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„Respiration, production, and growth efficiency of marine pelagic fungi“

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Marilena Heitger, BSc

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Abstract

Despite their important role as degraders of organic matter in terrestrial ecosystems, fungi have been understudied in the open ocean. Recent studies, however, suggest that marine fungi are ubiquitous in oceanic systems and are actively involved in the degradation of numerous compounds. Moreover, their role as parasites on diatoms suggest an important link in the food web. Nevertheless, the role of fungi in the carbon cycle of the oceans is still not characterized, and fungal respiration and production have been hardly studied.

This study focused on determining fungal growth efficiencies and its susceptibility to temperature differences and nutrient concentration. Hence, respiration, and biomass production of three fungal isolates (*Rhodotorula mucilaginosa*, *Rhodotorula sphaerocarpa*, *Sakaguchia dacryoidea*) were measured in laboratory experiments at two temperatures and nutrient concentrations. We found that respiration and production rates differed among species, temperature, and nutrient concentration. These parameters were higher at higher temperatures, but the growth efficiency showed the opposite trend. Nutrient concentration affected the parameters, too, even though the influence differed among species.

Even though in experiments with pure cultures, the conditions can never mimic the ones in the ocean, it gives first insight into the dynamics of marine fungal growth efficiency. Our results provide relevant information about the role of fungi as source or sink of carbon during organic matter remineralization. More research in this field is necessary to understand the role of fungi in the marine carbon cycle, a topic that gains even more importance in times of increasing CO_2 concentrations and global warming.

Introduction

The global carbon cycle is driven by numerous biological processes on land and in the oceans (Field *et al.* 1998). The primary production of organic carbon by phytoplankton constitutes around 50 Pg C $year^{-1}$, which is almost equal to terrestrial ecosystems (Field *et al.* 1998; Worden *et al.* 2015).

The interconversions of organic and inorganic carbon compounds are at the core of fluxes and exchanges of carbon between ocean and atmosphere (Worden *et al.* 2015). The most important biological processes controlling this interplay are photosynthesis – which converts carbon dioxide (CO_2) to organic carbon, allowing it to enter the oceanic food web, and heterotrophic respiration –which converts the organic carbon back into CO_2 (Rivkin and Legendre 2001). Furthermore, the biological carbon pump exports part of the produced carbon out of the euphotic zone into the deeper layers of the ocean – with a recent model predicting an export flux of around 9 Pg C $year^{-1}$ (DeVries and Weber 2017).

Since the introduction of the microbial loop hypothesis by Azam *et al.* (1983), it has been widely accepted that microbes are the key players of the processes governing the ocean's carbon cycle. Heterotrophic microorganisms produce new carbon biomass while growing, and simultaneously consume oxygen and respire CO_2. A means to relate these two processes is the growth efficiency, which determines how much biomass is produced per unit of assimilated carbon substrate (Del Giorgio and Cole 1998).

Even though the individual parameters to calculate the growth efficiency are often hard to quantify, they are necessary to model the carbon cycling through the microbial loop (Anderson and Ducklow 2001). Surveys of the global bacterial growth efficiency resulted in a high variability and mean values of 11 % in oceanic systems and 24 % in estuaries (Moran *et al.* 2022). Bacterial and zooplankton respiration are furthermore generally depicted as major carbon sinks (Anderson and Ducklow 2001).

The current warming of the oceans and the coupled deoxygenation and acidification stresses even more the importance of investigating microbial respiration as a crucial factor balancing the carbon storage in the ocean. Nevertheless, it's understanding is still limited by methodology and sufficient surveys, since in-situ respiration is difficult to measure accurately (Del Giorgio and Cole 1998; Robinson 2019).

Whereas the role of marine bacteria has been studied quite intensively since the beginning of the 21st century, the diversity and metabolic potential of aquatic fungi have been largely overlooked (Grossart and Rojas-Jimenez 2016). Even though fungi are found in nearly every marine habitat, their contribution to the open ocean food web and carbon cycle has gathered scientific interest only in the past decade (Amend *et al.* 2019).

On land, fungi are considered as crucial components of healthy ecosystems as they degrade and recycle plant material (Frac *et al.* 2018). Even though no other organisms can degrade as many different complex organic compounds, the knowledge about the diversity and metabolic potential of fungi is still limited (Grossart and Rojas-Jimenez 2016). Several studies suggest, however, that fungi play a substantial role in the pelagic biogeochemical cycles and – for instance – contribute to degrading marine snow particles in the bathypelagic realm of the ocean (Bochdansky *et al.* 2017; Morales *et al.* 2019).

The majority of free living planktonic fungi (mycoplankton) in the open ocean belongs to the divisions of Ascomycota and Basidiomycota in the subkingdom of Dikarya (Gao *et al.* 2010; Wang *et al.* 2014; Morales *et al.* 2019), whereas Chytridiomycota have been described as parasites on phytoplankton in productive coastal ecosystems (Gutiérrez *et al.* 2016). A recent study first described the "fungal shunt", which states that parasitic chytrids alter the fate of photosynthetic carbon and integrates fungi in the marine food web (Klawonn *et al.* 2021). These findings add to the previous description of the "mycoloop", where the infection of phytoplankton by chytrids was found to transfer nutrients directly from the infected cells to zooplankton (Kagami *et al.* 2014).

Planktonic members of Ascomycota and Basidiomycota in the subkingdom of Dikarya on the other hand have been described to be present in phytoplankton blooms, contributing to the degradation of the algal polysaccharides by utilizing extracellular enzymes (Cunliffe *et al.* 2017). Fungal interactions can lead to the fragmentation or aggregation of organic matter, which – for example – derives from plant material. This influence on the carbon pump in aquatic systems was termed as "mycoflux" (Grossart *et al.* 2019). A study focusing on fungal glycoside hydrolases transcripts furthermore states them as saprotrophs in the pelagic ocean (Chrismas and Cunliffe 2020). Recent observations in the North Sea underlined the active role of Dikarya in spring phytoplankton blooms (Priest *et al.* 2021). Moreover, the undeniable yet still largely

unexplored role of pelagic fungi in the global carbon cycle has been highlighted in a global-ocean multi-omics analysis of all carbohydrate-active enzymes (Baltar *et al.* 2021).

Irrespective of the presence and metabolic potential of pelagic fungi, the fungal contribution to microbial respiration has not been yet quantified (Grossart *et al.* 2019). So far, there is only one laboratory study available investigating the respiration of different pelagic fungal species isolated off the coast of Chile by Fuentes *et al.* (2015). Moreover, there is, to our knowledge, no data on the production rates or growth efficiency of marine fungi published. Their reported presence in phytoplankton blooms (Gutiérrez *et al.* 2010; Cunliffe *et al.* 2017), however, stresses that these data would be crucial to understand if fungal respiration rather acts as carbon sink or source.

Hence, this study is a first attempt to investigate respiration and production rates, and the growth efficiency of marine fungi. Since this is a novel research topic, the first aim was to develop a method to measure these parameters. After conducting preliminary experiments with different fungal species and several adaptions of the methods, a protocol to measure the desired parameters was established. Then, the three marine yeast species *Rhodotorula sphaerocarpa, Rhodotorula mucilaginosa*, and *Sakaguchia dacryoidea* (all in the division of Basidiomycota and the subdivision Pucciniomycotina) were grown in axenic cultures at different temperatures and nutrient conditions. Fungal respiration was investigated via optical oxygen sensors and the biomass production was estimated by measuring the particulate organic carbon content of the fungal cultures. Subsequently, the growth efficiency was determined by combining fungal production and respiration. To account for the effect of temperature on the metabolic rates of pelagic fungi we calculated the Q_{10} factor, which is the change of a metabolic rate with a temperature change of 10°C (Wohlers *et al.* 2009). In the ocean, where temperature shifts occur at large spatial and temporal scales (Arnosti *et al.* 2014), the response of organisms and metabolic processes is of special interest in the light of global warming. Finally, we determined the impact of nutrient concentration on the growth, production, respiration, and growth efficiency of the isolates.

Material and methods

Initial trials and experimental design

The initial idea was to measure the production and respiration of the fungal cultures at the same time (i.e., under the same conditions) to achieve the most accurate and comparable results. A basic requirement for measuring the consumption of oxygen in liquids is using air-tight bottles, excluding any external supply of oxygen. Several trials with different nutrient conditions were conducted to measure an increase in biomass during the same time and in the same bottles as the decrease of oxygen was measured. No such production of biomass could be detected during these experiments, indicating the demand of sufficient oxygen supply during the growth of the fungal cultures. Thus, the measurements had to be decoupled and different incubation times had to be applied, depending on the respiration and production of the cultures, respectively.

The experimental design resulting from these observations comprised culturing the fungal species in bottles with headspace, assuring sufficient oxygen supply (details are described below). At two timepoints during the exponential growth phase, subsamples were taken in triplicate to measure fungal abundance, biomass, and respiration.

For each species, two rounds of experiments were conducted: the first in a media with high nutrient concentration (details are given in the next section), simultaneously at 5°C and 15°C (Experiment 1), and the second at 15°C in a media with a low nutrient concentration (Experiment 2), as summarized in Table 1.

Table 1. Summary of conducted experiments per species.

	Experiment 1		**Experiment 2**
Nutrient concentration	0.5 g L^{-1}		0.05 g L^{-1}
Temperature	5°C	15°C	15°C

Cultivation of fungal isolates

The fungal isolates *Sakaguchia dacryoidea* and *Rhodotorula sphaerocarpa* were obtained from the Austrian Centre of Biological Resources (ACBR), whereas *Rhodotorula mucilaginosa* was isolated during the "Poseidon" Cruise across the Atlantic Ocean in 2019. The isolates were grown in the dark at room temperature on plates filled with an agar medium that contained glucose, peptone, yeast extract, malt extract, sea salts, agar, Milli-Q water, and antibiotics (Chloramphenicol); concentrations are shown in Table 2. The medium was autoclaved before filling the plates. Part of the fungal biomass was transferred to a new plate with a sterile cell spreader approximately one week before starting the actual experiment in the liquid culture.

For the liquid cultures, a growth medium containing the nutrients glucose, yeast extract, and peptone was prepared, using the according amounts for each experiment (see Table 2). The salinity of 30 was either obtained by adding a sea salt mixture (Sigma Aldrich) or by using artificial seawater (ASW), which was prepared prior to adding the nutrients. The ASW contained (per L Milli-Q water): 24.54 g NaCl, 4.09 Na_2SO_4, 0.7 g KCl, 0.2 g $NaHCO_3$, 0.1 g KBr, 0.003 g H_3BO_3, 0.003 g NaF, 11.1 g $MgCl_2 \cdot 6H_2O$, 1.54 g $CaCl_2 \cdot 2H_2O$, and 0.017, $SrCl_2 \cdot 6H_2O$. After adjusting the pH to 8.1, the medium was autoclaved, and 0.5 g L^{-1} Chloramphenicol were added to avoid bacterial contamination.

The fungal biomass was first diluted in autoclaved Milli-Q water until an optical density of 1 (measured in a spectrophotometer (VWR) at 660 nm) was achieved, and afterwards, the pure cultures were inoculated into the media (1 mL culture per 100 mL media). After thoroughly mixing, the culture was distributed to six Schott bottles per experiment and temperature in aliquots of 200 mL, which were incubated in a shaking incubator (Lab Companion) at 140 rpm and at 5°C and 15°C, respectively.

