The structure and diversity of microalgae-microbial consortia isolated from various local organic wastes.

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Abstract

Pure microalgae cultivation in organic wastes may be hampered by their low adaptation to extreme growth conditions and by the risk of microbial contamination. This work aimed to isolate self-adapted microalgae-microbial consortia able to survive in organic wastes characterized by extreme conditions, to be then proposed for technological application in removing carbon and nutrients from wastes' streams. To do so, sixteen organic wastes with different origins and consistency were sampled. Twelve microbial consortia were isolated from wastes and their eukaryotic and prokaryotic compositions were analyzed by next generation sequencing. Eight eukaryotic communities were dominated by Chlorophyta, led by *Chlorella*, able to

survive in different wastes regardless of chemical-biological properties. *Tetradesmus*, the second most represented genus, grew preferentially in substrates with less stressing chemical-physical parameters. *Chlorella* and *Tetradesmus* were mostly isolated from cow slurry and derived wastes which proved to be the best local residual organic source.

Key words: Eukaryotic; Microalgae; Next generation sequencing (NGS); Organic wastes; Prokaryotic.

1. Introduction

The amount of agricultural, industrial and municipal wastes have been increasing rapidly over recent decades due to the rising of global population, urbanization and economic development. Recent data (EUROSTAT, 2021) indicated for the EU a total production of 12.97 Mt of animal faeces, urine and manure, 13.01 Mt of industrial effluent sludges and 9.12 Mt of sludges and liquid wastes from wastewater.

It is important to dispose of organic wastes with suitable treatments by turning them into renewable energy and products enhancing recycling efficiency, as well as to avoid environmental impacts, natural resources depletion and health issues (Oliveira et al., 2017). Wastewater treatment producing sewage sludge, composting producing compost, and anaerobic digestion producing digestate, have been proposed to treat organic waste streams allowing nutrient and organic matter to be recovered (Niedzialkoski et al., 2021). Compost, sewage sludge and digestate have been used-as

organic amendments and fertilizers, replacing the use of mineral fertilizers, improving soil organic matter and thus contributing to a more sustainable agriculture (Riva et al., 2016).

Organic wastes were also reported as excellent sources of energy-rich organic C-molecules as well as of macro- and micro-nutrients for microalgae cultivation (Stiles et al., 2018). However, microalgae cultivation has been successful when wastewater was used to supply mineral nutrients and organic carbon for their growth (Mohsenpour et al., 2021).

Previous studies have proven that organic waste such as wastewater could be a suitable growth medium for the cultivation of certain pure microalgae species i.e., *Chlorella*, *Tetradesmus* and *Scenedesmus* (Goswami et al., 2021). Nevertheless, only a relatively small number of microalgae species have been developed and used extensively because of the necessity to control the stability of the microalgae populations and the risks of culture contamination (Bani et al., 2021). Bacteria, fungi, cyanobacteria and other microalgae establish mutualistic or competitive relationships with the inoculated microalgae strain, depending on the different species and environmental conditions (Brenner et al., 2008). Therefore, rather than attempting to combat the contamination of monocultures by unwanted and detrimental species, a different approach can be taken by cultivating native microalgae consortia isolated directly from local wastes, without a strict control of the microbial population stability. The ability of microalgae to grow in a substrate depends on both the chemical-biological parameters characterizing the substrate and the organisms' ability to colonize

the substrate (Agrawal, 2009). Research showed that microalgae are capable of producing highly resistant spores which enable them to survive in adverse conditions (Agrawal, 2009; Cheregi et al., 2019). Different algae have a relatively large tolerance range for changes in environmental conditions. For example, *Nostoc punctiforme* and *Anabaena circinalis* can germinate in the dark in the presence of organic carbon acting as a suitable source of energy (Agrawal, 2009; Cheregi et al., 2019). The brown alga *Macrocystis integrifolia* sporophyte can grow even at low temperature, i.e. 8 °C (Barsanti and Gualtieri., 2005)

Florentino (2019) found 21 microalgal genera, such as *Aphanocapsa*, *Planktothrix*, *Chlorella* and *Euglena* surviving in six waste stabilization ponds, demonstrating that algae are tolerant to environments characterized by high organic carbon and nutrients contents. Specifically, algae have been used to treat either primary or secondary waste effluents such as human sewage, livestock wastes, agro-industrial wastes, industrial wastes and piggery effluent (Mohsenpour et al., 2021). Algae-based systems for the removal of toxic minerals such as Pb, Cd, Hg, Sc, Sn, As and Br are also being developed (Goswami et al., 2021).

Therefore, microalgae growing on wastes are well adapted to nutrient-rich substrates, which will lead to further technological applications such as waste nutrient and carbon recovery (Caprio et al., 2018).

This work falls within a broad project funded by Lombardy Region (North Italy) aiming at both studying and isolating indigenous autochthonous algae-microbial consortia (ACs) to be then used for technological applications to treat organic wastes,

removing nutrients and carbon and producing useful biomass.

This first paper reports the initial step of the project, consisting in investigating and describing the presence of self-adapted algae-microbial consortia (ACs) in sixteen different organic wastes, locally collected, characterized for extreme growth conditions for microalgae because of high nutrient and organic matter contents. Molecular metabarcoding characterization was applied to identify the algae and bacterial taxa and their relative abundance and to provide information on the composition of microalgae-microbial communities obtained from the different wastes.

Isolated ACs will be then further studied for their ability growing in organic wastes (animal slurries derived products) measuring growth performance, biochemical composition, and nutrients recovery ability at lab scale using closed reactors (Technology Readiness Level - TRL – of 4) allowing the choosing of the best performing ACs. These latter will be then tested in open reactors at demonstration level (TRL 5-6) aiming to understand how an algal community is influenced by shifting the cultivation mode from closed reactors to open reactors, as well as to evaluate whether the dominant microalgae species from closed reactors could still be dominant in open reactors or whether they could be colonized by other species/bacteria/predators.

2. Materials and Methods

2.1 Waste sampling

Sixteen organic wastes samples with different origins (cow slurry, urban municipal wastewater, sewage sludge) and types (solid/slurry/liquid fractions) were sampled from plants/farms located in the Lombardy Region of northern Italy around noon in January

(Table 1). The waste samples were marked as S1 to S16. All collected samples were immediately brought to the laboratory and stored at 4˚C before further analysis.

2.2 Waste chemical and biological characterization

The waste samples were dried for 24 h at 105˚ C (APHA 2005), shredded in a blender and passed through a 2-mm mesh. Total solids (TS), volatile solid (VS) and total organic carbon (TOC) were determined according to standard procedures (APHA 2005). The pH and electrical conductivity (EC) were measured potentiometrically using an Orion-520A pH-meter and a WTW-LF537 (GE) conductivity electrode, respectively. Total Nitrogen (TKN), Ammonia nitrogen (NH_4^+-N), pH, volatile fatty acids (VFA) and alkalinity (ALK) were determined on fresh materials by using the analytical method for wastewater sludge (IRSA CNR, 1994). Optical density was measured as absorbance at 750 nm by using a Jeneway 7350 UV-visible spectrophotometer. Macro and microelement concentrations including Na, Mg, K, Ca, P, Mn, Fe, Cu, Zn, Cr, Co, Ni, As, Se, Mo, Cd, Pb were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, Aurora M90 BRUKER), preceded by microwave assisted (Multiwave ECO, Anton Paar GmbH) nitric acid digestion (EPA, 2007) of fresh samples. All chemical analyses were performed in triplicate. The biological property of samples, i.e. biological stability, was determined by the anaerobic biogas production (ABP) (Schievano et al., 2008). All biological tests were performed in duplicate.

2.3 Original biomasses cultivation

2.3.1 Experimental design

Preliminary experiments were performed in order to assess the best conditions able to isolate the greatest number of algae from the sixteen organic wastes. To do so, four groups of experiments were performed. First deionized water (experiment 1) and BG-11 (experiment 2) medium were used as nutrient solutions for the isolation of algae. In brief 2 g of waste were put into 500 mL sterilized Erlenmeyer flasks and 200 mL of deionized water or BG-11 were added. The blend was mixed and agitated manually for 10 minutes before putting it into the incubator. Subsequent approach considered CA Medium (CA), Bold's Basal Medium (BBM) and BG-11 as growing media adopting an optical density of 0.1 (experiment 3) and of 0.3 (experiment 4) (see supplementary material).

Diluted samples coming from all experiments were then maintained in the incubator under constant aeration and mixed by using filtered air (filter of 0.2 µm) with a continuous illumination of 50 µE m^{-2} s^{-1}, provided by fluorescent white tubes, at a controlled temperature of 22 ± 1°C for over 8 weeks.

The BG-11 nutrient solution at 0.3 optical density resulted in the isolation of the most microalgae consortia (original biomass – OB) (see supplementary material). BG-11 nutrient solution contains: 247.09 mg L^{-1} $NaNO_3$; 7.11 mg L^{-1} K_2HPO_4-P; 17.95 mg L^{-1} K_2HPO_4-K; 7.39 mg L^{-1} $MgSO_4 \cdot 7H_2O$; 9.81 mg L^{-1} $CaCl_2 \cdot 2H_2O$; 1.02 mg L^{-1} $(NH_4)_5[Fe(C_6H_4O_7)_2]$; 8.68 mg L^{-1} Na_2CO_3; 0.50 mg L^{-1} H_3BO_3; 0.50 mg L^{-1} $MnCl_2 \cdot 4H_2O$; 0.05 mg L^{-1} $ZnSO_4 \cdot 7H_2O$; 0.15 mg L^{-1} $Na_2MoO_4 \cdot 2H_2O$; 0.03 mg L^{-1} $CuSO_4 \cdot 5H_2O$; 0.01 mg L^{-1} $Co(NO_3)_2 \cdot 6H_2O$.

The microalgae consortia isolated from wastes were then cultivated in Photo Bio

Reactors (PBRs) of 0.5 L working volume. pH was set at up at 8 and it was maintained by using pure CO_2 injection adopting an "on-demand" modality. Room temperature (25° C) and constant air flux (10 L min^{-1}) were provided, as well as light that was provided by cold fluorescent lamps at irradiance of 312 μE m^{-2} s^{-1} at PBR surface, adopting a 12h:12h photoperiod regime. The OB (original biomass) was dosed at 10% v/v, i.e., 0.2-0.3 g L^{-1} was placed into the reactors and culture medium (BG-11) was added to start the trials to obtain cultivated biomass (CB). Two replicates/microalgae consortium were carried out.

To monitor the culture growth, biomasses' dry weights (TS) were determined by sampling 10 mL of biomass suspension from each PBR every 2 days. The samples were centrifuged at 4,000 rpm for 10 min and then washed with an equivalent volume of distilled water to remove salts. Biomass samples were then filtered by 1.2-μm filter (GF/C, Whatman Ltd., Maidstone, UK), dried overnight at 80 °C and weighed.

2.4 Microalgae consortia molecular characterization

In order to investigate the diversity and population of microbial consortia, DNA extraction was carried out on both the original biomass (OB) and cultivated biomass (CB) during the exponential growth phase. The consortia biomasses were collected by centrifugation at 4,000 rpm for 10 min and 8,000 rpm for another 10 mins and then stored at -80° C until further analysis. Then the lyophilized biomass samples were collected for DNA extraction by DNeasy plant mini kit Qiagen, following the procedure described by the manufacturer. The extracted DNA samples were stored at -20˚ C for

173 further use. DNA concentration and purity were determined by a nanodrop 1000
174 spectrophotometer (Thermo Fisher Scientific Inc., USA).

175 For next generation sequencing (NGS), a library for 16S and 18S marker genes was
176 prepared following Illumina Protocol. NGS can be used to sequence entire genomes
177 and to generate extensive data from diverse microbial communities in a timely manner.
178 For the 16S, the hypervariable V3-V4 region was amplified using the 341F and 805R
179 primers while for 18S, the V9 region was amplified using the 1389F and 1510R primers
180 both modified with the required Illumina sequencing adaptors. 16S and 18S PCR
181 amplification was performed on a total volume of 25 µl: 12.5 µl of appTaq RedMix
182 (Appleton Wood Ltd., UK), 1 µl of forward and 1 µl of reversed primers modified with
183 Illumina over-hanger (10 uM) (IDT, Belgium), 2.5 µl of extracted DNA and 8 µl of
184 PCR grade water (Merck, Germany). Thermal protocol for 16S gene was as follows: i.
185 95° C for 3 mins; ii. 30 cycles at 95° C for 15s; iii. 57° C for 15s; iv. 72° C for 30s; v.
186 72° C for 7 mins. For 18S marker gene the thermal protocol was as follows: i. 98° C
187 for 3 mins; ii. 30 cycles at 98° C for 10s; iii. 56° C for 30s; iv. 72° C for 15s; v. 72° C
188 for 7 mins. PCR products were cleaned using Agencourt AMPure XP PCR Purification
189 beads (Beckman Coulter), following the manufacturer's instructions. 2.5 µl of purified
190 PCR product was used in a short secondary PCR, to attach Nextera XT indices, in the
191 presence of 2.5 µl of Nextera i5 and i7 index, 12.5 µl Appletonwood Taq and 5 µl of
192 PCR water. Thermal cycling conditions consisted of an initial denaturation step of 3
193 min at 95° C followed by 8 cycles each of 30 s at 95° C, 30s at 55° C and 30 s at 72° C
194 followed by a final extension step of 5 min at 72° C. PCR products were purified using

Agencourt AMPure XP PCR Purification beads as described previously. PCR products were quantified using PicoGreen® dsDNA quantification assays (Thermo Fisher Scientific), on a POLAR star Omega (BMG Labtech) plate reader. Nextera XT amplicons were then pooled in equimolar concentration. The length of amplicons was verified with Agilent bioanalyzer DNA kit (Agilent, USA). Final quantification of the pooled amplicon library was determined with the NEBNext® Library Quant Kit for Illumina® (New England BioLabs) prior to sequencing on the Illumina MiSeq (2 X 300 bp) at the University of Essex (UK).

