Microbial communities in seawater from an Arctic and a temperate Norwegian fjord and their potentials for biodegradation of chemically dispersed oil at low seawater temperatures

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Keywords: Oil; biodegradation; Arctic; microbial communities; dispersants

Abstract

Biodegradation of chemically dispersed oil at low temperature (0-2°C) was compared in natural seawater from Arctic (Svalbard) and a temperate (Norway) fjords. The oil was premixed with a dispersant (Corexit 9500) and small-droplet oil dispersions prepared. Faster biotransformation of *n*-alkanes in the Arctic than in the temperate seawater were associated with the initially higher abundance of the alkane-degrading genus *Oleispira* in the Arctic than the temperate seawater. Comparable transformation of aromatic hydrocarbons was further associated with the late emergences *Cycloclasticus* in both seawater sources. The results showed that chemically dispersed oil may be rapidly biodegraded by microbial communities in Arctic seawater. Compared to oil biodegradation studies at higher seawater temperatures, longer lag-periods were experienced here, and may be attributed to both microbial and oil properties at these low seawater temperatures.

1. Introduction

The estimated occurrence of undiscovered oil and gas north of the Arctic Circle may be as much as 90 billion barrels of oil and 47 trillion cubic meters of natural gas, most of it in offshore areas (Bird et al., 2008). In addition, reduced ice coverage in the Arctic will result in higher transport activities in this region than today. Strict regulations of oil exploration and production and transport in the Arctic are imposed by responsible governmental bodies, but accidental releases of oil may occur and cause impacts on local marine environments. Oil spilled to the marine environment undergoes a number of weathering processes like evaporation, water-in-oil (w/o) emulsification, dispersion, dissolution of small and charged compounds, and photo-oxidation (NRC 2003).

Biodegradation is an important weathering process that may result in complete mineralization of hydrocarbons (HCs). Oil spills to marine environments may result in blooms of oil-degrading bacteria, increasing dramatically in their abundance (Braddock et al., 1995; Brakstad and Lødeng, 2005; Bælum et al., 2012; Dubinsky et al., 2013; Hazen et al., 2010; Yakimov et al., 2007). Most of these are affiliated to the classes Alphaproteobacteria or Gammaproteobacteria (Yakimov et al., 2007), and several of them are obligate hydrocarbonoclastic, exclusively transforming HCs (Yakimov et al., 2007). Aliphatic HC-degrading bacteria like *Alcanivorax* are typically succeeded by bacteria like *Cycloclasticus*, which attack more slowly biodegradable oil compounds like polycyclic aromatic HCs (PAH) (Kasai et al., 2002; Röling and van Bodegom, 2014). Several studies have shown that also Arctic seawater (SW) and marine ice contains hydrocarbonoclastic bacteria with the abilities to biotransform oil HCs (Bagi et al., 2014; Bowman and McCuaig, 2003; Brakstad et al., 2008; Deppe et al., 2005; Garneau et al., 2016; Gerdes et al., 2005; McFarlin et al., 2014; Yakimov et al., 2004). In cold SW, alkane degradation is often associated with high abundances of psychrophilic *Oceanospirillales*, like *Oleispira antarctica*, while

Cycloclasticus is associated with degradation of aromatic HCs both in temperate and cold SW (Coulon et al., 2007; Dong et al., 2015). Members of the genus *Colwellia* are associated with oil-contaminated marine ice and cold SW, Antarctic sediments, and were also abundant in the deep-sea oil plume after the Deepwater Horizon oil spill (Brakstad et al., 2008; Powell et al., 2006; Redmond and Valentine, 2012). Single-cell genomic studies have revealed that *Oceanospirillales* are associated with *n*-alkane and cycloalkane degradation pathways, while *Colwellia* may be associated with gaseous, and simple aromatic HC degradation (Mason et al., 2014; Mason et al., 2012).

Effective stimulation of bacterial degradation depends on the bioavailability of the oil compounds, as compounds in dissolved or dispersed fractions. Chemical dispersants are used as an oil spill response method to remove oil slicks from the sea surface, by generating dispersions with small droplet size and near-to neutral buoyancies in the seawater column. Despite some controversy about the effect of dispersants on oil biodegradation (Kleindienst et al., 2015b; Lindstrom and Braddock, 2002; Rahsepar et al., 2016), most studies have shown that efficient use of dispersants enhances the biodegradation (Brakstad et al., 2014; Bælum et al., 2012; Lee et al., 2013; McFarlin et al., 2014; Prince et al., 2013; Siron et al., 1995; Techtmann et al., 2017; Venosa and Holder, 2007). Even in Arctic SW at very low temperatures (-1°C), the use of dispersants facilitated oil biodegradation (McFarlin et al., 2014). However, since the oil dispersibility is related to viscosity and pour point (Brandvik and Faksness, 2009), the dispersibility of many oils become reduced in cold seawater.

Since dispersant treatment may be a relevant oil spill response (OSR) treatment in the Arctic to prevent the oil from stranding or drifting into ice-covered areas, it is essential to investigate the effect of this OSR treatment on oil biodegradation in Arctic SW. We therefore compared biodegradation of chemically dispersed oil in Arctic and temperate SW at low

temperature (0-2°C) and the relations between community successions and biotransformation of oil compound groups in the SW sources.

2. Materials and Methods

2.1 SW sampling

Svalbard (SVB) SW (80L) was collected beneath the ice in the Van Mijen fjord (77°56′N, 16°43′ E) on April 21, 2016. Holes were drilled in the ice (ice drill) and appr. 10 L SW filled on each of 8 x 20-L Teflon-bags (5-gallon Pail Liners and Lid Protectors made by 2.5 mill modified PTFE film, Welch Fluorocarbon, Dover, NH, USA). The bags were closed with double sets of pull-ties and each bag placed in a 12-L lacquered-lined drum and closed with a locking ring (Air Sea Containers Ltd., Birkenhead, UK). The seawater was stored overnight at 4-5°C (airport in Longyearbyen), transported by plane the next day, and arrived at our lab in the afternoon of April 22. The SW was then acclimated at 0-2°C for 5 days until the biodegradation experiment started. Triplicate SW samples (2 L) were also filtered on site (Svalbard) through 0.22 µm Durapore filters (Merck KGaA, Darmstadt, Germany), and the filters transported together with seawater. Volumes of SW (2 L) were filtered through 0.22 µm Durapore filters (triplicate) after arrival to our lab to determine if transport had affected the composition of the microbial communities.

SW (80 L) was also collected from 80 m depth in a temperate fjord, Trondheimsfjord (TRD; 63°26'N, 10°23'E) through a pipeline system supplying our labs (SINTEF Sealab, Trondheim, Norway) with continuous seawater. This SW is collected below thermocline and is expected to have a temperature of appr. 5-6°C from previous measurements (Brakstad et al., 2004). This SW was collected at the same day as the SVB SW (April 21, 2016), stored at 4°C until the SVB water arrived at the lab. The TRD SW was then acclimated as described

above for the SVB SW. Triplicate SW samples of TRD SW (2 L) were filtered through 0.22 µm Durapore filters when sampled and after storage overnight at 4°C.

2.2 Biodegradation experiment

Fresh Troll naphthenic oil (batch 2007-0087) and Corexit 9500A dispersant (Nalco, Sugar Land, TX, USA) were used in this experiment. This oil had low viscosity (27 mPas;13°C), a density of 0.900 g/cm^3, pour point of -18°C, and low wax (2.0 vol%) and asphaltene (0.2 wt%) contents.

The oil was pre-mixed with Corexit 9500 in a dispersant-to-oil ratio (DOR) of 1:100, and oil dispersions prepared in an oil droplet generator (Brakstad et al., 2015a; Nordtug et al., 2011). Two oil droplet stock dispersions (concentration of 200 mg/L and median droplet size of 10 µm) were prepared in the droplet generator system, with acclimated SW from SVB or TRD. Based on oil droplet concentration measurements (Coulter Counter; see below), each stock dispersion was diluted in natural acclimated SW (0-2°C) from their respective source (SVB or TRD) to reach final nominal concentrations of 2 mg/L oil droplets. This oil concentration did not require additional mineral nutrient amendment, as previously shown (Brakstad et al., 2015a; Prince et al., 2013). The dispersions were distributed in baked (450°C) and autoclaved flasks (2 L; Schott), completely filled and capped without headspace or air bubbles, and flasks were mounted on a carousel system with slow continuous rotation (0.75 r.p.m), as previously described (Brakstad et al., 2015a). The carousel system was maintained at 0-2°C for 64 days in the dark. Triplicate flasks of dispersions in natural SW from both SVB and TRD were sacrificed for analyses after 30 minutes incubation (0 days). Flasks were then sacrificed for analyses after 7, 14, 21, 28, 42 and 64 days of incubation as described for 0-day samples. Flasks with seawater blanks without oil or dispersant, were

incubated at the same conditions as the oil dispersions, and one flask of each SW blank sacrificed at the same times as the dispersions.

2.3 Microbiology analyses

2.3.1 Total cell concentrations and most probable number determinations

Total prokaryote concentrations were determined by epifluorescence microscopy analyses of samples stained by the nucleic acid stain 4',6-diamidino-2-phenylindol (Porter and Feig, 1980). Most probable number (MPN) concentrations of heterotrophic prokaryotes (HP) and oil-degrading prokaryotes (ODP) were determined as previously described (Brakstad et al., 2008), except for the incubation conditions. Incubations were performed at 0-2°C for 7 days, followed by 20°C for 3 days (HP) or 7 days (ODP).

2.3.2 16 S rRNA gene amplicon sequencing

Seawater blanks and oil dispersions (approximately 500 ml) were filtered through 0.22 µm filters (Millipore), and DNA was extracted from filters by employing FastDNA Spin kit for soil (MP Biomedicals), according to the manufacturer's instructions. DNA quantification was performed by Qubit 3.0 fluorometer (Thermo Fisher Scientific Waltham, MA, USA), with dsDNA High Sensitivity kit (ThermoFisher Scientific, MA, USA).

16S rDNA amplicons were generated according to Illuminas "16S Metagenomic Sequencing Library Preparation" protocol using S-D-bact-0341-b-S-17 and S-bact-0785-a-A-21 primer set (Klindworth et al., 2013). Amplicons generated by PCR were isolated using magnetic beads (Agencourt Amoure XP Beads). Libraries have been quantified using Quant iT Picogreen Dye and the Fragment Analyzer (Advanced Analytical), as well on Agilent's

Bioanalyzer. All amplicons were pooled equimolar and then sequenced paired-end on the Illumina MiSeq platform, 2x300nt, following the manufacturer instructions.

Raw pair-end reads were assembled with fastq-join in QIIME 1.9.1 (Caporaso et al., 2010b). Assembled sequences were demultiplexed and quality filtered to remove low quality reads (Phred score < 20; -q 19). UCHIME was employed for chimera detection on assembled quality filtered reads (Edgar et al., 2011). Operational Taxonomic Units (OTUs) were determined by clustering assembled sequences on 97% nucleotide identity using UCLUST (Edgar, 2010) with open reference clustering option. Representative sequences were aligned with PyNAST (Caporaso et al., 2010a), and taxonomy assignment was performed with RDP classifier (Wang et al., 2007), based on SILVA-123 database (Klindworth et al., 2013). To evaluate for potential differences in the dynamics of microbial communities between different samples and sample groups at separate time points, multivariate statistics in the form of principal coordinate analysis (PCoA), based on un-weighted UniFrac distance metrics was carried out. Prior to that, relative abundances of OTUs were calculated, and OTUs with < 0.01% of relative sequence abundance were removed. Statistical analysis was performed within the Phyloseq package v.1.12.2 (McMurdie and Holmes, 2013) in R-studio v.3.2.2. For visualization of taxonomical composition, for each taxon (on genus or family level) cut-off of 3 % was applied for incubation samples, while for source water samples cut-off was set at 2% of relative sequence abundance. All ambiguously assigned sequences, where a query sequence matches a sequence in the reference database that has no annotation, and sequences that have no match at all in the database, have been merged into one group called “Unassigned”. In the supplemental material the “Unassigned” group is described in more detail (Fig. S1), and only OTUs having >5% of relative abundance are represented.

Nucleotide sequence data for 16S rRNA amplicon sequences were deposited to the European Nucleotide Archive (ENA), and the sequences can be found under study accession number PRJEB24364 entitled “PETROMAKS E#12”.

2.4 Chemical analyses and data treatments

Samples of oil dispersions and seawater blanks were solvent-solvent extracted (dichloromethane) for measurements of semivolatile organic compounds (SVOC) by gas chromatographic methods. The flask glass walls were also rinsed with DCM after removal of dispersions to extract material attached to the glass walls. Extracts of dispersions and glass walls were pooled. Total extractable organic carbon (TEOC) was analysed on a gas chromatograph coupled to a flame ionization detector (GC-FID; Agilent 6890N with 30 mDB1 column; Agilent Technologies), while quantification of 87 targeted compounds or compound groups (*n*C10-*n*C36 n-alkanes, decalins, phenols, 2- to 5-ring polycyclic aromatic HCs (PAH) and 17α(H),21β(H)-Hopane) was performed by a gas chromatograph coupled to a mass spectrometer (GC-MS; Agilent 6890 plus GC coupled with an Agilent 5973 MSD detector, operated in Selected Ion Monitoring [SIM] modus; Agilent Technologies), as previously described (Brakstad et al., 2014). Target analytes were normalized against 17α(H),21β(H)-Hopane (Prince et al., 1994; Wang et al., 1998). Samples were acidified (pH<2) for analyses of 35 VOC compounds in a Purge & Trap unit (Teledyne Tekmar Atomx; Mason OH, U.S.A.) coupled to a GC-MS (Agilent 6890N GC and an Agilent 5975B MSD detector; Agilent Technologies) (P&T GC-MS). In both GC-MS analyses of SVOC and P&T GC-MS analyses of VOC compounds, response values for individual target analytes were determined, and based on a signal-to-noise ratio of > 10, the lower limit of detections (LOD) was from 0.01 µg/L to 0.01 µg/L for SVOC and VOC compounds.

