1   **ORIGINAL ARTICLE**

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3   **Hunters or gardeners?**

4   **Linking community structure and function of trap-associated microbes to the nutrient**
5   **acquisition strategy of a carnivorous plant**

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Abstract

All higher eukaryotes live in a relationship with diverse microorganisms which colonize their bodily surfaces; plants are no exception. However, we still lack a satisfactory understanding of how these loosely associated microbiomes with immense diversity and functional potential interact with their hosts or how these interactions shape processes within populations and ecosystems. There is considerable similarity between microbial communities colonizing plant surfaces such as roots, and those of the animal gut. This often overlooked parallel allows us to look at microbial as well as host ecophysiology from a fresh perspective. The traps of carnivorous plants are sophisticated digestive organs and interface environments between the supply and the demand for nutrients. We selected the miniature ecosystem in the traps of aquatic carnivorous *Utricularia* plants as our model system. By assessing the trap-associated microbial community structure, diversity, function, as well as the nutrient recycling potential of bacterivory, we gained insight into the nutrient acquisition strategies of the *Utricularia* hosts. We conclude that trap ecophysiological function is in many aspects highly analogous to that of the herbivore gut and centers around complex microbial consortia, which act synergistically to covert complex organic matter, often of algal origin, into a source of nutrients for the plants.

Key words: algae, bacteria, ciliate bacterivory, digestive mutualism, fungi, herbivory, nutrient turnover, plant–microbe interactions, protists, *Utricularia* traps.

Introduction

49 Recent progress in our understanding of the interdependencies between complex multicellular
50 organisms and their associated microbes has led to an increasing awareness that these
51 interactions are neither rare nor specialized. Instead, they represent fundamentally important
52 aspects of biology and ecology (McFall-Ngai *et al.*, 2013).
53   Plant-associated microorganisms have long been recognized as key partners in
54 enhancing plant nutrient acquisition, mitigating plant stress, promoting growth, or facilitating
55 successful defense mechanisms against pathogens or grazers (for review, see Berg *et al.*,
56 2014). Apart from the well-studied and relatively "simple" symbioses such as mycorrhizal
57 and rhizobial interactions, there is a large pool of diverse microorganisms in varying degrees
58 of association to different plant surfaces and tissues. These often highly complex microbial
59 communities clearly play a significant role in plant ecophysiology, but the underlying
60 mechanisms governing these associations are largely unexplored (Rinke *et al.*, 2013).
61 The liquid-filled traps of carnivorous plants and phytotelmata of other species, such as tank-
62 bromeliads, represent an environment with conditions highly suitable for microbial
63 colonization and growth. They have often been used in the past as model systems for
64 ecological research, especially focused on microbial food-web structure and related ecological
65 questions (Cochran-Stafira and Von Ende, 1998; Koopman *et al.*, 2010; Gray *et al.*, 2012).
66 However, the information on the relationship between microbial communities associated with
67 these environments and their plant hosts has been scarce (Adlassnig *et al.*, 2011).
68   Recently, the hypothesis that microbial communities inhabiting the traps of rootless
69 aquatic carnivorous *Utricularia* plants may be closely coupled to plant metabolism, nutrient
70 acquisition, and growth was proposed (Sirová *et al.*, 2009). The exudation of large amounts of
71 bioavailable photosynthates into *Utricularia* traps and their subsequent rapid utilization by the
72 microorganisms present has been experimentally confirmed and represents a direct link
73 between the plant host and associated microbiota (Sirová *et al.*, 2010, 2011). *Utricularia* are
74 among the most numerous and cosmopolitan genera of carnivorous plants, attractive to
75 researchers, due to their extremely small and unusual genomes (e.g., Carretero-Paulet *et al.*,
76 2015; Silva *et al.*, 2016). Depending on the species and growth conditions, a single
77 *Utricularia* plant may bear hundreds to thousands of traps, usually on highly segmented
78 leaves (Figure 1). These are tiny (1–5 mm long) liquid-filled bladders, whose lumen is
79 isolated from the ambient environment by a two-cell thick trap wall and is generally anoxic or
80 anaerobic (Adamec, 2007). The presence of various microbial taxa actively colonizing the
81 lumen of *Utricularia* traps has previously been detected and roughly quantified (Sirová *et al.*,
82 2009, 2011; Caravieri *et al.*, 2014, Alcaraz *et al.*, 2016). Likewise, it has been noted that the
83 external surfaces of *Utricularia* plants tend to be covered in thick algal periphyton, exceeding
84 in abundance that of other aquatic species (Díaz-Olarte *et al.*, 2007). Although *Utricularia*-
85 associated microorganisms are thought to play an important role in the plant's ecophysiology,
86 a deeper insight into their community structure and ecology is lacking.
87   *Utricularia* were long thought to be classical examples of the carnivorous habit (Darwin,
88 1875). However, their nutrient acquisition strategy is the subject of debate and the importance
89 of carnivory in their nutrition has been questioned (Richards, 2001; Sirová *et al.*, 2009). It has
90 been proposed that algae, frequently observed and abundant in both the plant periphyton and

91 traps (Sirová *et al.*, 2009; Płachno *et al.*, 2012), rather than metazoan plankton, are the main
92 source of nutrients for the plants (Peroutka *et al.*, 2008; Alkhalaf *et al.*, 2009).
93 Here, we present the results of next generation sequencing of the microbiome, including
94 the periphytic communities, associated with two *Utricularia* species – *U. australis* and *U.*
95 *vulgaris*. Microbial community structure, diversity, the nutrient recycling potential by
96 bacterivorous protists as well as their importance for plant growth are assessed in *U. reflexa*.
97 It has been well established in both vertebrates and invertebrates that microbial diversity in
98 the digestive tract reflects closely the composition of the prevailing food source (Nalepa *et al.*,
99 2001; Barboza *et al.*, 2010). The traps of *Utricularia* species also function as digestive
100 systems and are interfaces between the "food" supply and the demand for nutrients of the
101 rootless plants. The main nutrient source in *Utricularia*, however, has remained ambiguous
102 and its direct verification at *in-situ* conditions technically challenging (Adamec, 2011).
103 During trap lifespan, only a minority of traps capture a macroscopic prey, while all of them
104 contain communities of microbial commensals (Friday, 1989; Richards, 2001).
105 Based on the observations of trap-associated microbial community structure and
106 behavior and reports from literature, we hypothesize here that aquatic *Utricularia* traps are
107 structures able to process and utilize organic material of algal (and/or plant) origin. To test
108 this hypothesis, we assessed the functional capabilities of trap-associated microorganisms
109 with respect to gene expression, and compared the microbial community structure with
110 similar datasets from pitcher traps of other carnivorous plants, gut microflora of various
111 vertebrates and invertebrates, including carnivores and detritivores, the rumen microbiota of
112 various herbivores, and environmental samples from the soil, rhizosphere, and freshwater. By
113 combining molecular methods, microscopy, and other approaches, we were able to shed light
114 onto the ecology of this highly specific and unique microbial niche which, surprisingly, seems
115 to bear closest analogy to mammalian rumen ecosystems.

Material and Methods

117 *Plant material and sampling*
118 To assess the presence and diversity of trap associated microbiota, the ecophysiologically
119 well characterized aquatic species *U. vulgaris* and *U. australis* were selected. Adult *U.*
120 *australis* plants (40–50 cm in length) were collected from a mesotrophic bog at Ruda fishpond
121 (South Bohemia, see Sirová *et al.*, 2010 for details). The 0.8 m^2 polypropylene experimental
122 container, where *U. vulgaris* plants were cultivated, contained *Carex* sp. litter and
123 approximately 250 liters of dystrophic water, closely simulating natural conditions (Adamec
124 *et al.*, 2010a; Borovec *et al.*, 2012). For the assessment of microbial community structure, *U.*
125 *vulgaris* and *U. australis* plants were divided into three sections of increasing age (young,
126 mature, old). From each of these segments approximately 70 larger, excised, functional traps
127 without visible metazoan prey and trapless leaves with periphyton were collected
128 (approximately 200 mg FW).
129 To assess the actively transcribed microbial gene pool, excised *U. vulgaris* traps from the
130 entire shoot without visible metazoan prey were collected randomly (approximately 250 mg
131 FW), with pooled traps from a single plant considered a replicate; three biological replicates

132   were collected in total. All collected plant material was immediately placed into liquid N_2 and
133   samples were stored at –80°C until further processing.
134   For the estimation of the protozoan grazing rates, a different species with larger traps –
135   the tropical *U. reflexa* – was selected, because relatively large volumes of trap fluid are
136   needed for this analysis. The plants were cultivated indoors in 3-liter aquaria; in dystrophic
137   cultivation water closely simulating natural conditions (see Adamec 2007).

