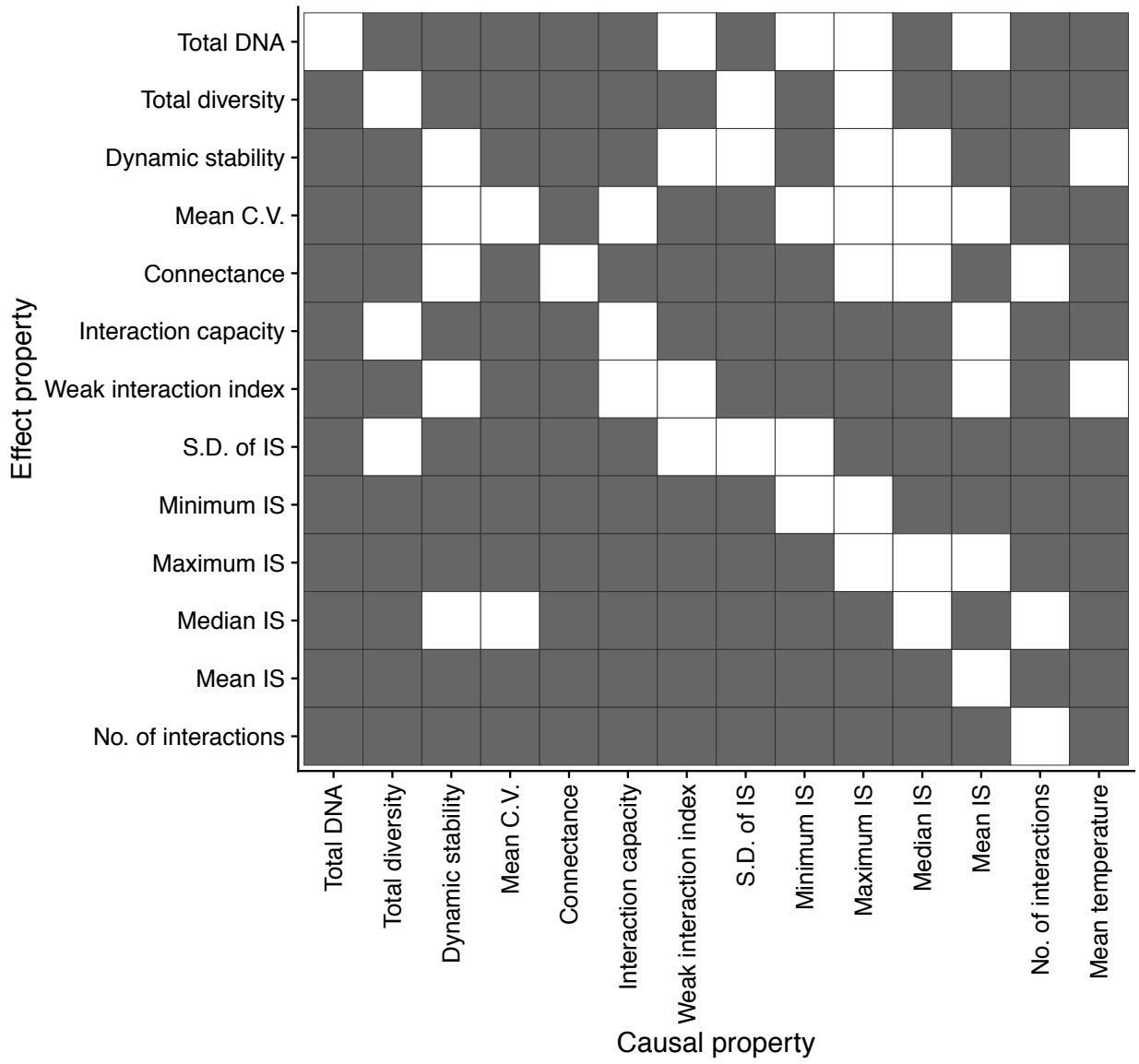

Figure S8 | Causal relationships among the network properties. A cell filled with gray colour indicates that there is a causal influence from a column variable ("Causal property") to a row variable ("Effect property"), which is detected by convergent cross mapping.