Non-linear regression analyses were performed by the option "plateau followed by one-phase decay" in GraphPad Prism vs. 6.0 (GraphPad Software Inc., La Jolla, CA, U.S.A). The

plateau period included the non-responsive lag-period before start of the biodegradation. Rate coefficients ($k1$) were determined for the degradation period, and half-lives were determined from the rate coefficients ($t1/2 = 0.693/k1$).

One-way ANOVA analyses were performed by GraphPad Prism vs. 6.

2.5 Oil droplet analyses

Oil droplet concentrations and size distributions in 20 ml samples (triplicate) were determined by Coulter Counter measurements (Beckman Multisizer 4; Beckman Coulter Inc., Brea, CA, USA) fitted with 100 µm aperture, for measurement of droplets size and concentrations within a diameter range of 2-60 µm. Filtered (0.22 µm) SW was used as electrolyte. All droplet sizes reported here are expressed as median droplets diameter.

2.6 Other analyses

SW analyses on site were performed with CTD instrumentation with additional units for dissolved oxygen (DO) and chlorophyll A. DO and water temperatures in the lab were determined by a DO meter (YSI, Inc., Yellow Springs, OH, USA).

Nutrient analyses of SW included total Nitrogen (internal procedure), NO_3+NO_2-N (ISO 13395), NH_4-N (ISO 11732), *o*-PO_4-P and total Phosphorous (both ISO 15681-2), Iron (ISO 17294-2:2016), total organic carbon (TOC) (EN 1484), and Fe (method ISO 17294m:2016). All analysed by Eurofins Environment Testing Norway, Bergen, Norway.

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3. Results and Discussions

3.1 Seawater characterization

The SW from SVB, which was sampled beneath the ice coverage, had a temperature of ÷1.7°C, a salinity of 34.3 PSU, a DO concentration of 12.5 mg/L, and a chlorophyll A concentration of 4.5 µg/L. Chlorophyll A concentrations also showed diurnal algal migration in the SW column, with the highest concentrations in the upper layer beneath the ice coverage in the afternoon, when the SW was sampled. The TRD SW was collected below thermocline at 80 m depth. The temperature below thermocline in this fjord at depths corresponding to the SW inlet has been shown to be 7.7-7.9°C (Børsheim et al., 1999), while we measured the temperature at the SW outlet at the laboratory to vary between 5.9 and 7.4°C over a period of 42 days (Brakstad et al., 2004). Upon sampling for the current experiment, the TRD SW had a temperature at the pipeline outlet of 5.6°C, salinity of 34.5 PSU and a DO concentration of 9.1 mg/L.

The concentrations of mineral nutrients, Fe and TOC in the SWs were measured in both SWs, showing comparable concentrations of organic carbon, total P, *o*-PO_4-P, NO_2/NO_3-P and Fe, while NH_4-P was higher in the TRD than the SVB SW (Table S1, Supplementary Information (SI)). High ammonium concentrations in the TRD SW indicated significant biological activity in this water, for instance by decomposition of N-containing organic matter, which may have been related to algal spring bloom (Børsheim et al., 1999).

Microbial communities were compared in the two SW sources by 16S rDNA amplicon analyses (Fig. 1). In the TRD SW sampled from the pipeline system, 82.3±0.5 % of the sequences (cut-off at 2 %) were identified. Abundant families in this SW included the families *Rhodospirillaceae* (9.4±0.3%), *Flavobacteraceae* (7.9±0.9%), *Rhodobacteraceae* (4.6±0.3 %) and *Nitrospinaceae* (3.9±0.2%) (Fig. 1A). In the SW from SVB filtered directly from beneath

the ice, 94.5 % of the sequences were identified, including 62.4 % sequences associated with *Flavobacteraceae* (15.2±0.1%), *Oceanospirillaceae* (12.7±0.6 %), *Rhodobacteraceae* (11.9±0.6%) and *Colwelliaceae* (8.1±0.3%) (Fig. 1B). In addition, chloroplast sequences related to *Cyanobacteri*a were abundant in the samples from both TRD and SVB (2.0-2.4 %). On genus level, the SVB water showed abundances of candidatus *Pelagibacter* (5.3±0.8), *Polaribacter* (6.9±0.1 %), *Colwellia* (8.3±0.3%) and *Balneatrix* (12.3±0.6) (see Fig. S2, Supplementary Information), all genera associated with Arctic SW or fjord ice (Brakstad et al., 2008; Groudieva et al., 2004; Jain and Krishnan, 2017; McFarlin et al., 2017; Zeng et al., 2013). The TRD SW showed abundances of candidatus *Pelagibacter* (6.8±0.5%) and *Nitrospina* (3.7±0.2%) (Fig. S2). Candidatus *Pelagibacter*, being abundant in both SWs, has been reported to be the most abundant group of heterotrophic bacteria in the oceans, representing approximately one quarter of all rRNA genes identified in clone libraries from marine environments (Morris et al., 2002). *Balneatrix*, the predominant *Oceanospirillaceae* genus in the SVB SW, was observed to be abundant in Arctic waters of the Pacific Ocean (Han et al., 2014), as well as in the North Sea waters during winter-spring season, following planktonic blooms (Han et al., 2014; Kassabgy, 2011). Interestingly, in the study by Jain and Krishnan (2017) performed in the waters of Svalbard, *Balneatrix* was found to be one of the major genera associated with particles, adding to the conclusion that this genus probably thrives on algal bloom by-products. *Colwellia* has been associated with HC biodegradation in cold seawater, from polar regions and the deep sea (Bagi et al., 2014; Bælum et al., 2012; Redmond and Valentine, 2012), and has even been shown to be stimulated in oil-polluted marine ice (Brakstad et al., 2008). The *Bacteroidetes* genus *Polaribacter* has been associated with Arctic SW and marine ice (Brakstad et al., 2008; Jain and Krishnan, 2017; McFarlin et al., 2017; Zeng et al., 2013), and members of this genus may harbour *alk*B genes involved in alkane biodegradation (Nie et al., 2014). The family *Nitrospinaceae* (genus *Nitrospina*),

271 which was abundant in the TRD SW, includes nitrite-oxidizing bacteria involved in the
272 oxidation of ammonium to nitrate (Levipan et al., 2014), in accordance with the high
273 ammonium concentration in the TRD SW. Chloroplast sequences of Cyanobacteria have been
274 detected by 16S rDNA analyses in Arctic SW and ice (Brakstad et al., 2008; Jain and
275 Krishnan, 2017). Plastids are believed to be an early offshoot of the cyanobacterial
276 evolutionary line (Nelissen et al., 1995), and may therefore be detected by 16S rDNA primers
277 (Ghyselinck et al., 2013; Nübel et al., 1997). The abundances of the cyanobacterial
278 chloroplast sequences were in accordance with the chlorophyll A concentrations at the site in
279 the SVB SW.

280 After transport of the SVB SW, higher abundances of *Oceanospirillaceae* (24.1±3.7%)
281 and *Colwelliaceae* (15.6±30.9%) was measured, when compared to the data from the on-site
282 filtration (Fig. 1C). The predominant genera were *Balneatrix* (22.5±3.5%) and *Colwellia*
283 (13.8±0.7%) (Fig S1).

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285 *3.2 Temperature, DO and oil droplets*

286 The temperature was kept below 1°C in the SW during the biodegradation experiment,
287 except for a period between day 33 and day 39 (Fig. S3). Due to a failure in the temperature-
288 controls system, the carousel system was temporarily moved to a temperature-controlled room
289 holding 5°C for 6 days, and then moved back to the original rom holding 0-1°C. The
290 temperature never increased above 4.3°C in the SW (Fig. S3). DO saturation was maintained
291 above 60% saturation in the dispersions during the biodegradation period (Fig. S3).

292 Initial oil droplet concentrations in the dispersions were 3.03±0.14 mg/L and 2.98±0.04
293 mg/L in the SVB and TRD dispersions, respectively, i.e. very close to the nominal
294 concentrations of 3 mg/L. The oil droplet concentrations within the Coulter Counter

295 measurement range (2-60 µm) decreased to 0.46±0.05 mg/L (SVB) and 0.59±0.32 mg/L
296 (TRD) at the end of the biodegradation period (Fig. 2A). The decrease was faster in the SVB
297 than the TRD dispersions. The median oil droplet sizes at the start of the experiment were
298 17.3±0.2 µm (SVB) and 15.1±0.2 µm (TRD), and decreased to 4.6±0.4 µm (SVB) and
299 4.1±0.5 µm (TRD) after 64 days (Fig. 2B). The initial oil droplet sizes were in accordance
300 with typical median oil droplet sizes achieved after efficient dispersant treatments of spilled
301 oil (Brakstad et al., 2014; Lunel, 1993). The droplet sizes decreased faster in the SVB than the
302 TRD dispersions (Fig. 2B), in accordance the emergences of compact 'floc' particles in the
303 SVB SW (Fig. S4).

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305 *3.3 Hydrocarbon biodegradation*

306 At the start of the biodegradation experiment, the TEOC concentrations in the dispersions
307 were 1.75±0.07 mg/L (TRD) and 1.56±0.05 mg/L (SVB), i.e. 52-59 % of the initial oil droplet
308 concentrations (Fig. S5). TEOC was reduced by 11.9 ± 3.2 % and 17.8± 4.1 % at the end of
309 the experiment in SW from TRD and SVB, respectively. This reduction in TEOC was
310 considerably lower than determined with 10 µm dispersions of a paraffinic oil (Macondo) at
311 5°C, which resulted in 79 % TEOC reduction after 64 days of incubation in a carousel system
312 (Brakstad et al., 2015a). Interestingly, the depletion in the SVB and TRD SWs were also
313 lower than losses of HCs (48-61% loss) from dispersed Alaskan North Slope (ANS) oil in
314 natural Arctic SW from the Chukchi Sea (2.5 mg/L oil concentrations) (McFarlin et al.,
315 2014).

316 Biotransformation of *n*C14-*n*C36-alkanes, naphthalenes, and 2- to 3-PAH in the
317 dispersions were determined after normalization against 17α(H),21β(H)-Hopane, as
318 previously described (Prince et al., 1994). The biotransformation of *n*-alkanes was faster in

the dispersions with SVB SW than TRD SW (Fig. 3A). After 14 days of incubation, 61.8±1.3 % of the *n*-alkanes in SVB was biotransformed, compared to only 3.4±0.8 % in the TRD dispersions. Also after 28 days of incubation, biotransformation was higher in the SVB (77.6±0.6 %) than in the TRD (16.0±9.8 %) dispersions. However, after 64 days depletions were comparable in the dispersions (93%). These differences were caused by faster degradation of the *n*C14-*n*C27 alkanes, while biotransformation of the *n*-alkanes with longer chains (*n*C28-*n*C36) were comparable between SVB and TRD (Fig. S6).

However, the biotransformation of semivolatile organic compounds (naphthalenes and 2- to 6-ring PAH) were comparable between the two SW sources (Fig. 3B and Fig. 3C). After 64 days of incubation, > 99% of the naphthalene/PAH group was depleted. Analyses of individual target compounds showed that naphthalenes were completely biotransformed at the end of the experiment in dispersions from both SW sources, while depletion of the PAH depended on the alkyl substitution (Fig. S7).

Biotransformation of individual volatile alkanes (C5-C9 alkanes) and monoaromatic HCs (BTEX) were also comparable between dispersions in the two SW sources, although initial depletion was faster in the SVB than the TRD dispersions (Fig. 3C), with >99% depletion at the end of the experiment. However, C5-alkanes were depleted faster in the SVB than the TRD dispersions (Fig. S8).

Previous oil biodegradation studies have demonstrated the ability of indigenous bacteria in Arctic SW to biodegrade oil HCs at low temperature. Naphthalene was biomineralized faster in Arctic (Svalbard) than temperate (Norwegian fjord) SW at temperatures of 0.5 and 4°C. Interestingly, the Arctic SW also mineralized naphthalene faster than the temperate SW at temperatures of 8°C and 15°C (Bagi et al., 2014). Fresh or weathered (20% evaporated) ANS oil chemically and physically dispersed in natural SW from the Chukchi Sea (2.5 mg/L oil) showed near complete biotransformation (-1°C, incubation time 56 days) of *n*C17- and

*n*C18-alkanes and C0-C4-alkylated naphthalenes, while phenanthrenes and dibenzothiophenes were biotransformed from near completion to approximately 20 % depending on their alkyl-substitution (McFarlin et al., 2014). These results were similar to our results after 64 days of incubation (Fig. S6 and Fig. S7). In a biodegradation study of a light crude naphthenic oil (Draugen) in SW from western Greenland (Disko Bay) at 2°C, all *n*-alkanes (*n*C13-*n*C30) were biotransformed after 71 days of incubation (Scheibye et al., 2017), in agreement with our results. However, biotransformation of naphthalenes and 2- to 4-ring PAH in the Greenland SW was poor (Scheibye et al., 2017), compared to our results (Fig. S7). Oil biodegradation studies have also been performed in Antarctic SW, showing that the slow-release fertilizer Inipol EAP 22 enhanced the oil biodegradation potential of indigenous bacteria in Antarctic and sub-Antarctic SW (Delille et al., 1998; Delille et al., 2009).