138   *SSU rRNA amplicon sequencing for the taxonomical evaluation of the* Utricularia-*associated*
139   *microorganisms*
140   Nucleic acid extractions were conducted according to a modified bead-beating protocol
141   (Urich *et al.*, 2008). Total DNA was quantified fluorometrically using SybrGreen (Leininger
142   *et al.*, 2006). The PCR primers (515F/806R) targeted the V4 region of the SSU rRNA,
143   previously shown to yield accurate phylogenetic information and to have only few biases
144   against any bacterial taxa (Liu *et al.*, 2007; Bates *et al.*, 2011; Bergmann *et al.*, 2011). Each
145   sample was amplified in triplicate, combined, quantified using Invitrogen PicoGreen and a
146   plate reader, and equal amounts of DNA from each amplicon were pooled into a single 1.5-ml
147   microcentrifuge tube. Once pooled, amplicons were cleaned up using the Ultra Clean PCR
148   clean up kit (MO BIO Laboratories). Amplicons were sequenced on the Illumina MiSeq
149   platform at the Institute of Genomics and Systems Biology, Argonne National Laboratory,
150   Argonne (Chicago, USA). Paired-end reads were joined using Perl scripts yielding
151   approximately 253bp amplicons. Approximately 1.8 million paired-end reads were obtained
152   with average 66.000 reads per sample. Quality filtering of reads was applied as described
153   previously (Caporaso *et al.*, 2010). Reads were assigned to operational taxonomic units
154   (OTUs, cut-off 97% sequence identity) using an open-reference OTU picking protocol with
155   QIIME implemented scripts (Caporaso *et al.*, 2010). Reads were taxonomically assigned
156   using Green Genes database, release 13.08 as reference. Those reads which were assigned as
157   "chloroplast" and "mitochondrion" were excluded from further analyses.
158
159   *Meta-analyses of prokaryotic communities from different habitats*
160   To compare the composition of *Utricularia* trap-associated microbial communities, data
161   (OTU tables in biom format) from 9 relevant studies represent different habitats were
162   analyzed. Seven of the studies were downloaded from Qiita (https://qiita.ucsd.edu/) database,
163   16S rDNA sequences of the *Nepenthes* pitcher microbiome from the SRA archive (project ID
164   PRJNA225539) and *Sarracenia* pitcher sequences from the Genebank database (accession
165   numbers JF745346–JF745532 and JN368236–JN368422) (Supplementary Table 1).
166   Altogether, 4221 samples were included in the meta-analysis. The Qiita database works with
167   the closed reference OTU picking algorithm, we have therefore processed our sequences and
168   also the sequences from *Nepenthes* and *Sarracenia* pither microbiomes in the same way as the
169   Qiita pipeline, to ensure comparability with Qiita OTU tables. All OTU tables were then
170   merged together using Qiime scripts and analyzed as one dataset. Non-metric
171   multidimensional scaling (NMDS) using Bray-Curtis dissimilatory metrics was used for
172   computing distances between samples. Because the large amount of samples reduced clarity
173   of the graphical representation of results, for illustrative purposes only, we randomly chose 30
174   samples from each study to construct a dendrogram (Figure 2a) using the UPGMA algorithm.

175   To obtain a more function-related point of view, three *Utricularia vulgaris*
176   metatranscriptomes were compared to 43 metagenomes/metatranscriptomes from 6 different
177   habitats. The sequences from these studies were obtained from the MG-RAST server (Table
178   S1)
179
180   *RNA-seq analysis for functional profiling of the* U. vulgaris *trap-associated microbiome*
181   To assess the actively transcribed microbial gene pool, total RNA was extracted from the *U.*
182   *vulgaris* trap samples (n=3), using the protocol identical to that described in detail previously
183   (Sirová *et al.*, 2014). Briefly, DNA was removed from the extracts and two transcriptomic
184   libraries, eukaryotic and prokaryotic, were created at the Institute of Genomics and Systems
185   Biology, Argonne National Laboratory, Argonne (Chicago, USA) using standard Illumina
186   TruSeq RNA library preparation kits. The ribosomal RNA as well as eukaryotic (plant) RNA
187   fraction was removed in order to enrich prokaryotic transcripts, and, *vice versa*, eukaryotic
188   transcript enrichment was performed in parallel, to capture transcripts from fungi, protists,
189   and other eukaryotic microorganisms. Enriched mRNA from both libraries was reverse
190   transcribed to create metatranscriptomic libraries and sequenced using Illumina HiSeq
191   platform (100×100 cycle paired-end run). We obtained approximately 40 million paired-end
192   reads per sample. Reads were quality checked, low quality reads and reads with ambiguous
193   bases were filtered out. Reads from all three replicates (approx. 120 million sequences) were
194   then assembled with Velvet Optimizer (Zerbino and Birney, 2008) which resulted in
195   approximately 500,000 contigs. In this step, we filtered out the potential *Utricularia*
196   transcripts by blasting contigs against our *Utricularia* reference transcriptome (Bárta et al.
197   2015). All contigs which gave significant hit (e-value <0.0001, min score 80) were excluded
198   from further analyses. Contigs were also blasted against the SILVA database (release 111) to
199   identify ribosomal RNAs (rRNAs). Those sequences that gave BLAST bit score greater than
200   80 were marked as potential rRNAs and extracted from the dataset. Reads were than mapped
201   back onto the remaining contigs using bowtie algorithm with default parameters (Haas *et al*.,
202   2013). To identify potential prokaryotic functional gene transcripts, the remaining contigs
203   without rRNAs were blasted against the nr database with e-value of 0.001.
204
205   *Microbial network analyses*
206   The relative abundances of OTUs were square-root transformed and the 300 most abundant
207   OTUs in *U. vulgaris* samples were selected, to avoid the spurious correlations of rare OTUs.
208   The resulting OTU tables, separately for the trap (15 samples) and the periphyton (16
209   samples) were used for microbial network analyses. The analysis was done in Cytoscape 3.0.2
210   with the CoNET application (Shannon *et al.*, 2003; Faust *et al.*, 2012). The parameters and
211   settings for network analyses in CoNET application were: -parent_child_exclusion, -
212   row_minocc 8, -correlations (Spearman, Pearson, mutual information, Bray–Curtis and
213   Kullback–Leibler dissimilatory). The threshold for edge selection was set to 1,000 top and
214   bottom. During randomization, 100 iterations were calculated for edge scores. In the
215   following bootstrap step, 100 iterations were calculated, unstable edges were filtered out. The
216   Brown method was chosen as the P-value merging method and the Benjamini–Hochberg
217   procedure was selected for multiple test correction. The resulting network was visualized and

218 analyzed (i.e. degree of nodes, betweenness centrality, closeness centrality) in Cytoscape
219 3.0.2. Potential keystone OTUs were identified (Berry and Widder, 2014).
220   For the estimation of unique microbiomes of *U. australis* and *U. vulgaris* traps and
221 periphyton, the OTUs were grouped at the genus level. Genera presented only in *U. australis*
222 or *U. vulgaris* and respective trap or periphyton samples were classified as unique
223 microbiomes.