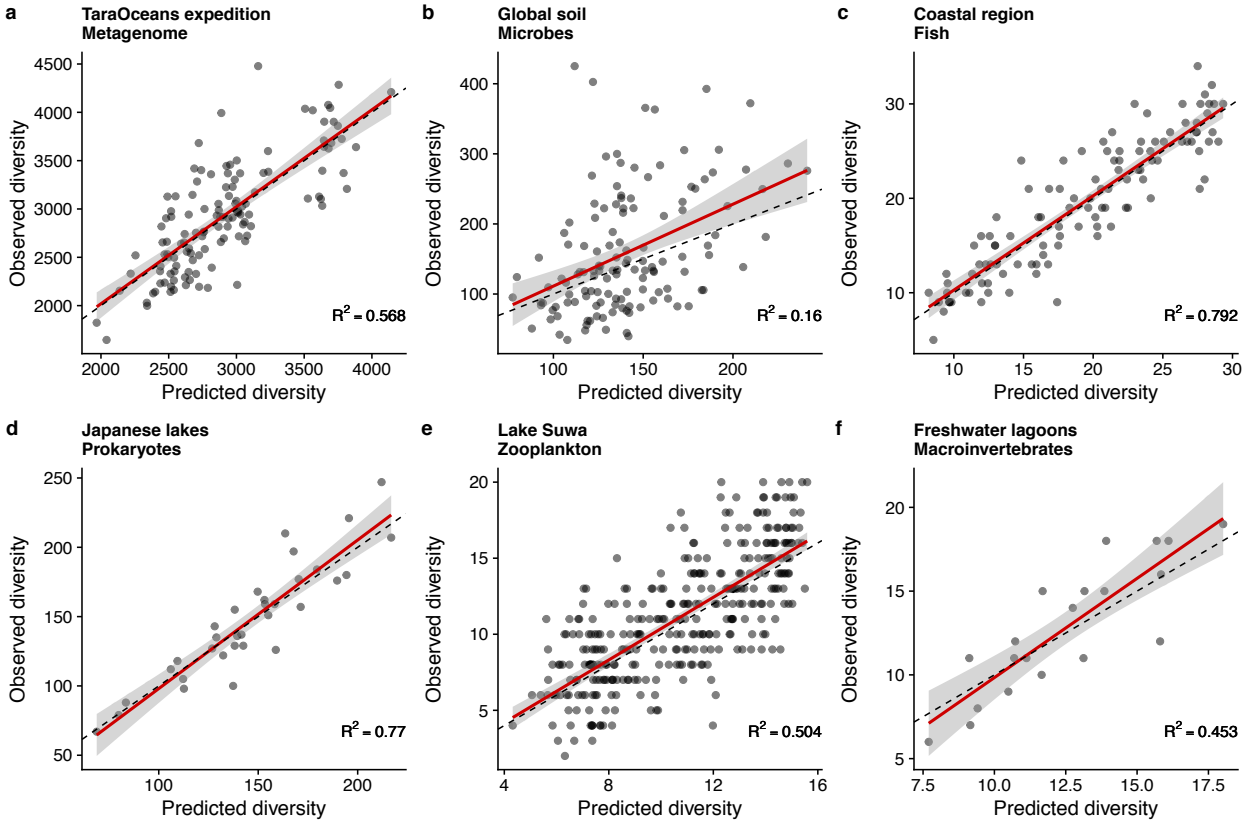

Figure S9 | Predicted and observed diversity based on the interaction capacity hypothesis. a-f Predicted and observed diversity for metagenomic data generated by Tara Oceans research expedition (**a**), global soil microbial community (**b**), a fish community in a marine costal region in Kyoto, Japan (**c**), prokaryotic communities in Japanese lakes (**d**), zooplankton communities in Lake Suwa, Japan (**e**) and macroinvertebrate communities in freshwater tributary lagoons surrounding Lake Biwa, Japan (**f**). Values at the bottom right in panels indicate adjusted R^2 of GAM.

Table S1| Statistical results of GAM between network properties.