Table 2. Ingredients of the media for the agar plates and liquid cultures of Experiment 1 and 2.

Compound	Agar plates [g L^{-1}]	Experiment 1: High nutrient media [g L^{-1}]	Experiment 2: Low nutrient media [g L^{-1}]
Glucose	10.00	0.50	0.05
Malt extract	5.00	-	-
Yeast extract	3.00	0.50	0.05
Peptone	5.00	0.50	0.05
Agar	20.00	-	-
Sea Salts	30.00	30.00	30.00
Chloramphenicol	0.50	0.50	0.50

The growth of the cultures was tracked by measuring the optical density (OD) of one control bottle in a spectrophotometer (VWR) at a wavelength of 660 nm. Previous experiments allowed to estimate the growth phase of the culture depending on the OD (values are shown in Table 3).

Table 3. Optical density (OD) values for estimating the growth phase of the fungal liquid cultures in the high nutrient (Experiment 1) and low nutrient media (Experiment 2).

Estimated growth phase	OD (660 nm) in Experiment 1	OD (660 nm) in Experiment 2
Lag phase	< 0.199	< 0.100
Exponential phase	0.200 – 0.599	0.100 – 0.154
Stationary phase	> 0.600	> 0.154

Quantification of the fungal biomass

For collecting the fungal biomass in the cultures, 5 to 20 mL of each triplicate were vacuum filtered through pre-combusted glass microfiber filters (Whatman, 25 mm

diameter, 0.7 µm) at <200 mbar. The filters were wrapped in combusted aluminium foil and stored at -20°C for further analyses.

To estimate the fungal carbon biomass in the culture, the content of particulate organic carbon (POC) was measured, following the Joint Global Ocean Flux study (JGOFS) protocol (UNESCO 1994). After thawing, the filters were dried with silica gel for 24 h, followed by fumigation in a desiccator for 24 h with 37 % hydrochloric acid (HCl) to remove inorganic carbon. Subsequently, the filters were dried again for 24 h with silica gel and afterwards prepared for the analysis in a CHNS elemental analyser by wrapping them in small tin capsules. For each sampling point and species, the adsorption of dissolved organic carbon from the growth media on the filters used to quantify fungal carbon biomass was determined by measuring the carbon content of only the media, which was then subtracted from the respective triplicates. To estimate fungal production, the mean POC content of the triplicates from the first sampling point was subtracted from the one from the second sampling point and the result was divided by the time between measurements.

When measurement errors of the CHNS analyser occurred while measuring samples of the *Sakaguchia dacryoidea* culture of Experiment 1, the POC concentration was estimated by a regression of OD and POC from preliminary growth experiments under the same conditions.

Fungal abundance

Simultaneously to collecting the fungal biomass, 2 mL of each culture were fixed with Glutaraldehyde (2 % final concentration) and stored at -80°C. These samples were used to measure cell abundance in the fungal cultures with a flow cytometer (BD Accuri C6™) by adapting a protocol for measuring bacterial abundance in seawater samples. Due to the measurement limit of maximum 1000 cells μL^{-1}, the samples were diluted with pre-filtered (0.2 µm) Milli-Q water by a factor of 2, 4, 8, or 50 to a final volume of 500 µL. To stain the cells, 5 µL of SYBR-Green were added to the samples and incubated for 10 minutes. After that, the cells were counted for 2 minutes in the flow cytometer, and total cell numbers were estimated using the respective size spectra of the fungal cells. Each triplicate was measured two times and the mean of these technical triplicates was used to calculate the mean and standard deviation of the biological triplicates.

Fungal respiration

Oxygen concentrations in the samples were measured via optical sensors. Founded on the principle of luminescence quenching by molecular oxygen, the sensors can be used in different applications for in-situ measurements (Bittig *et al.* 2018). To allow continuous oxygen measurements, optical sensor spots (PreSens Precision Sensing GmbH) were attached to the inner wall of 120 mL BOD bottles with silicon glue. Before each measurement, a two-point calibration of the sensors was conducted, following the manual of the company (Precision Sensing GmbH 2018). Briefly, oxygen free water (0 %) was produced by dissolving 1 g of sodium sulphate (Na_2SO_4) in 100 mL MilliQ-water and air-saturated water (100 %) was derived by a 20-minute aeriation of 100 mL MilliQ-water. Since the solubility of oxygen is strongly temperature dependent, the calibration was conducted close to the respective incubation temperature (5°C and 15°C, respectively).

For measuring the oxygen consumption of the fungal cultures, the bottles were filled completely, closed airtight and put into a water bath set to the respective temperature of 5 or 15°C. After an adaptation period of 10 minutes, as suggested by Warkentin *et al.* (2007), the oxygen concentration was measured with an oxygen meter (Fibox 4 trace, PreSens GmbH) that was connected via a glass fibre cable to the optode from outside the bottle. The oxygen concentration as well as the temperature of the water bath was measured continuously every 10 seconds. Additionally, the oxygen concentration of only the growth media was measured for around 20 hours *a priori*, to exclude any chemical oxygen consumption by the ingredients of the media, or a contamination of the culture.

Since the Fibox 4 trace can only analyse one sample at a time, it was required to switch the sensor between the replicates every 20 to 30 minutes. The samples were kept in the water bath and measured continuously until the oxygen concentration was below 50 µmol L^{-1}. After removing the non-linear parts of the oxygen decrease – which occurred mainly in the first 10 minutes or towards lower concentrations – the respiration rates were calculated from the slope of the regression line from oxygen decrease over time.

A respiratory quotient (RQ) of 1 was applied to convert the final oxygen consumption rates into carbon units, since it is the most widely used RQ for heterotrophic microorganisms (Robinson 2019).

Calculation of fungal growth efficiency and Q_{10}

Fungal growth efficiency (FGE) was calculated using the formula $FGE = \frac{FP}{FP+FR}$ where FP is the fungal production and FR the fungal respiration. To calculate FGE, the average respiration rate of both sampling points was used in order to achieve the most accurate results.

The Q_{10} value was calculated for respiration, cell-specific respiration, production, and FGE as the quotient of the results at 5°C and 15°C. For each species, the sampling points with the most similar OD values at 5°C and 15°C, respectively, were chosen.

Data analyses and statistics

The linear regression for calculating the respiration rates as well as production and the growth efficiency were conducted in MS Excel 2019 and RStudio (version 4.0.3), respectively. Statistical analyses to compare the parameters were performed in RStudio and the data was visualised using the packages ggplot2 (version 3.3.5), gridExtra (version 2.3) and ggpubr (version 0.4.0). After testing normality of the data with Shapiro-Wilk tests, and homogeneity of variances with Bartlett's tests, analyses of variances (ANOVAs) were performed to test for differences between the species. Subsequently, differences between the specific groups were tested by TukeyHSD post-hoc tests. Student's t-tests were applied when only two groups were compared.

Results

Growth dynamics of the fungal cultures

In Experiment 1 (0.5 g L^{-1} of nutrients, i.e., glucose, yeast extract, and peptone), *Rhodotorula mucilaginosa* was the fastest growing species at both incubation temperatures (see Figure 1 and 2): at 5°C, the lag phase lasted five days (d), contrasting

to only 1.25 d at 15°C. The exponential growth phase, where the samples for measuring production and respiration were taken, lasted for 1.5 d at 5°C and only approximately 8 hours (h) at 15°C. *Rhodotorula sphaerocarpa* was the second fastest species, with a lag phase of 7 d at 5°C and 2 d at 15°C. The exponential growth phase lasted 3 d at 5°C and one day at 15°C. *Sakaguchia dacryoidea* was the slowest growing species at both temperatures. At 5°C, the lag phase was 11 d long and 4 d at 15°C. The exponential phase lasted for 4 d at 5°C, and for 1.5 d at 15°C.

In Experiment 2 (0.05 g L^{-1} of nutrients), the growth yield was lower, however, all three species reached the stationary phase faster than in Experiment 1 and the exponential phase lasted only a few hours – as depicted in Figure 3. *Sakaguchia dacryoidea* was the first species to reach the exponential phase (28 h), followed by *Rhodotorula mucilaginosa* (36 h) and *Rhodotorula sphaerocarpa* (40 h).

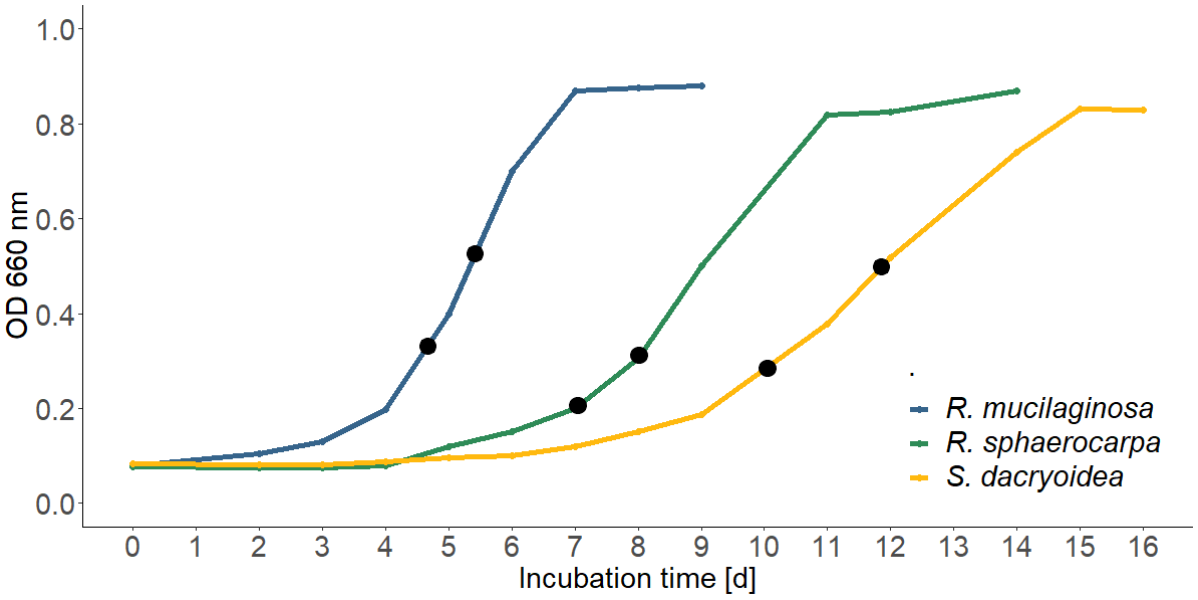

Figure 1. Growth curves of *Rhodotorula mucilaginosa, Rhodotorula sphaerocarpa,* and *Sakaguchia dacryoidea* at 5°C in Experiment 1; measured via optical density (OD; 660 nm). The black dots indicate the sampling points during the exponential growth phase.