224 *Quantification of trap-associated eubacterial and fungal communities*
225 Quantification of bacterial and fungal SSU rRNA genes was performed using the FastStart
226 SybrGREEN Roche® Supermix and Step One system (Life Technologies, USA). Each
227 reaction mixture (20 µl) contained 2 µl DNA template (~1–2 ng DNA), 1 µl each primer (0.5
228 pmol µl^{-1} each, final concentration), 6 µl dH$_2$O, 10 µl FastStart SybrGREEN Roche®
229 Supermix (Roche, France) and 1 µl BSA (Fermentas, 20 µg µl^{-1}). The qPCR conditions for
230 bacterial quantification were as follows: initial denaturation (3 min, 95°C) was followed by 30
231 cycles of 30 s at 95°C, 30 s at 62°C, 15 s at 72°C, and completed by fluorescence data
232 acquisition at 80°C used for target quantification. Product specificity was confirmed by
233 melting point analysis (52°C to 95°C with a plate read every 0.5°C) and amplicon size was
234 verified with agarose gel electrophoresis.
235   Bacterial DNA standards consisted of a dilution series (ranging from 10^1 to 10^9 gene
236 copies µl^{-1}) of a known amount of purified PCR product obtained from genomic *Escherichia*
237 *coli ATCC 9637* DNA by using the SSU gene-specific primers 341F/534R (Muyzer *et al.*,
238 1993). The R^2 values for the standard curves were >0.99. Slope values were >3.37 giving an
239 estimated amplification efficiency of >93%. The qPCR conditions for fungal quantification
240 were as follows: initial denaturation (10 min, 95°C) followed by 40 cycles of 1 min at 95°C, 1
241 min at 56°C, 1 min at 72°C, and completed by fluorescence data acquisition at 72°C used for
242 target quantification. Fungal DNA standards consisted of a dilution series (ranging from 10^1
243 to 10^7 gene copies µl^{-1}) of a known amount of purified PCR product obtained from genomic
244 *Aspergillus niger* DNA by using the SSU gene-specific primers nu-SSU-0817-5' and nu-
245 SSU1196-3' (Borneman and Hartin, 2000). R^2 values for the fungal standard curves were >
246 0.99. The slope was between −3.34 to −3.53 giving estimated amplification efficiency
247 between 95 and 93%, respectively.
248   Detection limits for the various assays (i.e. lowest standard concentration that is
249 significantly different from the non-template controls) were less than 100 gene copies for
250 each of the genes per assay. Samples, standards, and non-template controls were run in
251 duplicates. To deal with potential inhibition during PCR, the enhancers (BSA, DMSO) were
252 added to the PCR mixture. Also several dilutions (10×, 20×, 50×, 100×, and 1000×) for each
253 sample were tested to see the dilution effect on Ct values.

254 *Quantification of trap-associated methanogenic and methanotrophic communities*
255 Quantification of the *mcrA* gene (methanogens) was performed using the FastStart
256 SybrGREEN Roche® Supermix and Step One system (Life Technologies, USA). Each
257 reaction mixture (20 µl) contained 2 µl DNA template (~1–2 ng DNA), 0.1 µl each primer
258 (0.3 pmol µl^{-1} each, final concentration), 6 µl dH$_2$O, 10 µl FastStart SybrGREEN Roche®
259 Supermix (Roche, France) and 0.4 µl BSA (Fermentas, 20 µg µl^{-1}). Primers ME1 (5'-GCM

260 ATG CAR ATH GGW ATG TC-3') a MCR1R (5'-ARC CAD ATY TGR TCR TA-3')
261 producing amplicon length of 280bp (Hales *et al.*, 1996). The qPCR conditions for *mcrA* gene
262 were as follows: initial denaturation (10 min, 95°C) followed by 35 cycles of 30s at 95°C, 1
263 min at 50°C, 1 min at 72°C, and completed by fluorescence data acquisition at 72°C used for
264 target quantification. Standards consisted of a dilution series (ranging from 10^1 to 10^7 gene
265 copies μl^{-1}) of a known amount of purified PCR product obtained from genomic DNA of
266 *Methanosarcina barkeri* DSM-800.
267 Quantification of *pmoA* gene (methanotrophs) was performed using the FastStart
268 SybrGREEN Roche® Supermix and Step One system (Life Technologies, USA). Each
269 reaction mixture (20 µl) contained 2 µl DNA template (~1–2 ng DNA), 0.24 µl each primer
270 (0.5 pmol μl^{-1} each, final concentration), 5.6 µl dH$_2$O, 10 µl FastStart SybrGREEN Roche®
271 Supermix (Roche, France), 0.5 µl DMSO and 0.4 µl BSA (Fermentas, 20 µg μl^{-1}). Primers
272 A189-F (5'-GGNGACTGGGACTTCTGG-3') a M661-R (5'-
273 GGTAARGACGTTGCNCCGG-3') producing amplicon length of 491bp (Kolb *et al.*, 2003).
274 The qPCR conditions for *pmoA* gene were as follows: initial denaturation (10 min, 95°C)
275 followed by 35 cycles of 30 s at 95°C, 20 s at 57°C, 45 s at 72°C, and completed by
276 fluorescence data acquisition at 72°C used for target quantification. Standards consisted of a
277 dilution series (ranging from 10^1 to 10^7 gene copies μl^{-1}) of a known amount of purified PCR
278 product obtained from genomic DNA of *Methylobacter luteus*.
279
280 *Sequence deposition*
281 Raw sequences of 16S rDNA, ITS1 amplicons, and raw sequences of all three
282 metatranscriptomes were deposited in European Nucleotide Archive (ENA) under study ID
283 PRJEB19666. Annotated metatranscriptomic sequences can be found at the following
284 website: http://utricularia.prf.jcu.cz/
285 *Bacterial and protozoan quantification and the estimation of protozoan grazing rates*
286 Ten *U. reflexa* plants were divided into three segments of increasing age (young, mature, old).
287 Each segment contained 6 leaf whorls. Trap fluid was collected from the traps in each
288 segment (see Sirová *et al.*, 2009) and a pooled sample (~750 µl for each age category) from
289 all ten plants was made. Bacterial and protozoan counts in the trap fluid samples were
290 estimated using epifluorescence microscopy, according to methods described previously
291 (Šimek *et al.*, 2000).
292 The protist grazing rates were estimated using fluorescently labelled bacteria (FLB) as
293 a tracer. The FLB were prepared from the strain *Limnohabitans planktonicus*, as detailed in
294 Šimek *et al.* (2017). Cell sizes of the strain are comparable to that of bacterial cells commonly
295 occurring within the *U. reflexa* traps. The FLB uptake rates were determined in short-term
296 triplicate experiments, where tracer FLB were added to the trap-fluid samples to constitute 6-
297 8% of the total bacterial concentration. For further details on sample fixation, protist staining
298 and enumeration, and tracer ingestion determinations, see Šimek e*t al.* (2017). At least 45
299 ciliates were inspected for FLB ingestion in each replicate sample. To estimate total protist
300 grazing, we multiplied average uptake rates of protozoa by their *in-situ* abundances as
301 previously described (Šimek *et al.*, 2000).

8

Results and Discussion

Microbial diversity

Our results show that *Utricularia* plants harbor microbial communities rich in both taxonomic composition and metabolic capabilities. We have identified over 4,500 distinct OTUs in the traps and periphyton (see the complete OTU list in Supplementary Table 2a, 2b). Microbial (prokaryotic) alpha diversity in two studied *Utricularia* species was significantly higher than that found in the traps of other carnivorous plants and was comparable to that observed in other high-diversity systems: the rhizosphere and the cow gut (Table 1). A comparison with datasets from other environments with regards to composition (Figure 2a) revealed that the *Utricularia* trap microbiomes are species-specific, but similar between the two studied species. Out of the different environments selected, they most closely resembled the communities inhabiting the pitchers of a terrestrial carnivorous plant, *Sarracenia*, and those found inside the guts of herbivorous iguanas. When comparing the metatranscriptomic data from the traps of *U. vulgaris* with other systems, the highest similarity (more function-related) was with freshwater microbial communities (Figure 2b).

When comparing the trap and periphytic communities associated with one *Utricularia* species, the bacterial communities in both environments were dominated by Proteobacteria (58% and 54% of assigned sequences in periphyton and trap, respectively; p=0.013) and both had very similar bacterial composition at the family level. Over half of the taxa were shared even at the genus level (Figure 3). The Comamonadaceae were the most abundant group, constituting similar proportion (>10%) of both the trap and the periphytic communities. The close similarity of periphytic and trap communities suggests strong links between *Utricularia* external surfaces and the internal trap environment, with periphyton being the most likely source of inoculum for microbial colonization of traps (Sirová *et al*., 2009; 2011).

