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Do inferences about freshwater phytoplankton communities change when based on microscopy or high-throughput sequencing data?

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Abstract

1. Microscopy and high-throughput sequencing (HTS) detect and quantify algae differently. It is not known if microscopy-based abundance or biomass better compare to HTS data or how methodological differences affect ecological inferences about the phytoplankton communities studied.
2. We investigated methodological ($abundance_{microscopy}$ vs. $abundance_{HTS}$, $biomass_{microscopy}$ vs. $abundance_{HTS}$), habitat (littoral, pelagic, deep hypolimnion), and year (2014 vs. 2017) differences for phytoplankton communities of Lake Tovel (Italy) using ANOVA. Specifically, we tested the hypothesis that depending on comparing $abundance_{microscopy}$ or $biomass_{microscopy}$ to $abundance_{HTS}$ different effects would be indicated; we called this the metric effect. Furthermore, using samples from 2014 to 2017, we investigated environment–community relationships by a redundancy analysis based on $abundance_{microscopy}$, $biomass_{microscopy}$, and $abundance_{HTS}$, and compared the results.
3. Approximately 9 times more operational taxonomic units were reported with HTS ($n_{2014} = 819$, $n_{2017} = 891$) than algal taxa with microscopy ($n_{2014} = 90$, $n_{2017} = 109$) in 2014 and 2017. While microscopically assessed algal taxa were evenly distributed among phyla, the vast majority of operational taxonomic units were attributed to Chrysophyta (2014 = 54%, 2017 = 62%) and Bacillariophyta (2014 = 19%, 2017 = 17%). A metric effect for method differences was generally observed comparing $abundance_{microscopy}$ to $abundance_{HTS}$ with Chlorophyta, Cryptophyta, and Dinophyta showing higher % abundance with microscopy while richness and Chrysophyta showed higher values with HTS. Almost no metric effects were found in 2014, but they were common across phyla in 2017. Bacillariophyta and Eustigmatophyta showed the same habitat differences when comparing $biomass_{microscopy}$ to $abundance_{HTS}$.
4. Dinophyta showed habitat differences only with microscopy, while Chyrsophyta showed habitat differences only with HTS; these results were probably related to technical bias and strengths of HTS, respectively.
5. Habitat differences of phyla were reasonably related to their ecological niche and linked to factors such as temperature and feeding preferences; furthermore, phyla often showed a significant 2014-versus-2017 year effect. The year 2014 was very

wet while 2017 had a dry winter, and we attributed the patterns found to allochthonous nutrient input by rain and decreased turbulence. Redundancy analyses based on phytoplankton communities assessed with microscopy and HTS, respectively, equally indicated the importance of hydrology, nutrients, and temperature for phytoplankton communities and discriminated the littoral from the deep hypolimnion. However, variance explained was higher with HTS, and the pelagic was similar to the deep hypolimnion with microscopy but to the littoral with HTS.

6. Despite the different strengths of microscopy and HTS for biodiversity assessment, both datasets outlined similar large-scale patterns linked to strong environmental control of phytoplankton communities as they related to habitat and year differences. According to our hypothesis, metric effects were common; however, no general rule was found as to whether $abundance_{microscopy}$ or $biomass_{microscopy}$ should be compared to $abundance_{HTS}$. Notwithstanding metric effects, HTS-based data provided similar and more detailed information than microscopy, supporting the promise of HTS becoming the tool of the future for biodiversity research.

KEYWORDS

18S rRNA, habitat differences, high-throughput sequencing, Lake Tovel, redundancy analysis

1 | INTRODUCTION

Freshwater and marine phytoplankton play a pivotal role in the food web and in biogeochemical cycles (Litchman et al., 2015). The characterisation of plankton biodiversity is at the basis of any assessment of ecosystem state and environmental change, and phytoplankton-based methods are commonly used (e.g. Water Framework Directive, 2000/60/EC). Algal taxa are traditionally identified and counted with a microscope, a time-consuming process requiring expert knowledge. In this process, many algal species are too small to be identified by light microscopy, while others are rare and/or are cryptic species (i.e. do not have readily distinguishable morphological characteristics). However, high-throughput sequencing (HTS) providing millions of reads can revolutionise biodiversity assessment (Bush et al., 2019; Keck et al., 2017; Taberlet, Coissac, Pompanon, Brochmann, & Willerslev, 2012).