For each original biomass (OB) there were three replicates and 2 replicates for each cultivated biomass (CB), so that in total four replicates for the CBs. Only S-2 and S-7 had 2 replicates, and only biomasses derived from S2 and S7 had two replicates.

2.5 Data analysis

2.5.1 Multivariate analysis

Multivariate analyses were used to identify environmental parameters that were most strongly associated with each other, and to define environmental factors to eukaryotes and prokaryotes species associations. Principal component analysis (PCA) was used to identify trends between highly correlating chemical-biological parameters. This analysis provides information on the most meaningful parameters, which describe the whole dataset with minimum loss of original information. Analyses were performed by XLSTAT version 2016.02.28451.

2.5.2 Metabarcoding statistical analysis.

Reads were processed as described in Dumbrell et al. (2016). Briefly, quality filtering was carried out in Sickle, followed by error correction in SPAdes. Reads were paired ended using PEAR inside Pandaseq. Chimera check and clustering at 97% of similarity was performed using the vsearch algorithm. Repsets were imported in Qiime2 and taxonomy was assigned using sklearn classifier using SILVA database for 16S while for 18S taxonomy has been assigned using blastn algorithm.

Statistical analyses were performed in R studio. Figures were generated using gglpot2 library. Richness data were log transformed to meet the normality criteria and the equality of the variance (car package). A pairwise t-test was used to test if the richness in batch/original was significant. Phyloseq package was used to generate relative abundance and for the NMDS plot. Sequence data from this study were deposited in the SRA archive under the project accession numbers: PRJNA752495 for eukaryotic communities (18S) and PRJNA752492 for bacterial sequences (16S).

3 Results and Discussion

3.1 Organic wastes characterization

Many factors such as light, pH, and nutrients influence microalgae survival and growth; thus it is critical to understand the original substrates' features. To do so, the selected organic wastes were characterized for chemical-physical and biological parameters and results are reported in Table 2-4, reporting data referred to the fresh materials to better describe the real growth conditions.

Results show that wastes differed greatly from each other (Table 2-4), thus providing

a wide range of substrates. TS varied from 15 ± 1 g kg^{-1} (S4) to 257 ± 10 (S6) g kg^{-1} while VS was from 8 ± 0.2 g kg^{-1} (S2) to 221 ± 0.6 g kg^{-1}(S3). TS and VS reflected indirectly the waste turbidity, color, and absorbance properties, which would directly affect light availability for microalgae growth. pH varied from 6.4 ± 0.3 (S12) up to 9.1 ± 0.2 (0.1) (S3 and S6). Regarding pH, maintaining a suitable pH condition is critical for algae, as the tolerated pH range for most algal species has been reported to be between 7 and 9, with the optimum range being 8.2-8.7, though there are species that dwell in more acid/basic environments (Barsanti and Gualtieri, 2005).

Total alkalinity (TA)-varied widely between 2.1 ± 0.1 g (S9) and 18.7 ± 0.5 (S10) $CaCO_3$ kg^{-1}. TA affecting pH could affect algae spore germination that is reported to be optimal at neutral or slightly alkaline pH (Agrawal 2009). Above pH 8.3, alkalinity is mostly in the form of carbonate and below pH 8.3 and above 4.5 it is mostly in the form of bicarbonate. Both bicarbonate and carbonate can be used as carbon sources for algae growth in organic wastes.

Furthermore, three macronutrients, i.e., carbon, (TOC) (important for heterotrophic and mixotrophic algae growth), nitrogen (N) and phosphorus (P) also showed wide differences. In particular (Table 2), we found the following range of variation for C, N, $N\text{-}NH_4$ and P: 5 ± 0 g kg^{-1} (S4) to 110 ± 0.2 g kg^{-1} (S6), 1.5 ± 0 g kg^{-1} (S4) to 8.8 ± 0.11 g kg^{-1} (S13), 0.3 g kg^{-1} (S9) to 3.5 g kg^{-1} (S10) and 5 ± 0 g kg^{-1} (S2) to 299 ± 4 g kg^{-1} (S13), respectively. Moreover, apart from C, N and P, also the elements S, K, Na, Fe, Mg, Ca and trace elements such as B, Cu, Mn, Zn, Mo, Co, V and Se, that are essential nutrients for microalgae, showed a wide variability depending on feedstock

composition (Table 3-4) (Stiles et al., 2018).

Temperature is one of the significant environmental factors regulating survival and reproduction of algae and producing a shift in algal population and composition. Every alga has its own optima temperature and temperature tolerance limit for vegetative survival, spore formation, spore germination and growth (Agrawal 2009). The waste samples in this work were collected at around noon in January. The ambient temperature during the sampling day ranged from 2° C to 8 °C (Table 1), while samples temperatures ranged between 8° C and 20 °C depending on the storing system (Table 1). However, literature reported that algae are able to survive at a wide range of temperatures (Cheregi et al., 2019) and that spores can germinate when they are put under optimal conditions, as has been done in this work of isolating ACs.

3.2 Taxonomic profiling of original biomasses

3.2.1 Eukaryotic Communities

For eukaryotic communities a total of 3,565 operational taxonomic unit (OTUs) were obtained but only 2,183 OTUs could be assigned to the eukaryotic domain (100% eukaryotic). At phylum level (Figure 2a), the original biomasses were dominated by Chlorophyta (from 60% in S8_OB to 99% in OB-S1) except for samples S2_OB, S9_OB, S13_OB and S16_OB which were characterized by the abundant presence of the phyla Opisthokonta (S2_OB, 55% ± 3) and Discosea (S16_OB 68% ± 1). Contrarily, there was no dominant phylum in Samples S9_OB and S13_OB which were characterized by a mix of phyla i.e., S9_OB: Heterolobosea 37% ± 2, Ciliophora 23%

± 2, Chorophyta 18% ± 1 and Opisthokonta 14% ± 1; and S13_OB: Chlorophyta 42% ± 3, Discosea 27% ± 0. At genus level, *Chlorella* was the most common microalga found in both original and cultivated biomasses, followed by *Tetradesmus* (S7_OB, S8_OB and S11_OB), both belonging to Chlorophyta. Other important genera found were the microalgae predators *Colpoda* and *Vahlkampfia* (Wahi et al., 2018). Original biomasses obtained could be divided into three groups (Groups 1-3) for genus composition (Figure 2a Originals): Group 1 (S1_OB, S3_OB, S4_OB, S6_OB and S10_OB) mainly dominated by the presence of *Chlorella*; and Group 2 (S7_OB, S8_OB and S11_OB) dominated by *Tetradesmus*. Group 3 (S2_OB, S9_OB, S13_OB and S16_OB) did not show any dominating microalgal genus, but these samples were characterized by mixtures of different protozoa and by only a small number of different algae.

3.2.2 Prokaryotic Communities

641 OTUs were assigned to bacteria after removing OTUs shared with the controls and the ones that were assigned to Chloroplast and Mitochondria (862 OTUs were produced before any cleaning). The bacterial community (100% OTU) was dominated by Cyanobacteria, Proteobacteria and Planctomycetota which are commonly found in both wastewater and digestate media (Caprio et al., 2018). A first group of OB samples was dominated by Cyanobacteria, which almost reached 50% of prokaryotic content, i.e. S2_OB (53% ± 4), S6_OB (59% ± 13), S8_OB (65% ± 7), S11_OB (66% ± 2), S3_OB (44% ± 5) and S9_OB (47% ± 4). The most abundant genus of this phylum was *Synechocystis* that accounted for 47% ± 4 in S9_OB becoming the dominant genus in

S6_OB in which it accounted for 70% ± 7 of relative abundance (Figure 2b). Cyanobacteria, also known as photosynthetic bacteria, are prokaryotes able to survive in waste due to their capability to tolerate high levels of pollutants, to degrade highly persistent organic contaminants and to remove heavy metals such as Cr, Co, Cu and Zn. They are the only planktonic group capable of utilizing atmospheric nitrogen via biological N_2 fixation, and as such, can circumvent N-limited conditions. They are also capable of using alternate pathways for the acquisition of carbon and nitrogen to counteract the reduction of photosynthesis efficiency in an unfavorable environment (Yu et al., 2013). Additionally, Cyanobacteria make a good combination with green algae because of their ability to produce some growth promoting substances that result in a symbiotic relationship (Gutierrez-Wing et al., 2012). As can be seen in Figure 1a and Figure 1b, S2_OB (*Chlorella* 23%, *Muriella* 13% of Eukaryotes, *Synechocystis* 53% of Prokaryotes), S3_OB (*Chlorella* 84% and *Synechocystis* 44%), S6_OB (*Chlorella* 84% and *Synechocystis* 70%), S8_OB (*Tetradesmus* 59% and *Synechocystis* 65%), S9_OB (*Scenedesmus* 14% and *Synechocystis* 47%) and S11_OB (*Tetradesmus* 96% and *Synechocystis* 66%) were good examples. *Synechocystis 6803,* one strain of Cyanobacteria, demonstrates adaptable growth ability under photoautotrophic, mixotrophic and heterotrophic conditions (Vermaas, 1996). They have developed sophisticated regulatory systems to adapt cellular processes and maintain metabolic homeostasis in response to many environmental fluctuations, such as nutrient availability and the ambient chemical-biological properties (Spät et al., 2015).

Proteobacteria and Planctomycetota co-dominated the remaining communities (Figure 2b) with the exceptions of samples S4_OB, S7_OB and S10_OB, in which Proteobacteria represented the main phylum. No clear dominance could be found for all these samples at genus level; for example S13_OB was dominated by *Caedibacter*

(59% ± 6) which included an endosymbiont of *Paramecium* which is commonly present in sewage sludge (Beier et al., 2002). Other common genera found were: *Pirellula* (S16_OB 48% ± 4) and *Paludisphaera* (S16_OB 36% ± 10), which are commonly found in wastewater samples due to their high metabolic flexibility in using multiple compounds (Chouari et al., 2003). Furthermore, small amounts of *Sandaracinus* and *Luteimonas* (data not shown) were found in OB cultures, above all in digestate-derived substrates, since they are able to degrade both simple or recalcitrant organic molecules (Akyol et al., 2019). Moreover, due to the fact that the media (wastes) were enriched in N compounds, many N oxidizing bacteria were present in the initial community, e.g., *Nitrolancea* that is a nitrite oxidizing bacterium present in different OBs (S6_OB 13% ± 2 vs 4% ± 1 in batch). These bacteria were then lost in the CBs because, probably, of the effect of both temperature batch growth temperature (25° C), which was lower than their optimum (37° C), and low ability to compete with other bacteria in the consortia (Spieck et al., 2020).

3.3 Chemical-biological features of wastes vs. ACs isolation, and eukaryotic and prokaryotic community composition.

The driving forces structuring microbial communities are concurrently regulated by both external factors such as chemical-biological parameters of the growth media and internal factors such as the interaction between eukaryotes and prokaryotes (Bani et al., 2020).

In this study, 27 chemical-biological parameters characterizing the original organic wastes (Table 1) were determined (Tables 2-4) to understand how waste properties affected microbial and algae population. To do so, PCA was performed to describe

chemical-biological parameters vs. dominating microalgae (Figure 2a) and vs. dominating prokaryotes (Figure 2b). Two main factors (PC) were found to cover 61.8% of the total variance. PC1 correlated to heavy metals (Fe, Cu, Cr, Ni, As, Cd and Pb), TKN and P contents, while PC2 correlated to TS, VS, TOC, ABP, Mg, pH and Mo. In particular, PC1 (in forward direction) carried inorganic nutrients and PC2 (forward direction) carried organic matter contents (TS, VS and TOC) and its quality, i.e. biological stability (ABP), and pH, VFA, EC and Na did not play important roles as their positions were near to the centre.

Chlorella survived in almost all conditions regardless of the wide range of TS, pH, nitrogen, macro and microelements concentrations of original organic wastes, which accounted for its high abundance, i.e., 78-99.9 % of Eukaryotes (Figure 1a). The exception was for S7_OB, that was characterized by the strong presence of *Tetradesmus* (60 % of Eukaryotes); S2_OB in which the presence of *Muriella* (13 % of Eukaryotes) was also reported; and S13_OB that was characterized by an organic substrate rich in heavy metals (HMs), which probably limited the growth of algae.