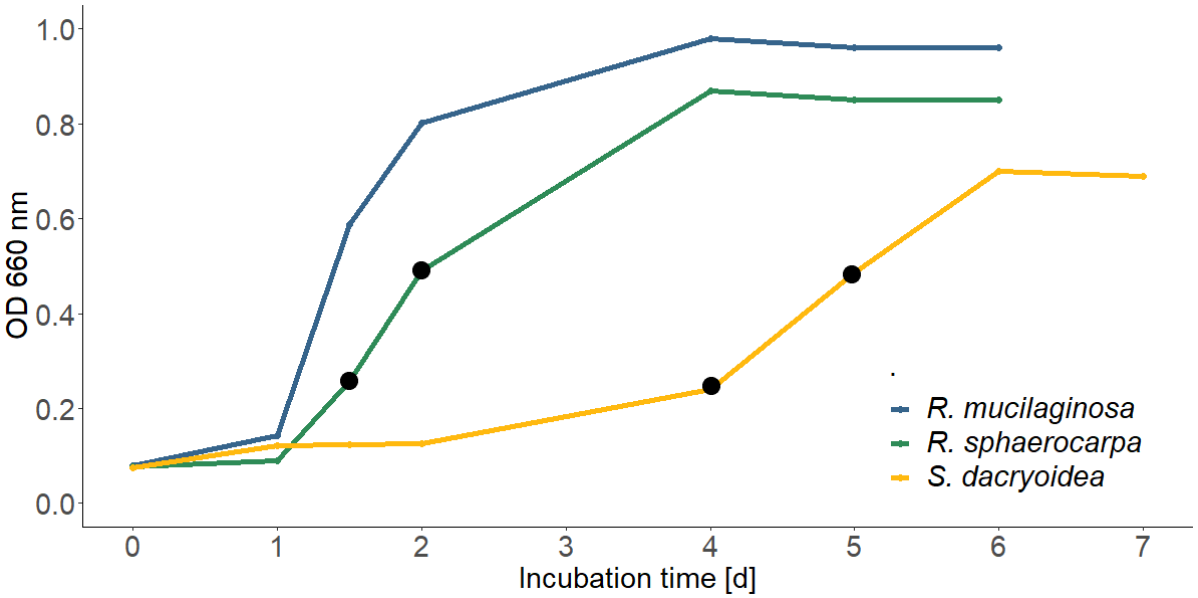

Figure 2. Growth curves of *Rhodotorula mucilaginosa, Rhodotorula sphaerocarpa,* and *Sakaguchia dacryoidea* at 15°C in Experiment 1; measured via optical density (OD; 660 nm). The black dots indicate the sampling points during the exponential growth phase.

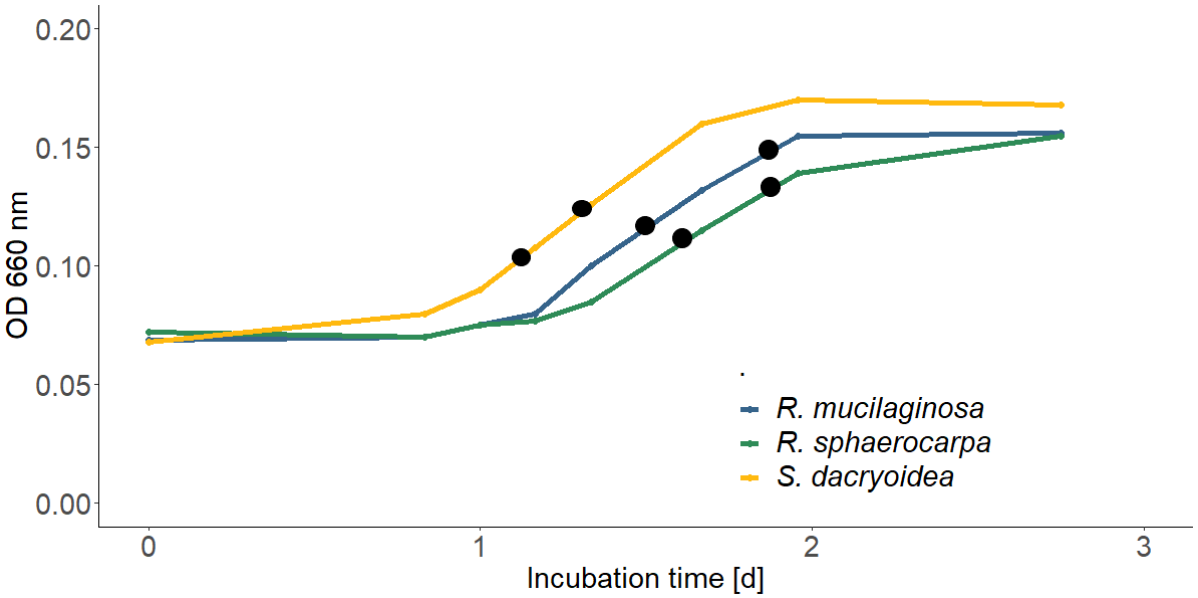

Figure 3. Growth curves of *Rhodotorula mucilaginosa, Rhodotorula sphaerocarpa,* and *Sakaguchia dacryoidea* at 15°C in Experiment 2; measured via optical density (OD; 660 nm). The black dots indicate the sampling points during the exponential growth phase.

Fungal biomass and production (FP)

The production rates of all species and both Experiments are depicted in Figure 4. During the exponential growth phase of Experiment 1 at 5°C, the POC concentration in the *Rhodotorula mucilaginosa* culture increased from 16.38 ± 8.53 mg C L^{-1} to 174.19 ± 23.21 mg C L^{-1} during 24.5 hours, which resulted in a biomass production rate of 6.44 mg C L^{-1} h^{-1}. The biomass of *Rhodotorula sphaerocarpa* increased from 37.92 ± 2.24 mg C L^{-1} to 78.18 ± 2.38 mg C L^{-1} in 24 hours with a production rate of 1.68 mg C L^{-1} h^{-1}.

The regression of OD and POC of *Sakaguchia dacryoidea* from preliminary growth experiments resulted in a slope of 118.53, an intercept of 1.55, and an R^2 of 0.63. Using this equation ($y = 118.53x + 1.55$) to calculate the POC concentration of *S. dacryoidea* revealed a biomass increase from 30.51 ± 5.51 mg C L^{-1} to 67.33 ± 3.86 mg C L^{-1} in 53 hours and the lowest production rate of 0.70 mg C L^{-1} h^{-1}.

At 15°C, the biomass of *R. sphaerocarpa* increased from 69.82 ± 6.29 mg C L^{-1} to 116.92 ± 12.43 mg C L^{-1} during 6 hours, resulting in a production rate of 7.85 mg C L^{-1} h^{-1}. *S. dacryoidea* increased from 28.53 ± 0.79 mg C L^{-1} to 56.42 ± 0.54 mg C L^{-1} in 27.5 hours, producing 1.01 mg C L^{-1} h^{-1}. There was almost no oxygen left in the beginning of the exponential growth phase of the *R. mucilaginosa* culture, hence no measurements were performed.

In Experiment 2, the POC concentration in the cultures was lower, and – as described above – the exponential growth phase was so short that the time between the sampling points was between only 3.5 to 7 hours. The biomass of *R. mucilaginosa* increased from 19.12 ± 0.23 mg C L^{-1} to 20.93 ± 0.36 mg C L^{-1} in 5 hours, which resulted in a production rate of 0.36 mg C L^{-1} h^{-1}. The *R. sphaerocarpa* culture produced 0.48 mg C L^{-1} h^{-1}, with a biomass increase from 15.91 ± 2.41 mg C L^{-1} to 19.18 ± 0.34 mg C L^{-1} in 6.75 hours. The highest production rate of Experiment 2 was measured in the *S. dacryoidea* culture: the biomass increased from 17.72 ± 1.22 mg C L^{-1} to 21.99 ± 4.16 mg C L^{-1} in only 3.5 hours, which resulted in a production rate of 1.22 mg C L^{-1} h^{-1}.

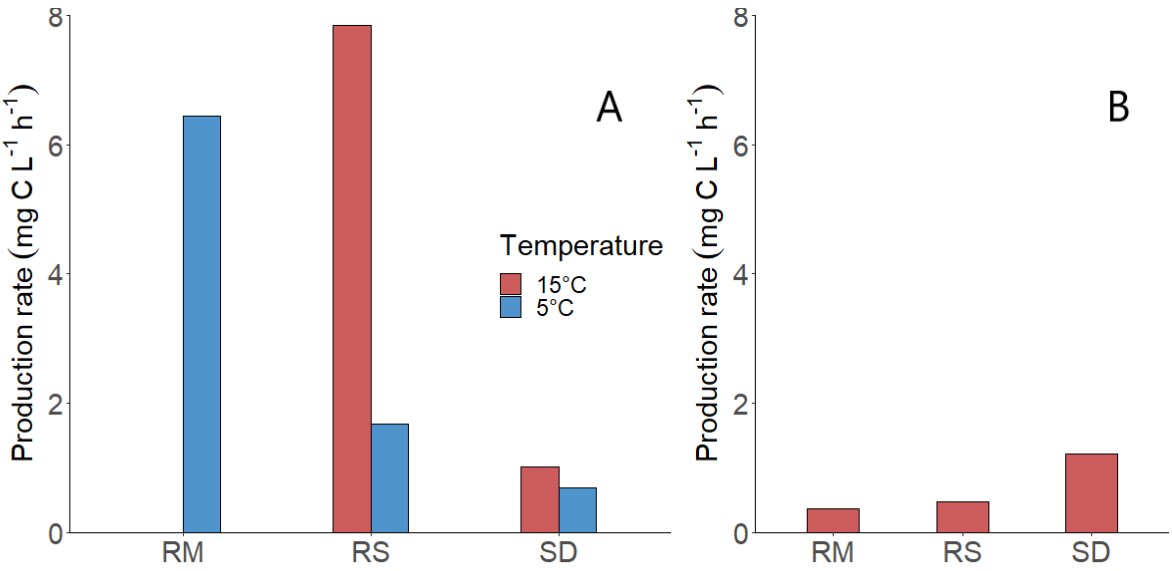

Figure 4. Fungal production rates of the three species *Rhodotorula mucilaginosa* (RM), *Rhodotorula sphaerocarpa* (RS), and *Sakaguchia dacryoidea* (SD) in Experiment 1 at 5°C and 15°C (A) and Experiment 2 (B). At 15°C, there was almost no oxygen left in the beginning of the exponential growth phase of the *R. mucilaginosa* culture, hence no measurements were performed. The bars represent the estimated production rates, which was calculated by subtracting the mean of triplicates at the first sampling point from the one at the second sampling point and dividing the result by the time between measurements (see section Methods; Quantification of fungal biomass for further details).

Fungal abundance

The cell abundance of all species at the first and second sampling point (see black dots in Figures 1-3) are depicted in Figure 5 (Experiment 1) and 6 (Experiment 2).

The highest abundance was measured in the *Rhodotorula mucilaginosa* culture at 5°C (Experiment 1) which increased from 1.07 x 10^{10} ± 1.28 x 10^{9} cells L^{-1} to 1.5 x 10^{10} ± 1.9 x 10^{9} cells L^{-1}. *Rhodotorula sphaerocarpa* increased from 1.92 x 10^{9} ± 3.49 x 10^{8} cells L^{-1} to 2.61 x 10^{9} ± 1.06 x 10^{9} cells L^{-1} at 5°C and from 7.16 x 10^{9} ± 9.63 x 10^{8} cells L^{-1} to 1.25 x 10^{10} ± 7.13 x 10^{8} cells L^{-1} at 15°C. The abundance of *Sakaguchia dacryoidea* changed from 8.85 x 10^{8} ± 5.22 x 10^{8} cells L^{-1} to 3.08 x 10^{9} ± 6.06 x 10^{8} cells L^{-1} at 5°C and from 1.18 x 10^{9} ± 2.14 x 10^{8} cells L^{-1} to 8.39 x 10^{9} ± 1.47 x 10^{9} cells L^{-1} at 15°C.