Rate coefficients, non-responsive lag-periods and half-lives of the *n*-alkanes, naphthalene/PAH and VOC groups were determined by first-order rates (Table 1). The lag-period of the *n*-alkanes was considerably shorter in the SVB than the TRD dispersions, while the subsequent rate coefficients and half-lives showed more similarity. However, for the naphthalene/PAH and VOC groups, the lag-periods, rate coefficients and half-lives were highly comparable between the SVB and TRD dispersions. In the current study at 0-2°C, the *n*C14- to *n*C27-alkane lag-periods in the SVB and TRD SWs were longer than in a chemically dispersed paraffinic oil incubated at 5°C (Brakstad et al., 2015a). However, the subsequent rate coefficients and half-lives were to some extent comparable to the study at 5°C in the SVB SW (Brakstad et al., 2015a), although slower biotransformation was measured in the TRD SW in the current study. The lag-periods of the naphthalene/PAH and VOC groups were also considerably longer at in this 0-2°C than at previous studies at 5°C in Norwegian or Gulf of Mexico SW, while subsequent rate coefficients and half-lives were more comparable between the temperatures (Brakstad et al., 2015a; Wang et al., 2016). These long non-responsive lag-

periods in the current studies at 0-2°C may partly be explained by the physical properties of the oil. The oil becomes more viscous in seawater when temperatures are reduced, resulting in reduced PAH solubility and bioavailability (Gold, 1969; Margesin and Schinner, 2001; Payne et al., 1991). Slow biotransformation of *n*C28-*n*C36 alkanes in both SVB and TRD SW, when compared to results at 5°C (Brakstad et al., 2015a), may have been a result of increased oil viscosity and paraffin wax formation (Srivastava et al., 1993). These results further confirm results from previous biodegradation results SW at 0°C and 5°C in our lab, showing that differences in degradation between these temperatures were high, and indicating that physical oil properties could have influenced oil biodegradation (Bagi et al., 2013; Brakstad and Bonaunet, 2006).

3.4 Stimulation of microbial growth

The total cell concentrations (epifluorescence microscopy) at the start of the biodegradation were 1.2-2.1 x 10^5 cells/ml in the TRD and 1.0-1.5 x 10^5 cells/ml at the start of the biodegradation experiment (Fig. 4A), and the differences in prokaryote concentrations were therefore insignificant between the SW sources after the 5 days acclimation period at 0-2°C. Peak concentrations levels were determined after 42 days of incubation (5.5-8.1 x 10^5 cells/ml), but the decline in concentrations was low at the end of the experiment, with higher concentrations in the dispersions (1.6-2.6 x 10^5 cells/ml) than in the SW without oil (1.1-1.3 x 10^5 cells/ml).

MPN concentrations of HP increased until day 28 in both dispersions (TRD and SVB), being 60 (TRD) and 100 (SVB) times higher than in SW without oil after day 28 (Fig. 4B). The HP concentrations then decreased by time, and were reduced from day 28 to day 64 by factors of 21 (TRD) and 100 (SVB), close the concentrations in the SWs without oil (Fig. 4B). MPN concentrations of ODP also increased, but peaked later than HP, and with higher concentrations in the SVB than the TRD dispersions (Fig. 4C).

394 The optimal concentrations of HP and ODP appeared later in the current studies than in
395 oil biodegradation at higher SW temperatures. When different oils were biodegraded in SW at
396 13°C, the highest concentrations of total microbes, HP and ODP were observed after 7 to 14
397 days of incubation (Brakstad et al., 2018). Several other studies have also shown that low
398 temperature may reduce bacterial growth, as well as result in extended degradation lag-
399 periods (e.g. Bagi et al., 2014; Brakstad and Bonaunet, 2006; Felip et al., 1996). Studies with
400 psychrotolerant bacteria have shown a tendency of limited substrate uptake at low
401 temperatures (Nedwell and Rutter, 1994), which may have affected bacterial stimulation and
402 be part of the explanation for the extended lag-period experienced in our study. However,
403 microbes in Arctic SW have also been shown to respond faster than microbes in temperate
404 SW to HC pollution at low temperatures (Bagi et al., 2014), as observed in our studies.

405

406 *3.5 Bacterial communities associated with hydrocarbon biotransformation*

407 The bacterial communities in the SVB dispersions at the start of the experiment (day 0
408 samples after the 5-days acclimation period) resembled mainly the community structures in
409 the original SWs after arrival at SINTEF's laboratories, (Fig. 1). As shown in Fig. 5A, the
410 SVB 0-day samples were predominated on family level by *Colwelliaceae* (29.4±8.0%
411 abundance), *Oceanospirillaceae* (25.3±4.2% abundance), *Flavobacteraceae* (10.2±0.8%
412 abundance) and *Rhodobacteraceae* (9.6±2.5% abundance), while *Rhodospirillaceae*
413 (10.0±0.8% abundance) and *Flavobacteraceae* (7.5±0.9% abundance) were the predominant
414 families in the 0-day samples from the TRD dispersions. On genus level, the SVB 0-day
415 samples were predominated by *Colwellia* (*Colwelliaceae*), *Balneatrix* (*Oceanospirillaceae*),
416 *Polaribacter* (*Flavobacteraceae*), *Loktanella* (*Rhodobacteraceae*), *Sulfitobacter*
417 (*Rhodobacteraceae*), and Candidatus *Pelagibacter* (*Alphaproteobacteria*), while the genera
418 *Nitrospina*, Candidatus *Pelagibacter*, and the SAR-92 clade (*Gammaproteobacteria*), were

abundant in the TRD dispersions (Fig. 5B). In the SVB samples, the abundances of *Colwelliaceae*, *Flavobacteraceae* and *Rhodobacteraceae* were maintained during the first 28 days of incubation in oil dispersions and seawater controls, with average (triplicate) abundances of *Colwelliaceae* ranging from 28.4±3.6% to 39.2±2.6 % in the dispersions and 17.7 to 37.1% in the SW controls. The corresponding results for *Flavobacteriaceae* during the same period were 10.2±0.8% to 26.3±2.3 % (oil dispersions) and 2.5 to 45.8 % (SW controls) and for *Rhodobacteracea* 5.0±0.1% to 9.6±2.5 % (oil dispersions) and 2.1 to 8.5 % (SW controls). The most obvious differences in the SVB SW between oil dispersions and controls without oil during the first 21 days of incubation were the relatively abundances of *Oceanospirillaceae*, increasing from 16.7±8.9% after 7 days to 30.3±2.3 % after 21 days in the oil dispersions, while decreasing from 24.6% to 6.7% in the SW controls during the same period (Fig. 5A). The increased abundances of *Oceanospirillaceae* in the oil dispersions corresponded to the period of extensive *n*-alkane depletion in the SVB dispersions (Fig. 3A). Within the *Oceanospirillaceae*, *Balneatrix* was enriched in source seawater, but after 14 days of incubation abundances declined to <10% in the dispersions, while the genus *Oleispira* increased and reached the peak at day 21 (23.9±2%) (Fig. 5B). In SW controls the abundances of *Oleispira* remained <1% during the complete biodegradation period (Fig. 5A). Members of this genus are typical psychrophilic and primarily aliphatic HC-degrading bacterium with optimal growth at 2-4°C (Yakimov et al., 2003) and have been associated with oil alkane biodegradation in cold marine environments (Coulon et al., 2007; Golyshin et al., 2010). After 42 and 64 days of incubation, a shift in the bacterial communities was observed in the SVB oil dispersions, with low abundances of *Oceanospirillaceae* (1.6±0.6% abundances), while the abundances of *Rhodobacteraceae* increased (22.6±12.1% abundances; Fig. 5A). The high abundances of *Flavobacteriaceae* were maintained in the SVB dispersants (Fig. 5A), also during this last period of the biodegradation (43.8±15.5% abundance). The genera

Sulfitobacter and *Polaribacter* were associated with the high abundances of *Rhodobacteraceae* and *Flavobacteriaceae*, respectively (Fig. 5B). Both these genera are associated with oil biodegradation in seawater or oil-contaminated marine environments (Brakstad and Lødeng, 2005; Brakstad et al., 2008; Deppe et al., 2005; Dubinsky et al., 2013; Guibert et al., 2012). Members of *Polaribacter* have been commonly detected in Arctic marine environments like seawater and ice (Brakstad et al., 2008; Gerdes et al., 2005; McFarlin et al., 2017), and members of the genus have also been shown to harbour *alk*B genes involved in alkane degradation (Guibert et al., 2016). After 28 days of incubation, the *Piscirickettsiaceae* genus *Cycloclasticus* started to proliferate in SVB dispersions, reaching its maximum abundance at day 42 (17.8±1.5%). This increase in abundance coincided with depletion of naphthalenes/2- to 6-ring PAHs and VOC (Fig. 3B and 3C). *Cycloclasticus* is considered to be cosmopolitan (Teramoto et al., 2010) and is detected repeatedly in biodegradation studies where it is associated with mineralization of aromatic compounds, both BTEX and PAH (Brakstad et al., 2015b; Dubinsky et al., 2013; Geiselbrecht et al., 1998; Hazen et al., 2010; Kleindienst et al., 2015a; Redmond and Valentine, 2012).

While *Oceanospirillacea* and *Colwelliaceae* represented more than 50 % of the sequences in the SVB dispersions at the start of the experiment (day 0), the abundances of these families in the 0-day TRD dispersions were < 5% (3.3±0.8 % *Oceanospirillaceae* and 1.0±0.1% *Colwelliaceae*). However, the abundances of these families in TRD oil dispersions were increased considerably after 7 days of incubation (26.7%±3.1% of *Oceanospirillaceae* and 12.5±4.6% *Colwelliacea*). *Oceanospirillaceae* then declined, while *Colwelliacea* continued to increase in abundance up to 40.4±6.5% after 28 days (Fig. 5A). However, both families were also abundant in the SW controls (7.7-9.3% abundance of *Oceanospirillaceae* and 24.3-26.1% abundance of *Colwelliaceae* between 7 and 28 days). The *Colwelliaceae* family were mainly represented by the genus *Colwellia*, which showed a high response in

both, oil incubations and control samples. *Colwellia*, nevertheless, decreased in abundance after 42 days in oil incubations (<10%), but remained with high abundance in control sample (21.7%) (day 64). This experiment was started in April, and *Colwellia* often proliferates in cold local seawater during winter-spring season, while abundances may be lower in during summer-autumn in temperate SW (Oberbeckmann et al., 2016). *Colwellia* is associated with oil mineralization in cold seawater, from polar regions and the deep sea (Bagi et al., 2014; Bælum et al., 2012; Redmond and Valentine, 2012). However, the substantial increases in *Colwellia* abundances in both TRD dispersions and SW blanks may indicate that the prominent members of the *Colwellia* were psychrophilic bacteria that were triggered and bloomed as a result of low temperature rather than by HC source. The increased levels of *Oceanospirillaceae* in the TRD oil dispersions were associated with several genera, with *Oleispira* as the most prominent (increasing from 1.7±0.6% abundance after 7 days, to 7.7±2.4 % after 28 days), while the abundances of this genus remained low in TRD SW controls (0.9-2.5%), as shown in Fig. 5B. However, this potentially psychrophilic genus showed lower abundances in the TRD than the SVB dispersions, which may explain slow *n*-alkane degradation in TRD dispersions (Fig. 3A). *Flavobacteriaceae* showed a moderate response as well in TRD dispersions, with similar abundances to source seawater throughout the experiment (about 9%), while SW controls revealed higher abundances (days 28 and 64). *Rhodospirillaceae* increased in abundance towards the end of experiment in the TRD dispersions, similar to SVB dispersions, with the highest abundances recorded for days 42 and 64 (27.3%±4.6% and 24.8%±1.1%, respectively). No increase in abundance in SW control samples was detected. Unlike in SVB dispersions, the *Rhodobacteraceae* family in TRD dispersions was comprised of many genera exhibiting less than 3% in sequence abundance. Finally, similar to SVB dispersions, the *Piscirickettsiaceae* genus *Cycloclasticus* proliferated starting at day 28 in the TRD dispersions, but reached maximum abundance at day 42 and 64

494 with 22.9%±17.3% and 28.4%±15.8%, respectively. The comparable abundances of
495 *Cycloclasticus* in both SVB and TRD dispersions were related to the similar
496 biotransformation rates of lag-periods and degradation rates of naphthalenes/PAH and VOC
497 in these dispersions (Table 1; Fig. 3B and C). Increase in abundance of *Rhodobacteraceae*
498 and *Cycloclasticus* coincided with depletion pattern of PAHs (Fig. 3B).

499 PCoA plot of the microbial community structure showed that replicate samples generally
500 clustered together (Fig. 6). Typically, the successions moved in one direction in the
501 dispersions from both SWs, from incubation day 0 to day 64. The communities in SVB and
502 TRD dispersions clustered apart, confirming distinct community compositions. The
503 community changes from day 28 to day 42 and 64 were apparent in both dispersions
504 compared to initial incubations. Community shifts in SW control samples were observed as
505 well. This may be influenced by the static experimental conditions, or by temperature stress,
506 since both SWs were incubated outside their natural ambient temperature.

507

508 ## 4. Conclusions

509 The results from these studies showed that Arctic microbial communities have the
510 capacity to biotransform alkanes and aromatic hydrocarbons in oil that has been efficiently
511 dispersed to small droplets. The microbial communities from an Arctic SVB SW degraded *n*-
512 alkanes in dispersed oil in a naphthenic crude oil faster than communities from a temperate
513 TRD SW at a low temperature (0-2°C), while aromatic hydrocarbons were biotransformed
514 similarly by the communities from the two SW sources. The faster *n*-alkane degradation in the
515 SVB SW was primarily associated with higher initial abundances of the typical psychrophilic
516 alkane-degrading *Oceanospirillaceae* genus *Oleispira* in the SVB than the TRD dispersions.
517 The faster *n*-alkane degradation in the SVB than TRD SW at the low temperature may
518 therefore have been affected by the indigenous microbial communities in different SW

519 sources. Larger *n*-alkanes ($\geq nC_{29}$) were only slowly depleted in both dispersions, when
520 compared to previous studies at 5°C SW temperatures (Brakstad et al., 2015a).
521 Biotransformation of aromatic HCs and VOCs were comparable between the two SW sources,
522 and related to high abundances of the *Piscirickettsiaceae* genus *Cycloclasticus* emerging late
523 in the biodegradation period. The biotransformation rates of the aromatic HCs and VOC at the
524 low SW temperature were to some extent comparable to results from a higher temperature
525 (5°C) (Brakstad et al., 2015a), after extended lag-periods.