Explained variable	Smooth term (explaining variable)	Effective d.f.	*F* value	*P* value	Adjusted R^2
Connectance	Total ASV diversity	**4.7**	**6.4**	**2.35×10^{-6}**	**0.06**
Connectance	Mean air temperature	**4.8**	**8.4**	**1.55×10^{-8}**	**0.079**
Connectance	Total DNA concentrations	**5.1**	**42.9**	**2.68×10^{-48}**	**0.319**
Dynamic stability	Mean C.V.	1.8	0.8	0.426	0.002
Dynamic stability	Total ASV diversity	**6.5**	**2.7**	**9.91×10^{-3}**	**0.029**
Interaction capacity	Total ASV diversity	**3.7**	**24.6**	**3.21×10^{-21}**	**0.169**
Interaction capacity	Mean air temperature	**4.3**	**10.8**	**3.36×10^{-10}**	**0.089**
Interaction capacity	Total DNA concentrations	**6.9**	**40.2**	**6.01×10^{-57}**	**0.356**
Interaction capacity	Connectance	**6.1**	**177.9**	**1.79×10^{-235}**	**0.694**
Maximum IS	Total ASV diversity	3.2	1	0.404	0.005
Maximum IS	Mean air temperature	1	2.7	0.0983	0.003
Mean C.V.	Total ASV diversity	**4.8**	**10.8**	**2.34×10^{-11}**	**0.094**
Mean IS	Total ASV diversity	**5.4**	**52.5**	**1.30×10^{-61}**	**0.376**
No. of interactions	Total ASV diversity	**5**	**129.5**	**9.13×10^{-146}**	**0.581**
Total ASV diversity	Total DNA concentrations	**6.5**	**4.9**	**1.29×10^{-5}**	**0.054**
Total DNA concentrations	Mean air temperature	**5.8**	**10.6**	**1.42×10^{-12}**	**0.107**

Supplementary information for “Interaction capacity underpins community diversity”

Masayuki Ushio

Contents:

1. Thermal cycle profiles

Common protocols

The first-round PCR (first PCR) was carried out with a 12-μl reaction volume containing 6.0 μl of 2 $\times$ KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, WA, USA), 0.7 μl of forward primer, 0.7 μl of reverse primer (each primer at 5 μM used in the reaction; with adaptor and six random bases), 2.6 μl of sterilized distilled H_2O, 1.0 μl of DNA template and 1.0 μl of internal standard DNA mixture. Thermal cycle profile, primer set and interanal standard DNA sequences are provided in following subsections. Triplicate first PCRs were performed, and these replicate products were pooled in order to mitigate the PCR dropouts. The pooled first PCR products were purified using AMPure XP (PCR product : AMPure XP beads = 1:0.8; Beckman Coulter, Brea, California, USA). The pooled, purified, and 10-fold diluted first PCR products were used as templates for the second-round PCR.

The second-round PCR (second PCR) was carried out with a 24-μl reaction volume containing 12 μl of 2 $\times$ KAPA HiFi HotStart ReadyMix, 1.4 μl of each primer (each primer at 5 μM in the reaction volume), 7.2 μl of sterilized distilled H_2O and 2.0 μl of template. Different combinations of forward and reverse indices were used for different templates (samples) for massively parallel sequencing with MiSeq. The thermal cycle profile after an initial 3 min denaturation at 95°C was as follows (12 cycles): denaturation at 98°C for 20 s; annealing at 68°C for 15 s; and extension at 72°C for 15 s, with a final extension at 72°C for 5 min.

Twenty microliters of the indexed second PCR products were mixed, and the combined library was again purified using AMPure XP (PCR product: AMPure XP beads = 1:0.8). Target-sized DNA of the purified library (*ca.* 440 bp for prokaryote 16S rRNA; *ca.* 320 bp for eukaryote 18S rRNA; *ca.* 470-600 bp for fungal ITS; *ca.* 510 bp for animal COI) was excised using E-Gel SizeSelect (ThermoFisher Scientific, Waltham, MA, USA). The double-stranded DNA concentration of the library was quantified using a Qubit dsDNA HS assay kit and a Qubit fluorometer (ThermoFisher Scientific, Waltham, MA, USA). The double-stranded DNA concentration of the library was then adjusted using MilliQ water and the DNA was applied to the MiSeq (Illumina, San Diego, CA, USA). The prokaryote 16S rRNA, eukaryote 18S rRNA, fungal ITS and animal COI libraries were sequenced using MiSeq Reagent Kit V2 for 2 $\times$ 250 bp PE, MiSeq Reagent Kit V2 for 2 $\times$ 150 bp PE, MiSeq Reagent Kit V3 for 2 $\times$ 300 bp PE and MiSeq Reagent Kit V2 for 2 $\times$ 250 bp PE, respectively.

Primer sequences

For the first PCR, taxa-specific univarsal primers were combined with the MiSeq sequencing primers and six random bases (Ns) to improve the quality of MiSeq sequencing. For the second PCR, MiSeq adaptor and sequencing primers were combined with index sequences (eight bases denoted by X in the following table) to identify each sample. See sample metadata for index sequences of each sample (available in https://github.com/ong8181/ecolnet-int-capacity/SpplementaryData).