For HTS, specific barcodes are selected for different microalgae. The 16S rRNA gene is used for cyanobacteria (e.g. Liu, Yang, Yu, & Wilkinson, 2015; Obertegger, Bertilsson, Pindo, Larger, & Flaim, 2018) and also for eukaryotic microalgae (e.g. Bennke, Pollehne, Müller, Hansen, Kreikemeyer, & Labrenz, 2018), the rbcL gene for benthic diatoms (e.g. Rimet et al., 2018a; Vasselon et al., 2017), the tufA gene for green algae (Saunders & Kucera, 2010; Vieira et al., 2016), and the 18S rRNA gene for protists (e.g. Bradley, Pinto, & Guest, 2016; Piredda et al., 2017). The sequences obtained by these specific barcodes are linked to species names from well-curated and open-access reference libraries (Table 1). Despite the relative ease of obtaining sequences with HTS, downstream analyses that turn sequences into operational taxonomic units (OTUs) and assign taxonomic names require proper training (Shade, Dunivin, Choi, Teal, & Howe, 2019); this analytical bottleneck can be circumvented by specialised workshops (Shade et al., 2019) and the use of commercial (e.g. https://digitalinsights.qiagen.com/) or open-source software (e.g. https://usegalaxy.org/). The possibility of repeating bioinformatics analyses and taxonomic assignment, once sequences are obtained, is an advantage of HTS, hardly achieved with microscopy, which requires re-analysing stored samples. However, the polymerase chain reaction (PCR) of HTS-based methods inflates sequencing success and abundance estimation (Krehenwinkel et al., 2017; Nichols et al., 2018; Vasselon et al., 2018; Stern et al., 2018), and there is no easy solution on how to correct any technical bias (Pawlowski et al., 2018); in contrast, microscopy has the advantage of providing both abundance and biomass and information about algal morphology (e.g. size, life stages), impossible as yet to assess with HTS.

Microscopy- and HTS-based methods vary in their abilities to detect and quantify algal taxa (Boopathi & Ki, 2016; Eiler et al., 2013; Groendahl, Kahlert, & Fink, 2017; Zimmermann, Glöckner, Jahn, Enke, & Gemeinholzer, 2015). As many researchers emphasise (e.g. Gran-Stadniczeñko et al., 2019; Hardge et al. 2018; Xiao et al., 2014), HTS-based methods can process over 50 times the sample volume with respect to standard microscopy-related methods, and thus, not surprisingly, HTS generally reports a higher diversity compared to microscopy (e.g. Abad et al., 2016; Boopathi et al., 2015; Groendahl, Kahlert, & Fink, 2017; Lara et al., 2015; Rimet, Vasselon, Barbara, & Bouchez, 2018; Xiao et al., 2014). However, many novel OTUs lack a taxonomic assignment, and many microbial eukaryotes with a taxonomic assignment lack a DNA identity (De Vargas et al., 2015; Eiler et al., 2013; Stern et al., 2018; Stoeck & Epstein, 2003), thus hindering taxonomic assignment of OTUs

TABLE 1 Overview of barcodes and open access reference libraries used for specific algal groups

Group	Barcode	Reference library with link		Reference	Note
Cyanobacteria, protists	**16S, 18S**	**SILVA**	**https://www.arb-silva.de/**	**Quast et al. (2013)**	
Diatoms	rbcL	diat.barcode	https://www6.inrae.fr/carrtel-collection_eng/Barcoding-database;	Rimet et al. (2018b)	
Photosynthetic eukaryotes	16S	phytoref	http://phytoref.sb-roscoff.fr/doblast	Decelle et al. (2015)	
Protists	18S	PR2	https://github.com/pr2database/pr2database/wiki	Guillou et al. (2013)	Includes EukRef (del Campo et al., 2018) with the new release
Planktonic foraminifera	18S	PFR2	http://pfr2.sb-roscoff.fr/	Morard et al. (2015)	
Green algae	tufA			Marcelino and Verbruggen (2017)	QIIME-friendly format