During Experiment 2, the abundance of *R. mucilaginosa* increased from 3.39 x 10^{9} ± 8.53 x 10^{7} cells L^{-1} to 3.75 x 10^{9} ± 2.18 x 10^{8} cells L^{-1}, and *S. dacryoidea* from 1.67 x 10^{9} ± 1.98 x 10^{8} cells L^{-1} to 3.01 x 10^{9} ± 1.16 x 10^{8} cells L^{-1}. In contrary, the abundance of *R.*

sphaerocarpa decreased from 2.97 x 10^9 ± 1.12 x 10^8 cells L^{-1} to 2.50 x 10^9 ± 3.60 x 10^8 cells L^{-1}.

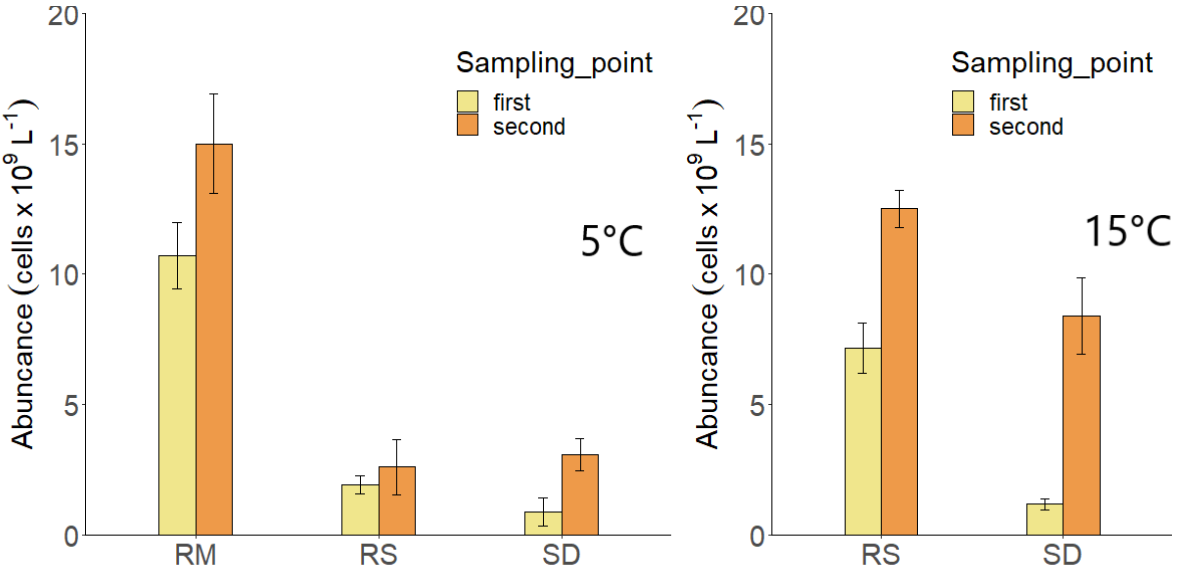

Figure 5. Cell abundance of the three species *Rhodotorula mucilaginosa* (RM), *Rhodotorula sphaerocarpa* (RS), and *Sakaguchia dacryoidea* (SD) in Experiment 1 at 5°C and 15°C in the first and second sampling point (see Figure 1 and 2 for reference). The bars show the mean abundance, and the error bars the standard deviation of biological triplicates. At 15°C, there was almost no oxygen left in the beginning of the exponential growth phase of the *R. mucilaginosa* culture, hence no measurements were performed.

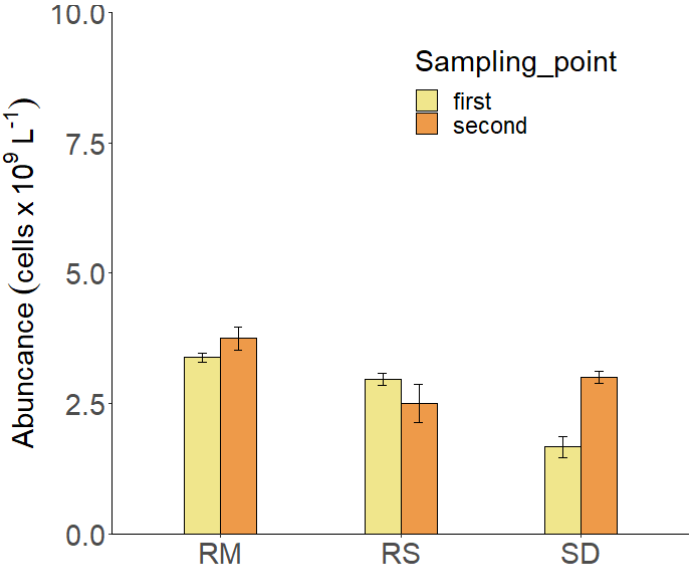

Figure 6. Cell abundance of the three species *Rhodotorula mucilaginosa* (RM), *Rhodotorula sphaerocarpa* (RS), and *Sakaguchia dacryoidea* (SD) in Experiment 2 at 15°C in the first and second sampling point (see Figure 3 for reference). The bars show the mean abundance, and the error bars the standard deviation of biological triplicates.

Fungal respiration (FR)

The continuous measurement of oxygen in the cultures revealed that the decrease of oxygen in all samples was linear until a concentration of 50 to 150 µmol L^{-1}. The initial oxygen concentration differed due to the temperature dependency of oxygen solubility and the fast consumption of the cultures at 15°C. Representative examples of the oxygen consumption of *Rhodotorula sphaerocarpa* in Experiment 1 (high nutrient media; 5°C and15°C), and Experiment 2 (low nutrient media; 15°C) are depicted in Figure 7.

Respiration was measured at the two sampling points during the exponential growth phase, where also the carbon content was measured to determine the production rate (see black dots in Figures 1-3). The oxygen consumption was almost always higher at the second sampling point, though in a few cases the culture had already reached the beginning of the stationary phase, which became apparent with lower oxygen consumption rates. To compare the respiration rates between species and temperatures, the sampling points with the most similar optical density values were chosen.

The highest respiration rate was measured in the *Rhodotorula sphaerocarpa* culture in Experiment 1 at 15°C at the second sampling point (211.87 ± 9.95 µmol O_2 L^{-1} h^{-1}); the first sampling point exhibited lower respiration with 136.33 ± 27.04 µmol O_2 L^{-1} h^{-1}. This was still higher than the respiration rate of *Sakaguchia dacryoidea* at the second sampling point, which was 111.08 ± 1.72 µmol O_2 L^{-1} h^{-1}, around double the rate of the first sampling point (53.13 ± 4.84 µmol O_2 L^{-1} h^{-1}). The mean oxygen consumption rates of the two species at the second sampling point, which resembled in terms of OD, differed significantly from each other (T-test; $p<0.01$; see Figure 8).

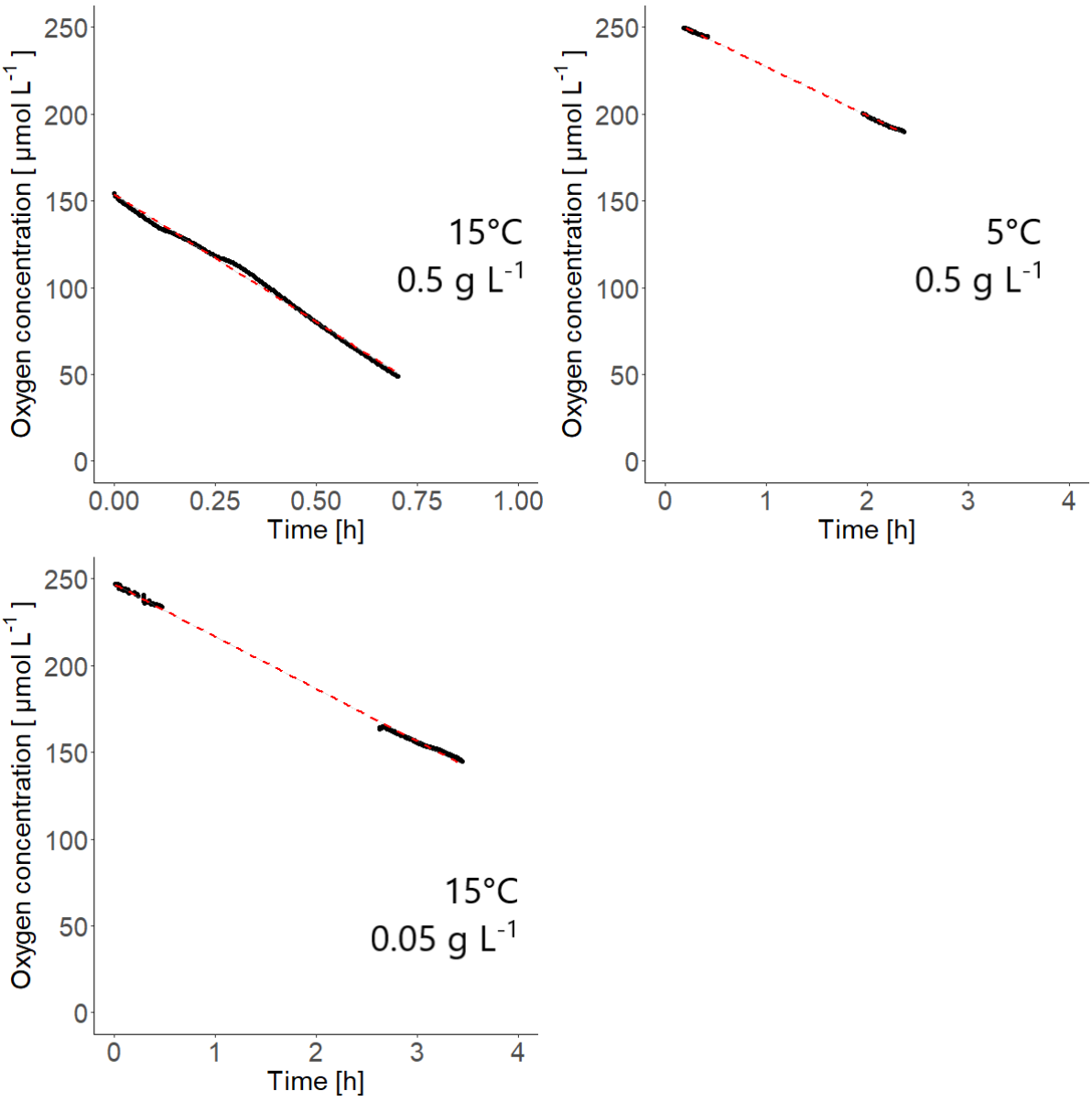

Figure 7. Representative examples of the decrease of oxygen concentration over time in selected replicates of the *Rhodotorula sphaerocarpa* cultures in the high nutrient media (0.5 g L^{-1} glucose, yeast extract, and peptone) at 5°C and 15°C (Experiment 1), and in the low nutrient media (0.05 g L^{-1} glucose, yeast extract, and peptone) at 15°C (Experiment 2). The black dots represent the oxygen concentration in µmol L^{-1} as measured by the optical oxygen sensors and the dashed red line was fitted by a linear regression model.