Taxa-specific 1st PCR primers

Target taxa	Primer name	Sequencing primer - NNNNNN - Universal primer
Prokaryote (16S rRNA)	515F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG NNNNNN GTGYCAGCMGCCGCGGTAA
	806R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG NNNNNN GGACTACNVGGGTWTCTAAT
Eukaryote (18S rRNA)	Euk_1391f	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG NNNNNN GTACACACCGCCCGTC
	EukBr	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG NNNNNN TGATCCTTCTGCAGGTTCACCTAC
Fungi (ITS)	ITS1_F_KYO1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG NNNNNN CTHGGTCATTTAGAGGAASTAA
	ITS_KYO2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG NNNNNN TTYRCTRCGTTCTTCATC
Animal (mitocondoria COI)	mlCOIintF	ACACTCTTTCCCTACACGACGCTCTTCCGATCT NNNNNN GGWACWGGWTGAACWGTWTAYCCYCC
	HCO2198	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT NNNNNN TAAACTTCAGGGTGACCAAAAAATCA

References for the taxa-specific 1st PCR primers are as follows: 515F-806R (Bates *et al.* 2011; Caporaso *et al.* 2011), Euk_1391f-EukBr (Amaral-Zettler *et al.* 2009), ITS1_F_KYO1-ITS_KYO2 (Toju *et al.* 2012), mlCOIintF (Leray *et al.* 2013) and HCO2198 (Folmer *et al.* 1994).

2nd PCR primers

Primer name		MiSeq adaptor - XXXXXXXX - Sequencing primer
2nd PCR primer for 16S, 18S and ITS	Forward	AATGATACGGCGACCACCGAGATCTACAC XXXXXXXX TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
	Reverse	CAAGCAGAAGACGGCATACGAGAT XXXXXXXX GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG
2nd PCR primer for COI	Forward	AATGATACGGCGACCACCGAGATCTACAC XXXXXXXX ACACTCTTTCCCTACACGACGCTCTTCCGATCT
	Reverse	CAAGCAGAAGACGGCATACGAGAT XXXXXXXX GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

Taxa-specific thermal cycle profiles of the first-round PCR

Prokaryote 16S rRNA (515F-806R)

The thermal cycle profile after an initial 3 min denaturation at 95˚C was as follows (35 cycles): denaturation at 98˚C for 20 s; annealing at 60˚C for 15 s; and extension at 72˚C for 30 s, with a final extension at the same temperature for 5 min.

Eukaryote 18S rRNA (Euk_1391f-EukBr)

The thermal cycle profile after an initial 3 min denaturation at 95˚C was as follows (35 cycles): denaturation at 98˚C for 20 s; annealing at 62˚C for 15 s; and extension at 72˚C for 30 s, with a final extension at the same temperature for 5 min.

Fungal ITS (ITS1_F_KYO1-ITS_KYO2)

The thermal cycle profile after an initial 3 min denaturation at 95˚C was as follows (35 cycles): denaturation at 98˚C for 20 s; annealing at 55˚C for 15 s; and extension at 72˚C for 30 s, with a final extension at the same temperature for 5 min.

Animal COI (mlCOIintF-HCO2198)

The thermal cycle profile after an initial 3 min denaturation at 95˚C was as follows (35 cycles): denaturation at 98˚C for 20 s; annealing at 55˚C for 15 s; and extension at 72˚C for 30 s, with a final extension at the same temperature for 5 min.

2. Internal standard DNA sequences

Five artificially designed and synthesized internal standard DNAs, which are similar but not identical to the corresponding region of any existing target organisms (e.g., the V4 region of prokaryotic 16S rRNA), were included in the library preparation process to estimate the number of DNA copies (i.e., quantitative MiSeq sequencing; Ushio 2019). They were designed to have the same primer-binding regions as those of known existing sequences and conserved regions in the insert region. Variable regions in the insert region were replaced with random bases so that no known existing

sequences had the same sequences as the standard sequences. The numbers of standard DNA copies were adjusted appropriately to obtain a linear regression line between the copy numbers of the standard DNAs and their sequence reads from each sample. Sequences and the copy numbers of the standard DNAs are listed in the following tables.