526 The results from this study in cold SW demonstrate that chemically dispersed oil may be
527 biodegraded in Arctic SW at very low temperatures. Prolonged lag-periods of saturate and
528 aromatic oil compounds 0-2°C incubations compared to studies at higher temperatures
529 (Brakstad et al., 2015a; Wang et al., 2016), may have been affected by lower bacterial growth
530 rates and slow substrate uptake, but also by physical oil properties like viscosity and wax
531 precipitation at the low SW temperatures used in these studies. These data will have
532 implications for the predictions of the fate, as well as the environmental risk, related to oil
533 spill in Arctic and other cold SW environments after treatment with chemical dispersants.

534

535

536 **Acknowledgements**

537 This study was supported by the Norwegian Research Council Petromaks2 program
538 (contract #228271-E30) and the oil companies Statoil Petroleum AS, ExxonMobil
539 Exploration and Production Norway AS, AkerBP ASA, TOTAL E&P Norge ASA, and
540 ConocoPhillips Skandinavia AS. We will thank Inger Steinsvik, Marianne Unaas Rønsberg
541 Inger K. Almås, Marianne Aas, Lisbet Støen, Marianne A. Molid and Bror Johansen for
542 chemical analyses and technical assistance.

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Tables and Figures

Table 1. Non-linear regression analyses (first-order rates with lag-periods) of n-alkanes, naphthalenes/2- to 3-ring PAH (Naph/PAH) and VOC in dispersions with SVB and TRD SW. Calculations were based on ratios of concentrations at each sampling (C) and the concentrations at the start of the experiment (C_0). The n-alkanes and PAH are normalized against Hopane. The results show lag-periods, biotransformation rate coefficients (k1), half-lives determined from rate coefficients (ln2/k1) and goodness of fit (R^2).

Compound group	SW source	Lag-periods (days)	(k1 ± SD)	Half-lives (days)	R^2
n-alkanes	SVB	6.9	0.1008 ± 0.0106	6.9	0.9507
	TRD	25.6	0.0667 ± 0.0066	10.4	0.9876
Naph/PAH	SVB	25.1	0.0774 ± 0.0086	9.0	0.9830
	TRD	27.0	0.0883 ± 0.0157	7.8	0.9709
VOC	SVB	20.0	0.0679 ± 0.0121	10.2	0.9099
	TRD	20.0	0.0499 ± 0.0112	13.9	0.8641

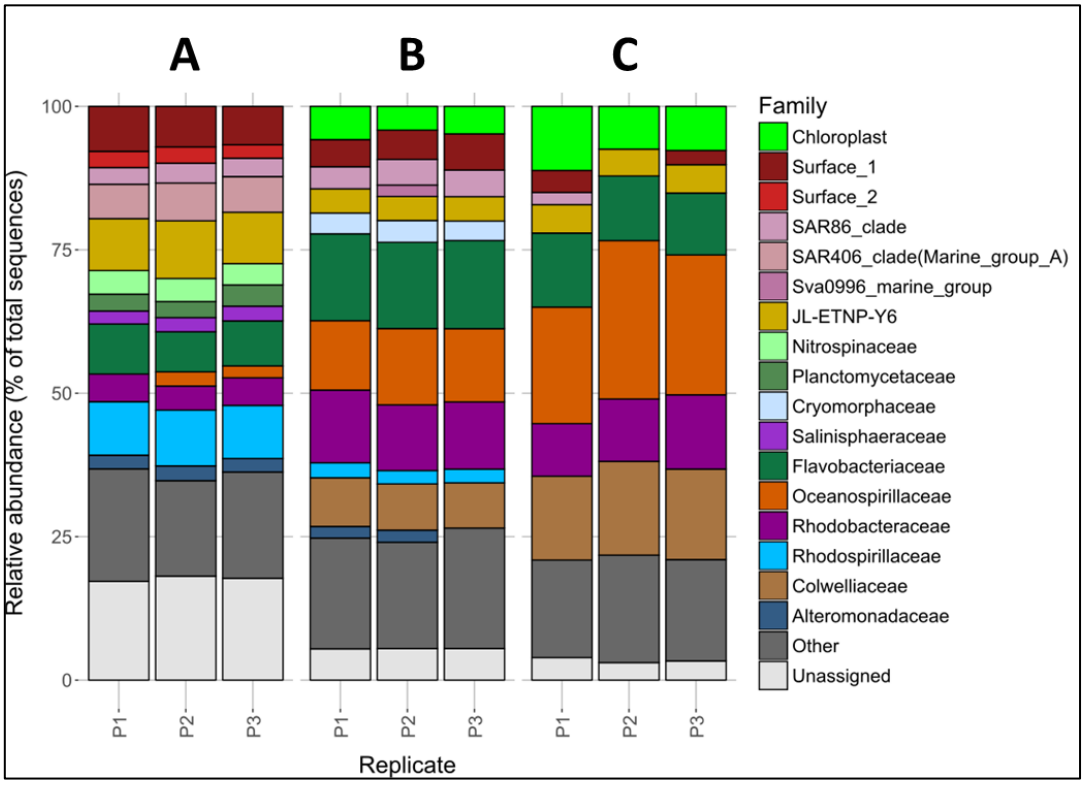
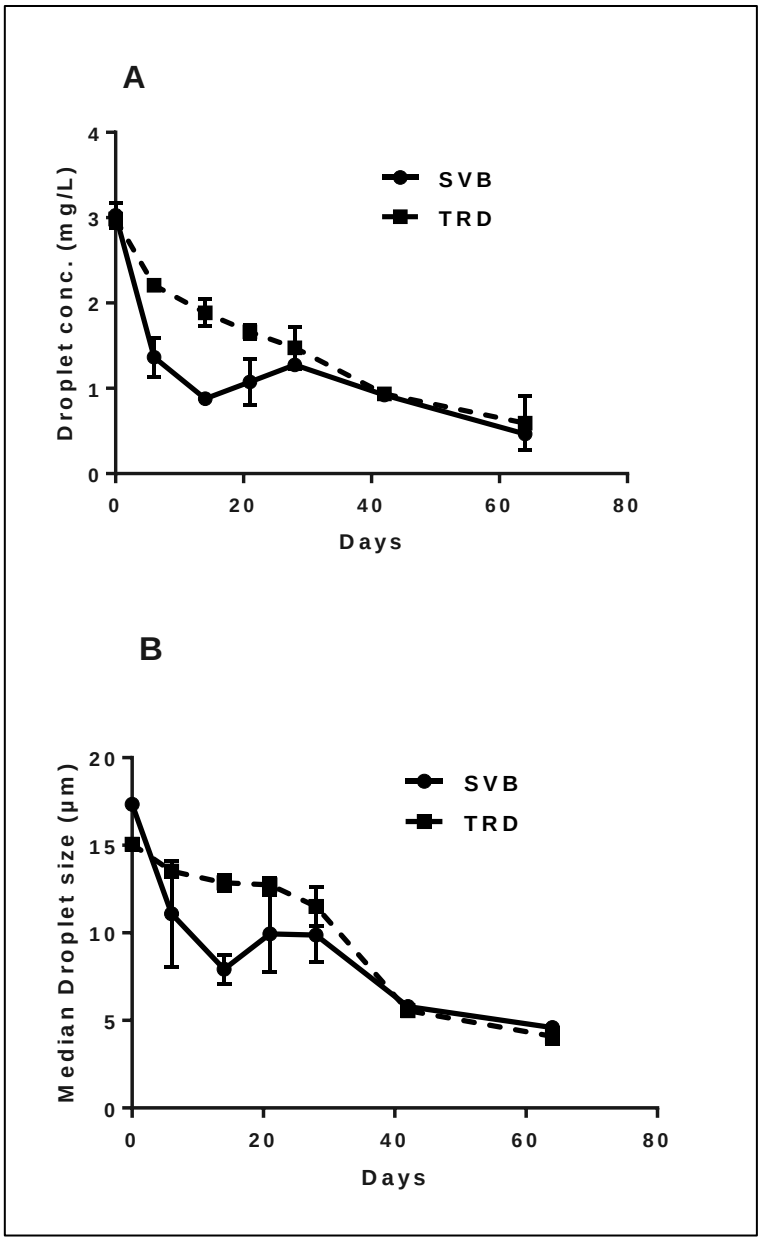


Fig. 1. Relative abundances of microbial families in three replicate samples of SW from TRD filtered immediately after sampling (A), from SVB filtered on site (B), and from SVB after transport to SINTEF's laboratories (C).



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803 Fig. 2. Oil droplet concentrations and median droplet size in dispersions. The error bars
804 represent SD of three replicates.
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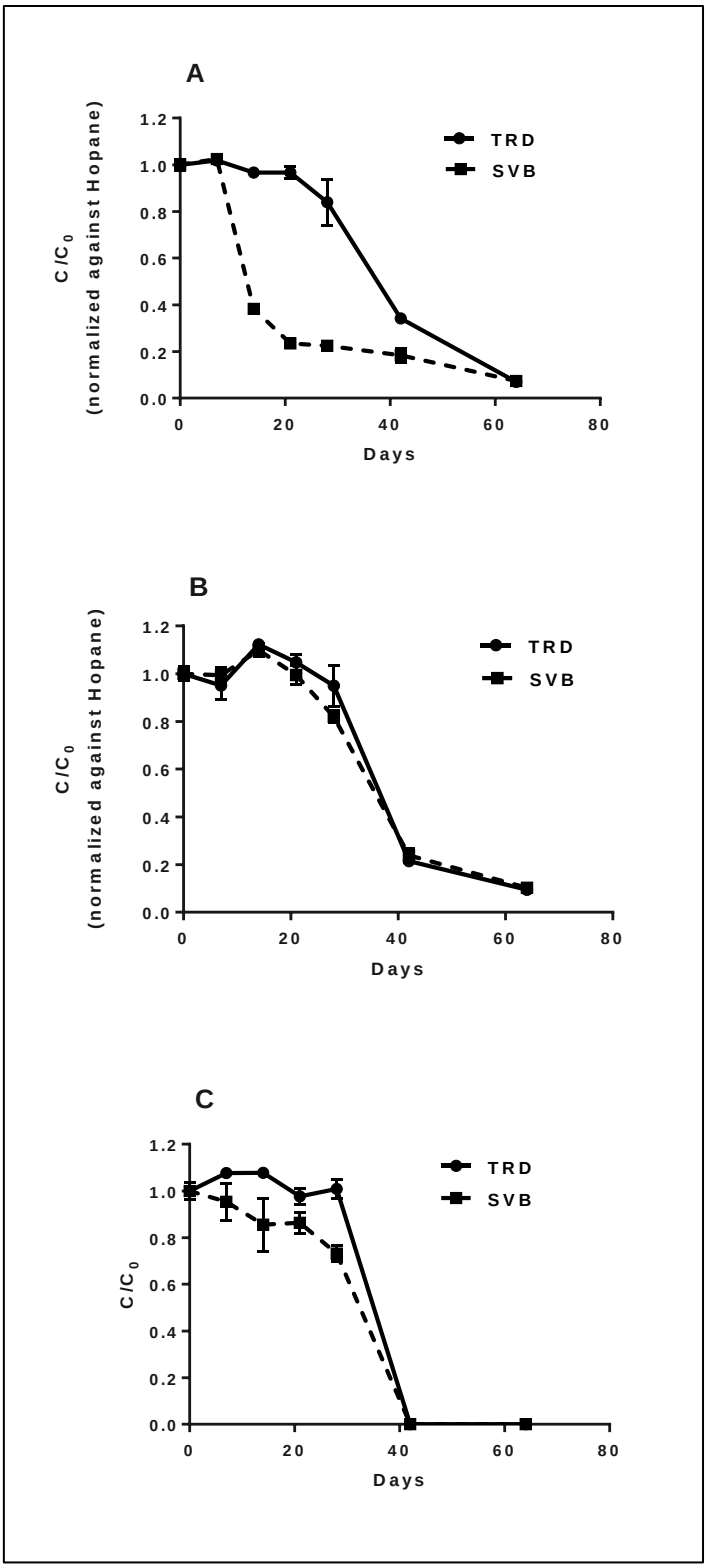
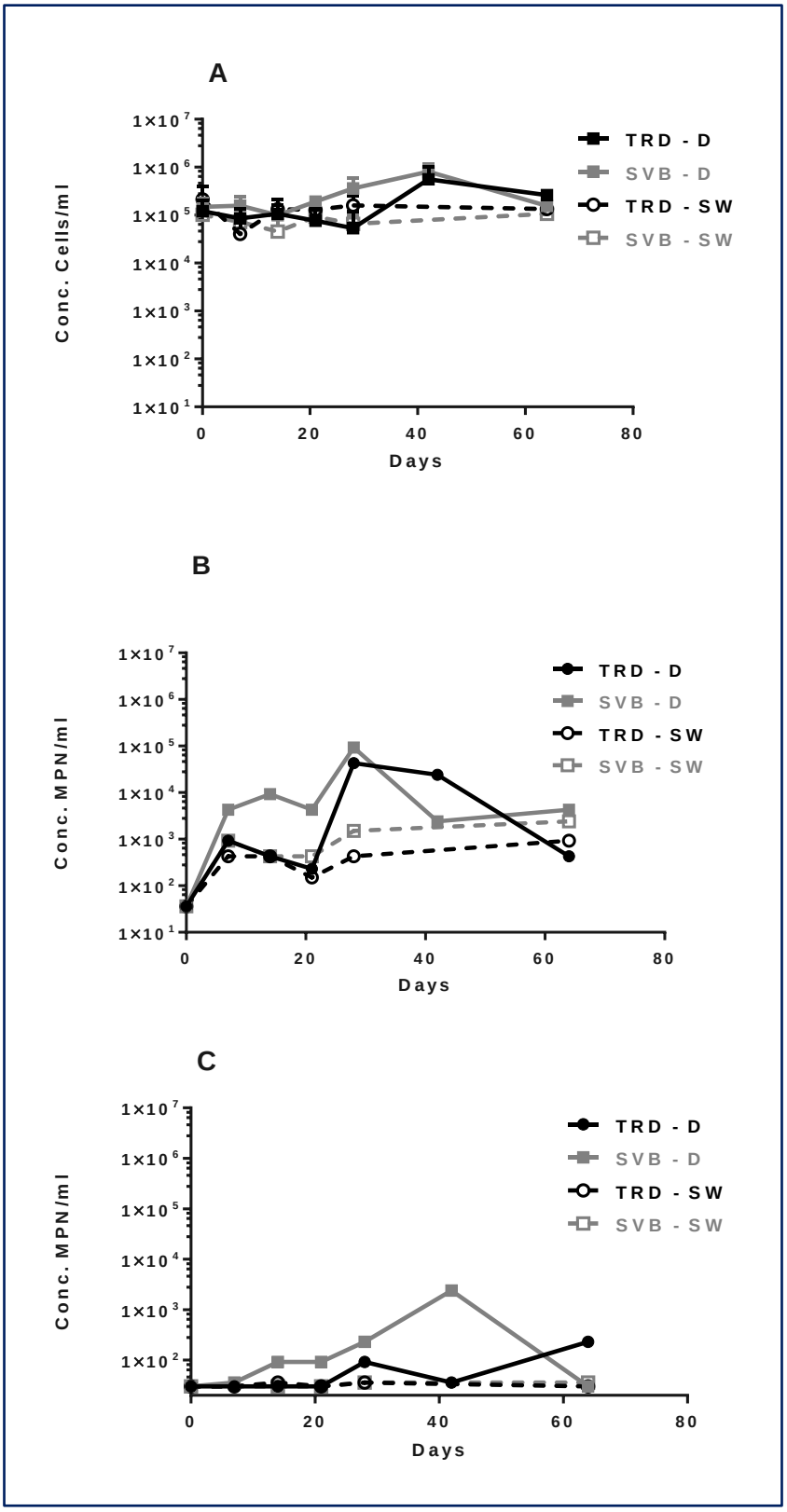


Fig. 3. Biotransformation of *n*-alkanes (A), naphthalenes/2- to 6-ring PAH (B), and VOC (C). The results are shown as the ratios of concentrations at each sampling (C) and the concentrations at the start of the experiment (C_0). The n-alkanes and PAH are normalized against Hopane. Error bars describe SD of three replicates.