No respiration rate could be calculated for the *Rhodotorula mucilaginosa* culture at 15°C in Experiment 1 since the oxygen concentration was already below 10 µmol L^{-1} when the measurement was started. At 5°C, however, it exhibited the highest respiration of the three species: 93.21 ± 4.53 µmol O_2 L^{-1} h^{-1} at the second sampling point. The OD,

however, was considerably higher than the one of *R. sphaerocarpa* (see Figure 1), which is why the first sampling point was used to compare between species, where the respiration rate was 55.90 ± 4.38 µmol O^2 L^{-1} h^{-1}. The same holds true for *S. dacryoidea*, which had a respiration rate of 47.44 ± 3.80 µmol O_2 L^{-1} h^{-1} at the second sampling point and 24.78 ± 7.24 µmol O_2 L^{-1} h^{-1} at the first. *R. sphaerocarpa* had a respiration rate of 19.60 ± 0.93 µmol O_2 L^{-1} h^{-1} at the first, and 30.38 ± 2.70 µmol O_2 L^{-1} h^{-1} at the second sampling point. As stated before, the second sampling point of this species was used for the comparison due to the resembling OD (see Figure 1). There was an overall significant difference between the species (ANOVA; $p<0.01$), however no significant different between the respiration rates of *R. sphaerocarpa* and *S. dacryoidea* (TukeyHSD post-hoc test; $p=0.42$; see Figure 8).

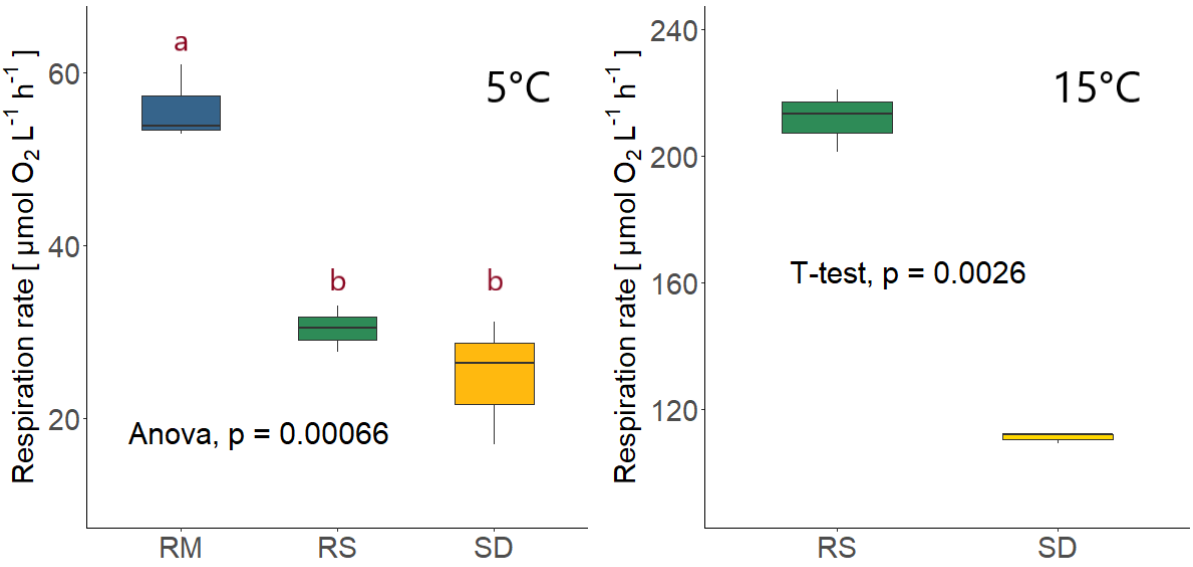

Figure 8. Respiration rates of Experiment 1 (high nutrient media) at 5°C and 15°C of the three species *Rhodotorula mucilaginosa* (RM), *Rhodotorula sphaerocarpa* (RS), and *Sakaguchia dacryoidea* (SD) with the respective statistical tests. The red letters indicate which species differed significantly from each other, as determined by a TukeyHSD post-hoc test. At 15°C, there was almost no oxygen left in the beginning of the exponential growth phase of the *R. mucilaginosa* culture, hence no measurements were performed.

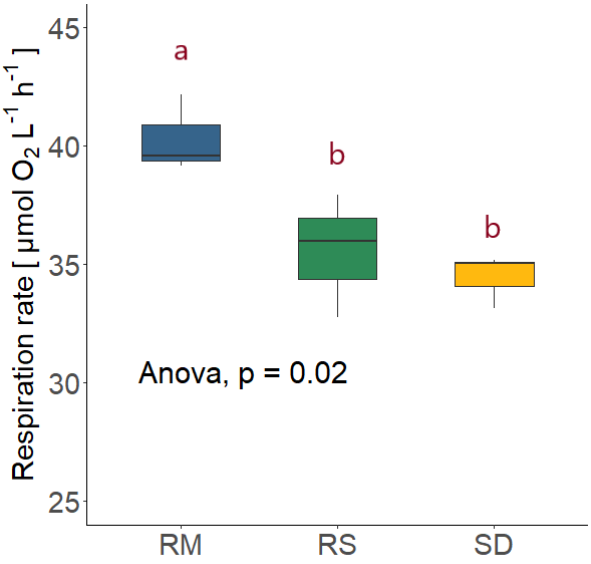

Figure 9. Respiration rates in Experiment 2 (low nutrient media) 15°C of the three species *Rhodotorula mucilaginosa* (RM), *Rhodotorula sphaerocarpa* (RS), and *Sakaguchia dacryoidea* (SD) with the respective statistical test. The red letters indicate which species differed significantly from each other, as determined by a TukeyHSD post-hoc test.

In Experiment 2 (low nutrient media, 15°C), the oxygen consumption rates were lower compared to the high nutrient media and in a similar range than the cultures of Experiment 1 at 5°C. The respiration of the first sampling point of all three species was used for comparison and again, *Rhodotorula mucilaginosa* exhibited the highest respiration rate (40.32 ± 1.63 µmol O_2 L^{-1} h^{-1}; at the second sampling point 37.91 ± 6.49 µmol O_2 L^{-1} h^{-1}), followed by *Rhodotorula sphaerocarpa* (35.36 ± 2.63 µmol O_2 L^{-1} h^{-1}; at the second sampling point 40.85 ± 5.25 µmol O_2 L^{-1} h^{-1}) and *Sakaguchia dacryoidea* (34.47 ± 1.12 µmol O_2 L^{-1} h^{-1}, at the second sampling point 25.16 ± 6.68 µmol O_2 L^{-1} h^{-1}). Again, the respiration rates of the three species differed significantly from each other (ANOVA; $p<0.05$), however, there was no difference between the respiration rates of *R. sphaerocarpa* and *S. dacryoidea* (TukeyHSD post-hoc test; $p=0.76$; see Figure 9).

Comparing the respiration rates of *R. sphaerocarpa* and *S. dacryoidea* at the different conditions of Experiment 1 (0.5 g L^{-1} of nutrients; 5°C and 15°C, respectively) and Experiment 2 (0.05 g L^{-1} of nutrients, 15°C) revealed that the values were always significantly higher in Experiment 1 at 15°C than at 5°C or in Experiment 2 (ANOVA; *R. sphaerocarpa*: $p<0.01$; *S. dacryoidea*: $p<0.01$; see Figure 10). However, the post-hoc

TukeyHSD test showed that the respiration rates did not differ significantly between Experiment 1 at 5°C and Experiment 2 (*R. sphaerocarpa*: p=0.58; *S. dacryoidea*: p=0.07).

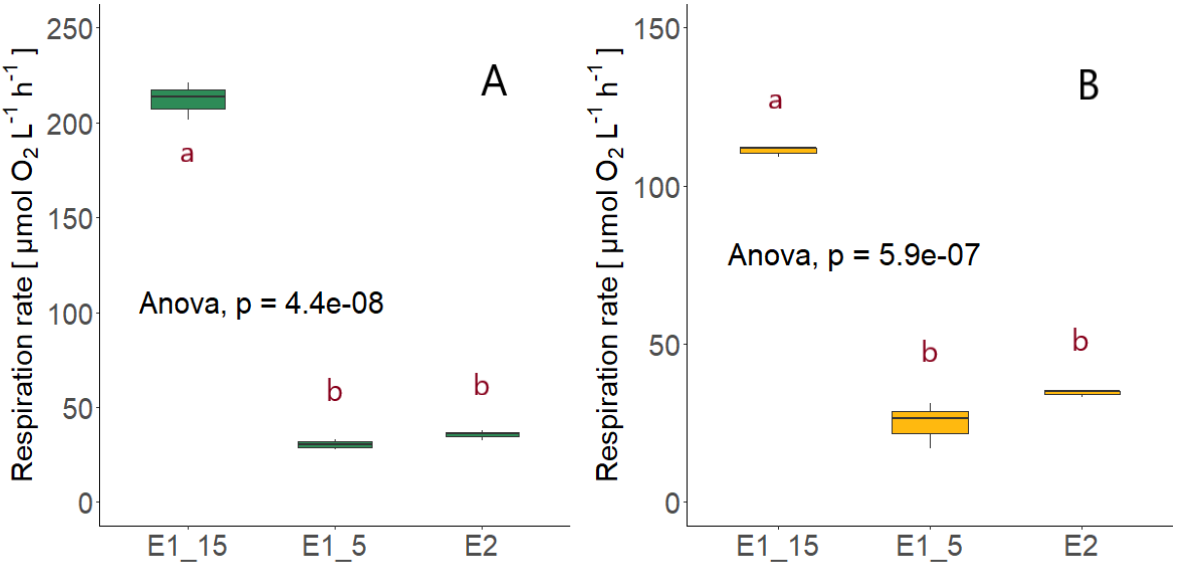

Figure 10. Respiration rates of *Rhodotorula sphaerocarpa* (A) and *Sakaguchia dacryoidea* (B) in Experiment 1 at 15°C (E1_15), and 5°C (E1_5), and in Experiment 2 (E2; see Methods for detailed information). The red letters indicate which treatments differed significantly from each other, as determined by a TukeyHSD post-hoc test.

Cell-specific respiration

Combining the respiration rates with the respective fungal abundance revealed the oxygen consumption per fungal cell, which differed only very slightly between the two sampling points of each species.

Contrary to the total respiration rates, *Sakaguchia dacryoidea* exhibited the highest cell-specific respiration in Experiment 1 at 5°C (15.69 ± 2.34 fmol O_2 cell^{-1} h^{-1}, see Figure 11), followed by *Rhodotorula sphaerocarpa* (12.80 ± 4.49 fmol O_2 cell^{-1} h^{-1}) and *Rhodotorula mucilaginosa* (8.77 ± 0.59 fmol O_2 cell^{-1} h^{-1}). The cell-specific respiration did not differ significantly between the species (ANOVA; p=0.073).