Ushio et al. (2018) showed that the use of standard DNAs in MiSeq sequencing provides reasonable estimates of the DNA quantity in environmental samples when analyzing fish environmental DNA. Indeed, the copy numbers of standard DNAs were highly correlated with the number of sequence reads in this study (for most samples, $R^2 > 0.9$; see figures in Github; https://github.com/ong8181/ecolnet-int-capacity), suggesting that the number of sequence reads is generally proportional with the number of DNA copies in a single sample. However, it should be noted that it corrects neither for sequence-specific amplification efficiency, nor for species-specific DNA extraction efficiency. In other words, the method assumes similar amplification efficiencies across sequences, and similar DNA extraction efficiencies across microbial species, which is apparently not valid for complex environmental samples. Therefore, we need careful interpretations of results obtained using that method.

Sequences and the copy numbers of standard DNAs of prokaryote 16S rRNA

Primer name	Sequence
STD_pro1 5,000 copies/μl	GTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTATCCGGAATTACTGGGCGTAAA GGGAAGGTAGGCGGAAGCTGAAGTCATGTGTGAAAACGCCTGGCTCAACTTAGCTCAGGG TCGCTGAAATCGTTGGATTCTCTGTGAACAAGGTCCTCGGAAATTTCTTGCTAGAGCAAT AAGGGCTGGCGGATAAGGCGAAACTTTGCGGCGAAGGCACGGAGCATAGGCAGGGCCCTA ACTGGACCGTTAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCC
STD_pro2 10,000 copies/μl	GTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTATCCGGAATTACTGGGCGTAAA GGGTATGTAGGCGGTAAAGGAAGTTACGAGTGAAATTACAGGGCTCAACCGATAAGTCGT GGCCAAGAGGCGGAGCCTGCAGTAATCGAGAGGTGGGCGGAATCGGGTAAAAGTCAACCT AAGGAAACAACTTTCAAAGGAAGCCAGATCGCGAAGGCGGCCTCGTAGTTAGGTCCTTCT CTGGACAAACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCC
STD_pro3 25,000 copies/μl	GTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTATCCGGAATTACTGGGCGTAAA GGGTTAGTAGGCGGGCAATTAAGTTATAGGTGAAAAGTAATGGCTCAACTTCGCGAACTC CGGACGCAGAAAGGTGCTGTGGCGTTAAAGGGGCTCCCGGAACCGAAGCAACAAACCTGA AAGCCGTTCCGATGTTTCGGAACTGCTAATGCGAAGGCTCAAGTCCTAGTAACTGGCGAT GTCCTAATCACAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCC
STD_pro4 50,000 copies/μl	GTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTATCCGGAATTACTGGGCGTAAA GGGAGTGTAGGCGGCCACGTAAGTTCCCGGTGAAATCGAGCGGCTCAACGTGCTCCTCGC CCGAGGTATCTAAGCCTTGTAACTCAGCCTAGGAACCCGGAAAGACTTTGTAGACATGCT AAAACGTGACCTGGTGTATGAATATCTCCAGCGAAGGCCGCACAACCGCACTCCACGCTT TAGTGGGTTTAAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCC
STD_pro5 100,000 copies/μl	GTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTATCCGGAATTACTGGGCGTAAA GGGCCGGTAGGCGGCTCCCGAAGTCGGTAGTGAAATTCTGAGGCTCAACTAAAACCACGA CATTGGAACGAGGTGTGTTAGCTTGATACCCGGCCAGTGGAACAGTGCTGCTCACTTCAT AACTCGGGTACGAAATAAGGAAGACGTCCCGCGAAGGCTTTGGCAGGGCATGCCAGGGCA TGGCTTTTGTTAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCC

Sequences and the copy numbers of standard DNAs of eukaryotic 18S rRNA

Primer name	Sequence
STD_euk1 10,000 copies/μl	GTACACACCGCCCGTCGCTCCTACCGATTGTGTGTGCGGGTGAAGGGACCGGATAGGTACT TGGGGTTGTTTCCCAAGCTATTAACTAGAGAACTTTACTAAACCAGCACACATAGAGGAAG GTGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCAGAAGGATCA
STD_euk2 5,000 copies/μl	GTACACACCGCCCGTCGCTCCTACCGATTGTGTGTGCGGGTGAAGCGGCAGGATAGGCCTC GAGGCTCGATTCCCAGATATGTCGACAGAGAACTTATCTAAACCGACACAAGTAGAGGAAG GTGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCAGAAGGATCA
STD_euk3 2,500 copies/μl	GTACACACCGCCCGTCGCTCCTACCGATTGCTTGTGCCGGTGAAGAGGATGGATGGGTGAG CAGGGGATATTCCCCAGGGCAGAAGGCGAGAACTTTCGTAAACCGTCGCATATAGAGGAAG GTGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCAGAAGGATCA
STD_euk4 1,250 copies/μl	GTACACACCGCCCGTCGCTCCTACCGATTGCCTGTGCGGGTGAAGAGGATGGATCGGCACT ACGGTGTTGTTCCCGTTTGGCCGGCGGGAGAATTTAAGTAAACCAGCGCAATTAGAGGAAG GTGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCAGAAGGATCA
STD_euk5 250 copies/μl	GTACACACCGCCCGTCGCTCCTACCGATTGTGTGTGCGGGTGAAGGGAAAGGATGGGGACT GCGGCGCTTTTCCCGGCAACACTTAAAGAGAAATTCACTAAACCTACACAATTAGAGGAAG GTGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCAGAAGGATCA

Sequences and the copy numbers of standard DNAs of fungal ITS

Primer name	Sequence
STD_asco1 20 copies/μl	CTCGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGG GATCATTACCGAGCGAGGGACGGAGATGAAGCCTTGACACTTTGTGTCCGACACGGTTTG CTTCGGGGGCGATTCTGCCGCAAAAGTTGCATTCCCCAAGATATTCGTCAAAACACTGCA TCAACACGTCGGAACTAACTGTTAATGTTTCAAAACTTTCAACAACGGATCTCTTGGTTC TGGCATCGATGAAGAACGCAGCGAA
STD_asco2 40 copies/μl	CTCGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGG GATCATTACCGAGCGAGGGAAGTCCCCTACTACGTCAGTCTTTGTGTCCTAGCAAAGTTG CTTCGGGGGCGACAATGCCGTACTCTCCGCATTCCCCTCATGCACGATCAAAACACTGCA CATAGACGTCGGGGTAATCCGGTAATTCTCGAAAACTTTCAACAACGGATCTCTTGGTTC TGGCATCGATGAAGAACGCAGCGAA
STD_asco3 100 copies/μl	CTCGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGG GATCATTACCGAGCGAGGGAACTTCTGTCCGCGGACCTCCTTTGTGATCCGAAGAGGTTG CTTCGGGGGCGATTTTGCCGGGGTTGACGCATTCCCCAGATACTATCTCAAAACACTGCA TGATGACGTCGGTAGAGTCCTAGAACGAAGTAAAACTTTCAACAACGGATCTCTTGGTTC TGGCATCGATGAAGAACGCAGCGAA
STD_asco4 200 copies/μl	CTCGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGG GATCATTACCGAGCGAGGGAGGTTGTCATGCGTCCCGAACTTTGTGAGTAGTTACTTTTG CTTCGGGGGCGAGTATGCCGAGGCGGAGGCATTCCCCCTAGCCGGGGTCAAAACACTGCA CCTTAACGTCGGGTGAACGACCAAAAGCACTAAAACTTTCAACAACGGATCTCTTGGTTC TGGCATCGATGAAGAACGCAGCGAA
STD_asco5 400 copies/μl	CTCGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGG GATCATTACCGAGCGAGGGAATTTAACGGAGTCTGGGGGCTTTGTGTCGGCTCCGGTTTG CTTCGGGGGCGATGCTGCCGGCGTGACGGCATTCCCCAGGTATCGCGTCAAAACACTGCA TATCGACGTCGGAGACCGTCAGTAAAGAGGCAAAACTTTCAACAACGGATCTCTTGGTTC TGGCATCGATGAAGAACGCAGCGAA