814

815 Fig. 4. Total concentrations of prokaryotic cells determined by epifluorescence microscopy
816 (A) and most probable number (MPN) concentrations of heterotrophic prokaryotes (B) and
817 oil-degrading prokaryotes (C). The results are shown for dispersions in SW from TRD (TRD-
818 D) and SVB (SVB-D) and in SW blanks from the two sources (TRD-SW and SVB-SW).

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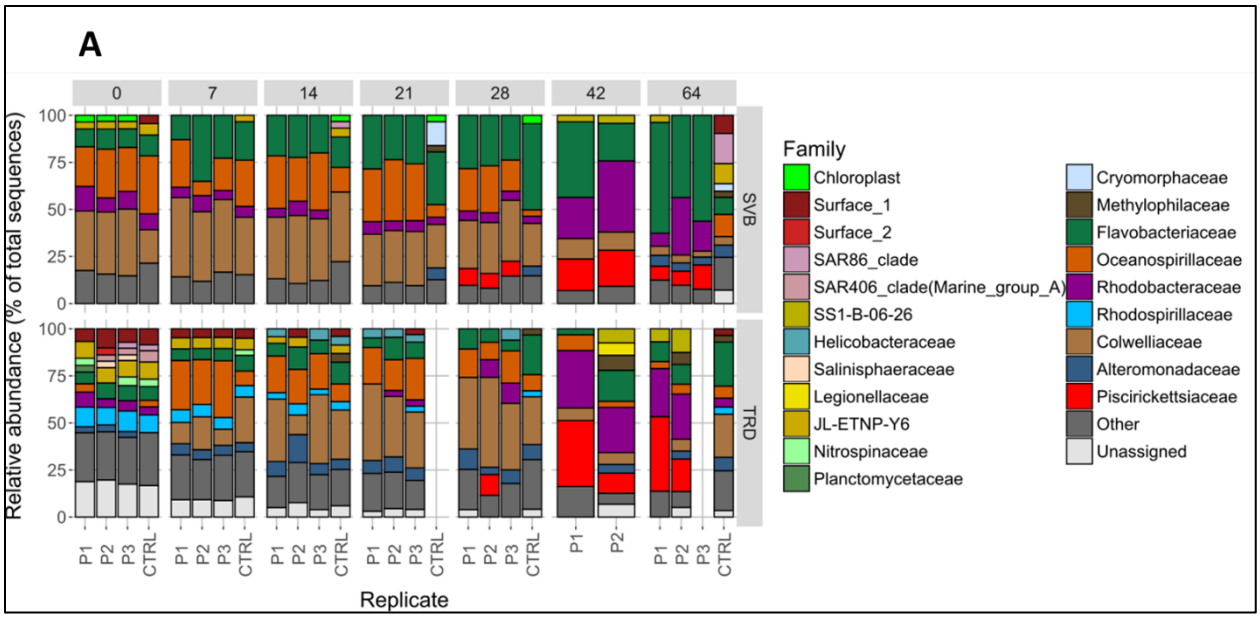


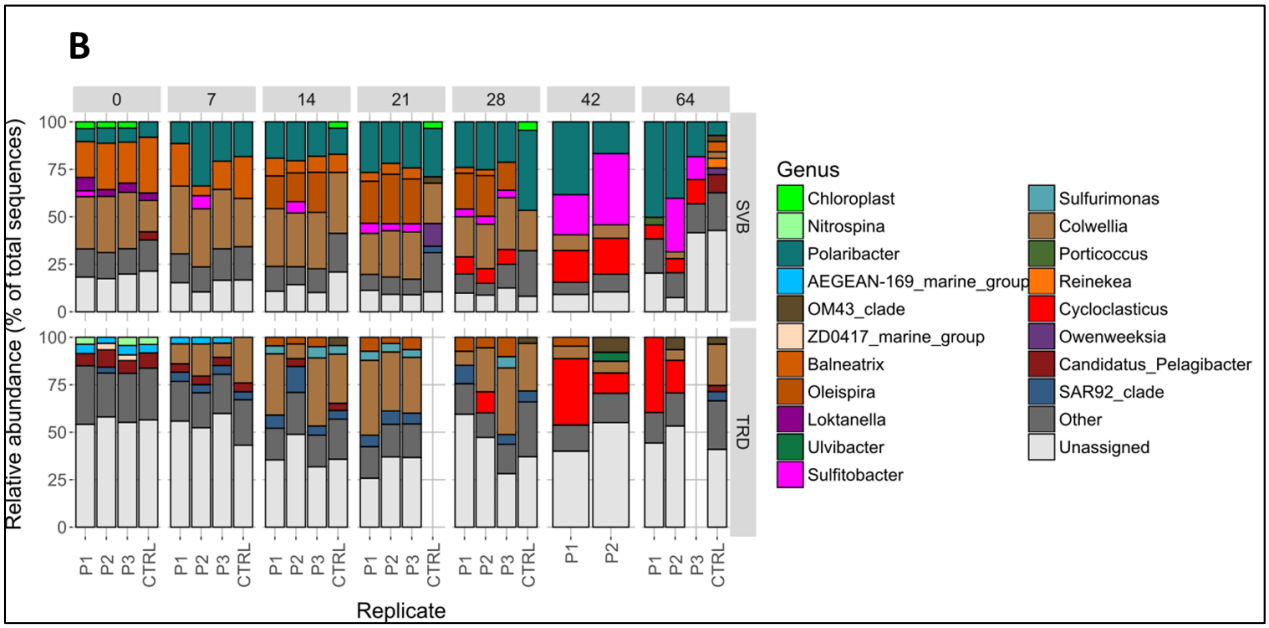
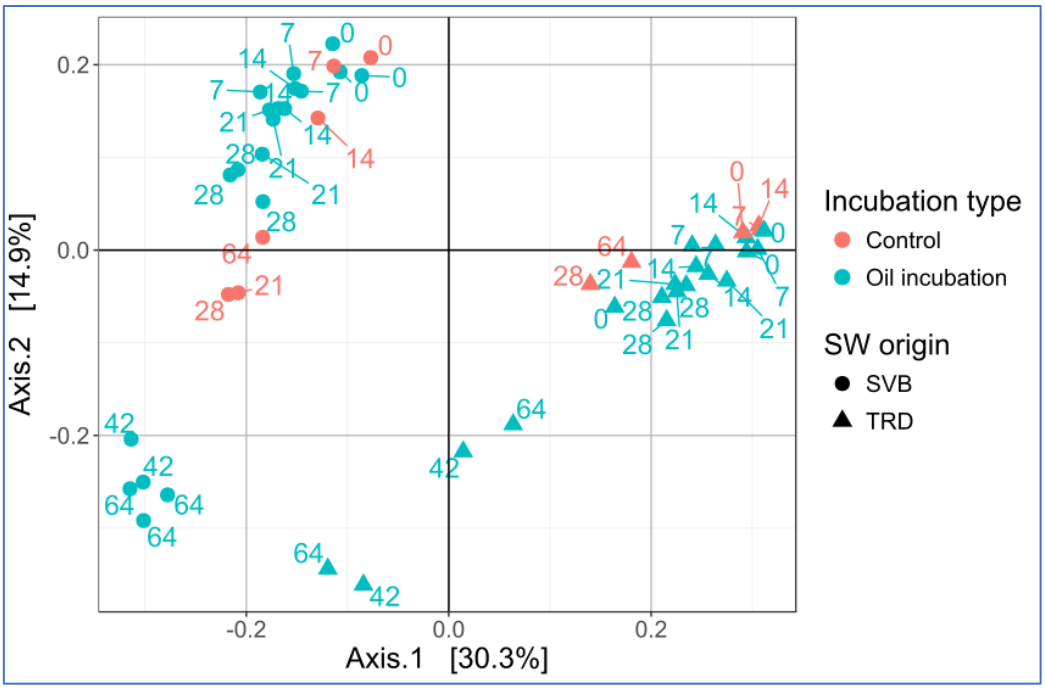


Fig. 5. Microbial communities on family (A) and genus (B) levels in replicate samples (P1-P3) of dispersions with SVB and TRD SW during the 64-days biodegradation period. SW controls without oil (CTRL) were also included for comparison. For closer examination of the "Unassigned" group on genus level, see Fig. S1 (Supplementary Information).



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828 Fig. 6. PCoA plot, based on un-weighted UniFrac distance metrics, of the microbial
829 community successions during biodegradation of dispersions with TRD and SVB SW. The
830 numbers describe the days if sampling. The arrows describe the movements of the microbial
831 successions during biodegradation.

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Figure 1

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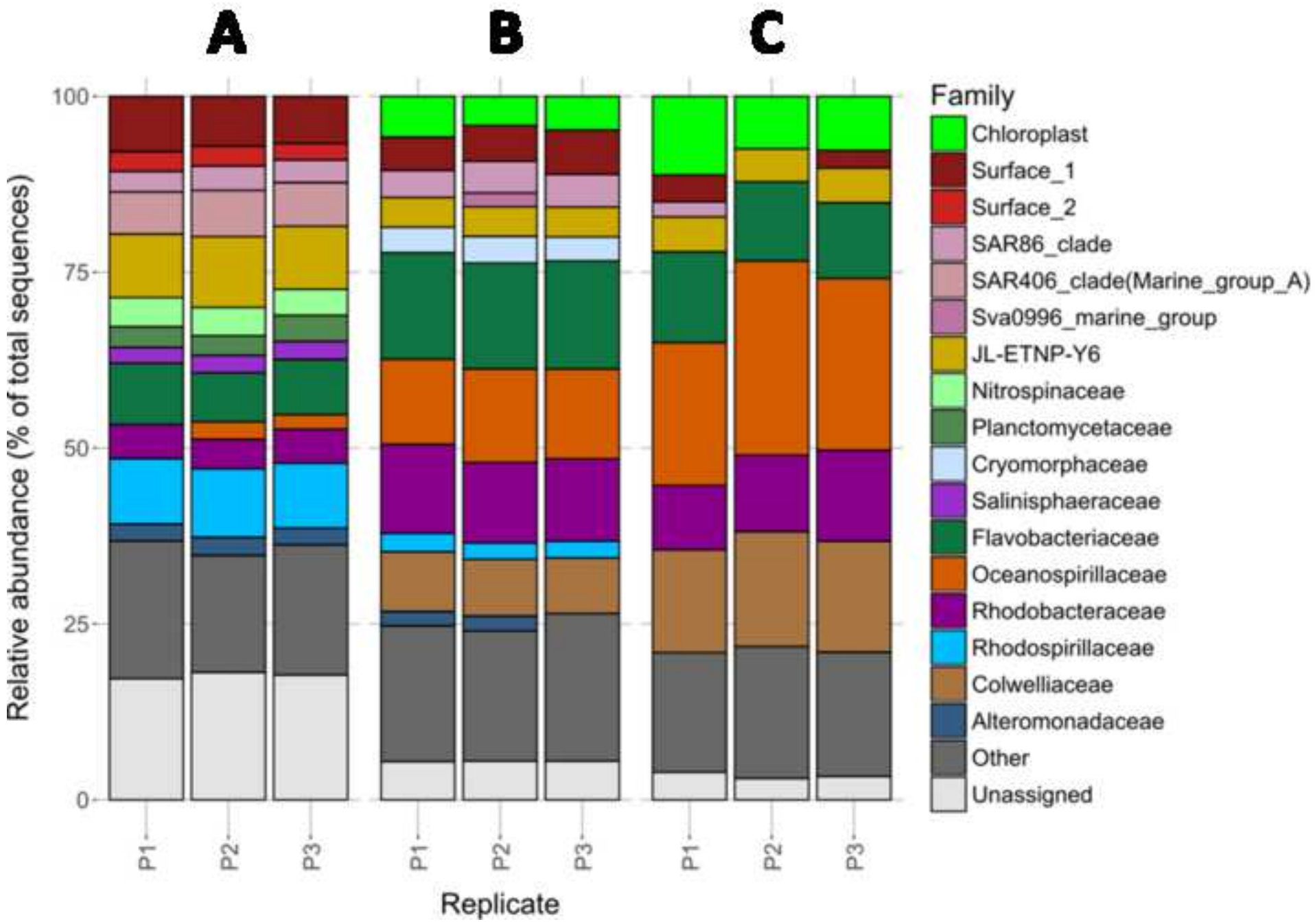