Chlorella is a small spherical (coccoid) green unicellular simple alga, that replicates exclusively by asexual reproduction, i.e., autospore formation; in addition it is easy to cultivate and it grows rapidly (Kumar et al., 2015). *Chlorella* is widely diffused and it has been reported to occur on damp soils, walls, bark of trees, freshwater pools, sewage and sewage treatment plants (Palmer, 1969).

Chlorella was reported as having high tolerance to non-ideal growing environments (Gacheva and Pilarski, 2008). According to Agrawal and Singh (2000), the vegetative

cell of *Chlorella* has high tolerance to dryness because of its small cell size and/or to the presence of sporopollenin protecting the cell wall. This fact can explain the presence of *Chlorella* in OB-samples S3_OB and S6_OB that, although characterized for organic substrate having the highest TS (242 and 257 g kg^{-1} FM), showed 84% of *Chlorella*. Again, previous findings reported that *Chlorella* is capable of surviving in different conditions, for example, in a wide pH range from pH 3 to pH 10.5 (Khalil et al., 2010).

Some HMs are essential for microalgae, but exposing microalgae to HMs beyond their tolerance range very likely affects their growth and metabolism (Expósito et al., 2021). *Chlorella* sp. was reported to be sensitive to As and Zn, Cu, Cr and Pb (Atoku et al., 2021). *Chlorella* can develop specific adaptive mechanisms to HMs (e.g. for Cu and Zn), thanks to the presence of anti-stress molecules such as brassinolide, an important hormone able to activate enzymatic and non-enzymatic systems responding to HM stress (Bajguz, 2010). The high HM contents (Table 3) reported for S13 and S14 may be the responsible for the low or null algae growth in S13 and S14 substrates (Figure 1a).

Some OB-samples were characterized by the presence of *Tetradesmus*, i.e. 96% in S11_OB, 60% in S7_OB and 59% in S8_OB, which was the second most abundant microalgae genus. *Tetradesmus* has been reported to be a robust species able to grow under unfavorable conditions (Dahiya et al., 2021); the finding of this algae in some of the organic wastes studied seemed to confirm this fact. Dahiya et al. (2021) found that *Tetradesmus wisconsinensis* was one of the dominant species in a wastewater plant in

India and *Tetradesmus obliquus* has been reported to grow in nitrogen and phosphorus-rich wastewater both heterotrophically and mixotrophically.

The multivariate analysis (PCA) performed for organic waste chemical-biological parameters (Figure 2a) indicated that *Tetradesmus* was mainly distributed in the lower part of the PCA axes (except for S16_OB, characterized by a much lower algae content, i.e. 26%) indicating that *Tetradesmus* preferred lower pH and TS content, and lower nutrient concentration than *Chlorella* (Figure 2a). Hodaifa et al. (2009) found the highest specific growth rate of *Tetradesmus obliquus* when the medium was maintained at a constant pH value of 7. Nevertheless, the S11 substrate that was characterized by a pH of 8 showed a eukaryotic OB composed 96% by *Tetradesmus*, but S11 also showed a very low TS content (TS of 1.7 ± 0.1). S16 substrate, that contained both high TS and pH, and high volatile fatty acids (VFAs), showed a low presence of *Tetradesmus* (26%), probably because this environment (VFAs) limited the growth of algae (Figure 1b).

Thus, the results described above seem to suggest that *Tetradesmus* survived in organic wastes that were not characterized for extreme chemical-biological parameters, unlike *Chlorella*, which seemed to be much less affected by chemical-biological parameters of the organic wastes. On the other hand, the presence of *Tetradesmus* excluded (or strongly reduced) the presence of *Chlorella*, suggesting that in the absence of extreme growing conditions the former, when present, grew better than the latter (Bani et al., 2020). This fact suggested that extreme chemical-biological parameters selected *Chlorella*. S12, that was characterized for both low TS and pH, did not show any algae growth, probably because of very high VFA (17,565 ± 208 g kg^{-1}FM) content

that inhibited algae growth. Nevertheless, sometimes, substrate features were not able to explain algae growth. For example, S2 and S7 substrates, for which algae growth was detected, showed similar characteristics (Table 2-4) to S5 and S15 that did not show any algae growth.

It is worth noting that *Chlorella* dominated in S1_OB, S3_OB, S4_OB, S6_OB and *Tetradesmus* dominated in S7_OB and S8_OB, were isolated from cow slurry and cow slurry derived wastes, such as liquid and/or solid digestate of cow slurry. Furthermore, *Chlorella* and *Tetradesmus* were also found isolated from organic fraction of municipal solid wastes (OFMSW) such as S10_OB and S11_OB. Therefore, it seemed that the best organic residue for isolating microalgae consortia was cow slurry and cow slurry derived wastes.

The chemical-biological parameters vs. dominating prokaryotes are shown in Figure 2b. As it can be seen from the Figure, *Synechocystis* tended to appear both at the top and at the bottom of PC2, showing its ability to co-exist with *Chlorella* (S2_OB, S3_OB and S6_OB), as well as *Tetradesmus* (S8_OB and S11_OB) and *Scenedesmus* (S9_OB).

3.4 Algae-bacteria consortia interactions

Besides chemical-biological parameters, bacterial communities are another major factor affecting algal communities, as previously reported (Choi et al., 2010). Algae and bacteria synergistically affect each other's physiology and metabolism (Bani et al., 2021). Interrelations between bacteria and microalgae are multifaceted and

complicated; for example, bacteria naturally can rely on photosynthetic phytoplankton to obtain the organic carbon needed to maintain their growth; in turn, phytoplankton can depend on bacteria to mineralize organic matter into inorganic substitutes, ultimately supporting the growth of algae (Yang et al., 2020). Figure 3 shows prokaryote distribution in the different samples represented by two PCs derived from PCA analysis and the relationships with eukaryote communities (algae). PC1 and PC2 that covered 26.6 % and 15.2% of the total variability, were able to separate OB-samples. Samples S1_OB, S3_OB, S4_OB, S6_OB and S10_OB, that showed the highest abundance of *Chlorella*, were preferentially distributed in the left part of PC1 and up part of PC2 (Figure 3). *Paludisphaera* (Planctomycetota) that accounted for 26.5% of the prokaryotic community of S1_OB, is a chemo-organotrophic aerobe capable of growth under micro-oxic conditions (Kulichevskaya et al., 2016), that makes it a good combination with *Chlorella* to get mutual benefits from each other. Samples S3_OB, S4_OB and S10_OB were characterized for the presence of Proteobacteria, such as *Roseomonas*, *Acinetobacter*, *Luteimonas* and *Porphyrobacter*, while S6_OB showed the presence of *Nitrolancea* (13%) in phylum Chloroflexi.

Porphyrobacter was present above all in S3_OB and S10_OB, i.e. 26% and 33%, respectively, while for S4_OB about 55% of undetectable genera made it impossible to define the most influential genus, although *Novosphingobium* (Proteobacteria) contributed 19%. *Porphyrobacter* is an aerobic and chemohetero-trophic bacterium with potential applications for hydrocarbon degradation, algalytic activity and bioleaching (Xu et al., 2018). *Porphyrobacter* (Xu et al., 2018) and *Novosphingobium*

(Thn, 2018) have both been commonly found in diverse and contaminated environments. *Novosphingobium* species can rearrange their genomes and functional profiles to adapt to local environments. As for S6_OB, Chloroflexi, also known as green filamentous bacteria, can grow photosynthetically under anaerobic conditions or in the dark by respiration under aerobic conditions (Jagannathan and Golbeck, 2009). Luis et al. (2017) demonstrated that Chloroflexi were dominant in the bacterial community of a biogas reactor fed by sludge and *Chlorella* biomass. Thus, *Chlorella*-dominated communities can be stable with various Proteobacteria and/or Planctomycetota, and also make a good combination with Cyanobacteria.

Tetradesmus, *Scenedesmus* and *Muriella* tended to group in the left part of axis PC1 (Figure 3). S11_OB (96% ± 2) and S16_OB (26% ± 1) were affected above all by the presence of *Pirellula* (Planctomycetota), accounting for 14% and 48% respectively. *Pirellula*, the bacteria that are responsible for nitrogen transformations, can utilize NO_2^--N to oxidize NH_4^+-N and generate N_2 under hypoxic or anaerobic environments. *Pirellula* removing ammonia would be inhibitory to algal growth (Choi et al., 2010), which can explain the fact that in the presence of more *Pirellula*, a lower *Tetradesmus* abundance was found. As for S13_OB, a significant abundance of *Caedibacter* (59%) appeared in the culture while *Chlorella* (42%) were found to be less dense. It is important to highlight that *Caedibacter* was probably not the reason for the scarcity of *Chlorella*, as it is reported to be capable to increasing its host's (in this work it refers to algae) fitness via manipulation of metabolic pathways and cell cycle control rather than negatively affecting the growth of its host (Dziallas et al., 2012).

The results seem to indicate that Planctomycetota presence was close to that of both *Tetradesmus* and *Chlorella*, unlike that of the Proteobacteria, of which the presence was generally found for OB characterized by *Chlorella* alone. The mixed populations can perform functions which are difficult or even impossible for individual strains or species (Brenner et al., 2008).

Compared to the unialgal culture, co-culture provides robustness to environmental fluctuations, culture stability, mutual benefits of nutrients distributions and resistance to invasion by other species (Subashchandrabose et al., 2011). However, it is worth stating that how positive or negative interactions modulate the dynamics of bacterial-eukaryotic communities is still far from being fully understood.

3.4 Original biomasses selected vs. cultivated biomasses

This paper aimed to investigate the presence of useful algae-microbial consortia able to grow on substrates rich in nutrients (N and P) and C, for subsequent processing production purposes. To do so, OBs isolated were successively cultivated under standardized batch conditions for algae growth (see section 2.4) and the cultivated microbial populations (CB) obtained were investigated.

Results indicate that S9_CB, S11_CB and S1_CB did not show any difference with respect to the original biomasses (OB) isolated from organic wastes (Figure 4a). For the other CBs, eukaryotes and bacterial communities were strongly influenced, as expected, by the original biomass (OB), as shown by the NMDS results (Figure 4a and Figure 5a) and supported by the PERMANOVA analyses (origin R^2= 0.61 and p-value < 0.05, condition R^2=0.07 and p-value < 0.05, origin*condition R^2= 0.19 and p-value

504 < 0.05).

505 Cultivated biomass richness (Figure 5a) did not seem to be affected by the batch
506 growth, with the exception only of S10_CB (pairwise t-test, p-value < 0.05). In batch
507 cultivated biomass samples, a *Chlorella* reads count reduction was usually associated
508 with an increase of *Colpoda* reads count (see for examples S6_CB, S2_CB and S4_CB
509 (Figure 2a)). However, recent studies had found that *Colpoda* sp. are also able to
510 prevent the collapse of *Chlorella* sp. in open ponds, as it eliminated bacterial cells that
511 could damage the microalgae (Haberkorn et al., 2020). Thus, the interaction between
512 algae and bacteria may be either beneficial or harmful to each other, depending on the
513 cultivation conditions.

514 Again, *Tetradesmus* decreasing, i.e., in S8_CB, from 59% to 42% and *Scenedesmus*
515 decreasing, i.e. in S9_CB from 14% to 8%, were both accompanied by *Colpoda* sp.
516 increase, i.e. from 10% in OBs to 35% and 69% in CBs, respectively. These results
517 supported once again the idea that understanding the interaction between the different
518 organisms is essential to tailor effective strategies for successful microalgae cultivation.

519 Bacterial communities, even if they maintain the phyla composition, as shown in
520 Section 3.2.2, have different community structures, as shown by Figure 4b, with the
521 only exceptions of S9_OB and S11_OB that were similar to S9_CB and S11_CB.
522 S9_OB communities were originally from wastewater while all the others were
523 sampled from digestate or slurry.

524 The bacterial community was influenced by both the origin of the samples (OB or
525 CB) and the type of inoculum (digestate, wastewater, manure etc.), as supported by the

PERMANOVA results (origin R^2= 0.44 and p-value < 0.05, condition R^2=0.07 and p-value < 0.05, origin*condition R^2= 0.25 and p-value < 0.05).

Alpha diversity for bacteria did not show variation between the OBs and the respective CBs, with the exception of only two communities (Figure 5b). S-6 is one of them (pairwise t-test, p-value< 0.005), however the difference can be easily explained as a drop in richness of *Synechocystis*, which was the main genus accounting for almost the totality of the community (see previous paragraph and Figure 2b).

4. Conclusions

The isolation of microalgae consortia from organic waste can be a winning approach in obtaining algae-microbial consortia (ACs) self-adapted to extreme conditions to be then used for cleaning wastes streams and producing useful biomass. In this work twelve consortia were successfully isolated from sixteen organic wastes. Cow slurry and derived products were the organic wastes from which most of the *Chlorella* and *Tetradesmus* dominant consortia were isolated. Isolated ACs will be further tested for their growing ability and chemical characteristics leading to the choice of the best performing ones which will then be used at full scale.