Sequences and the copy numbers of standard DNAs of animal mitochondorial COI

Primer name	Sequence
STD_mlCOI1 200 copies/μl	GGAACAGGATGAACAGTTTACCCTCCACTGTCAACCAGTATTGCTCACAGAGGAGCTTCC GTCGATTTAGCTATTTTTTCTTTACATTTAGCAGGCATCTCATCGATTATAGGGGCAATT AACTTTATTACAACCGTTATTAATATACGAATGTGATGCATAAACGCGGATCGAATACCT TTATTTGTTTGATCAGTCGTAATTACCAGGCTGATTTTTCTATTATCCCTACCCGTGCTA GCCAGAGTGATCTCTATGCTTTCGTCTGATCGTAACTTAAATACATCATTCTACGACCCT GCTGGTGGAGGGGACCCGATTTTGTATCAGCACCTATTTTGATTTTTTGGTCACCCTGAA GTTTA
STD_mlCOI2 100 copies/μl	GGAACAGGATGAACAGTTTACCCTCCACTCTCTTCACCAATTGCTCACCGCGGAGCATCA GTGGATTTAGCCATTTTTTCGTTTCATTTAGCAGGCATCTCCTCTATTTTAGGTGCATTA AACTTTATTACAACCGTTATTAATATACGATGACCAACAATAGTTAGAGATCGAATACCT TTATTTGTTTGATCAGTAGCTATTACCAGAGTCTTCTTTATATTATCACTACCAGTTCTA GCTAGAGTAATCTCTATGCTTTCGTCTGATCGTAACTTAAATACATCATTCTACGACCCG GCCGGGGGAGGAGACCCCATTTTCTATCAGCACCTATTTTGATTTTTTGGTCACCCTGAA GTTTA
STD_mlCOI3 50 copies/μl	GGAACAGGATGAACAGTTTACCCTCCACTATCCCCATCTATTGCACACCATGGAGCCTCT GTTGATTTAGCAATTTTTTCTTTACATTTAGCCGGATTGTCCTCTATTGTAGGGGCTATA AACTTTATTACAACCGTTATTAATATACGAATGTCCTCTATATAGGTAGATCGAATACCT TTATTTGTCTGATCTGTGGTCATTACGAGAGTGTTTCTAATTCTATCCCTACCGGTGCTA GCCAGAGTGATCTCTATGCTTTCGTCTGATCGTAACTTAAATACATCATTCTACGACCCT GCGGGAGGGGGCGACCCAATTTTGTATCAGCACCTATTTTGATTTTTTGGTCACCCTGAA GTTTA
STD_mlCOI4 25 copies/μl	GGAACAGGATGAACAGTTTACCCTCCACTTTCACCAAGTATTGCTCACGGGGGAGCCTCT GTCGATTTAGCAATTTTTTCTCTTCATTTAGCTGGTATTTCATCAATTTTAGGCGCACTT AACTTTATTACAACCGTTATTAATATACGAGCACCCGAGATATGCTGTGATCGAATACCT TTATTTGTTTGATCTGTGGAGATTACCAGGGTTATCCTACTTCTATCGCTACCCGTCCTA GCTAGAGTAATCTCTATGCTTTCGTCTGATCGTAACTTAAATACATCATTCTACGACCCG GCAGGTGGTGGGGACCCTATTTTTTATCAGCACCTATTTTGATTTTTTGGTCACCCTGAA GTTTA
STD_mlCOI5 5 copies/μl	GGAACAGGATGAACAGTTTACCCTCCACTTTCTCCTATAATTGCTCACTGAGGAGCATCT GTTGATTTAGCCATTTTTTCTCTACATTTAGCGGGTATATCATCTATTCTAGGAGCAGTA AACTTTATTACAACCGTTATTAATATACGAACGGCCTTAATAGTTCCGGATCGAATACCT TTATTTGTCTGATCAGTCGCCATTACGAGCGTCTTTCTTTTATTATCCCTACCCGTCCTA GCGAGAGTAATCTCTATGCTTTCGTCTGATCGTAACTTAAATACATCATTCTACGACCCG GCTGGTGGTGGTGACCCGATTTTGTATCAGCACCTATTTTGATTTTTTGGTCACCCTGAA GTTTA

3. Summary of sequence quality

I performed MiSeq run four times to generate sequences from the four metabarcoding regions (i.e., 16S rRNA, 18S rRNA, ITS and COI). Summary of the sequence runs is provided below.

MiSeq run name	Cycles	Target region	Pass filter (%)	>Q30 (%)	Yield (Gbp)	Total reads	Reads PF
RMR-076	500	16S rRNA	95.69	84.77	7.69	15,636,422	14,962,924
CMR-002	300	18S rRNA	89.64	94.86	5.67	20,158,344	18,070,998
RMR-078	600	ITS	95.13	79.73	16.08	27,533,984	26,191,796
RMR-099	500	COI	90.07	89.62	9.45	20,410,736	18,383,068
Total					38.89	83,739,486	77,607,998

Sequence reads from standard DNAs and non-standard DNAs are provided below.

MiSeq run name	Standard DNAs (all libs)	Non-standard DNAs (all libs)	Standard DNAs (sample only)	Non-standard DNAs (sample only)	Standard / Total
RMR-076	3,259,980	4,750,032	2,516,936	4,718,786	35%
CMR-002	5,391,480	7,437,546	4,485,282	7,394,159	38%
RMR-078	6,180,704	7,732,723	5,186,395	7,710,798	40%
RMR-099	3,343,130	7,693,367	2,759,167	7,472,133	27%
Per sample	26,418	40,136	24,305	44,384	35%

"all libs" indicates that sequence reads of all libraries including true samples, negative and positive controls are included. "sample only" indicates that sequence reads of only true samples are included (see the sample metadata in https://github.com/ong8181/ecolnet-int-capacity/SpplementaryData).