At 15°C, however, *R. sphaerocarpa* exhibited higher cell-specific respiration than *S. dacryoidea* (17.07 ± 1.67 fmol O_2 cell^{-1} h^{-1}; 14.09 ± 2.95 fmol O_2 cell^{-1} h^{-1}), but the difference was not significant (T-test; p=0.2, see Figure 11).

The overall highest cell-specific respiration was measured in the *S. dacryoidea* culture of Experiment 2, (20.76 ± 1.86 fmol O_2 $cell^{-1}$ h^{-1}), which was followed by *R. sphaerocarpa* (16.60 ± 3.57 fmol O_2 $cell^{-1}$ h^{-1}), and *R. mucilaginosa* (11.90 ± 0.21 fmol O_2 $cell^{-1}$ h^{-1}); the species differed significantly from each other (ANOVA; $p<0.05$). Whereas *R. sphaerocarpa* showed no significant difference from the other two species, *S. dacryoidea* and *R. mucilaginosa* differed significantly from each other (TukeyHSD post-hoc test; $p<0.01$, see Figure 12).

The cell-specific respiration rates of *R. sphaerocarpa* did not differ significantly between the different conditions of Experiment 1 (0.5 g L^{-1} of nutrients; 5°C and 15°C, respectively) and Experiment 2 (0.05 g L^{-1} of nutrients, 15°C) (ANOVA; $p=0.32$, see Figure 13A). *S. dacryoidea* exhibited significantly different cell-specific respiration rates among treatments (ANOVA; $p<0.05$), however the rate at 5°C in Experiment 1 was not different from Experiment 1 at 15°C ($p=0.71$) or from Experiment 2 ($p=0.09$), as was shown by a TukeyHSD post-hoc test (see Figure 13B).

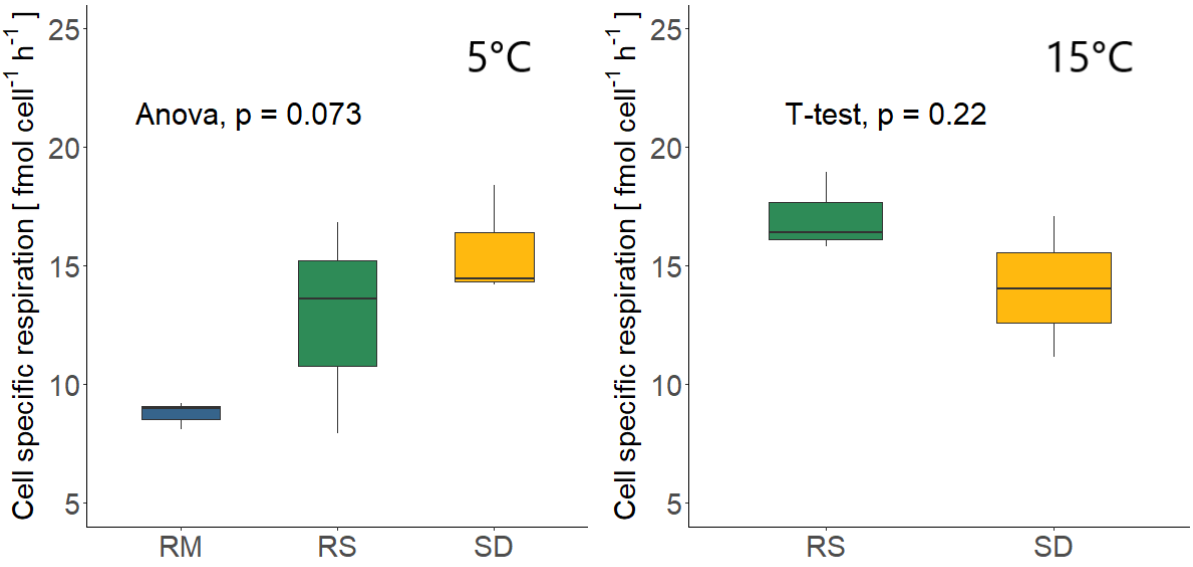

Figure 11. Cell-specific respiration rates in Experiment 1 (high nutrient media) at 5°C and 15°C of the three species *Rhodotorula mucilaginosa* (RM), *Rhodotorula sphaerocarpa* (RS), and *Sakaguchia dacryoidea* (SD) with the respective statistical tests. At 15°C, there was almost no oxygen left in the beginning of the exponential growth phase of the *R. mucilaginosa* culture hence no measurements were performed.

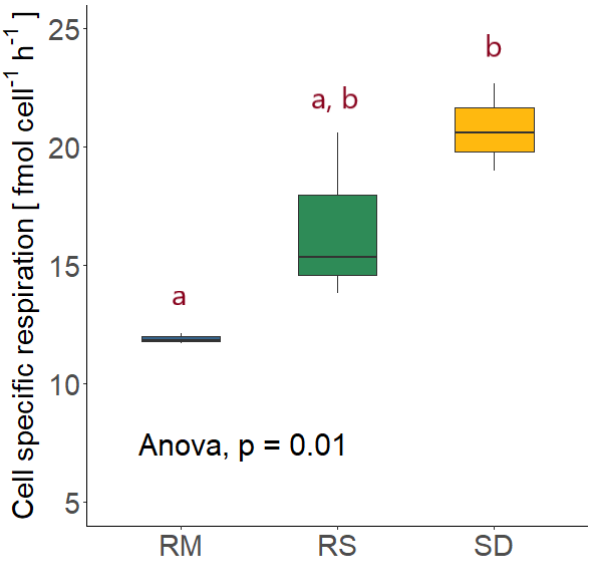

Figure 12. Cell-specific respiration rates in Experiment 2 (low nutrient media) 15°C of the three species *Rhodotorula mucilaginosa* (RM), *Rhodotorula sphaerocarpa* (RS), and *Sakaguchia dacryoidea* (SD) with the respective statistical test. The red letters indicate which species differed significantly from each other, as determined by a TukeyHSD post-hoc test.

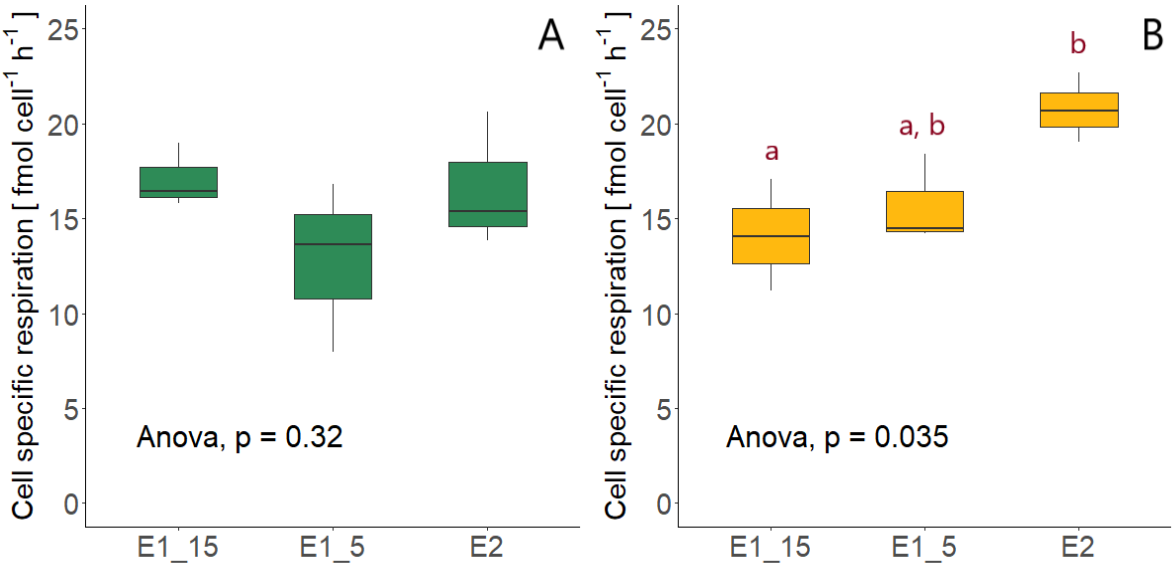

Figure 13. Cell-specific respiration rates of *Rhodotorula sphaerocarpa* (A) and *Sakaguchia dacryoidea* (B) in Experiment 1 at 15°C (E1_15), and 5°C (E1_5), and in Experiment 2 (E2; see Methods for detailed information). The red letters indicate which treatments differed significantly from each other, as determined by a TukeyHSD post-hoc test.

Fungal growth efficiency (FGE)

The highest FGE was calculated for the *Rhodotorula mucilaginosa* culture at 5°C in Experiment 1, which exhibited an FGE of 87 % (Figure 14). It was followed by *Rhodotorula sphaerocarpa* (85 %), and *Sakaguchia dacryoidea* (62 %). At 15°C, the growth efficiency of both *R. sphaerocarpa* and *S. dacryoidea* was lower with 78 % and 50 %, respectively. At the lower nutrient conditions of Experiment 2, *S. dacryoidea* exhibited the highest growth efficiency with 75 %. The FGE of *R. sphaerocarpa* was 51 % and *R. mucilaginosa* had an FGE of 43 % (Figure 15).

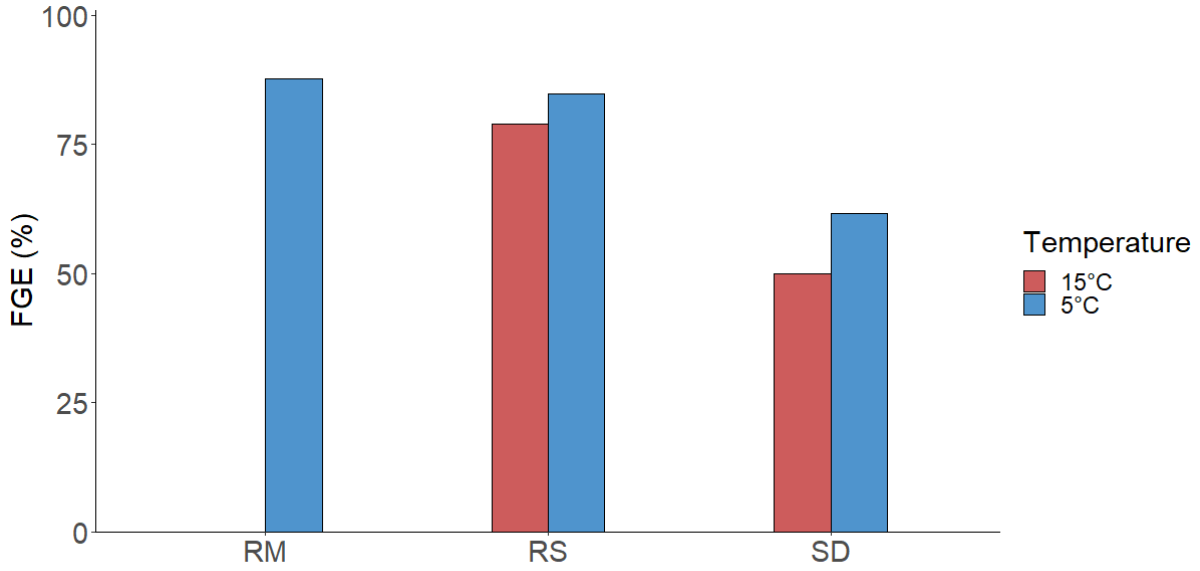

Figure 14. Fungal growth efficiency (FGE) of the three species *Rhodotorula mucilaginosa* (RM), *Rhodotorula sphaerocarpa* (RS), and *Sakaguchia dacryoidea* (SD) in Experiment 1 (high nutrient media) at 5°C (blue) and 15°C (red), respectively. At 15°C, there was almost no oxygen left in the beginning of the exponential growth phase of the *R. mucilaginosa* culture hence no measurements were performed. The bars represent the estimated growth efficiency calculated by the formula FGE=FP/(FP+FR), where FR is the mean respiration and FP the production rate, calculated by subtracting the average carbon content at the first sampling point from the one at the second sampling point and dividing this by the time between sampling points (see section Methods (Quantification of fungal biomass; Calculation of fungal growth efficiency) for further details).