Despite of the high similarity between the trap lumen and the periphyton in terms of prokaryotic community composition, when we compare the microbial co-occurrence analyses, we see two strikingly different systems with distinct "key-stone" taxa (Supplementary Table 3) and a distinct degree of interconnectedness (Supplementary Figures 1, 2) in each of the two environments. While the periphytic communities show a co-occurrence pattern (Supplementary Figure 1) typically observed in highly spatially and functionally interconnected microbial biofilms, the co-occurrence pattern of the trap community consists of several smaller, mutually disconnected microbial networks and implies a much more heterogeneous and fragmented environment inside the trap lumen (Supplementary Figure 2). This result is consistent with previously published observations showing progressing degree of microbial aggregation into flocks and multispecies biofilms with progressing *Utricularia* trap development (Sirová *et al*., 2009). In analogy, results coming from direct microscopic examinations of the rumen ecosystem and its contents (McAllister *et al*., 1994) show that microbial populations are largely attached to feed particles (>70% of total numbers, Russell *et al.*, 2009) and that bacteria from the ruminal fluid associate into micro-colonies forming multispecies biofilms. These metabolically related organisms then associate with their preferred substrates and produce the myriad of enzymes necessary for the digestion of chemically and structurally complex plant tissues, hence, creating a system of mutually disconnected micro-environments.

345   In terms of core microbiota, one of the most distinct groups, which were specific to
346   the *Utricularia* trap environment only, were members of the Peptostreptococcaceae family
347   (Clostridiales), which successfully colonized the lumen, but were rarely found in the
348   periphyton (Supplementary Table 2a). The group represents anaerobic fermenters often
349   associated with habitats such as animal guts and oral cavities, manure, soil, and sediments
350   (Slobodkin, 2014). In the rumen ecosystem, *Peptostreptococcus* spp. produce high amounts of
351   ammonia, but are not able to hydrolyze intact proteins and do not use carbohydrates as a
352   carbon source. Thus, they occupy a niche of peptide- and amino-acid-degrading
353   microorganisms and depend on proteolytic bacteria (Attwood *et al.*, 1998). The genes
354   expressed in the traps that were assigned to this family, such as pyrroline-5-carboxylate
355   reductase [EC:1.5.1.2] or threonine aldolase [EC:4.1.2.5] (Supplementary Table 4), are all
356   involved in the metabolism of amino acids. In ruminants, as much as 90% of the amino acids
357   reaching the small intestine are derived from ruminal microorganisms. The trap lumen
358   contains very high concentrations of ammonium ions (Sirová *et al.*, 2014) and many bacteria
359   able to hydrolyze proteins are present; it is therefore likely that a process highly similar to that
360   in the rumen involving Peptostreptococcaceae, is taking place therein.

361   *Methane metabolism*
362   Herbivore gut ecosystems generally tend to produce copious amounts of methane as a result
363   of the fermentation activity by the strictly anaerobic methanogenic Archaea (Hackstein and
364   Van Alen, 1996). Using gas chromatography, we have not detected the release of methane gas
365   from the *Utricularia* traps (data not shown), and methanogens were not found in our trap fluid
366   samples using the qPCR assay (Table 2). However, significant amounts of methanotrophs
367   were found, constituting up to 40% of total prokaryotic community (Supplementary Table
368   2c). These included active obligate methanotrophs, for example from the genus
369   *Methylococcus* (Gammaproteobacteria, Supplementary Table 2c). Moreover, methane
370   metabolism was also found to be one of the most expressed prokaryotic modules (KEGG) in
371   the trap fluid metatranscriptome from *U. vulgaris* (Table 2a). These somewhat paradoxical
372   results may be explained by a recent discovery (Repeta *et al*., 2016) that the degradation of
373   dissolved organic matter in the aquatic environment by commonly occurring bacteria, e.g.
374   *Pseudomonas* spp., can, even in the presence of oxygen, result in the release of methane,
375   ethylene and propylene.
376   We speculate that this process can explain the presence and activity of methanotrophs
377   in the *Utricularia* traps, which may metabolize all of the produced methane, thus preventing
378   its detection. This hypothesis, however, needs to be experimentally verified.
379
380   *Degradation of complex algal/plant polymers*
381   We have detected the presence of bacterial genera in the traps, which are considered to
382   include cellulolytic species capable of degrading complex organic material of plant (algal)
383   origin (Supplementary Table 2a), for example *Clostridium*, *Ruminococcus*,
384   *Caldicellulosiruptor*, *Butyrivibrio*, *Acetivibrio*, *Cellulomonas*, *Erwinia*, *Thermobifida*,
385   *Fibrobacter*, *Cytophaga*, and *Sporocytophaga*. Also notable is the significant presence of
386   active myxobacteria (*Cystobacter* spp.), which are known to include cellulolytic species and

387 are frequently isolated from systems with high decomposing plant material content (Shimkets
388 *et al.*, 2006).
389   Bacterial degradation of cellulose-rich material is more restricted to biomass
390 containing low amounts of lignin, as bacteria are generally poor producers of lignanases.
391 Plant and algal biomass produced in aquatic systems contains little amounts of lignin and is
392 typically degraded by bacteria which are better adapted for the aquatic environment than
393 fungi. Overall numbers of bacterial cells found in the *U. reflexa* trap fluid were similar to
394 those reported previously (Sirová *et al.*, 2009; 2011), being an order of magnitude higher in
395 the old traps compared to the young and mature (Table 3). Bacterial degradation of cellulose-
396 rich material is more restricted to biomass containing low amounts of lignin, as bacteria are
397 generally poor producers of lignanases. Plant and algal biomass produced in aquatic systems
398 contains little amounts of lignin and is typically thought to be degraded mostly by bacteria,
399 which are better adapted for the aquatic environment than fungi.
400   Fungi, however, were also found to be present in the traps and represented a
401 significant proportion of the total microbial community, as quantified by qPCR (Table 2).
402 Fungi, especially the anaerobic chytrids (Chytridiomycota), are frequently found in the
403 gastrointestinal tracts of ruminants and non-ruminant herbivores, either associated with
404 digested organic material, or as free-swimming zoospores in the fluid phase, playing
405 significant role in the digestive process of complex plant polymers. Many fungal taxa whose
406 presence inside of the *Utricularia* traps was determined by SSU rRNA sequencing, (e.g.
407 *Chrysomphalina* sp., Agaricales, Basidiomycota, Supplementary Table 2b) were most
408 probably entrapped as spores from the ambient environment and do not represent trap-
409 associated microbes as such, but rather a potential source of nutrients. Others, most notably
410 the saprotrophic *Basidiobolus* sp. (Basidiobolales, Zygomycota) abundant in the traps of all
411 ages (up to 45% of total OTUs), or species belonging to the above mentioned
412 Chytridiomycota (Supplementary Table 2b) found to be actively growing in the traps
413 (Supplementary Table 4), are likely a component of the trap microbial network and contribute
414 to the nutrient release and assimilation by the plant host.
415   pH is considered a key factor affecting the efficiency of organic matter degradation
416 and nutrient release in the rumen and herbivore digestive tracts as both fungal and bacterial
417 enzymes are sensitive to pH changes (Russel and Wilson, 1996). It has been shown that the
418 trap pH in aquatic *Utricularia* is highly stable, usually acidic, species specific (Sirová *et al.*,
419 2003, 2009) and its stability likely contributes to the optimal development of the trap-
420 associated microbial communities and their activities.

421 *Protistan grazing and nutrient turnover*
422 A third important group of microbes in the traps are the protists. Their numbers in the *U.*
423 *reflexa* traps were extremely high, rising steadily with increasing trap age, up to
424 approximately 50,000 cells per milliliter of trap fluid in the oldest traps. This means that a
425 single trap, depending on age, harbored tens to hundreds of individuals (Table 3). Such high
426 population densities are rarely found in natural environments and are comparable only to
427 those found in the mammalian rumen or in the activated sludge systems (e.g. Towne *et al.*,
428 1990; Abraham *et al.*, 1997). The protist community in *U. reflexa* traps, however, had very
429 low diversity and essentially consisted of a monoculture composed of various euglenid

430   species and a single conspicuous zoochlorellae-bearing ciliate (Figure 4). This organism has

431   recently been described as a new species – *Tetrahymena utriculariae* – and has not yet been

432   found in any other environment (Pitsch *et al.*, 2017; Šimek e*t al.,* 2017).

433   Ciliates are important bacterial predators, mainly in nutrient-rich freshwater

434   environments (e.g. Šimek e*t al.,* 1998, 2000; Thouvenot *et al.,* 1999), and are generally

435   considered one of the key agents ensuring nutrient recycling and transfer to other trophic

436   levels (e.g. Laybourn-Parry, 1992). We have confirmed and quantified bacterivory in *T.*

437   *utriculariae*, whose grazing rates were comparable to literature reports (Neurer and Cowles,

438   1995). Due to their abundance, the ciliates were able to turn over the entire bacterial standing

439   stock in the trap fluid extremely fast: 4–5 times in 24 hours in the younger traps (Table 3).