4. Rarefaction curve

Rarefying sequence reads is a common approach to evaluate microbial diversity. I examined rarefaction curves and found that the sequencing captured most of the prokaryotic diversity (Figure S2). Also, a previous study suggested that simply rarefying microbial sequence data is inadmissible (McMurdie & Holmes 2014). Another important point is that my analyses involve conversion of sequence reads to DNA copy numbers and thus are different from other commonly used analyses in microbiome studies. Considering these conditions and the previous study, sequence reads were subjected to most of the downstream analysis in the present study without performing further corrections using rarefaction or other approaches.

5. Negative control results

Prior to the library preparation, work-spaces and equipment were sterilized. Filtered pipet tips were used, and separation of pre- and post-PCR samples was carried out to safeguard against cross-contamination. To monitor cross-contamination during field sampling and library preparation process, 10 PCR-level negative controls without internal standard DNAs, 22 PCR-level negative controls with internal standard DNAs, and 30 field-level negative controls were employed for each MiSeq run. PCR-level negative controls were made by adding MilliQ water instead of extracted DNA. For field-level negative controls, a 500-ml plastic bottle containing MilliQ water was carried to the field with the other five 500-ml plastic bottles, and taken back to the laboratory after the water sampling. Then, the MilliQ water in the bottle were filtered using the cartridge filter (ϕ 0.22-μm and ϕ 0.45-μm). The filter cartridges were treated in an identical way with the other samples during the DNA extraction and library preparation processes. Field-level negative controls were collected consecutively during the first 15 sampling events (from 24 May 2017 to 6 June 2017, and the first pre-monitoring sampling on 22 May 2017). After the first 15 sampling events, field-level negative controls were collected once in a week (i.e., on Monday).

All of the negative controls generated negligible sequences compared with the field samples and positive controls for the four MiSeq runs (Figure S2), suggesting that there was no serious cross-contamination during the water sampling, DNA extraction and library preparation processes.

6. Positive control results

Positive control samples were taken to monitor for possible degradation during the sample storage. Water samples were collected from Plot 2 on 23 May 2017 before planting rice seedlings. Ten sub-samples were separately filtered using the cartridge filter (ϕ 0.22-μm and ϕ 0.45-μm), and stored at -20°C in the same way as the other samples. DNAs of the positive controls were extracted subsequently and analyzed in the same way as the other samples. DNA extraction dates for the positive controls were as follows: 23 May 2017, 27 June 2017, 12 July 2017, 9 August 2017, 22 August 2017, 11 September 2017, 29 September 2017, 11 October 2017, 25 October 2017, and 8 November 2017 (i.e., from an immediate DNA extraction to 5.5 months storage at -20°C).

The 10 most abundant ASVs were extracted and their abundances were visualized in Figure S2i-l. The most abundant ASVs were robustly detected from most of the positive samples and there were no dramatic reductions in the DNA copy numbers if DNAs were extracted before 3 months after water sampling (red points in the figures). DNAs of the other samples were extracted within 1-2 months after water sampling in the present study, suggesting that there is no serious DNA degradation during the sample storage.

7. Additional discussion on the meta-analysis

The simple, nonlinear regression using temperature and abundance (or biomass) were separately applied to each data set, and explained biodiversity surprisingly well for the five aquatic data sets (Figure S9; adjusted R^2 = 0.453-0.792) and somewhat worse for global soil data (Figure S9b; adjusted R^2 = 0.16). Connectance may be difficult to predict from abundance in spatially heterogeneous ecosystems such as soil, which may account for the lower predictability of the regression model of the soil data. Also, interaction capacity may be influenced by species identity (e.g., Figure S7b), and in that case interaction capacity may not be simply a function of temperature and/or

abundance. Thus, the accuracy with which diversity can be predicted from temperature and abundance may differ for target organisms and ecosystems. Nonetheless, biodiversity is surprisingly well explained only by a nonlinear regression using temperature and abundance, suggesting that the interaction capacity hypothesis might be applicable to a wide range of taxa and ecosystems.

8. Sample metadata

Sample metadata is provided as a separate CSV file (see SI_Table_MetaDataSummary.csv in https://github.com/ong8181/ecolnet-int-capacity/SpplementaryData).

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