E-supplementary data for this work can be found in e-version of this paper online

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Table 1. Raw wastes sampling and origin details

Sample Name	Origin	Storge Mode	Digestion Type	Location	Plant/farm Scale	Plant Volume m^3	Samp Tempe °C
Cow slurry	Cattle Stables	Open lagoon	-	Mantova	150 animals	-	8
digestate of cow slurry	Biogas Plant	Open storage	Mesophilic	Suzzara	1 MW, 5 digesters	12000	15
digestate of cow slurry	Biogas plant	Open storage	Mesophilic	Suzzara	1 MW, 5 digesters	12000	10
digestate of cow slurry	Biogas plant	Open storage	Mesophilic	Pegognaga	1 MW, 4 digesters	10000	20
Cow slurry	Cattle Stables	Open lagoon	-	Pegognaga	100 animals	-	9
digestate of cow slurry	Biogas plant	Open storage	Mesophilic	Pegognaga	1 MW, 4 digesters	10000	10
Cow slurry	Cattle stables	Open lagoon	-	Lodi	400 animals	-	10
gestate of cow slurry	Cattle stables	Open storage	Mesophilic	Bologna	-	-	15
Wastewater	Urban wastewater treatment plant	Open lagoon	-	Peschiera Borromeo	$5.2x10^5$ equivalent inhabitants	154095 m^2	10
gestate of OFMSW[a]	Biogas plant fed by OFMSW	Open storage	Thermophilic	Lodi	1 MW, 4 digesters	8000	20
d fraction of OFMSW	Biogas plant fed by OFMSW	Closed lagoon	-	Lodi	1 MW, 4 digesters	8000	10
OFMSW	Biogas plant fed by OFMSW	Closed lagoon	-	Lodi	1 MW, 4 digesters	8000	10
Sewage Sludge	Biogas Plant	Open lagoon	-	Vellezzo Bellini (PV)	1.6 MW, 3 digesters	13500	15
state of sewage sludge	Biogas Plant	Closed storage	Thermophilic	Vellezzo Bellini (PV)	1.6 MW, 3 digesters	13500	20
Cow slurry	Cattle stables	Open storage	-	Bologna	-	-	8
Cattle manure	Cattle stables	Open storage	-	Lodi	-	-	10

[a]OFMSW: Organic fraction of municipal solid wastes

Table 2. Chemical Characterization of raw wastes

Waste sample	TS g kg^{-1} FM	VS g kg^{-1} FM	pH	TN g kg^{-1} FM	N-NH_4 g kg^{-1} FM	TVFAs g kg^{-1} FM	TA g $CaCO_3$ kg^{-1} FM	TOC g kg^{-1} FM	TP g kg^{-1} FM	EC ds m^{-1}	ABP ml g^{-1} FM
S-1	78 ± 1	57 ± 0.6	8.4 ± 0.2	4.1 ± 0.05	1.6 ± 0.1	276 ± 30	17.7 ± 0.2	34 ± 1	63 ± 2	29.7 ± 0.9 [c]	20.5 ± 1.6
S-2	19 ± 3	8 ± 0.2	8.6 ± 0.1	2.5 ± 0.01	1.3 ± 0	1749 ± 94	8.7 ± 2.4	6 ± 0.1	5 ± 0	26 ± 1.3 [c]	2.8 ± 0.1
S-3	242 ± 2	221 ± 0.6	9.1 ± 0.2 [b]	4.2 ± 0.09	0.9 ± 0	154 ± 12	7.1 ± 0	95 ± 0.4	159 ± 10	21.3 ± 1.2 [d]	52.6 ± 2.8
S-4	15 ± 1	9 ± 0.1	7.5 ± 0.1	1.5 ± 0	0.9 ± 0	1334 ± 64	6.9 ± 0.1	5 ± 0	9 ± 1	20 ± 1.6 [c]	3.9 ± 0.2
S-5	46 ± 1	29 ± 0.1	7.9 ± 0	3.7 ± 0.11	1.5 ± 0.1	718 ± 10	12. 9 ± 0.6	17 ± 0.8	60 ± 1	29.9 ± 4 [c]	7.4 ± 1.1
S-6	257 ± 10	218 ± 1.3	9.1 ± 0.1 [b]	5.4 ± 0.26	0.9 ± 0	623 ± 20	6.7 ± 0	110 ± 0.2	151 ± 14	23 ± 1.9 [e]	68 ± 3.6
S-7	65 ± 1	58 ± 0.4	7.2 ± 0	3.1 ± 0.14	1.0 ± 0	7469 ± 183	13.3 ± 0.5	27 ± 0	47 ± 4	23.8 ± 1.2 [c]	30.2 ± 2.6
S-8	66 ± 0	37 ± 0.1	7.8 ± 0	5.9 ± 0.06	2.6 ± 0.1	408 ± 7	11.8 ± 0.1	21 ± 1	156 ± 8	27.6 ± 1.7 [c]	14.9 ± 1.1
S-9	16 ± 0	13 ± 0.1	6.7 ± 0.2	1.2 ± 0.05	0.3 ± 0	598 ± 40	2.1 ± 0.1	6 ± 0.3	54 ± 2	2.8 ± 0.6 [c]	4.6 ± 0.4

S-10	20 ± 3	12 ± 0.1	8.1 ± 0.1	5.6 ± 0.43	3.5 ± 0.4	64 ± 0	18.7 ± 0.5	7.8 ± 0.4	18 ± 1	43.1 ± 1.7 [c]	5.7 ± 0.2
S-11	17 ± 1	10 ± 0.1	8.1 ± 0.3	5.5 ± 0.07	3.3 ± 0.1	513 ± 92	16.6 ± 0	4 ± 0.2	12 ± 0	44 ± 0.1 [c]	5.3 ± 0.2
S-12	87 ± 11	66 ± 0.1	6.4 ± 0.3	7.7 ± 0.01	3.4 ± 0	17565 ± 208	16.1 ± 0.1	35 ± 0.3	71 ± 6	30.4 ± 2.3 [c]	42.7 ± 2.3
S-13	119 ± 0	85 ± 0.4	7.4 ± 0.1	8.8 ± 0.11	2.4 ± 0.1	10631 ± 190	5.7 ± 0.1	41 ± 0.3	299 ± 4	23.9 ± 1.1 [c]	33.6 ± 3.4
S-14	97 ± 1	58 ± 0.6	8.0 ± 0.2	7.7 ± 0.07	2.9 ± 0.1	3309 ± 411	7.0 ± 0.2	23 ± 1	289 ± 0	24.7 ± 2.6 [c]	19.2 ± 1.1
S-15	40 ± 7	29 ± 0.2	7.6 ± 0.2	2.8 ± 0.02	0.9 ± 0.2	277 ± 9	7.4 ± 0.3	14 ± 1	46 ± 0	19.5 ± 0.6 [c]	9.4 ± 0
S-16	162 ± 5	136 ± 2	8.7 ± 0 [b]	5.8 ± 0.06	1.4 ± 0.1	766 ± 171	12.1 ± 0.1	7 ± 0.5	109 ± 4	32.3 ± 0.8 [d]	52.5 ± 1.6

[a] FM: fresh materials
[b] pH dilution rate sample: water =1:10
[c] EC dilution rate sample: water =1:5
[d] EC dilution rate sample: water =1:10
[e] EC dilution rate sample: water =1:25

Table 3. Element composition of raw wastes

	Na	Mg	K	Ca	Fe	Mn	Cr	Cu
				mg kg^{-1} FM[a]				
S-1	104 ± 11	70 ± 2	421 ± 12	2156 ± 71	27 ± 1	1.6 ± 0.1	0.1 ± 0	0.6 ± 0.1
S-2	64 ± 2	11 ± 0	368 ± 31	792 ± 12	4 ± 0	0.2 ± 0	u.d.l[b]	0.1 ± 0
S-3	84 ± 3	146 ± 10	421 ± 69	1059 ± 128	20 ± 1	2.1 ± 0.1	0.3 ± 0	0.5 ± 0.1
S-4	54 ± 4	16 ± 2	185 ± 14	2155 ± 34	1 ± 0	0.1 ± 0	u.d.l	0.1 ± 0
S-5	85 ± 0	63 ± 0	375 ± 5	2681 ± 47	11 ± 0	0.8 ± 0.7	0.1 ± 0	0.4 ± 0
S-6	90 ± 7	138 ± 12	378 ± 16	1031 ± 227	24 ± 2	2.2 ± 0.2	0.2 ± 0	1.0 ± 0.4
S-7	63 ± 1	51 ± 2	223 ± 5	1495 ± 35	8 ± 0	0.9 ± 0.1	0.1 ± 0	0.3 ± 0
S-8	205 ±7	43 ± 1	119 ± 0	2785 ± 110	80 ± 3	35 ± 0.1	0.3 ± 0	7.5 ± 0.1
S-9	12 ± 1	14 ± 0	14 ± 0	4334 ± 22	13 ± 1	0.2 ± 0	0.1 ± 0	0.4 ± 0
S-10	160 ± 1	2 ± 0	194 ± 3	894 ± 14	6 ± 0	0.1 ± 0	u.d.l	0.1 ± 0
S-11	130 ± 1	2 ± 0	166 ± 1	513 ± 2	3 ± 0	0.1 ± 0	u.d.l	0.1 ± 0
S-12	247 ± 28	31 ± 0	276 ± 8	2701 ± 83	27 ± 1	1.6 ± 0	0.1 ± 0	0.5 ± 0
S-13	36 ± 1	65 ± 2	58 ± 3	2701 ± 77	141 ± 2	3.1 ± 0.1	0.9 ± 0	3.7 ± 0.4
S-14	30 ± 1	66 ± 1	48 ± 1	3446 ± 28	227 ± 2	4.2 ± 0	1.1 ± 0.1	3.6 ± 0.2
S-15	38 ± 1	38 ± 0	225 ± 8	1884 ± 2	26 ± 1	1 ± 0	0.1 ± 0	0.3 ± 0
S-16	147 ± 24	146 ± 5	585 ± 3	2525 ± 246	34 ± 1	3.6 ± 0.2	0.3 ± 0	0.8 ± 0.1

[a]FM: fresh materials
[b]u.d.l: under detection level

Table 4. Element composition of raw wastes

	Zn	Ni	As	Se	Mo	Cd	
				mg kg^{-1} FM[a]			
S-1	2.7 ± 0.2	0.031 ± 0	0.018 ± 0	0.025 ± 0.011	0.045 ± 0.007	u.d.l	
S-2	0.3 ± 0	0.005 ± 0.001	0.005 ± 0	0.009 ± 0	0.021 ± 0	0.001 ± 0	
S-3	5.4 ± 0.3	u.d.l[b]	0.065 ± 0.024	0.103 ± 0.006	0.141 ± 0.014	u.d.l	0
S-4	0.2 ± 0	0.004 ± 0	0.005 ± 0.001	0.007 ± 0	0.007 ± 0	0.001 ± 0	
S-5	1.7 ± 0	0.07 ± 0	0.013 ± 0	0.019 ± 0.002	0.024 ± 0.001	0.002 ± 0	
S-6	3.5 ± 0.2	0.017 ± 0	0.035 ± 0.002	0.075 ± 0.033	0.122 ± 0.067	0.004 ± 0	
S-7	1.5 ± 0	0.018 ± 0.001	0.008 ± 0	0.014 ± 0.007	0.032 ± 0.005	u.d.l	
S-8	45.7 ± 0	0.182 ± 0.004	0.024 ± 0	0.148 ± 0	0.018 ± 0.014	0.005 ± 0	0
S-9	1.1 ± 0	0.068 ± 0	0.01 ± 0	0.008 ± 0	0.007 ± 0.003	0.002 ± 0	
S-10	0.5 ± 0	0.046 ± 0.002	0.009 ± 0	0.008 ± 0	0.017 ± 0	0.002 ± 0	0
S-11	0.3 ± 0	0.035 ± 0.002	0.006 ± 0.001	0.007 ± 0	0.003 ± 0	u.d.l	0
S-12	1.6 ± 0	0.061 ± 0	0.02 ± 0.005	0.029 ± 0.017	0.046 ± 0.006	u.d.l	
S-13	10.2 ± 0.4	0.552 ± 0.026	0.102 ± 0.001	0.047 ± 0.007	0.086 ± 0.010	0.016 ± 0	0

S-14	10.5 ± 0.5	0.65 ± 0.05	0.127 ± 0.004	0.052 ± 0.002	0.088 ± 0.044	0.021 ± 0	
S-15	1.3 ± 0.2	0.037 ± 0.01	0.004 ± 0	0.012 ± 0.001	0.022 ± 0.008	0.001 ± 0	
S-16	4.2 ± 0.3	0.1 ± 0.02	0.014 ± 0.001	0.039 ± 0.005	0.059 ± 0.025	u.d.l	

[a]FM: fresh materials
[b]u.d.l: under detection level

Caption Figures

Figure 1. Eukaryotic phylum (a) and genus (b), and prokaryotic phylum (c) and genus (d) composition associated to each treatment (only genera above 5% are shown). S-1 to S-16 represent the microalgae and bacteria consortia.