440   Turnover time increased markedly in the old traps, due to the large increase in bacterial

441   numbers. Obviously, the turnover of microbial mass in the *U. reflexa* traps and the release of

442   soluble mineral nutrients from microbial cells with their subsequent uptake by the plant from

443   the trap fluid are, to a large extent, facilitated by protist predation on bacteria. *Utricularia*

444   plants apparently depend on microbial activity for the supply of nutrients and thus the amount

445   of bacterial mass produced in the prey-free traps likely is as important to the plant host as the

446   amount of organic matter digested.

447

448   *Predatory bacteria*

449   Protists are, however, not the only predators affecting bacterial populations in the traps of

450   *Utricularia.* We have also detected the family Bacteriovoracaceae in the lumen, whose

451   members are known for being obligatory predators of other, especially Gram-negative,

452   bacteria. There have been few studies of the ecological roles of predatory bacteria. They are,

453   however, present in diverse habitats, which indicates that, like viruses, they are important

454   determinants of microbial community dynamics (Velicer and Mendes-Soares, 2009). In the

455   *Utricularia* traps, they are relatively abundant (Supplementary Table 2a), metabolically active

456   (Supplementary Table 4) and, therefore, likely to selectively influence the trap microbial

457   community dynamics through enhanced mortality rates of particular bacterial species in this

458   closed environment.

459   *Microbial activity*

460   When considering the overall activity of trap-associated microbiota, our results confirm

461   highly dynamic, rapidly growing communities of both prokaryotes and eukaryotes (Tables 4a,

462   4b). Expression profiles (Supplementary Table 4) indicate rapid growth, intensive protein

463   metabolism, respiration, DNA synthesis, and motility. Notable is the high expression of

464   bacterial UDP-glucose-6-dehydrogenase, which has been linked to the environmentally

465   regulated biosynthesis of exopolysaccharides (Petit *et al*., 1995), or that of transaldolase,

466   which is also one of the highly expressed proteins in the trap fluid and has been shown to be

467   an important colonization factor favoring the establishment of bacteria in the gut (González-

468   Rodríguez *et al*., 2012). This activity is likely linked to bacterial aggregation and attachment

469   to organic particles, which is typical for the rumen environment and has been observed in

470   *Utricularia* traps previously (Sirová *et al*., 2009).

471   The transcriptomic analysis also offers several clues indicating the ability of trap-
472 associated microbes to degrade complex organic material of algal origin. Algal cell walls and
473 other cellular structures are composed of various monosacharides, derived from glucose,
474 linked into polymers (cellulose and hemicellulose). These monosaccharides also include *D*-
475 galactose (Buchanan *et al.*, 2015). Three enzymes involved in galactose metabolism were
476 among the prokaryotic genes most expressed in *Utricularia* traps (Table 1a, Supplementary
477 Table 4): the α- and β-galactosidases, which catalyze the cleavage of the terminal *D*-
478 galactosyl residues of plant and algal hemicelluloses and their activity is often associated with
479 herbivore digestive systems (Yapi *et al.*, 2007; Lu *et al*., 2012), and UDP-glucose 4-
480 epimerase, which performs the final step in the Leloir pathway catalyzing the reversible
481 conversion of UDP-galactose to UDP-glucose. The high expression levels of these enzymes
482 underscore the importance of microbial galactose metabolism in the traps and are a further
483 indication that the trap microbes, especially bacteria, actively degrade complex algal material.

484 *Conclusions*
485 We conclude that, in analogy to herbivores, the aquatic *Utricularia* plants support the
486 development of a diverse and sophisticated microbial ecosystem inhabiting both their traps
487 and their large external surfaces, which allows them to digest and utilize complex organic
488 material of algal origin and thrive even at highly oligotrophic sites. Here, although metazoan
489 prey is usually scarce and prey capture rates correspondingly low (Richards, 2001), the supply
490 of algae growing in close proximity to the traps as part of the *Utricularia* periphytic
491 "gardens" is continuous and abundant (Płachno *et al*., 2012). In analogy to the digestive tracts
492 of herbivorous animals, feedstuffs enter the traps and are degraded to various extents by the
493 microbial populations colonizing them. Like the gut/ruminal ecosystems, traps harbor a
494 diverse, symbiotic population of bacteria, fungi, and ciliates that have adapted for survival
495 under low oxygen supply, high cell densities, and ciliate predation. Moreover, they have
496 evolved the capacity for efficient utilization of complex and often recalcitrant plant polymers,
497 such as cellulose and hemicellulose. As such, the *Utricularia*–microbe systems represent
498 unique biodiversity and activity hotspots within the nutrient-poor, dystrophic environments, in
499 which they grow (Figure 5), and should be considered as synchronized, mutually dependent
500 biological and ecological units in future research.
501

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507

508

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703

704   *Figure captions*

705

706   **Figure 1** Experimental *Utricularia vulgaris* shoot on a Petri dish. Segmented leaves bearing
707   traps and the growth tip are visible.

708

709   **Figure 2** Meta-analyses of prokaryotic communities from different environments (a) based on
710   16S rDNA gene sequencing (4421 samples, UPGMA tree, data from 9 relevant studies were
711   downloaded from the Qiita database (https://quiita.ucsd.edu/) and
712   metagenomes/metatranscriptomes (b) based on shotgun sequencing and KEGG annotations
713   (43 samples obtained from MG-RAST, Neighbor joining tree). For better visualization of the
714   UPGMA tree (b), only up to 30 samples per study were selected.

715

716   **Figure 3** Compositional overlap in *Utricularia*-associated microbiomes at the genus level,
717   computed for prokaryotic genera only. (a) Comparison between *U. australis* and *U.vulgaris*
718   microbiomes and (b) between *U. australis* and *U.vulgaris* periphyton and trap.

719

720   **Figure 4** *Tetrahymena utriculariae* under the epifluorescence microscope**.** Zoochlorellae are
721   visible in purple, the nucleus is stained blue, and fluorescently labeled bacteria in food
722   vacuoles show green fluorescence.