Figure 2A

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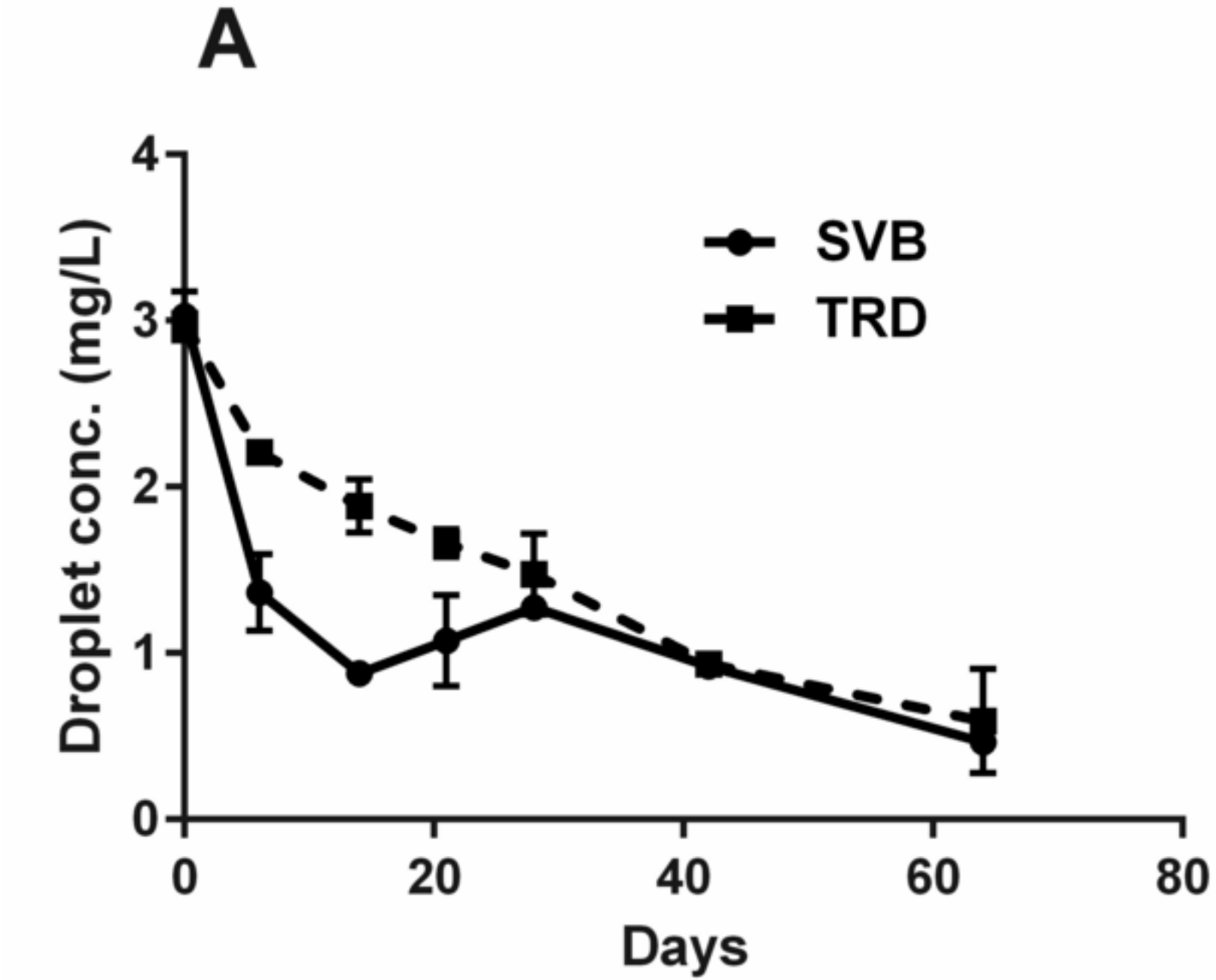

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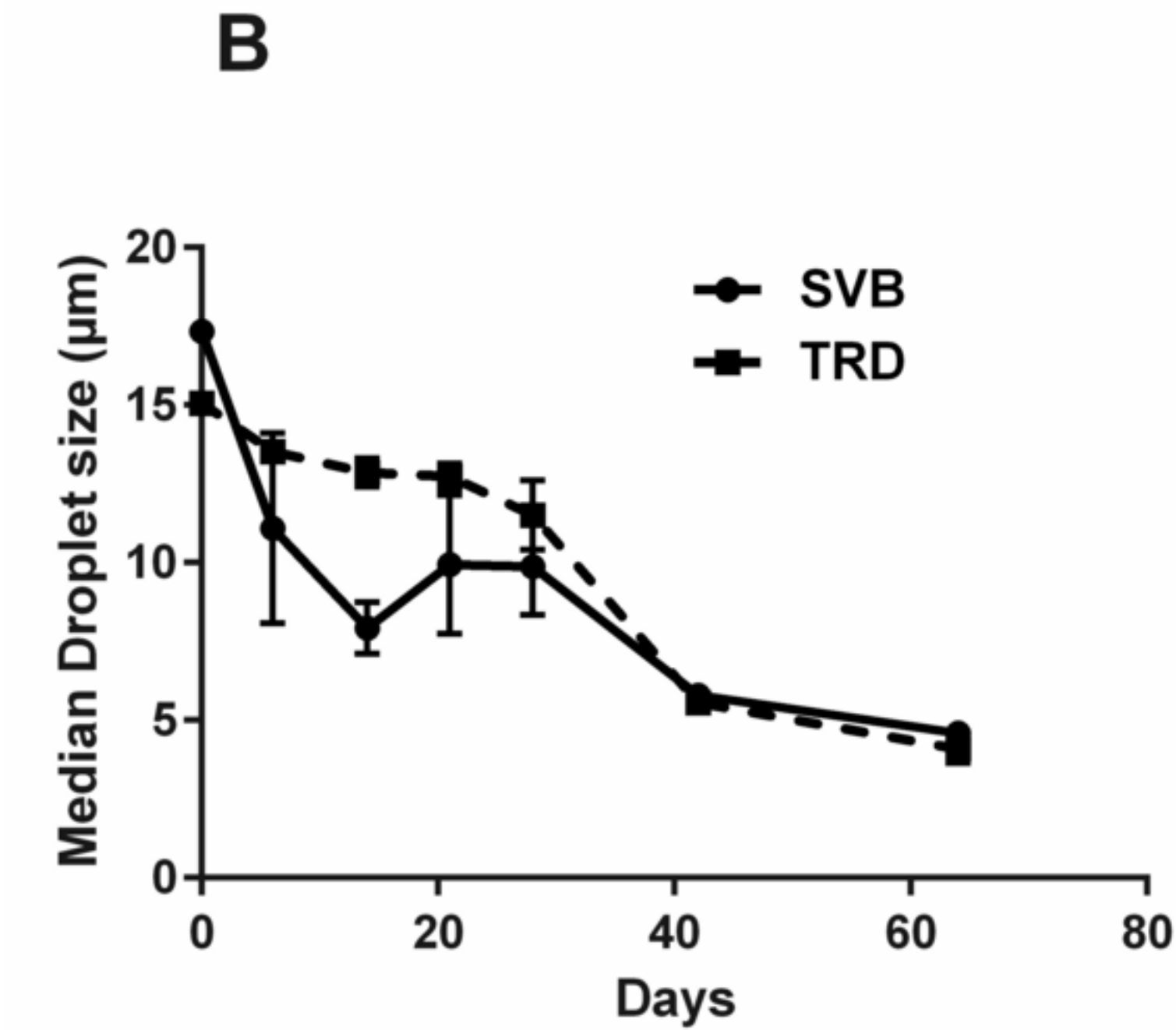

Figure 3A

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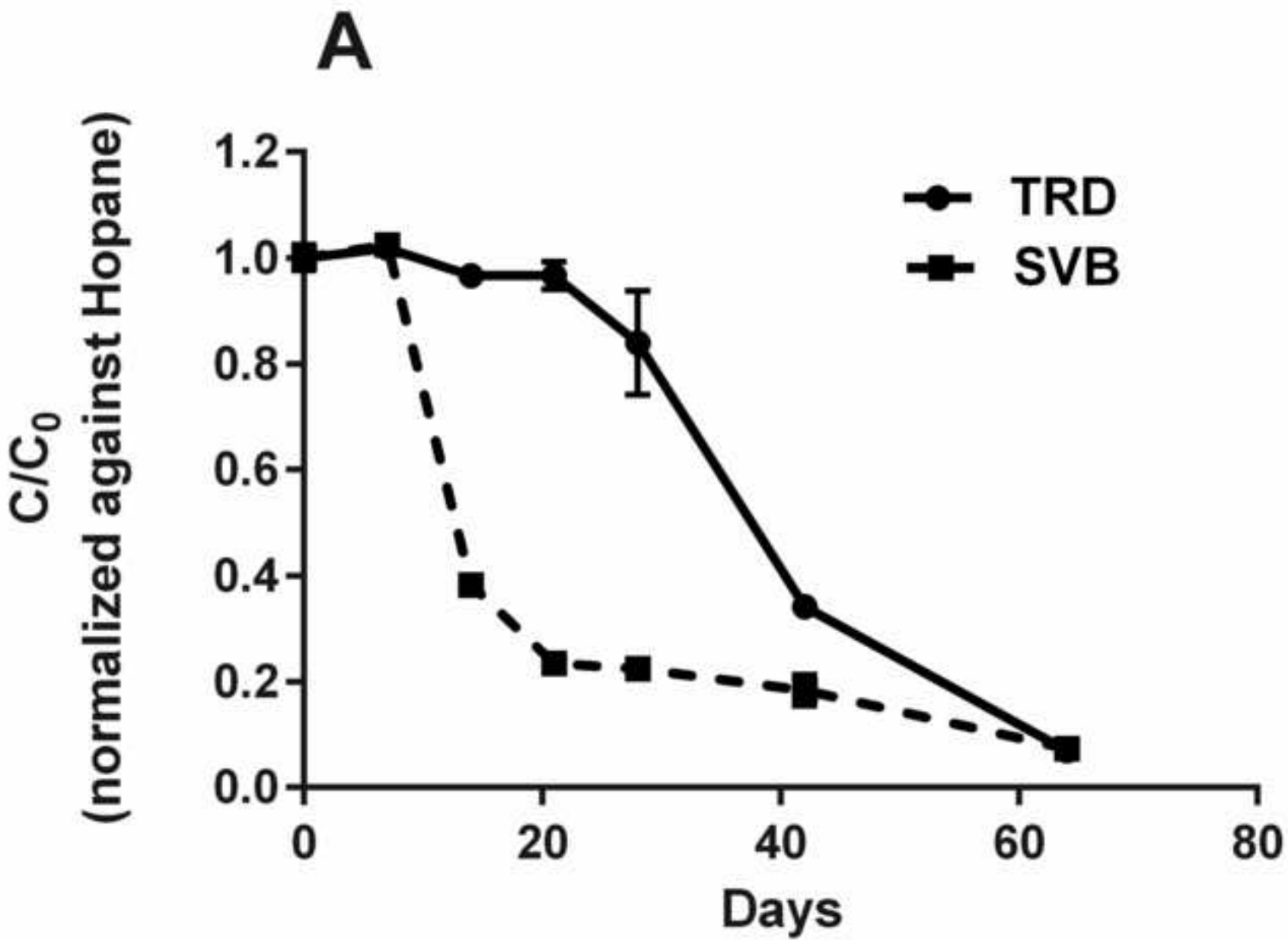

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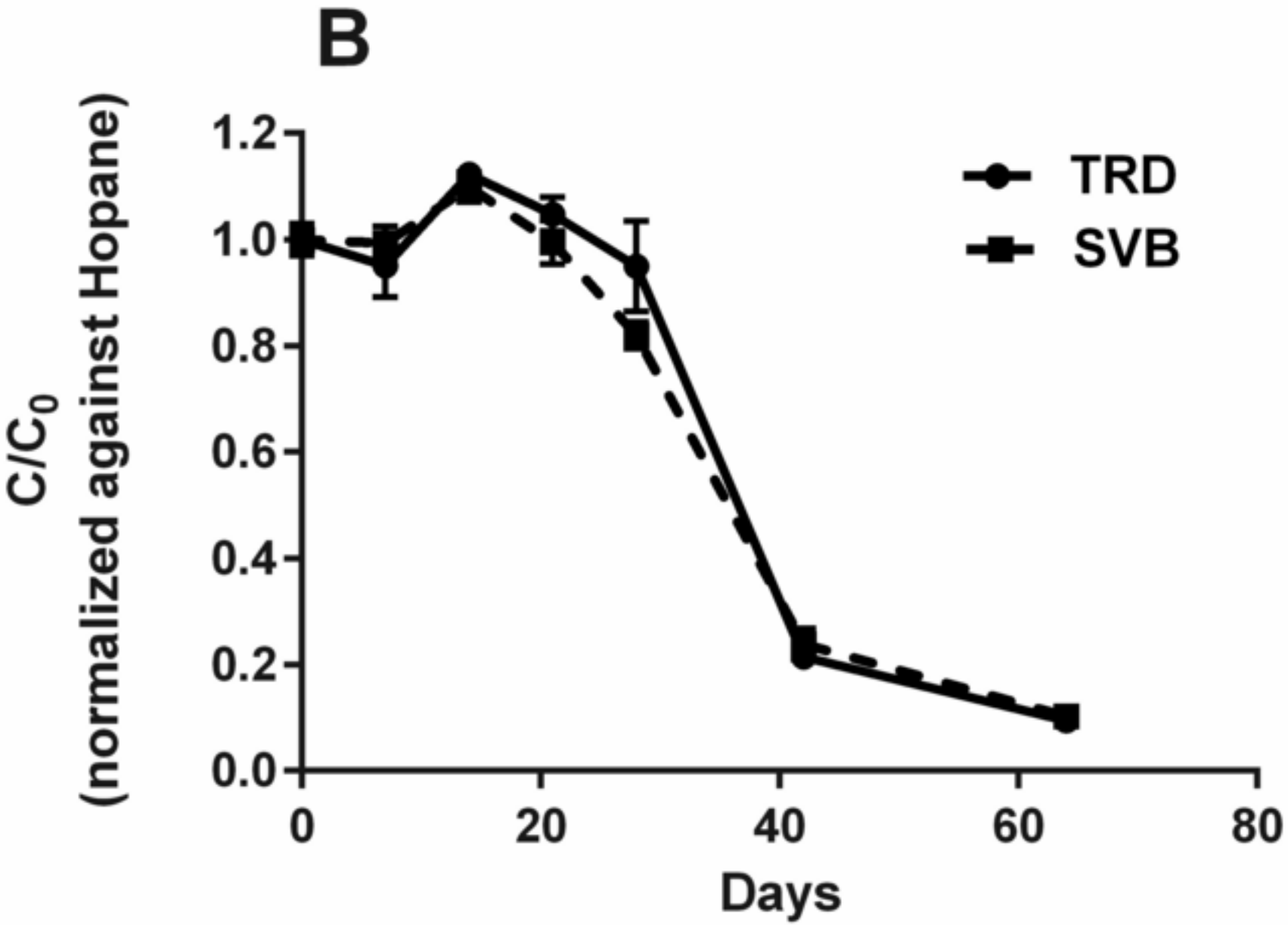