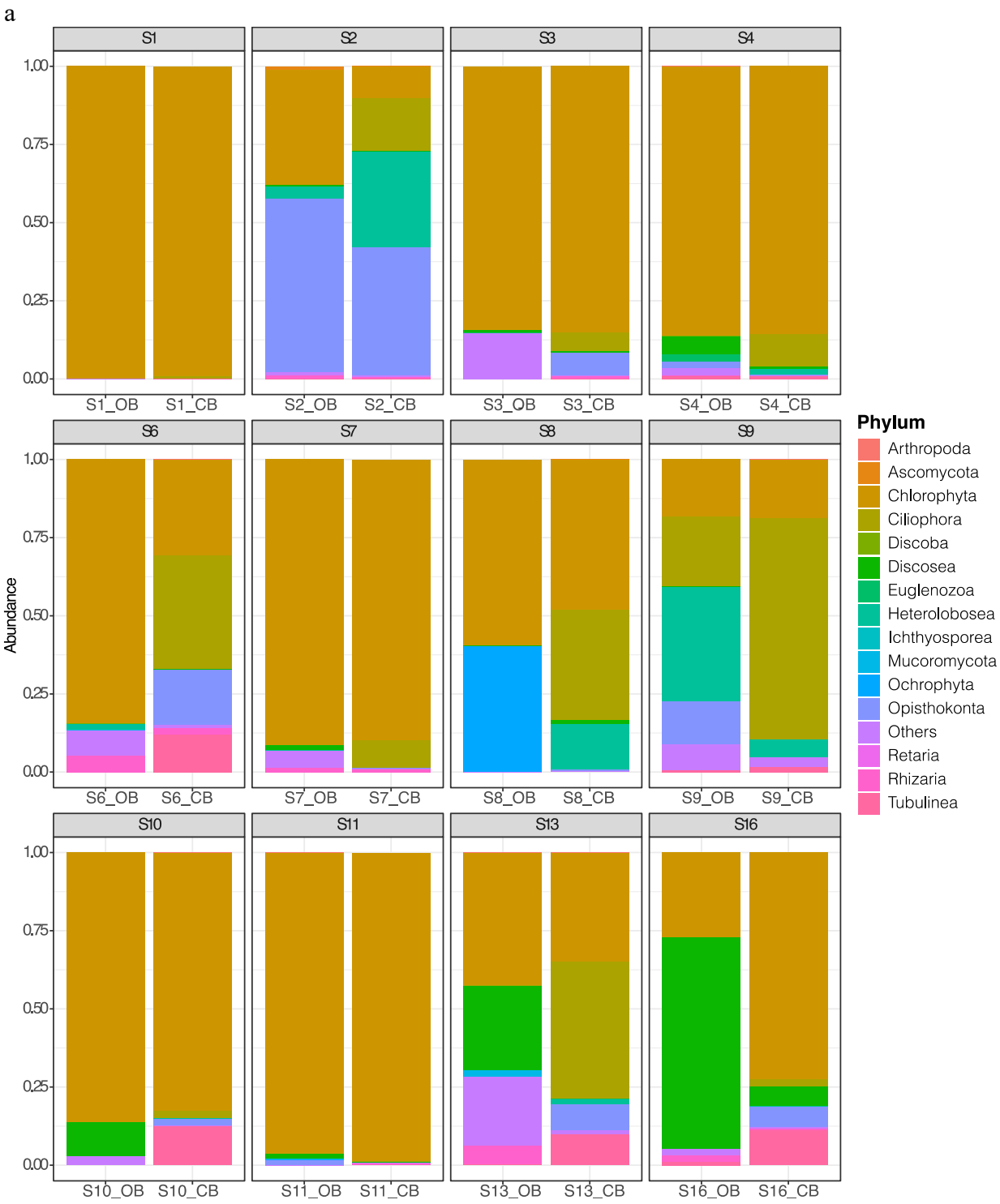
Figure 2. Principal component plot of waste chemical-biological parameters vs. eukaryotic microalgae (a), and prokaryotic bacteria and cyanobacteria abundance (b).

Figure 3. Principal component plot of prokaryotic bacteria and eukaryotes abundance.

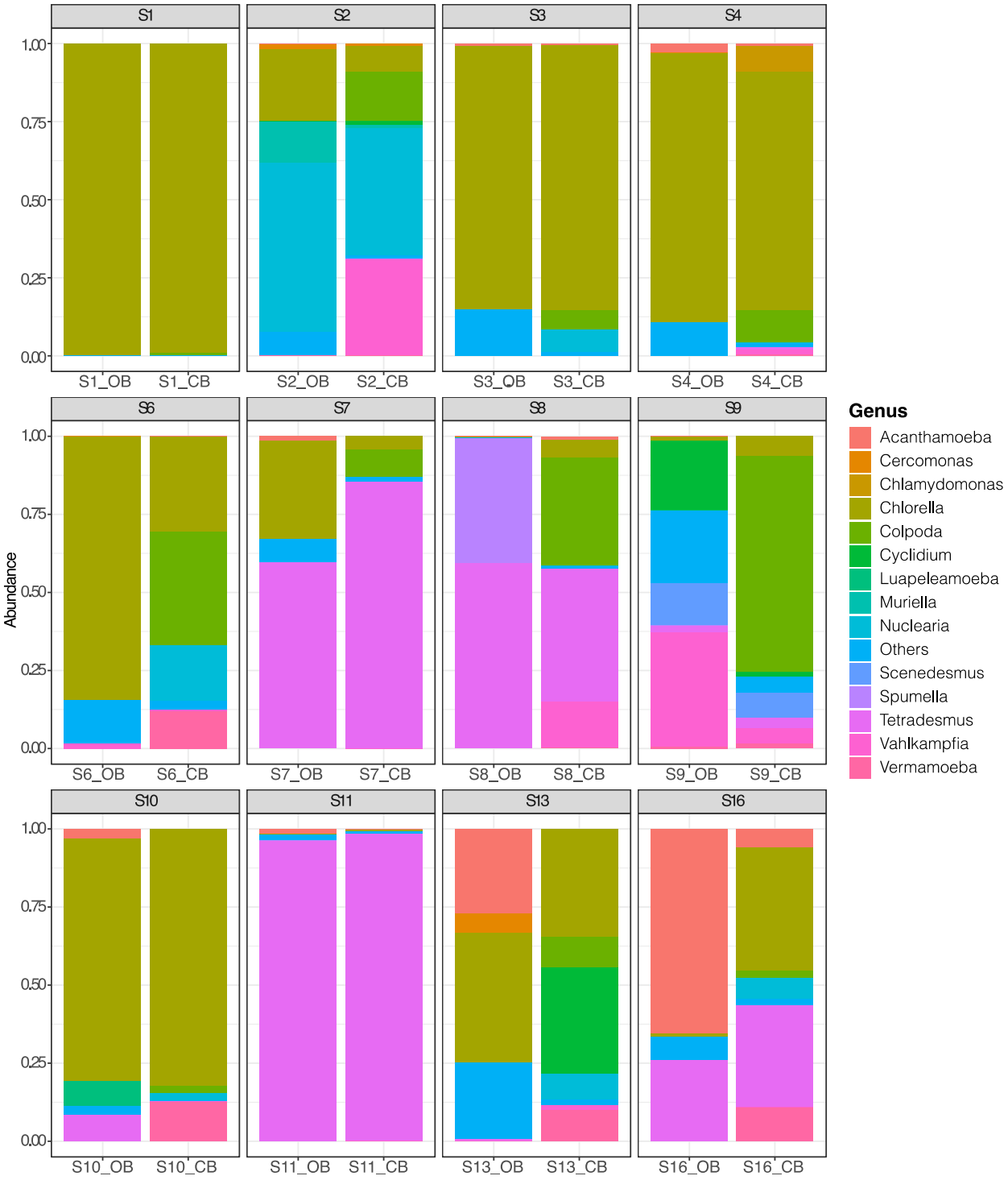
Figure 4. NMDS for eukaryotic community (a) and prokaryotic (b) community. Each panel represents organic wastes (different colors) from which microbial consortia have been obtained. Different shapes represent the different growth condition: (circle) original eukaryotic/prokaryotic community obtained from organic wastes (OB) and (square) eukaryotic/prokaryotic community cultivated starting from original community (CB from trial A and B).

Figure 5. Eukaryotic (a) and prokaryotic (b) community richness. Each panel represents a different original (S-_OB) and cultivated (S-_CB) biomasses.

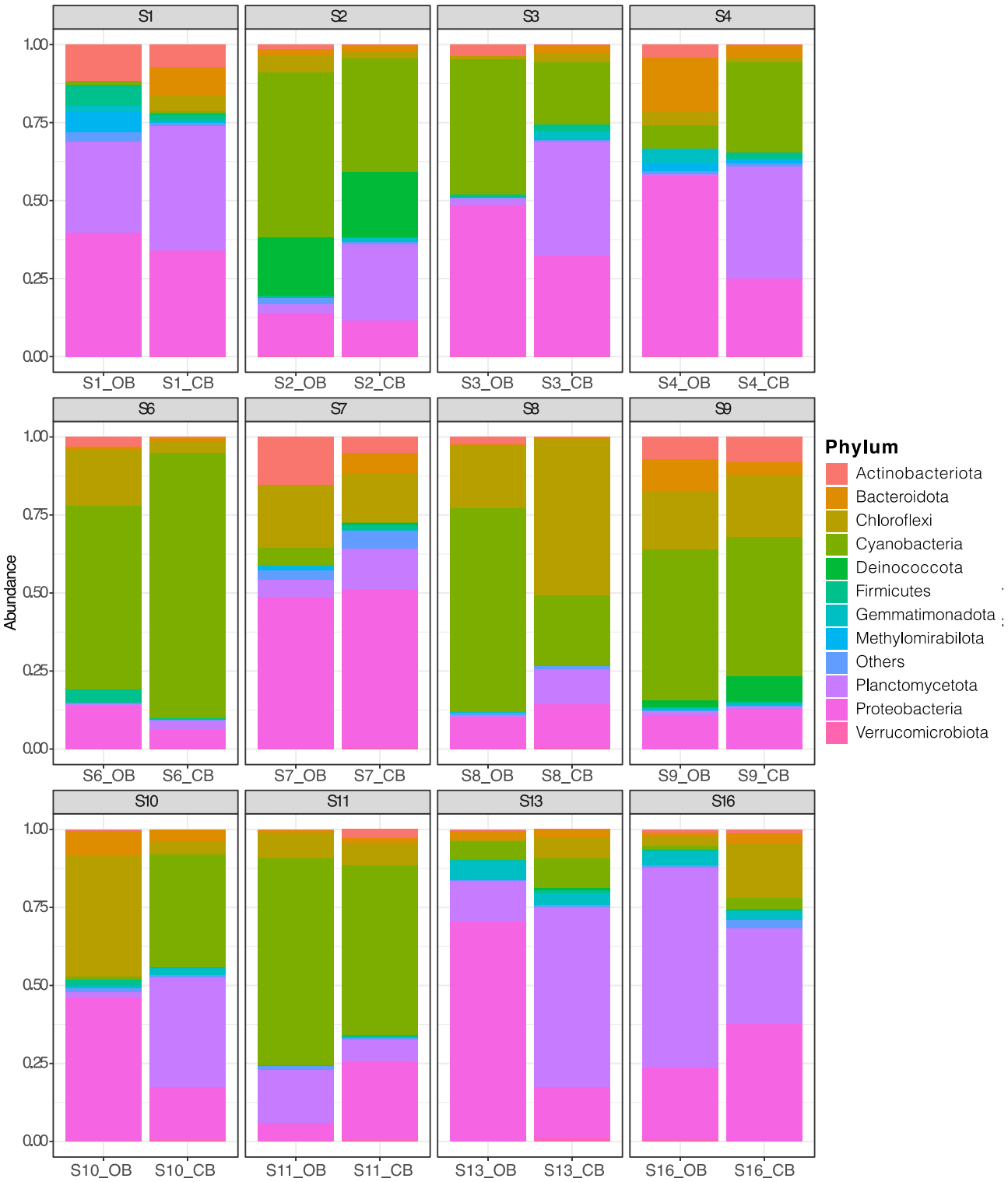
a


S1
S2
S3
S4
S6
S7
S8
S9
S10
S11
S13
S16
Abundance
1.00
0.75
0.50
0.25
0.00
S1_OB
S1_CB
S2_OB
S2_CB
S3_OB
S3_CB
S4_OB
S4_CB
S6_OB
S6_CB
S7_OB
S7_CB
S8_OB
S8_CB
S9_OB
S9_CB
S10_OB
S10_CB
S11_OB
S11_CB
S13_OB
S13_CB
S16_OB
S16_CB
Phylum
Arthropoda
Ascomycota
Chlorophyta
Ciliophora
Discoba
Discosea
Euglenozoa
Heterolobosea
Ichthyosporea
Mucoromycota
Ochrophyta
Opisthokonta
Others
Retaria
Rhizaria
Tubulinea