723

724   **Figure 5** Conceptual representation of the *Utricularia* trap ecophysiology: main microbe-
725   microbe and plant-microbe interactions are shown.
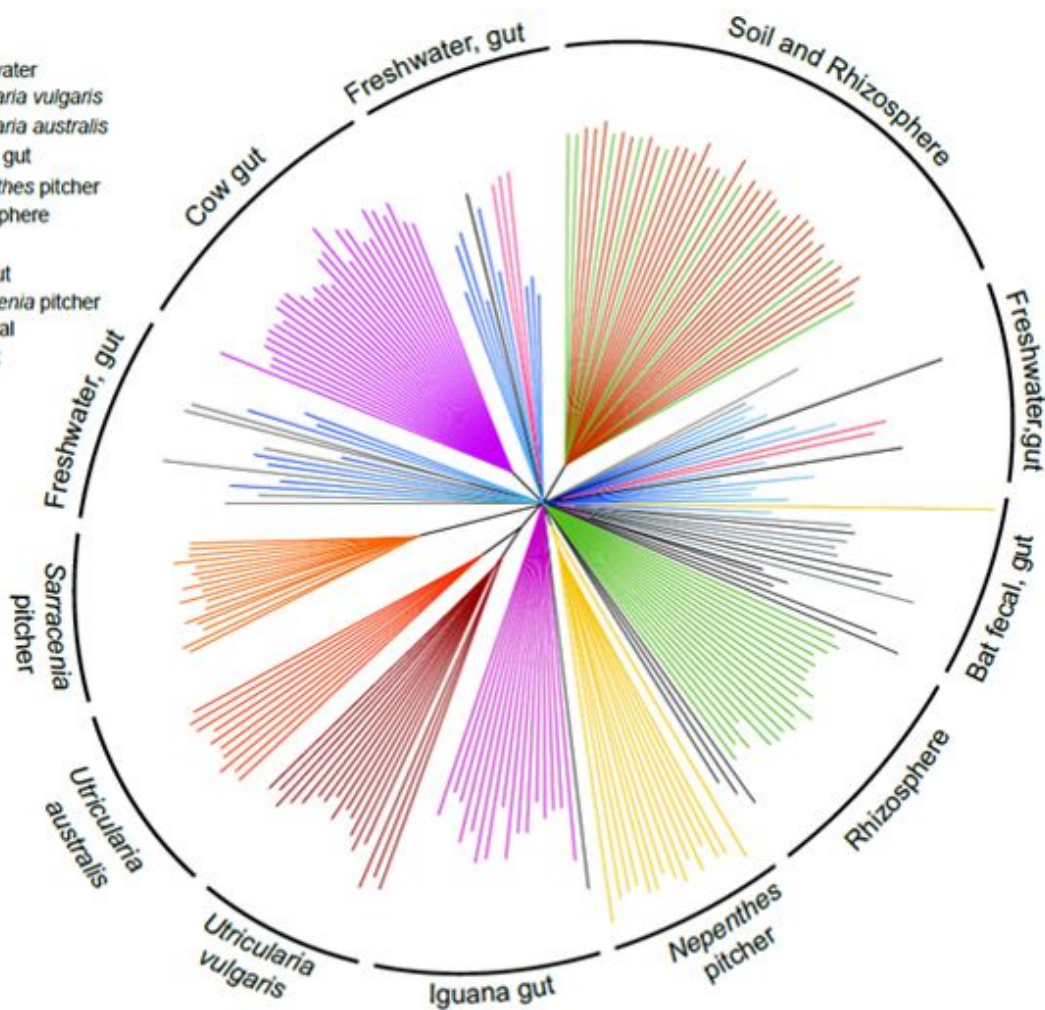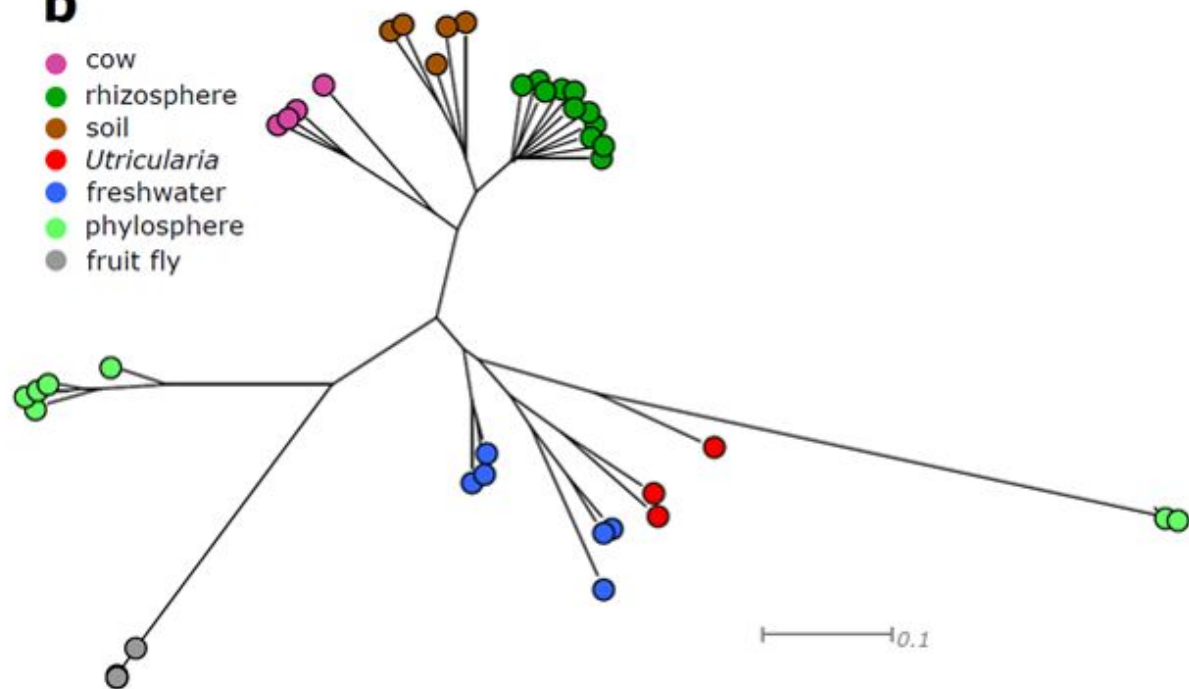
726   *Table legends*

727

728   **Table 1** Comparison of selected alpha diversity indexes for various 16S datasets from

729   different habitats (*N*=number of samples in study). All datasets were subsampled to 2000

730   sequences prior to analyses, more information on the data used can be found in

731   Supplementary Table 1.

732

733   **Table 2** Comparison of the abundance of total bacteria, methanothrophs, methanogens, fungi,

734   and fungal to bacterial ratio (F/B) in the trap and periphyton of *Utricularia* species.

735   Quatification of bacteria, fungi, methanotrophs and methanogens was done using the

736   16SrDNA, 18SrDNA, pmoA, and mcrA gene copy numbers, respectively. Quantity was

737   normalized to total amount of DNA.

738

739   **Table 3** The estimates of bacterial and ciliate numbers, the individual grazing rate (IGR) and

740   total grazing rate (TGR) of ciliates and the turnover rate of bacterial standing stock

741   (Turnover) in *U.reflexa* traps of different age is presented. Means of 3 technical replicates are

742   shown.

743

744   **Table 4** The 40 most expressed prokaryotic (a) and eukaryotic (b) functional KEGG modules.

745   Functional assignment was done in MEGAN, using the KEGG database. Abundances were

746   estimated in Trinity by mapping raw reads to assembled contigs, using the *bowtie2* algorithm

747

748

a

Legend:
- ● Freshwater
- ● *Utricularia vulgaris*
- ● *Utricularia australis*
- ● Iguana gut
- ● *Nepenthes* pitcher
- ● Rhizosphere
- ● Soil
- ● Cow gut
- ● *Sarracenia* pitcher
- ● Bat fecal
- ● Bat gut

Labels around circle: Cow gut; Freshwater, gut; Soil and Rhizosphere; Freshwater, gut; Bat fecal, gut; Rhizosphere; *Nepenthes* pitcher; Iguana gut; *Utricularia vulgaris*; *Utricularia australis*; *Sarracenia* pitcher; Freshwater, gut

0.05

b

Legend:
- ● cow
- ● rhizosphere
- ● soil
- ● *Utricularia*
- ● freshwater
- ● phylosphere
- ● fruit fly