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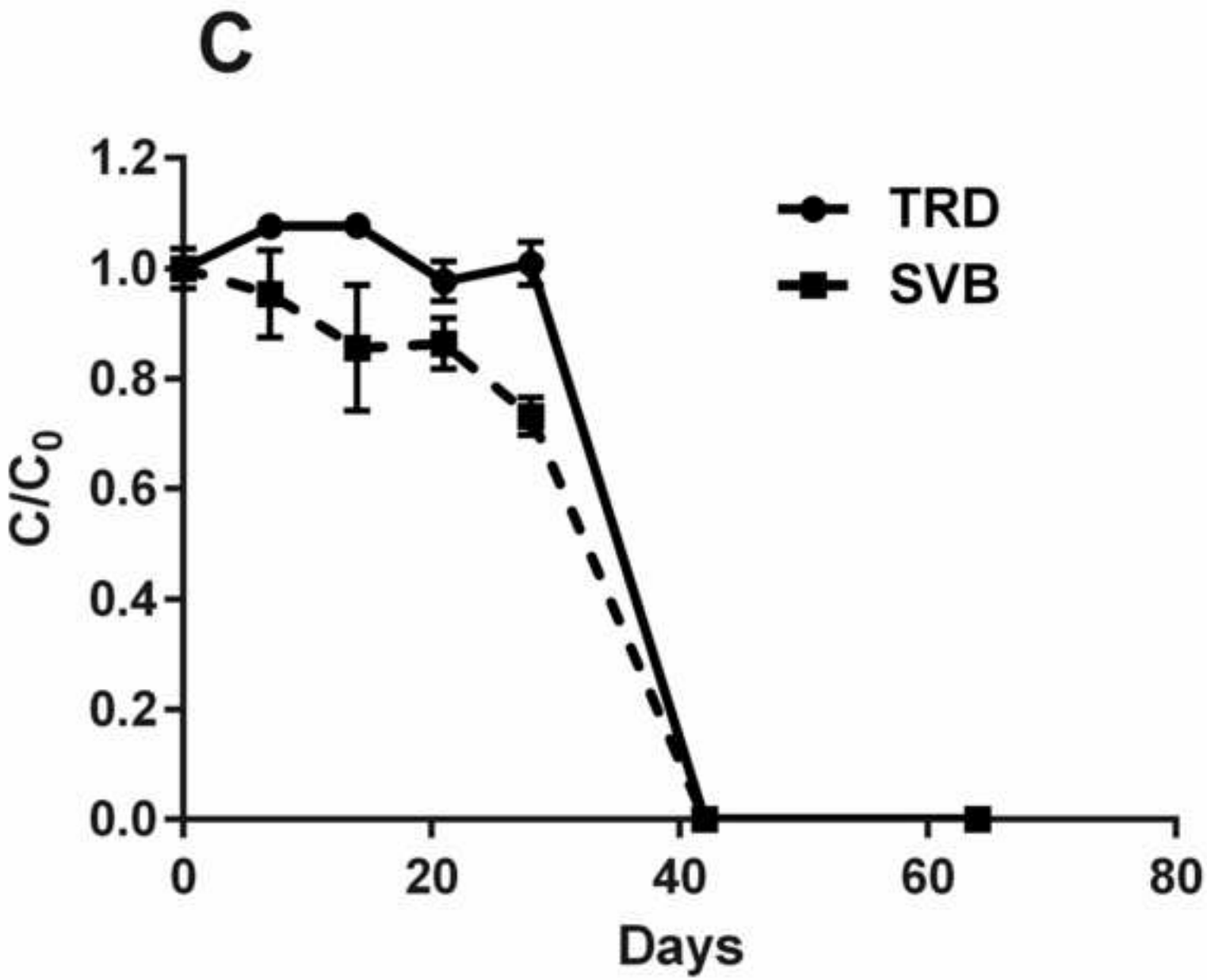

Figure 4A

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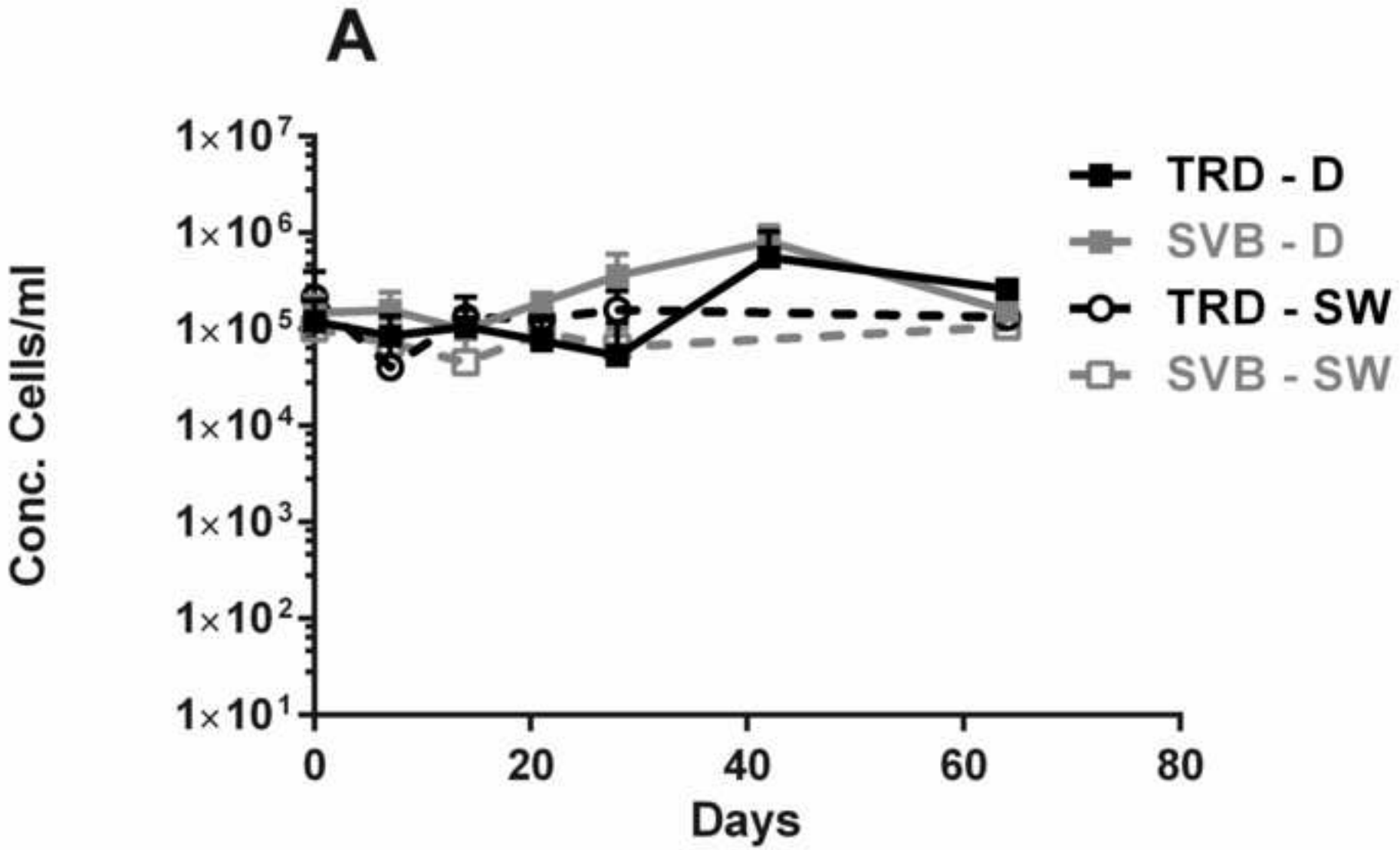

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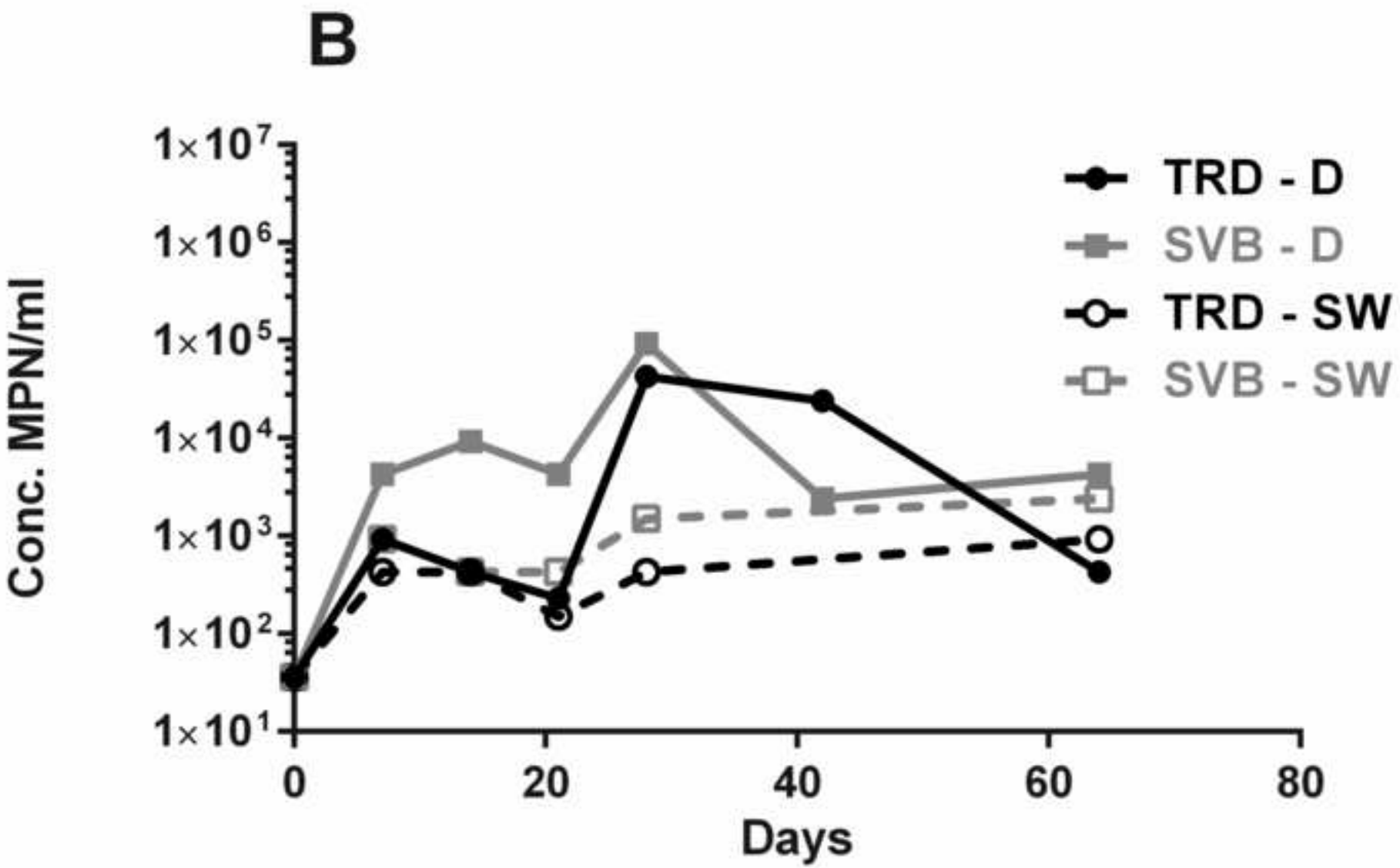