b


S1
S2
S3
S4
S6
S7
S8
S9
S10
S11
S13
S16
Abundance
1.00
0.75
0.50
0.25
0.00
S1_OB
S1_CB
S2_OB
S2_CB
S3_OB
S3_CB
S4_OB
S4_CB
S6_OB
S6_CB
S7_OB
S7_CB
S8_OB
S8_CB
S9_OB
S9_CB
S10_OB
S10_CB
S11_OB
S11_CB
S13_OB
S13_CB
S16_OB
S16_CB
Genus
Acanthamoeba
Cercomonas
Chlamydomonas
Chlorella
Colpoda
Cyclidium
Luapeleamoeba
Muriella
Nuclearia
Others
Scenedesmus
Spumella
Tetradesmus
Vahlkampfia
Vermamoeba

c


S1
S2
S3
S4
S6
S7
S8
S9
S10
S11
S13
S16
Abundance
1.00
0.75
0.50
0.25
0.00
S1_OB
S1_CB
S2_OB
S2_CB
S3_OB
S3_CB
S4_OB
S4_CB
S6_OB
S6_CB
S7_OB
S7_CB
S8_OB
S8_CB
S9_OB
S9_CB
S10_OB
S10_CB
S11_OB
S11_CB
S13_OB
S13_CB
S16_OB
S16_CB
Phylum
Actinobacteriota
Bacteroidota
Chloroflexi
Cyanobacteria
Deinococcota
Firmicutes
Gemmatimonadota
Methylomirabilota
Others
Planctomycetota
Proteobacteria
Verrucomicrobiota

d

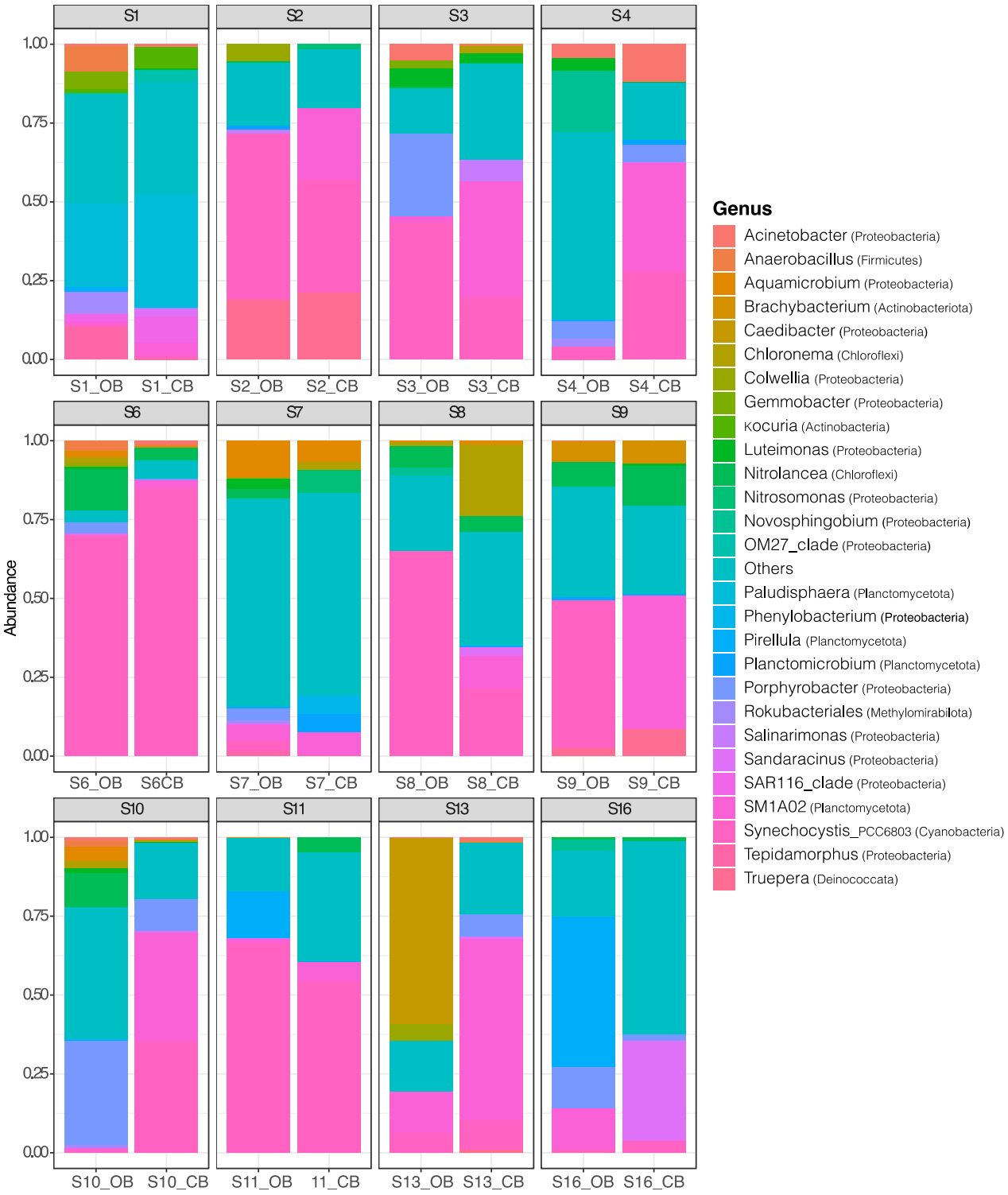
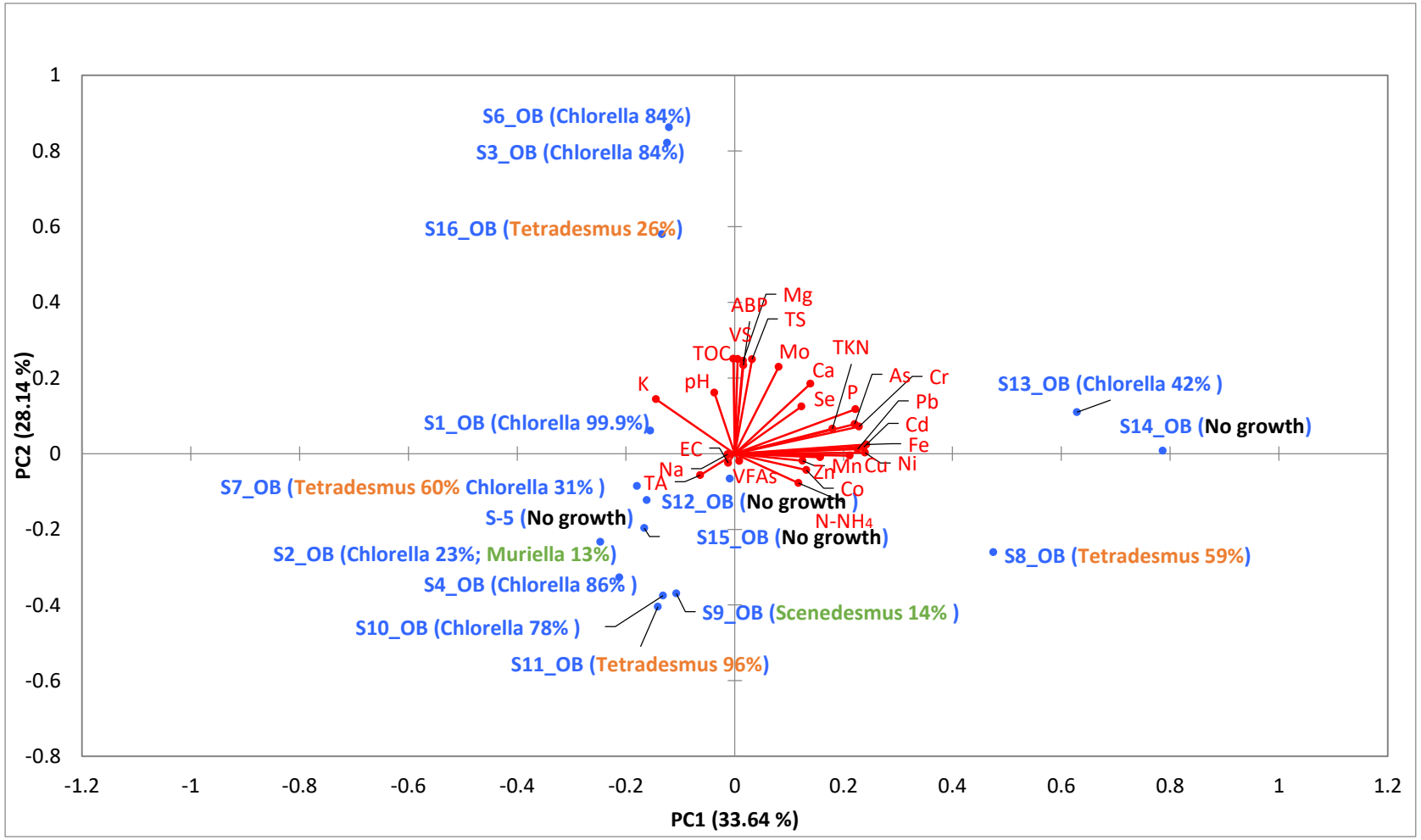

S1
S2
S3
S4
S6
S7
S8
S9
S10
S11
S13
S16
Abundance
1.00
0.75
0.50
0.25
0.00
S1_OB
S1_CB
S2_OB
S2_CB
S3_OB
S3_CB
S4_OB
S4_CB
S6_OB
S6CB
S7_OB
S7_CB
S8_OB
S8_CB
S9_OB
S9_CB
S10_OB
S10_CB
S11_OB
11_CB
S13_OB
S13_CB
S16_OB
S16_CB
Genus
Acinetobacter (Proteobacteria)
Anaerobacillus (Firmicutes)
Aquamicrobium (Proteobacteria)
Brachybacterium (Actinobacteriota)
Caedibacter (Proteobacteria)
Chloronema (Chloroflexi)
Colwellia (Proteobacteria)
Gemmobacter (Proteobacteria)
Kocuria (Actinobacteria)
Luteimonas (Proteobacteria)
Nitrolancea (Chloroflexi)
Nitrosomonas (Proteobacteria)
Novosphingobium (Proteobacteria)
OM27_clade (Proteobacteria)
Others
Paludisphaera (Planctomycetota)
Phenylobacterium (Proteobacteria)
Pirellula (Planctomycetota)
Planctomicrobium (Planctomycetota)
Porphyrobacter (Proteobacteria)
Rokubacteriales (Methylomirabilota)
Salinarimonas (Proteobacteria)
Sandaracinus (Proteobacteria)
SAR116_clade (Proteobacteria)
SM1A02 (Planctomycetota)
Synechocystis_PCC6803 (Cyanobacteria)
Tepidamorphus (Proteobacteria)
Truepera (Deinococcata)

Fig. 1

a


S6_OB (Chlorella 84%)
S3_OB (Chlorella 84%)
S16_OB (Tetradesmus 26%)
S1_OB (Chlorella 99.9%)
S7_OB (Tetradesmus 60% Chlorella 31%)
S-5 (No growth)
S2_OB (Chlorella 23%; Muriella 13%)
S4_OB (Chlorella 86%)
S10_OB (Chlorella 78%)
S11_OB (Tetradesmus 96%)
S9_OB (Scenedesmus 14%)
S15_OB (No growth)
S12_OB (No growth)
S8_OB (Tetradesmus 59%)
S13_OB (Chlorella 42%)
S14_OB (No growth)
ABP
Mg
TS
VS
TOC
Mo
TKN
Ca
As
Cr
K
pH
Se
P
Pb
Cd
Fe
EC
Na
TA
VFAs
Zn
Mn
Cu
Ni
Co
N-NH4
PC1 (33.64 %)
PC2 (28.14 %)
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1.2
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
1.2