0.1

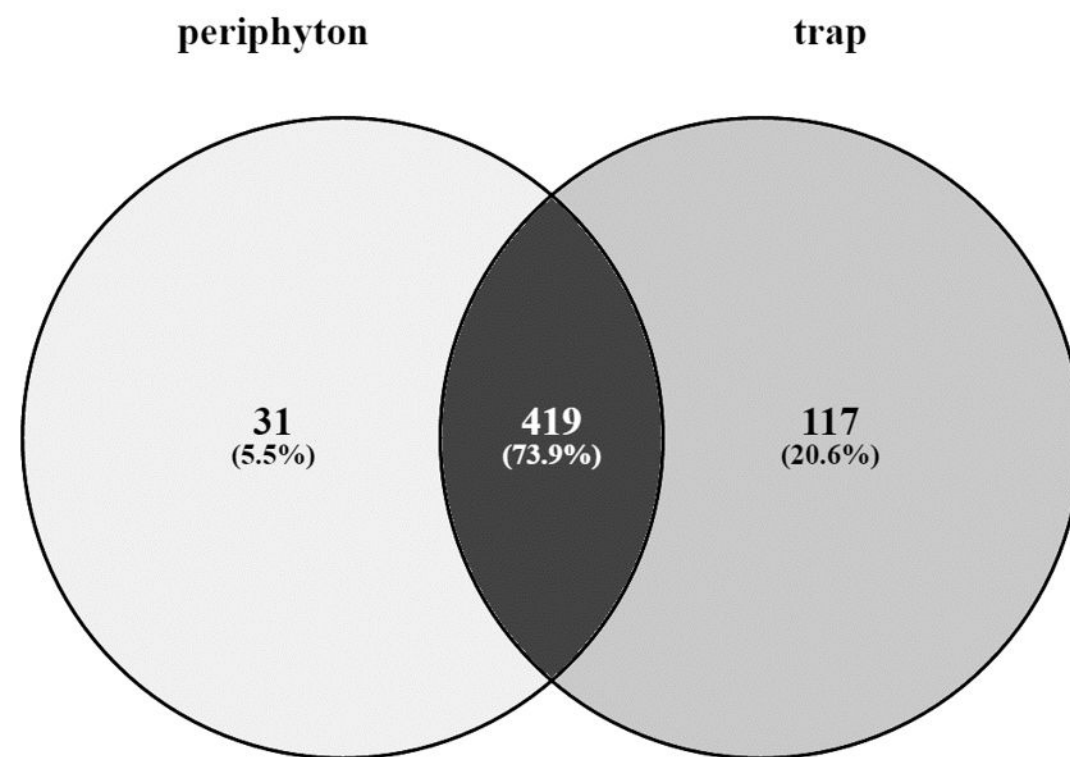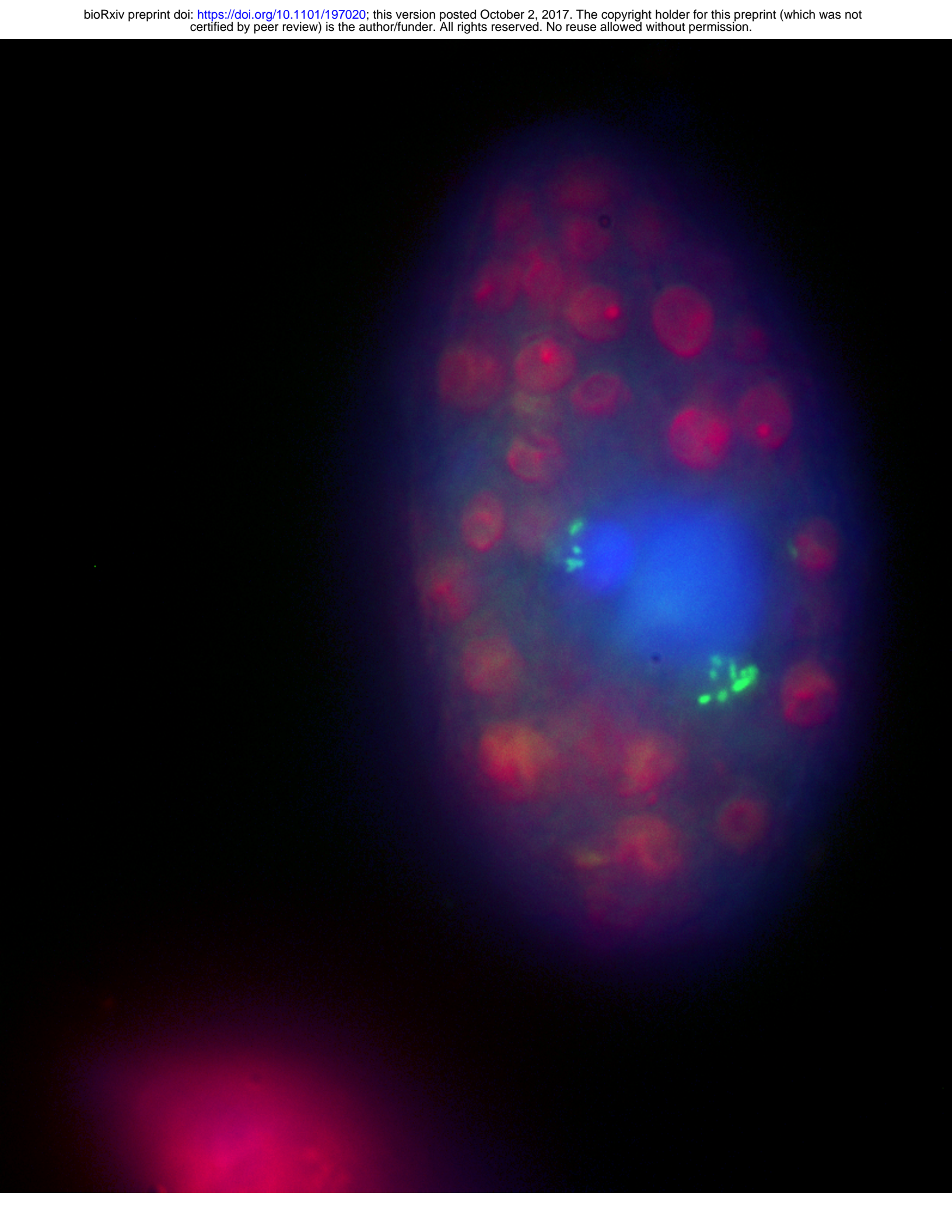**(a) Distinct bacterial assemblages**
- organic matter degradation
- exoenzyme/protein production
- ammonia oxidation
- methanotrophy

(b) Fungi (Ascomycota)
- saprotrophy
- degradation of complex org. matter

(c) Protozoan grazers
- organic matter turnover
- nutrient release
- osmotrophy

(d) Algae and cyanobacteria
- photosynthesis
- exoenzyme production
- decomposition - source of complex org. matter

(e) Predatory bacteria

(f) Periphytic mixed-species biofilm

1 Active transport of *Utricularia* photosynthates into trap fluid

2 Uptake of dissolved N and P by *Utricularia*

3 Input of organic matter and detritus through the *Utricularia* trap door

4 Particulate organic matter

	Trap fluid	Ambient env.
Dissolved N (mg/L)	5-25	0.02-0.05
Dissolved P (mg/L)	0.2-0.6	0.01-0.03
Bacterial numbers	10^9	10^6
Bacterial diversity	high	low
Eukaryotic diversity	low	high
Microenvironment	fragmented	continuous

enzymes

anoxic, nutrient rich trap fluid

grazing

predation

Trap door

Trigger hairs

oxic, nutrient poor environment

ammonia, phosphate

aminoacids, sugars,
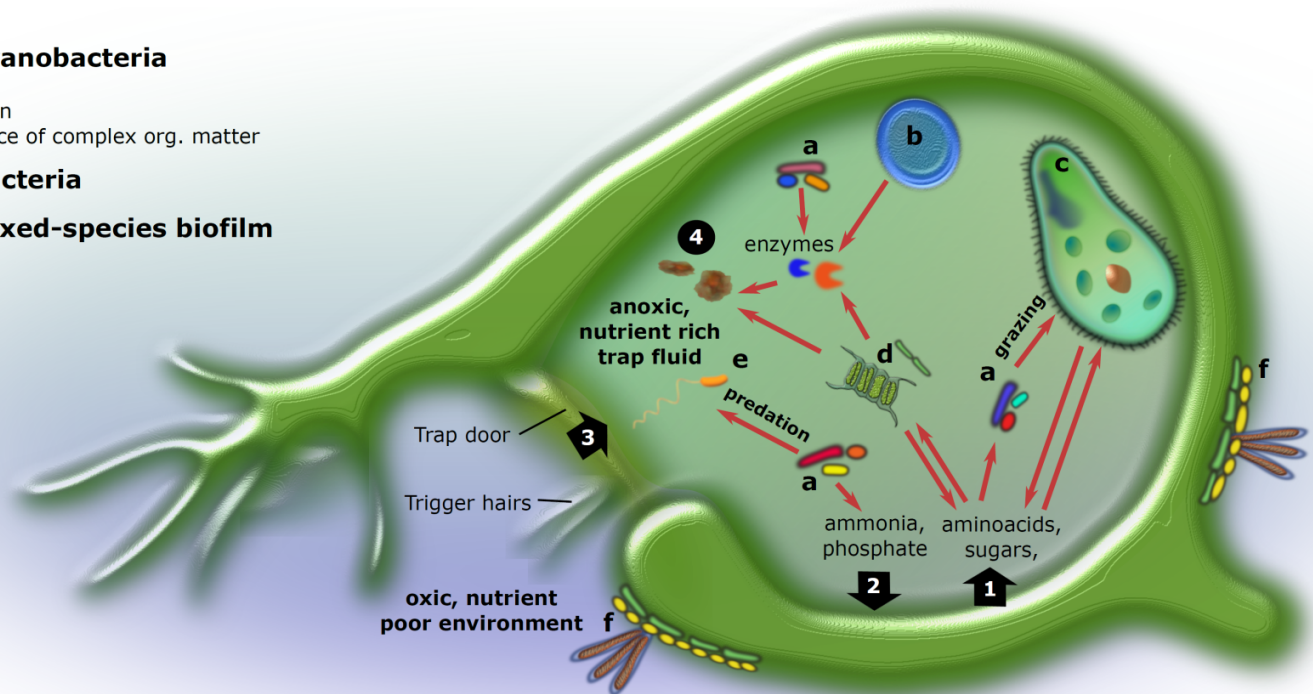
Table 1

Habitat	N	PD whole tree	ANOVA	Chao1 index	ANOVA	Obs. sp.	ANOVA	Shannon index	ANOVA
Soil	309	74.2	a	2238.5	a	1043.2	a	9.6	a
Rhizosphere	751	49.6	b	1505.3	b	675.5	b	7.3	b
Cow gut	188	35.0	c	778.5	c	480.1	c	7.6	b
Utricularia	22	34.2	c	816.4	c	422.5	c	7.0	b
Trap	16	35.0	c	859.2	c	439.3	c	7.1	b
Periphyton	6	32.2	c	702.2	c	377.7	c	6.9	b
Iguana gut	91	22.6	b	359.3	d	218.3	d	5.4	c
Freshwater	2635	21.1	b	262.9	d	206.9	d	5.4	c
Sarracenia pitchers	17	15.4	bd	270.1	d	169.4	de	5.3	c
Nepenthes pitchers	16	13.1	bd	211.2	d	132.8	de	4.5	c
Bat gut	51	11.9	d	185.4	d	108.4	e	3.0	de
Bat fecal	13	10.3	bd	163.4	d	87.8	de	2.6	e