in downstream analysis of HTS data. Furthermore, one gene does not always separate all species, as it is the case for green algae (Marcelino & Verbruggen, 2016) where the 18S rRNA gene is too conserved (Krienitz & Bock, 2012). Thus, it is very common that HTS detects phyla that remain undetected by microscopy (e.g. Eiler et al., 2013; Gao et al., 2018; Liu et al., 2009; Xiao et al., 2014), while microscopy detects species that remain undetected by HTS (Xiao et al., 2014). Furthermore, for certain phyla such as Dinophyta and Bacillariophyta, HTS shows a varying detection sensitivity with respect to microscopy; for example, fewer species (Dinophyta: Eiler et al., 2013), less percent dominance (Bacillariophyta: Piredda et al., 2017; Wright, Mitchelmore, Place, Williams, & Orano-Dawson, 2019), more species (Dinophyta: Xiao et al., 2014), and a higher percent dominance (Dinophyta: Piredda et al., 2017) are reported with HTS compared with microscopy. These differences could be linked to both bias in HTS (Pawlowski et al., 2018) and to varying taxonomic expertise of the operator providing algal counts and biomass values (Straile et al. 2015). Furthermore, similar relative abundance (Bacillariophyta: Banerji et al., 2018) and a positive correlation between biodiversity indices obtained with HTS and microscopy (Bacillariophyta: Rimet et al., 2018b; Rivera, Vasselon, Bouchez, & Rimet, 2020) is also reported.

Considering the different strengths inherent to microscopy and HTS, several studies (e.g. Abad et al., 2016; Boopathi & Ki, 2016; McManus & Katz, 2009; Xiao et al., 2014) advocate the parallel use of both methods, especially when aiming at characterising the whole phytoplankton community and not only specific algal groups. Few studies can afford this double approach because of financial, temporal, and personnel limits, and thus we know little about how results obtained by microscopy-based methods compare to results obtained by HTS-based methods investigating the whole phytoplankton community. While most comparative studies focus on completeness of taxonomical inventories, few studies (Abad et al., 2016; Amorim et al. 2015; Eiler et al., 2013; Gao et al., 2018; Gran-Stadniczeñko et al., 2019) compare ecological inferences made with both traditional and more innovative techniques, and they report contrasting or similar results. Microscopy and HTS indicate similar seasonal dynamics of phytoplankton composition (Eiler et al., 2013) and similar temporal differences in Bacillariophyceae among winter and early spring (Gran-Stadniczeñko et al., 2019). Phytoplankton based on microscopy and HTS equally discriminate sampling stations (Gao et al., 2018) and the ecological status of rivers using a diatom index (Amorim et al. 2015; Vasselon et al., 2017). While Abad et al. (2016) report a good agreement of spatial and temporal patterns of marine zooplankton communities assessed with microscopy and HTS in multivariate ordination, this was not the case for phytoplankton. Furthermore, few freshwater studies that investigate the whole phytoplankton community (Xiao et al., 2014; Gran-Stadniczeñko et al., 2019) compare traditional approaches with HTS for more than 1 year; most studies are snapshots of biodiversity (Charvet, Vincent, & Lovejoy, 2012; Stoeck et al., 2014; Groendahl et al., 2017) or cover few months (Abad et al., 2016; Eiler et al., 2013; Boopathi & Ki, 2016; Moreno-Pino et al., 2018). Thus, studies are needed that

compare inferences about the whole phytoplankton community made with microscopy- and HTS-based data over a multi-year period.