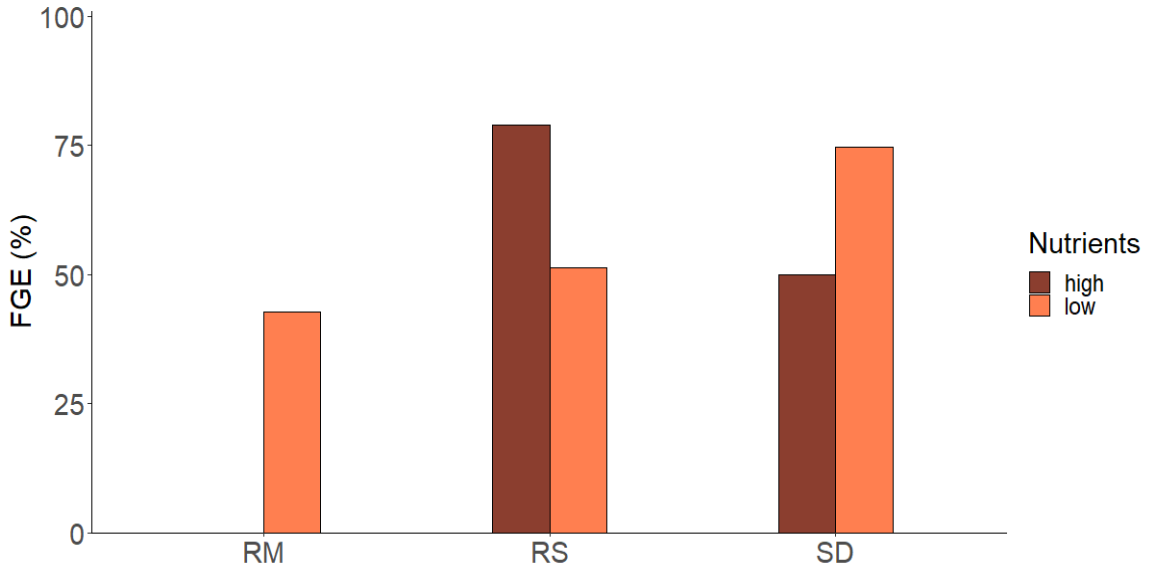

Figure 15. Fungal growth efficiency (FGE) of the three species *Rhodotorula mucilaginosa* (RM), *Rhodotorula sphaerocarpa* (RS), and *Sakaguchia dacryoidea* (SD) in Experiment 1 (high nutrient media; dark brown) and Experiment 2 (low nutrient media, light brown). In Experiment 1 at 15°C, there was almost no oxygen left in the beginning of the exponential growth phase of the *R. mucilaginosa* culture hence no measurements were performed. The bars represent the estimated growth efficiency calculated by the formula FGE=FP/(FP+FR), where FR is the mean respiration and FP the production rate, calculated by subtracting the average carbon content at the first sampling point from the one at the second sampling point and dividing this by the time between sampling points (see section Methods (Quantification of fungal biomass; Calculation of fungal growth efficiency) for further details.

Effects of temperature and nutrient concentration on FR, FP, and FGE

In Experiment 1, both species exhibited significantly higher total respiration rates at 15°C than at 5°C (*Rhodotorula sphaerocarpa*: $p<0.05$; *Sakaguchia dacryoidea*: $p<0.01$). The Q_{10} of respiration was 4.49 for *R. sphaerocarpa* and 2.14 for *S. dacryoidea*. On the contrary, the cell specific respiration was not dependent on the temperature (*R. sphaerocarpa*: $p=0.24$; *S. dacryoidea*: $p=0.51$). *R. sphaerocarpa* had a Q_{10} value of 1.33, however *S. dacryoidea* exhibited higher cell-specific respiration at 5°C, resulting in a Q_{10} of 0.89.

The production rates of both species were higher at higher temperature, as indicated by Q_{10} values of 4.68 (*R. sphaerocarpa*) and 1.46 (*S. dacryoidea*). Both species exhibited higher FGE at 5°C compared to 15°C (see Figure 14), resulting in Q_{10} values of 0.93 (*R. sphaerocarpa*) and 0.81 (*S. dacryoidea*). All Q_{10} values are summarized in Table 4.

Table 4. Q_{10} values of the total respiration, cell-specific respiration, production, and growth efficiency of *Rhodotorula sphaerocarpa* and *Sakaguchia dacryoidea*. The values derive from comparing the measurements at 5°C and 15°C of Experiment 1.

	Total respiration	Cell-specific respiration	Production	Growth efficiency
Rhodotorula sphaerocarpa	4.49	1.33	4.68	0.93
Sakaguchia dacryoidea	2.14	0.89	1.46	0.81

Discussion

Methodology and selection of fungal species

This study is a first attempt to assess the respiration, production, and growth efficiency of pelagic fungi to investigate their role in the marine carbon cycle. Since this is a novel research objective, the first part of the project was to find appropriate methods for measuring the required parameters.

The utilization of optical oxygen sensors delivered valuable insights into the oxygen consumption of the cultures. The "traditional" chemical method followed by titration established by Winkler (1888) is still the most widespread method for measuring oxygen in aquatic systems, whereas optical sensors were introduced in aquatic research more recently (Tengberg *et al.* 2006). A direct comparison of the two methods resulted in similar oxygen values and respiration rates which confirms that it is possible to achieve comparable results with either method (Bondyale-Juez *et al.* 2017). Compared to oxygen measurements only in the beginning and end of an incubation period (i.e., discrete measurements), the continuous measurement has the advantage to allow observing the oxygen consumption dynamics in real time (Stief *et al.* 2021). Applying the Winkler method could have led to false assumptions because the required incubation duration was not known and was proved to be different among the species, temperature, and nutrient concentration.

Hence, optical oxygen sensors can be considered as a suitable tool to assess the oxygen consumption of fungal cultures. Moreover, the oxygen concentration of the sterile media (controls) remained stable, as was also reported by Warkentin *et al.* (2007). The importance of thoroughly saturating the culture with air (in a sterile place, to avoid contamination) was highlighted by the observation that the *Rhodotorula mucilaginosa* culture consumed all oxygen in the culture before the measurement could be started. This could be further explained by the fast growth of the species and the high nutrient concentration in the media.

The high fungal respiration rates required the decoupling of measuring respiration and production. The results of the present study confirm the final experimental set-up as suitable means to answer the research objectives, even though it may have to be adapted when studying other species or applying different growth conditions.

All three species cultivated in this study belong to the division of Basidiomycota in the subkingdom Dikarya. The latter have been described as most abundant fungal subkingdom in the open ocean (Gao *et al.* 2010; Wang *et al.* 2014; Morales *et al.* 2019). A more recent study reports an abundance of 10^5 cells L^{-1} of yeast(-like) Dikarya during a phytoplankton bloom in the North Sea and states especially Basidiomycota as most prevalent fungal phylum (Priest *et al.* 2021). *Rhodotorula mucilaginosa* is a widespread species that is found in the open ocean but also in coastal areas; and both *Rhodotorula* species were identified in a survey studying fungal diversity in the Mid-Atlantic Ridge (Gadanho and Sampaio 2005). *S. dacryoidea* has furthermore been identified as one of the five most abundant species off the coast of Portugal (Gadanho *et al.* 2003). The high abundance of basidiomycetes yeasts in a phytoplankton bloom and their potential to utilize many different carbon sources, as reported by Priest *et al.* (2021) further underlines the importance of studying this phylum and its role in the ocean. Finally, novel results from a marine fungal biodiversity survey at the University of Vienna suggest that the three basidiomycete species are among the most abundant taxa along a transect through die Atlantic Ocean (Breyer pers. comm.). This finding emphasizes the isolates as suitable organisms for laboratory studies with the aim of gaining insight into the processes in the open ocean.

Significant differences between the species were found in most of the metabolic parameters studied. This leads to the suggestion that in natural conditions, the

composition of the fungal community can be critical for the community's activity. For example: in a community with *R. mucilaginosa* as dominant species, the respiration can be expected to be much higher than in a community dominated by *S. dacryoidea*. These suggestions are, however, still hypothetical since the investigation of many more species would be necessary.

Fungal growth and production

All fungal species exhibited fast growth curves in all experiments. Recently, marine basidiomycete yeasts have been suggested to behave as opportunistic r-strategists in phytoplankton blooms (Priest *et al.* 2021). The present study supports this and further suggests that in oceanic regions with high nutrient concentrations, these species would perform rapid growth.

Conducting the experiments under different conditions, revealed the surprising fact that the growth of the cultures was not significantly slowed down by nutrient limitation. To the contrary, the exponential growth phase of all three species was achieved faster and lasted shorter in Experiment 2 with the low nutrient medium. Nevertheless, the growth yield was considerably higher in Experiment 1 at higher nutrient concentrations. Temperature, on the other hand, slowed down the growth, and the production rate remarkably, as could be observed in Experiment 1. The growth yield, however, was comparable at both temperatures in terms of optical density and biomass (POC).

The production rates of the *Rhodotorula* species were higher in Experiment 1 with the high nutrient media. *Sakaguchia dacryoidea* on the other hand exhibited a higher production rate at lower nutrient concentrations (see Figure 4).

The measured FP (0.36 to 7.85 mg C L^{-1} h^{-1}) exceeded the production of batch cultures of a natural bacterial community from the Mediterranean, which exhibited only around 0.03 mg C^{-1} L^{-1} h^{-1} in nutrient enriched media (Motegi *et al.* 2009). Pure cultures of strains of marine bacteria *Roseobacter* and *Cytophaga* expressed production rates of 0.02 to 0.03 mg C^{-1} L^{-1} h^{-1} (Teira *et al.* 2012).

The Q_{10} of FP was 4.68 (*Rhodotorula sphaerocarpa*) and 1.46 (*Sakaguchia dacryoidea*). The results indicate that the production rate of *R. sphaerocarpa* is more temperature-dependent than *S. dacryoidea* and the latter can produce biomass at lower temperatures

almost as fast as at higher temperatures. The effect of temperature is comparable to the Q_{10} of other growth parameters such as the growth rates of prokaryotes (2.1 – 3.9; White *et al.* 1991), and nanoflagellates (2.66; Choi and Peters 1992).