Table 2

	Bacteria (10^6)	**Fungi** (10^3)	**Methanotrophs** (10^6)	**Methanogens**	**F/B**
Trap (n=16)	1.6	7.7	0.34	B.D.	0.0048
St.dev	*1.9*	*5.3*	*0.4*		
Periphyton (n = 6)	2.9	41	0.41	B.D.	0.0143
St.dev	*4.3*	*79*	*0.21*		

B.D. – below detection

Table 3

Trap age	Bacteria [10^6 ml^{-1}]	Protozoa [10^3 ml^{-1}]	IGR [bact. prot.$^{-1}$ h^{-1}]	TGR [10^6 bact. ml^{-1} d^{-1}]	Turnover [day^{-1}]
Young	44.5	35.4	273.4	223.1	5.0
Mature	65.3	46.5	263.1	290.0	4.4
Old	266.9	50.8	342.1	411.8	1.5

Table 4

a

KEGG level 1	KEGG level 2	KEGG level 3	aver.[a]	error
Cellular Processes	Transport and Catabolism	Endocytosis	57	37
	Cell Growth and Death	Cell cycle - Caulobacter	38	16
Environmental Inf. Processing	Signal Transduction	Two-component system	45	33
Genetic Inf. Processing	Translation	Aminoacyl-tRNA biosynthesis	224	165
	Translation	Ribosome	198	137
	Folding, Sorting and Degradation	RNA degradation	123	34
	Replication and Repair	Base excision repair	60	39
	Transcription	Spliceosome	40	34
Metabolism	Energy Metabolism	Oxidative phosphorylation	519	396
	Nucleotide Metabolism	Pyrimidine metabolism	338	210
	Energy Metabolism	Nitrogen metabolism	286	243
	Metabolism of Cofactors and Vitamins	Porphyrin and chlorophyll metabolism	236	187
	Carbohydrate Metabolism	Amino sugar and nucleotide sugar metabolism	234	166
	Energy Metabolism	Methane metabolism	216	162
	Nucleotide Metabolism	Purine metabolism	167	116
	Carbohydrate Metabolism	Galactose metabolism	164	127
	Amino Acid Metabolism	Glycine, serine and threonine metabolism	150	137
	Metabolism of Other Amino Acids	Glutathione metabolism	128	86
	Energy Metabolism	Photosynthesis	124	91
	Carbohydrate Metabolism	Citrate cycle (TCA cycle)	109	70
	Lipid Metabolism	Glycerophospholipid metabolism	105	107
	Carbohydrate Metabolism	Propanoate metabolism	104	124
	Amino Acid Metabolism	Arginine and proline metabolism	100	68

high expression
intermediate expression
low expression

Carbohydrate Metabolism	Pentose phosphate pathway	94	*49*
Energy Metabolism	Carbon fixation in photosynthetic organisms	92	*58*
Amino Acid Metabolism	Valine, leucine and isoleucine degradation	90	*68*
Carbohydrate Metabolism	Pyruvate metabolism	83	*42*
Biosynthesis of Other Secondary Metabolites	Penicillin and cephalosporin biosynthesis	69	*32*
Amino Acid Metabolism	Phenylalanine metabolism	69	*66*
Amino Acid Metabolism	Lysine biosynthesis	68	*63*
Carbohydrate Metabolism	Glycolysis / Gluconeogenesis	68	*70*
Amino Acid Metabolism	Alanine, aspartate and glutamate metabolism	66	*57*
Carbohydrate Metabolism	Starch and sucrose metabolism	63	*70*
Carbohydrate Metabolism	Glyoxylate and dicarboxylate metabolism	59	*24*
Amino Acid Metabolism	Phenylalanine, tyrosine and tryptophan biosynthesis	55	*45*
Amino Acid Metabolism	Histidine metabolism	48	*9*
Carbohydrate Metabolism	Butanoate metabolism	42	*17*
Amino Acid Metabolism	Tryptophan metabolism	40	*30*
Metabolism of Cofactors and Vitamins	Ubiquinone and other terpenoid-quinone biosynthesis	38	*25*
Carbohydrate Metabolism	Inositol phosphate metabolism	37	*41*

aFPKM normalized transcript abundances. Averages were calculated from 3 biological metatranscriptome replicates

Table 4

b

KEGG level 1	KEGG level 2	KEGG level 3	aver.[a]	error
Cellular Processes	Transport and Catabolism	Lysosome	1 622	904
	Transport and Catabolism	Regulation of autophagy	1 223	843
	Transport and Catabolism	Endocytosis	1 163	563
	Cell Growth and Death	Cell cycle	1 155	707
	Cell Growth and Death	Oocyte meiosis	1 093	603
	Cell Growth and Death	Meiosis - yeast	1 034	716
	Cell Growth and Death	Cell cycle - yeast	1 000	494
	Cell Communication	Gap junction	853	554
	Transport and Catabolism	Peroxisome	624	335
	Cell Communication	Tight junction	587	235
Environmental Inf. Processing	Signal Transduction	MAPK signaling pathway	1 143	669
	Membrane Transport	ABC transporters	1 091	569
	Signal Transduction	Calcium signaling pathway	786	359
	Signal Transduction	PI3K-Akt signaling pathway	624	349
	Signal Transduction	Phosphatidylinositol signaling system	562	321
Genetic Inf. Processing	Transcription	Spliceosome	2 980	1 680
	Translation	Ribosome	2 945	1 842
	Folding, Sorting and Degradation	Protein processing in endoplasmic reticulum	2 620	1 340
	Translation	Ribosome biogenesis in eukaryotes	2 255	1 258
	Folding, Sorting and Degradation	Ubiquitin mediated proteolysis	2 192	1 024
	Translation	Aminoacyl-tRNA biosynthesis	1 791	952
	Translation	RNA transport	1 475	726
	Folding, Sorting and Degradation	RNA degradation	1 109	538
	Folding, Sorting and Degradation	Proteasome	968	497
	Translation	mRNA surveillance pathway	893	378

high expression
intermediate expression
low expression

	Replication and Repair	Nucleotide excision repair	653	239
	Replication and Repair	Base excision repair	622	401
Metabolism	Nucleotide Metabolism	Purine metabolism	2 378	1 255
	Energy Metabolism	Oxidative phosphorylation	1 490	941
	Carbohydrate Metabolism	Amino sugar and nucleotide sugar metabolism	1 128	532
	Metabolism of Other Amino Acids	Glutathione metabolism	1 116	582
	Lipid Metabolism	Glycerophospholipid metabolism	898	499
	Glycan Biosynthesis and Metabolism	N-Glycan biosynthesis	770	318
	Nucleotide Metabolism	Pyrimidine metabolism	761	426
	Amino Acid Metabolism	Alanine, aspartate and glutamate metabolism	621	375
	Amino Acid Metabolism	Arginine and proline metabolism	605	363
	Glycan Biosynthesis and Metabolism	Other glycan degradation	598	316
	Energy Metabolism	Photosynthesis	532	301
	Xenobiotics Biodegradation and Metabolism	Metabolism of xenobiotics by cytochrome P450	498	213
	Carbohydrate Metabolism	Citrate cycle (TCA cycle)	495	284

[a]FPKM normalized transcript abundances. Averages were calculated from 3 biological metatranscriptome replicates