5

6

7 b

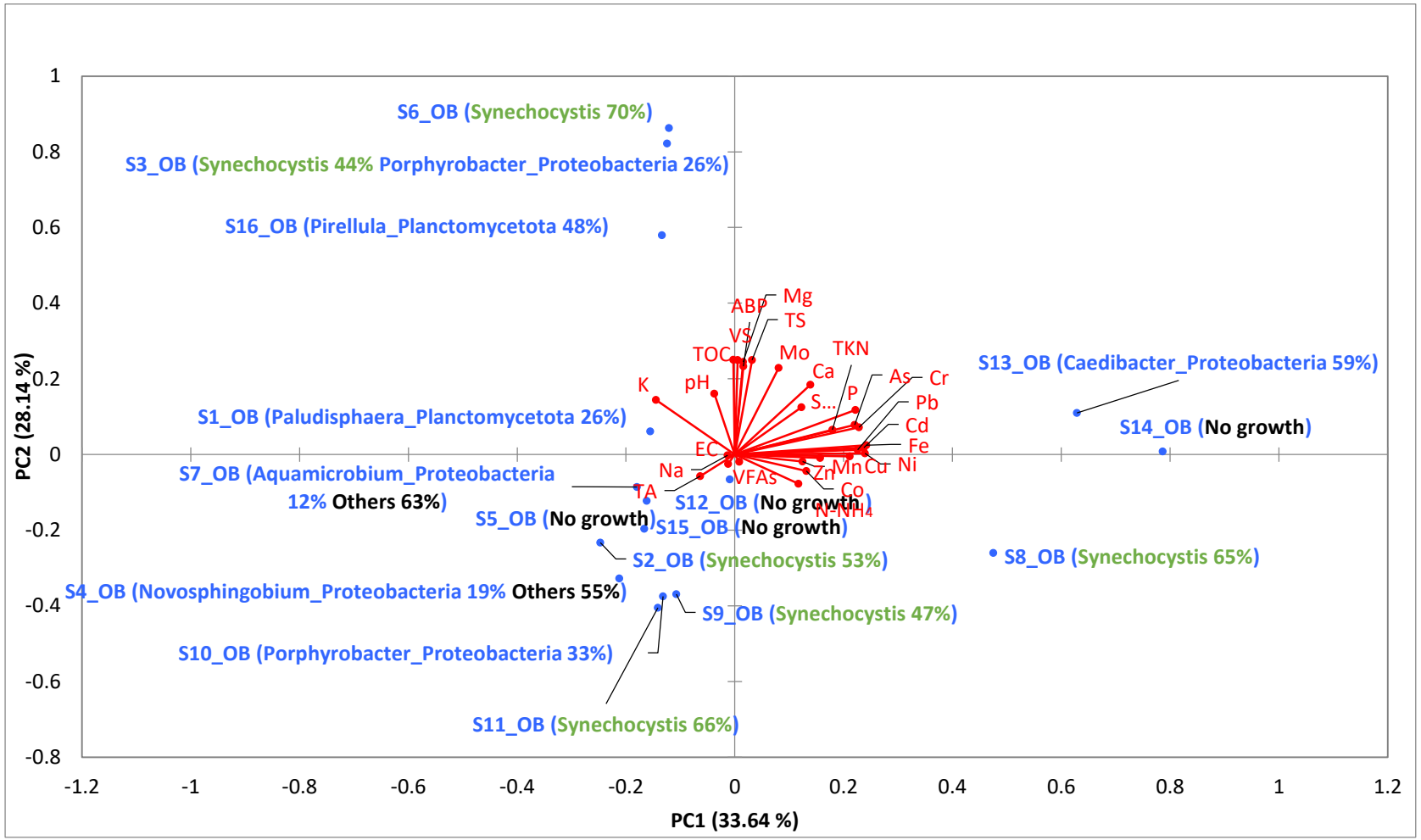

S6_OB (Synechocystis 70%)
S3_OB (Synechocystis 44% Porphyrobacter_Proteobacteria 26%)
S16_OB (Pirellula_Planctomycetota 48%)
S1_OB (Paludisphaera_Planctomycetota 26%)
S7_OB (Aquamicrobium_Proteobacteria 12% Others 63%)
S5_OB (No growth)
S4_OB (Novosphingobium_Proteobacteria 19% Others 55%)
S10_OB (Porphyrobacter_Proteobacteria 33%)
S11_OB (Synechocystis 66%)
S2_OB (Synechocystis 53%)
S15_OB (No growth)
S12_OB (No growth)
S9_OB (Synechocystis 47%)
S13_OB (Caedibacter_Proteobacteria 59%)
S14_OB (No growth)
S8_OB (Synechocystis 65%)
ABP
Mg
TS
VS
TOC
Mo
TKN
Ca
K
pH
As
Cr
S...
P
Pb
Cd
Fe
EC
Na
Zn
Mn
Cu
Ni
VFAs
TA
Co
N-NH4
PC1 (33.64 %)
PC2 (28.14 %)
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1.2
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
1.2

Fig 2.

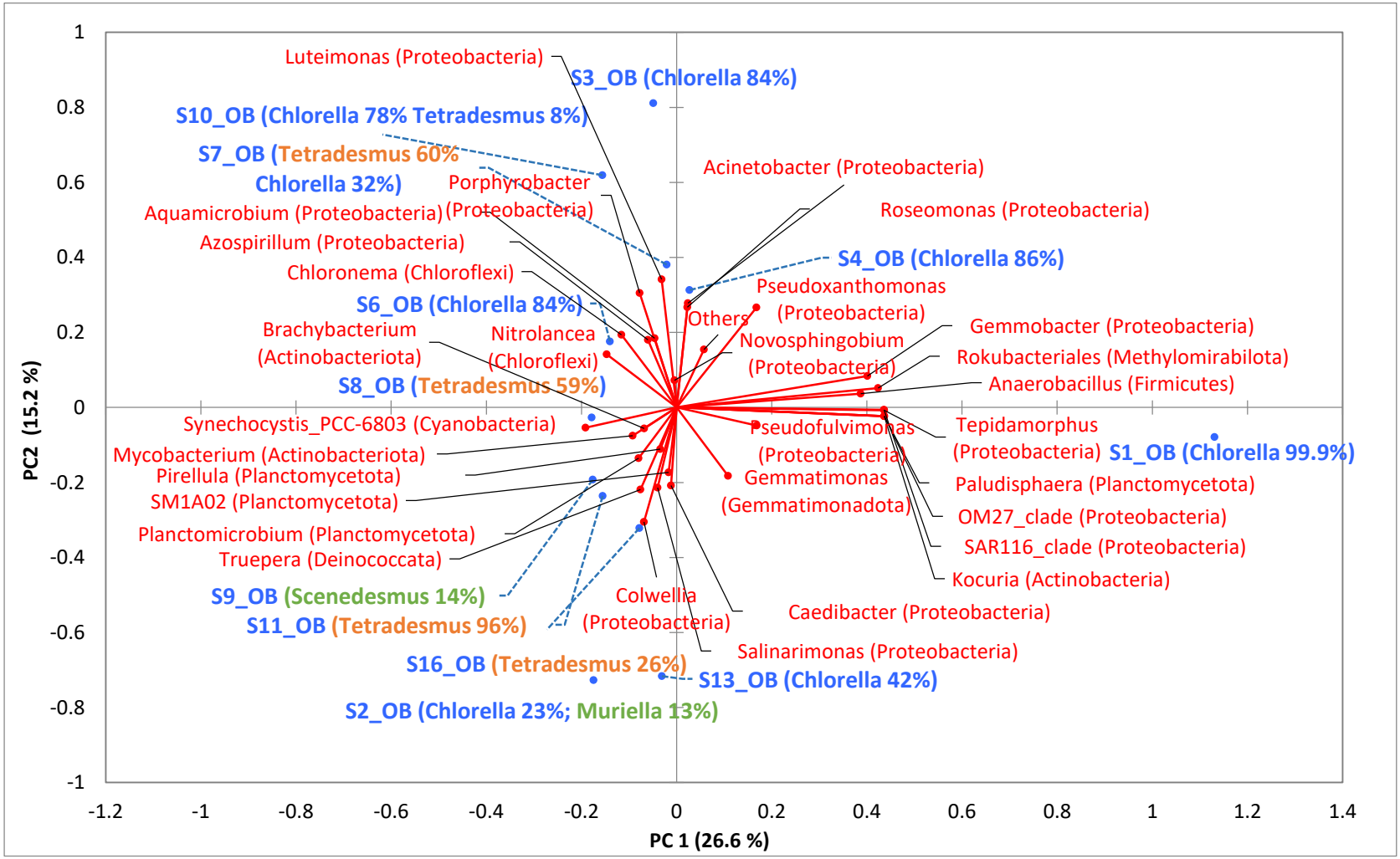
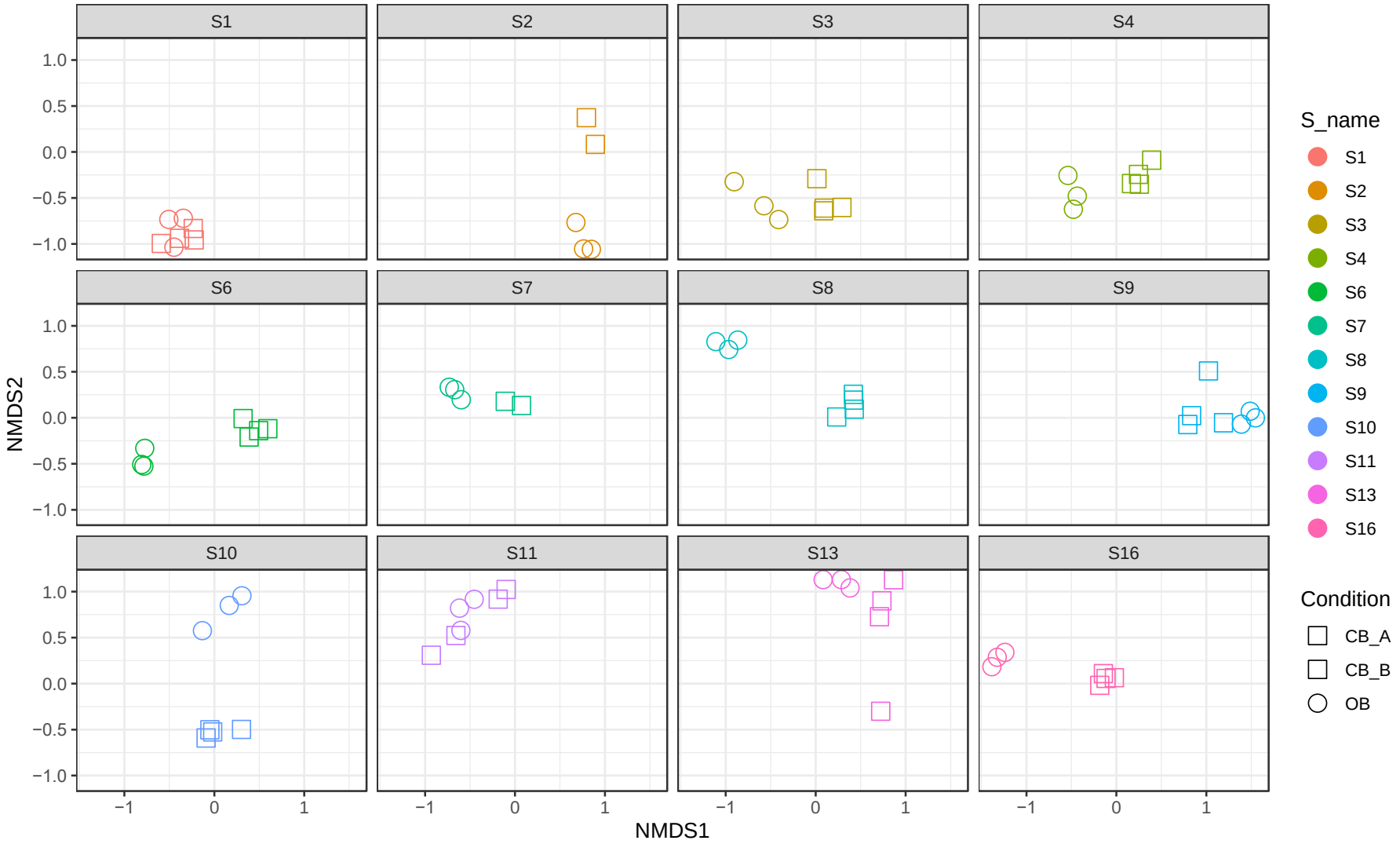
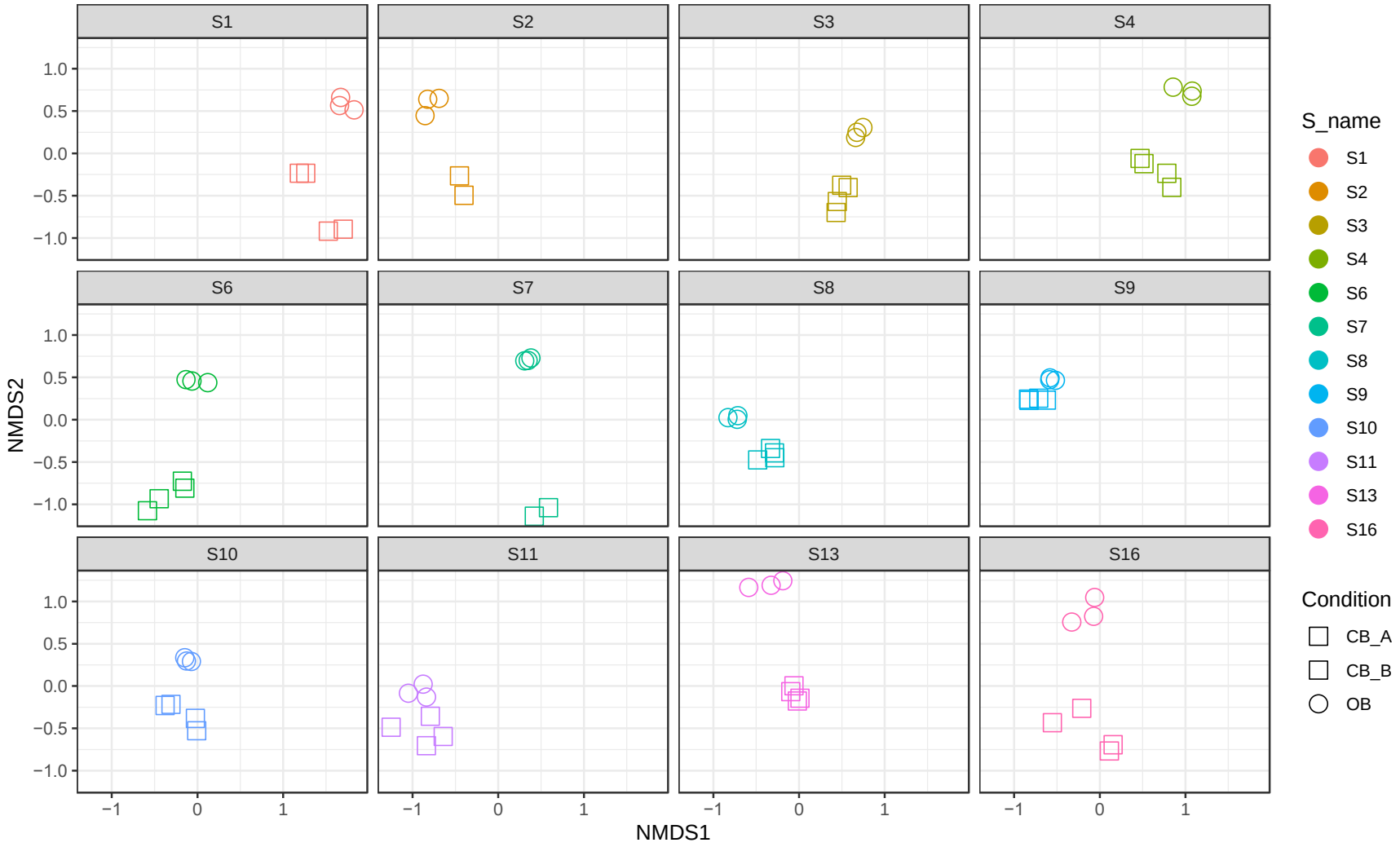