Fungal respiration

This study is one of the first to assess the open question about marine fungal respiration. In a previous study where five fungal isolates from the coast off Chile were cultivated at 13°C and a glucose concentration of 5 g L^{-1} (among other conditions), respiration rates between 156.4 and 273.1 µmol O_2 L^{-1} h^{-1} were measured (Fuentes *et al.* 2015). Even though the glucose concentration is ten-fold higher than in the present study, the numbers are in the same range. When the authors lowered the glucose concentrations to 0.1 and 0.01 g L^{-1}, respectively, the obtained respiration rates (at 20°C) were between 65.5 and 108.5 µmol O_2 L^{-1} h^{-1} (Fuentes *et al.* 2015). Given the 5°C difference, the numbers are again in a similar range as the respiration of the basidiomycete yeast species used in this study. The lack of more studies about marine fungal respiration makes it difficult to compare the numbers, while stressing the importance of research in this field even more.

When comparing the results to other heterotrophic organisms, the numbers were found to be also higher than a bacterial culture isolated from a eutrophic river and incubated at 20°C. It expressed respiration rates of up to 61.5 µmol O_2 L^{-1} h^{-1} (high bacterial abundance of 20 x 10^6 cells mL^{-1}), whereas natural plankton communities in the same river respired at a lower rate of 15.6 µmol O_2 L^{-1} h^{-1} (Warkentin *et al.* 2007).

The respiration of marine bacterial cultures is even lower, with reported respiration rates up to 2.2 µmol O_2 L^{-1} h^{-1} in nutrient enriched batch cultures (Motegi *et al.* 2009). Compared to cultures, the bacterial respiration in the ocean is obviously much lower: in surface waters, the average respiration rate is between 0.13 and 0.31 µmol O_2 L^{-1} h^{-1} (open ocean and coastal areas, respectively; Robinson and Williams 2005), and in the meso- and bathypelagic realm as low as 0.01 µmol O_2 L^{-1} h^{-1} (Reinthaler *et al.* 2006; Mazuecos *et al.* 2015). Even though fungal respiration is arguably higher than that of bacteria due to the mere size of the cells, it can be hypothesised to be much lower in natural oceanic systems than in the laboratory cultures.

The dependence of respiration rates on the nutrient concentration further points towards a considerable loss of CO_2 fuelled by FR during phytoplankton blooms, where high nutrient concentrations occur over a relatively short time span. While the bacterial contribution can be determined quite uncomplicated by filtration and makes up around 79 % of the total community respiration (Del Giorgio *et al.* 2011), the extent to which fungi contribute to the total respiration of oceanic systems is yet to be discovered.

The cell-specific respiration rates determined in this study (7 to 20 fmol $cell^{-1}$ h^{-1}) are considerably higher than rates reported for bacterial cultures originating from freshwater (1 to 2 fmol $cell^{-1}$ h^{-1}; Warkentin *et al.* 2007) or for a culture of a bacterial community from the Mediterranean Sea (0.1 to 2 fmol $cell^{-1}$ h^{-1}; Motegi *et al.* 2009). Marine bacteria in their natural environment exhibit even lower cell-specific respiration rates of around 0.003 fmol $cell^{-1}$ h^{-1} (Reinthaler *et al.* 2006; Baltar *et al.* 2009). While bacterial cell-specific respiration seemingly increases with depth (Baltar *et al.* 2009), the present study suggests that the cell-specific respiration of fungi is not majorly influenced by temperature or nutrient concentrations. *Sakaguchia dacryoidea* exhibited higher rates at lower nutrient concentrations and at the lower incubation temperature (Q_{10} = 0.89), pointing towards an adaptation of this species to nutrient-limited conditions as well as lower temperatures.

The Q_{10} of FR in this study – 2.14 (*S. dacryoidea*) and 4.49 (*R. sphaerocarpa*) – point towards an important influence of temperature on the total respiration of the cultures. The values from this study agree with a Q_{10} of 3.7 reported for microbial respiration in the mesopelagic zone of the South Atlantic and Indian Ocean (Mazuecos *et al.* 2015), while they are slightly higher than – for example – the Q_{10} of marine copepod respiration (1.8 - 2.1; Ikeda *et al.* 2001).

In general, respiration is accelerated by rising temperatures to a greater extent than primary production (Wohlers *et al.* 2009; Robinson 2019). The present results suggest that fungi are no exception, and the effect of temperature can be even more pronounced than for other organisms. This conclusion is also supported by Fuentes *et al.* (2015), who reported Q_{10} values of respiration between 2.2 and 6.8. In times of global warming, the increased respiration could further fuel climate change by increasing carbon losses to the atmosphere.

Fungal growth efficiency

The growth efficiency reported in this study represents the first ever measured FGE of marine fungi. Both *Rhodotorula* species exhibited high FGEs with values above 75 % in Experiment 1. This means, that over ¾ of the assimilated carbon is converted into biomass, whereas less than ¼ is respired to CO_2. In Experiment 2, both *Rhodotorula* species exhibited lower FGEs than in Experiment 1, though only *R. sphaerocarpa* can be directly compared due to the temperature. *Sakaguchia dacryoidea* revealed the surprising observation of a higher FGE in the low nutrient media of Experiment 2. This adds to the previous suggestion that this species is better adapted to lower nutrient concentrations and lower temperatures. *R. mucilaginosa* exhibited the highest FGE at 5°C in Experiment 1 and the lowest FGE in Experiment 2. Even though this species can grow rapidly at all conditions, the growth efficiency seems to depend strongly on the nutrient conditions.

To compare the fungal growth efficiency to the bacterial growth efficiency (BGE) of marine cultures, Table 5 reviews previous studies and summarizes growth efficiencies of different experiments. We found no reports of BGE higher than 70 %, so the FGE of *Rhodotorula mucilaginosa* (87 %) remains the maximum. Nevertheless, BGEs of around 50 % are commonly found in bacterial cultures, as well.

The BGE in the global ocean is lower than the one of cultures and ranges between less than 5 % in the open ocean to the exception of 60 % in nutrient-rich coastal waters (Del Giorgio and Cole 1998). A more recent study focusing on the north-eastern Pacific supports these findings with values as high as 20 % close to the coast and below 5 % towards the open ocean (Del Giorgio *et al.* 2011). In deep water masses, BGE values below 5 % were furthermore reported by Reinthaler *et al.* (2006).

According to this difference between bacterial cultures and observations from the open ocean, it can be presumed that the FGE in the open ocean would be lower than the FGE obtained in this study. A general nutrient dependency of FGE can not be suggested due to the different reactions of the species to the changing nutrient concentration. Nevertheless, a substantial FGE in nutrient rich environments like phytoplankton blooms can be expected.

Table 5. Review table of the growth efficiency of cultures of marine organisms from 1993 towards the present study.

Organism(s)	Culture conditions	Growth efficiency	Reference
Bacterial community from the Gulf of Mexico	Batch culture	61 %	Kroer 1993
Bacterial community of the Bothnian Sea	Batch culture *with* vs. without added nutrients	14 – 58 % 11 – 54 %	Zweifel *et al.* 1993
Bacterial community off the coast of Massachusetts	Batch vs. Continuous culture	34 – 70 % 43 – 58 %	Goldman and Dennett 2000
Marine bacterial isolate (*Vibrio harveyi*)	Pure culture with addition of glucose and iron	45 – 55 %	Kirchman *et al.* 2003
Bacterial community of the Mediterranean Sea	Seawater cultures with added phosphorus	17 – 70 %	Motegi *et al.* 2009
Marine bacterial isolates (*Roseobacter* and *Cytophaga*)	Pure culture in seawater and added Zobell medium	50 - 70 %	Teira *et al.* 2012
Marine bacterial isolates (*Vibrio splendidus* and *Phaeobacter gallaeciensis*)	Batch culture with glucose as carbon source	9 – 25 %	Goto *et al.* 2020
Marine fungal isolates (Basidiomycota)	Pure cultures in defined growth media	43 – 87 %	This study

The Q_{10} values of FGE obtained in this study were below 1, meaning that at higher temperatures, the conversion of assimilated carbon to biomass is less efficient than at lower temperatures. This agrees to the inverse relation of temperature and BGE across oceanic systems reported by Rivkin and Legendre (2001). While the production is accelerated by higher temperature, the respiration increases relatively more, which results in a lower FGE. Putting this again in the light of global warming, it could mean that with increasing temperature, the FGE decreases, and fungi act as sources of atmospheric CO_2 to a higher extent than they would do at lower temperatures.

Conclusion

This project is an important first step to achieve insight into the respiration of marine fungi. It combines two topics, that are rather difficult to access: the research on marine pelagic fungi, which gathered scientific interest only recently, and respiration, which measurement has always been viewed as difficult. The results suggest that fungal respiration, as well as production and growth efficiency are high when compared to bacterial cultures as well as natural bacterial communities. The nutrient concentration affects all mentioned parameters, but the response varies between species. Furthermore, the effect of temperature differences leads to the assumption, that with warming oceans, FR could increase and FGE could decrease, leading to an even higher loss of CO_2 to the atmosphere. Understanding the biological processes of oceanic systems is crucial to study the influence of climate change (Worden *et al.* 2015), and pelagic fungi can not be neglected from the processes governing the marine carbon cycle anymore. In the future, further research should focus on increasing the knowledge about respiration, production, and growth efficiency of marine pelagic fungi.

Zusammenfassung

Obwohl Pilze eine tragende Rolle im Abbau von organischem Material in terrestrischen und limnischen Ökosystemen innehaben, steht die Forschung über marine Pilze erst am Beginn. Kürzlich veröffentlichte Studien belegen jedoch ihr Vorkommen in allen marinen Lebensräumen sowie ihre Beteiligung am Abbau zahlreicher organischer Substanzen. Zusätzlich stellen parasitische Pilze an Diatomeen ein wichtiges Verbindungsglied im marinen Nahrungsnetz dar. Ihre Rolle im marinen Kohlenstoffkreislauf bleibt bisher jedoch unerforscht.

Das Ziel dieser Studie war, die Wachstumseffizienz von marinen Pilzen und deren Empfindlichkeit gegenüber Temperatur- und Nährstoffunterschieden zu untersuchen. Hierfür wurden sowohl der Sauerstoffverbrauch als auch die Biomasseproduktion von drei einzelligen, marinen Pilzarten (*Rhodotorula mucilaginosa*, *Rhodotorula sphaerocarpa*, und *Sakaguchia dacryoidea*) in Laborexperimenten bei zwei Temperaturen und Nährstoffkonzentrationen gemessen. Die beiden Parameter Respiration und Biomasseproduktion zeigten höhere Werte bei höherer Temperatur, während die Wachstumseffizienz das Gegenteil erwies. Die Nährstoffkonzentration beeinflusste die Parameter ebenso, jedoch zeigten die Arten unterschiedliche Reaktionen.

Während die Resultate solcher Laborversuche mit Reinkulturen niemals die im offenen Ozean vorherrschenden Bedingungen widerspiegeln können, sind sie doch ein wichtiger erster Einblick in die Dynamik der Wachstumseffizienz von marinen Pilzen. Die Untersuchung der genannten Parameter liefert wichtige Informationen über die Rolle von marinen Pilzen im Kohlenstoffkreislauf, besonders während Algenblüten, wo sie als Zersetzer von organischem Material agieren. Ob marine Pilze als Kohlenstoffquelle oder -senke agieren, gilt es zu erforschen, besonders im Hinblick auf im Zuge des Klimawandels steigende Temperaturen und Kohlendioxidkonzentrationen in der Atmosphäre.

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