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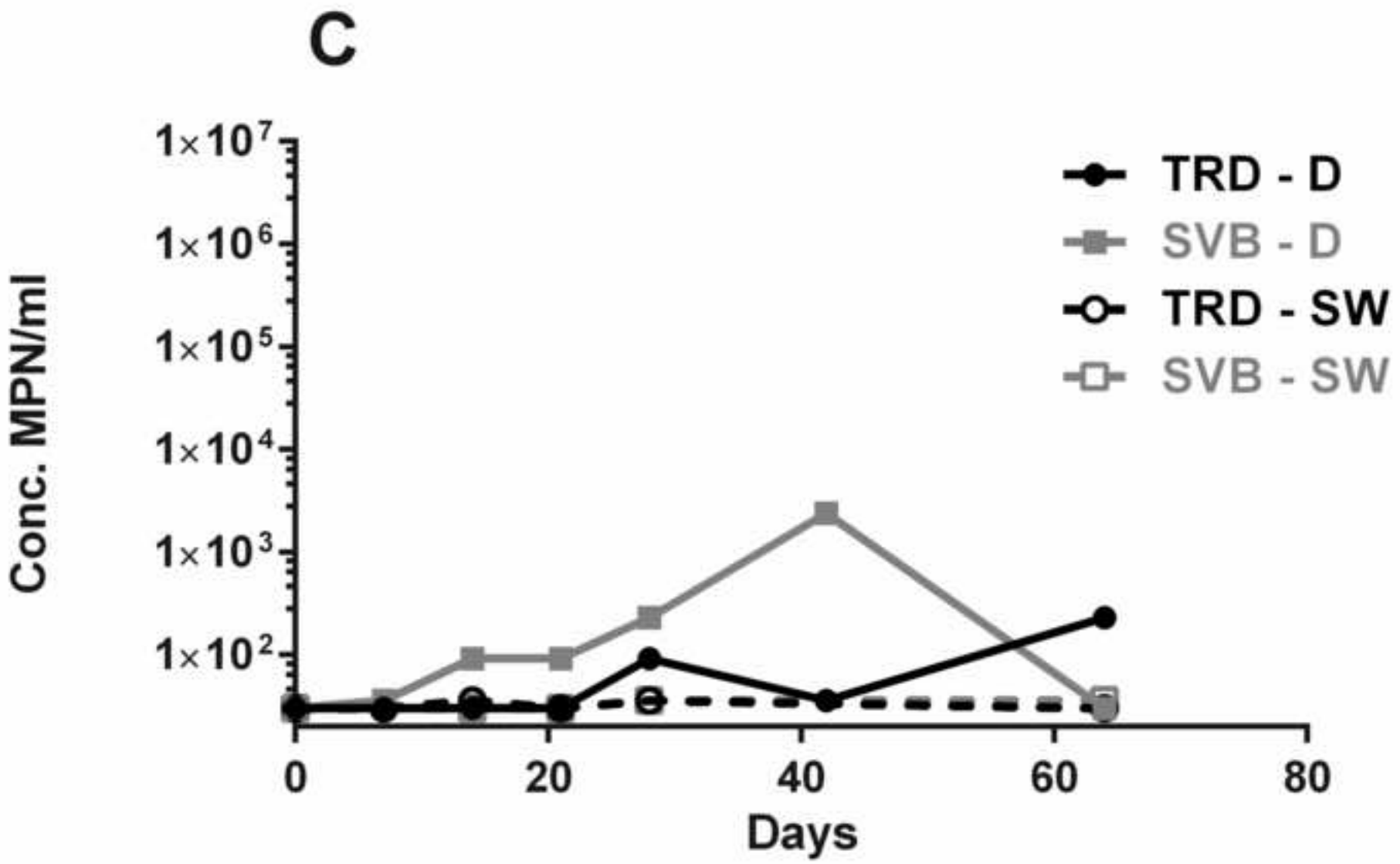

Figure 5A

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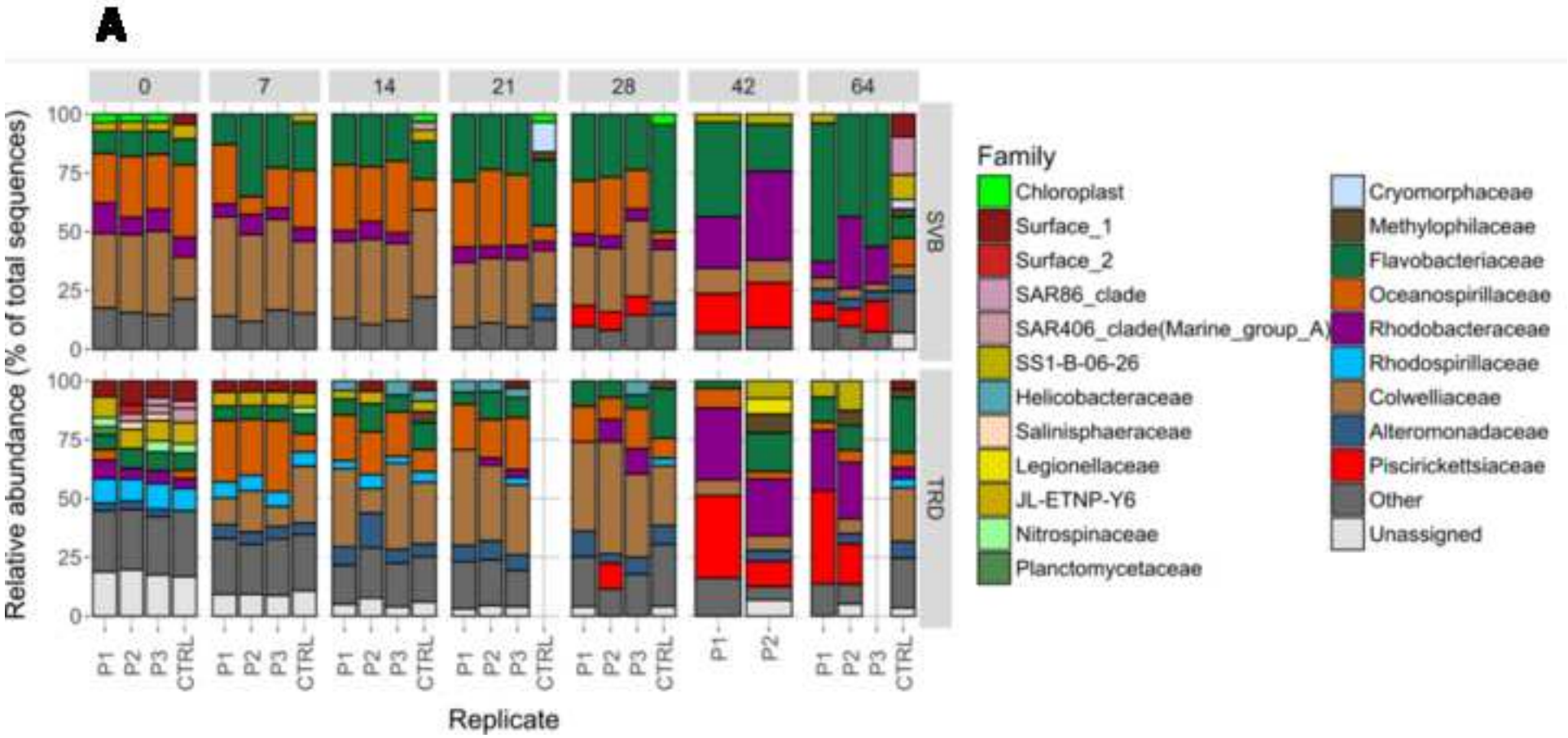

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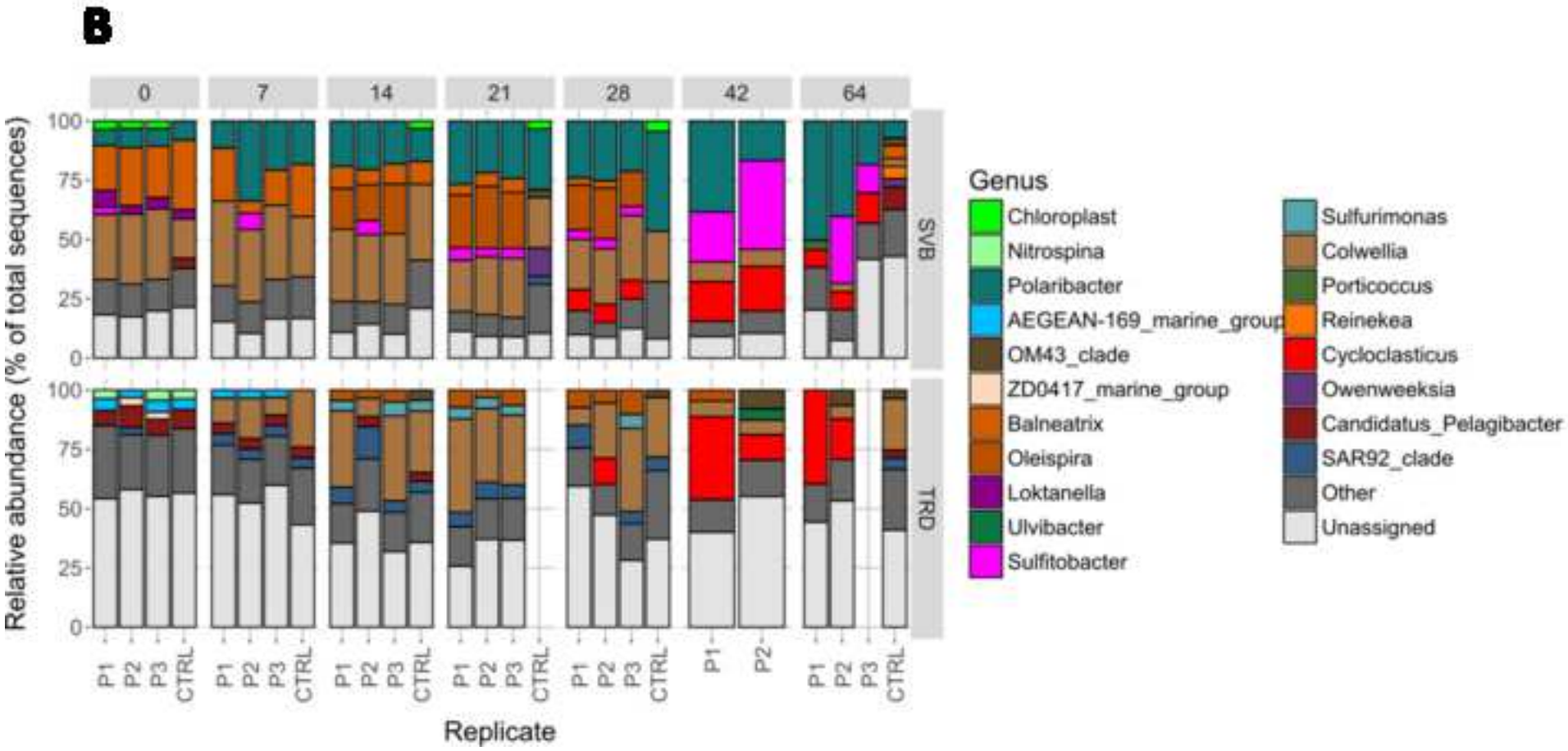

Figure 6

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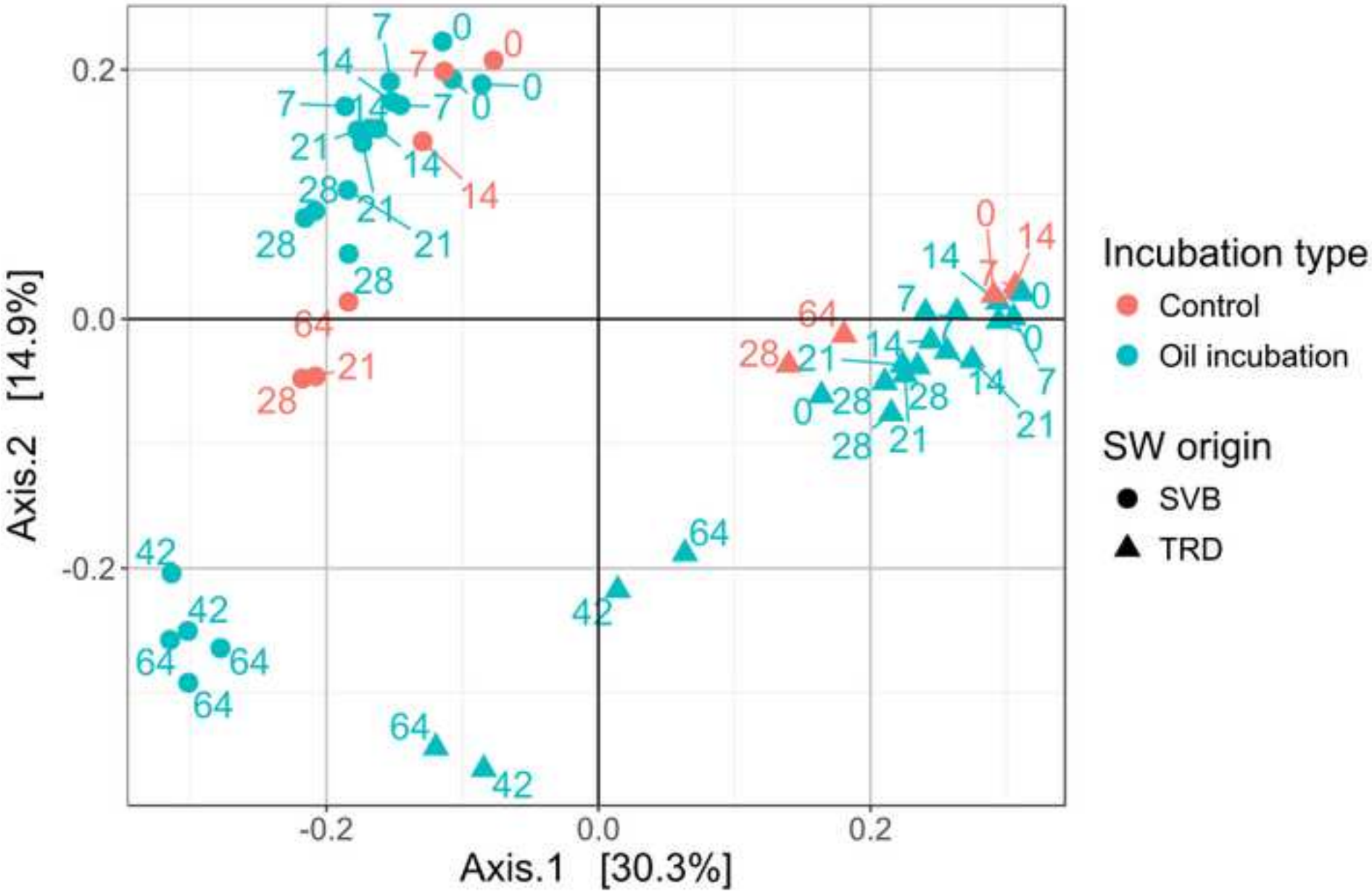

Supplementary Data

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