Luteimonas (Proteobacteria)
S3_OB (Chlorella 84%)
S10_OB (Chlorella 78% Tetradesmus 8%)
S7_OB (Tetradesmus 60%
Chlorella 32%)
Porphyrobacter
(Proteobacteria)
Acinetobacter (Proteobacteria)
Roseomonas (Proteobacteria)
Aquamicrobium (Proteobacteria)
Azospirillum (Proteobacteria)
S4_OB (Chlorella 86%)
Chloronema (Chloroflexi)
Pseudoxanthomonas
(Proteobacteria)
S6_OB (Chlorella 84%)
Others
Gemmobacter (Proteobacteria)
Brachybacterium
(Actinobacteriota)
Nitrolancea
(Chloroflexi)
Novosphingobium
(Proteobacteria)
Rokubacteriales (Methylomirabilota)
S8_OB (Tetradesmus 59%)
Anaerobacillus (Firmicutes)
Synechocystis_PCC-6803 (Cyanobacteria)
Pseudofulvimonas
(Proteobacteria)
Tepidamorphus
(Proteobacteria)
S1_OB (Chlorella 99.9%)
Mycobacterium (Actinobacteriota)
Pirellula (Planctomycetota)
Gemmatimonas
(Gemmatimonadota)
Paludisphaera (Planctomycetota)
SM1A02 (Planctomycetota)
OM27_clade (Proteobacteria)
Planctomicrobium (Planctomycetota)
SAR116_clade (Proteobacteria)
Truepera (Deinococcata)
Kocuria (Actinobacteria)
S9_OB (Scenedesmus 14%)
Colwellia
(Proteobacteria)
Caedibacter (Proteobacteria)
S11_OB (Tetradesmus 96%)
Salinarimonas (Proteobacteria)
S16_OB (Tetradesmus 26%)
S13_OB (Chlorella 42%)
S2_OB (Chlorella 23%; Muriella 13%)
PC2 (15.2 %)
PC 1 (26.6 %)
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
1.2
1.4

Fig. 3

13 a


S1
S2
S3
S4
S6
S7
S8
S9
S10
S11
S13
S16
NMDS2
NMDS1
1.0
0.5
0.0
−0.5
−1.0
−1
0
1
S_name
S1
S2
S3
S4
S6
S7
S8
S9
S10
S11
S13
S16
Condition
CB_A
CB_B
OB

b

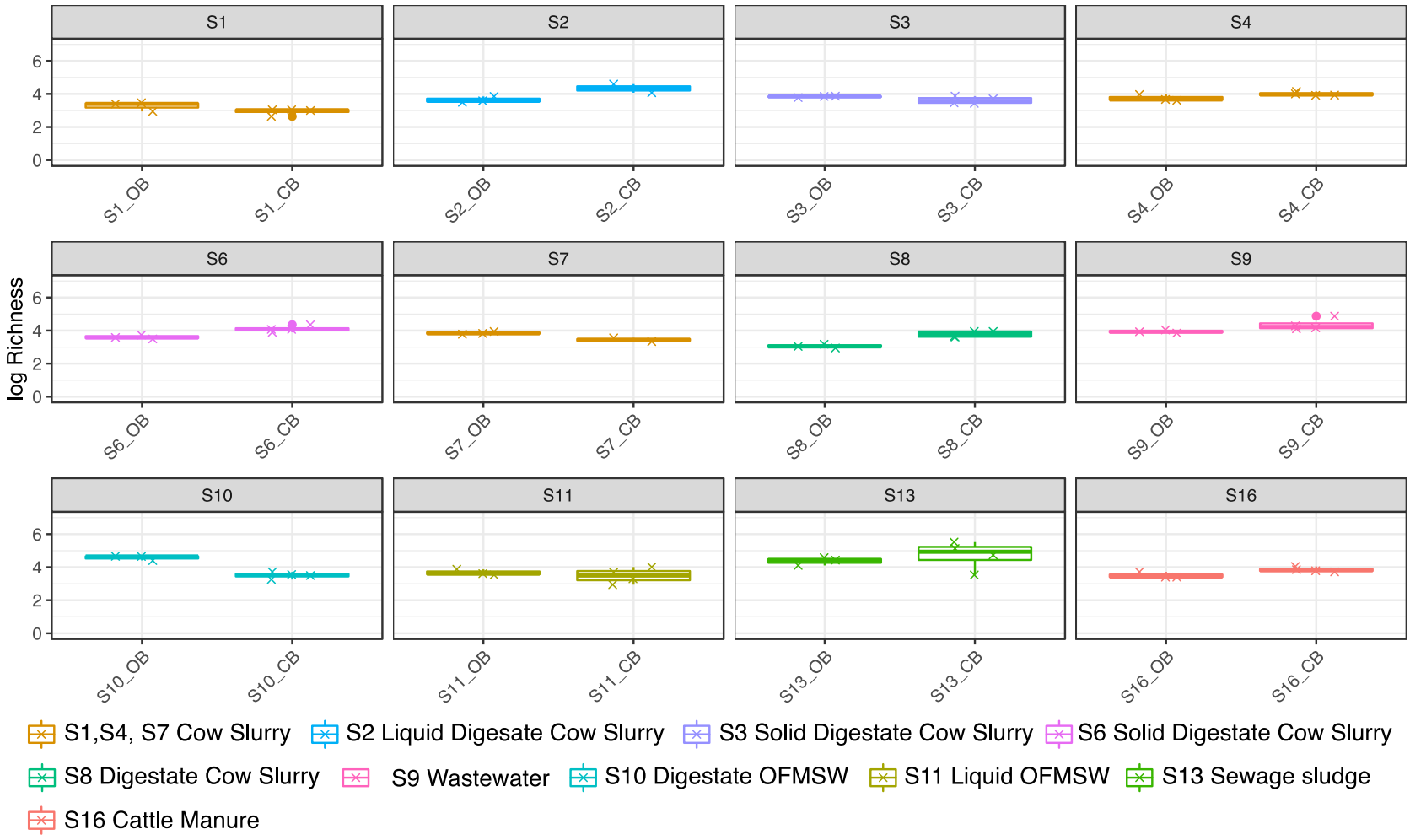

S1
S2
S3
S4
S6
S7
S8
S9
S10
S11
S13
S16
NMDS1
NMDS2
S_name
S1
S2
S3
S4
S6
S7
S8
S9
S10
S11
S13
S16
Condition
CB_A
CB_B
OB

30

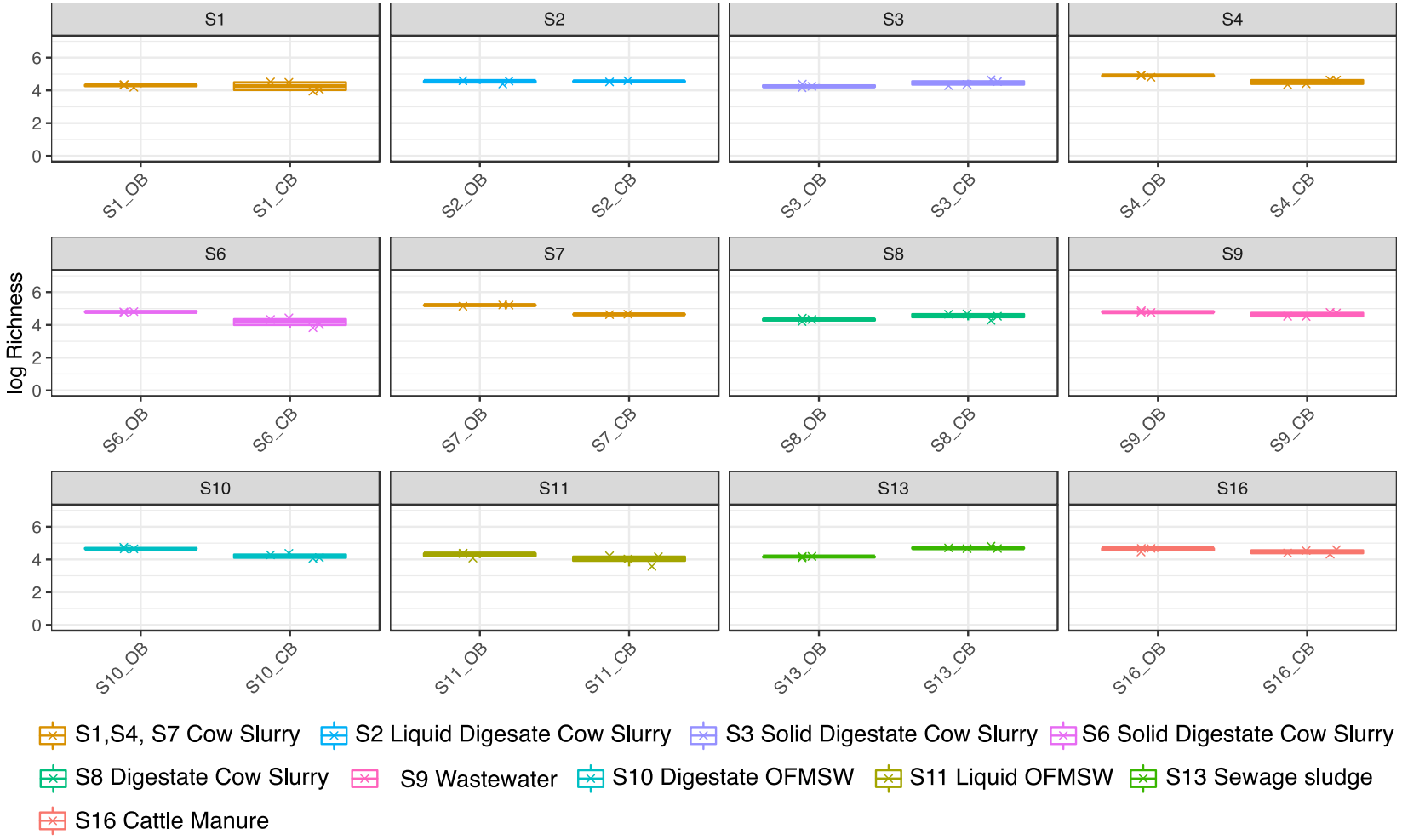
31 Fig. 4

32 a

52


S1
S2
S3
S4
S6
S7
S8
S9
S10
S11
S13
S16
log Richness
0
2
4
6
S1_OB
S1_CB
S2_OB
S2_CB
S3_OB
S3_CB
S4_OB
S4_CB
S6_OB
S6_CB
S7_OB
S7_CB
S8_OB
S8_CB
S9_OB
S9_CB
S10_OB
S10_CB
S11_OB
S11_CB
S13_OB
S13_CB
S16_OB
S16_CB
S1,S4, S7 Cow Slurry
S2 Liquid Digesate Cow Slurry
S3 Solid Digestate Cow Slurry
S6 Solid Digestate Cow Slurry
S8 Digestate Cow Slurry
S9 Wastewater
S10 Digestate OFMSW
S11 Liquid OFMSW
S13 Sewage sludge
S16 Cattle Manure

b


S1
S2
S3
S4
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S9
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S13
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log Richness
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S1_OB
S1_CB
S2_OB
S2_CB
S3_OB
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S6_CB
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S13_OB
S13_CB
S16_OB
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