Ecological studies on phytoplankton communities based on microscopy often report both abundance and biomass or biovolume, respectively (e.g. Zohary, 2004), and for the ecological status assessment of European lakes, total algal biomass is generally reported (Pasztaleniec, 2016). While some studies (e.g. Abad et al., 2016; Groendahl et al., 2017; Rimet et al., 2018) focus on abundance in their comparison with HTS, others focus on biomass or biovolume (e.g. Charvet et al., 2012; Gao et al., 2018; Gran-Stadniczeñko et al., 2019). Thus, no consensus exists on what to use in comparisons with HTS data. With microscopy, abundance data have their validity when focusing on similar-sized taxa or focusing on one species while biomass is preferred when focusing on the whole phytoplankton community because abundance is biased in favour of small cells. Apart from technical issues (e.g. primer choice, primer efficiency), one major biological issue in the comparison between microscopy and HTS data is the variation in gene copy number per cell for each species that affects sequence abundance per species (Vasselon et al., 2018). In laboratory experiments, cell length predicts the 18S gene copy number for 18 algal strains representing several eukaryotic classes (r^2 = 0.75; Zhu, Massana, Not, Marie, & Vaulot, 2005), and algal cell biovolume predicts the rbcL gene copy number for eight diatom species (r^2 = 0.94; Vasselon et al., 2018). For field samples, the total 18S gene copy number per sample predicts the total biovolume of diatoms (r^2 = 0.65; Godhe et al., 2008), and also ciliates show a higher concordance between sequence abundance and biomass rather than cell abundance (Pitsch et al., 2019). Even though HTS data of the whole phytoplankton community are better correlated with microscopically assessed abundance than biomass (Eiler et al., 2013), and studies may tend to prefer abundance in their comparisons, the latter results indicate that cell biovolume and biomass also are a valid proxy for gene copy number. In any case, no correlation between cell abundance and sequence abundance has also been found (Weber & Pawlowski, 2013; Stoeck et al., 2014). Thus, if HTS is used quantitatively and is now becoming an alternative method for environmental monitoring of the whole phytoplankton community (Hering et al., 2018), it is important to understand how HTS data relate to abundance and biomass assessed with microscopy in different habitats and in different years.

Here, we assessed microscopy abundance and biomass and HTS sequence abundance, and compared inferences on phytoplankton communities made with both approaches. For HTS, the 18S rRNA gene was used that covers a wide range of different microalgae and is the preferred barcode for phytoplankton (Bennke et al., 2018). Furthermore, we focused on three distinct habitats within Lake Tovel that reflect a varying degree of hydrological stability, light climate, and community composition; the littoral is the most hydrologically unstable because it receives >80% of the lake's water inflow through underground springs (Borsato & Ferretti, 2006) while the hypolimnion is considered the most stable because of its sheltered position from the in- and outflow, and the pelagic is intermediate (Obertegger et al., 2018). Furthermore, the littoral shows highest, the hypolimnion lowest, and the pelagic intermediate light transparency (Obertegger, Pindo, & Flaim, 2019), an important parameter for phytoplankton (Richardson, Beardall, & Raven, 1983). Planktonic communities respond to hydrological stability (Winder & Hunter, 2008; Shade, Jones, & McMahon, 2008; Obertegger et al., 2018) and changing light climate (Edwards, Thomas, Klausmeier, & Litchman, 2016), and varying community composition in different habitats should be equally indicated by microscopy and HTS data. Specifically, we (1) tested for method ($abundance_{microscopy}$ vs. $abundance_{HTS}$, $biomass_{microscopy}$ vs. $abundance_{HTS}$), habitat (littoral, pelagic, hypolimnion), and year differences (2014 vs. 2017) in phytoplankton community composition, and (2) tested the hypothesis that these differences were not equally indicated by microscopically assessed abundance and biomass, respectively, compared to sequence abundance; we call this the metric effect. The most fortunate situation is not finding any metric effect. However, when a metric effect is present, the best case is when microscopically assessed abundance or biomass, respectively, and sequence abundance indicate the same effect while the worst case is when an effect is indicated by only one method, microscopy or HTS. The latter result could be related to the technical strength or bias of microscopy or HTS. We (3) related HTS and microscopy data, both abundance and biomass, to environmental parameters in a redundancy analysis (RDA) and compared the results. Thus, our study fills a knowledge gap by providing a comprehensive overview on the comparability of microscopy and HTS-based data and on the strength of HTS to draw ecological inferences with respect to microscopy data; this is an important aspect when HTS will gradually substitute microscopy in the future.

2 | METHODS

2.1 | Site description

Lake Tovel (LTER site IT09-005-A; 46.261 N, 10.949 E; 1178 m above sea level) is a glacial lake (area: 0.4 km^2; maximum depth: 39 m; mean depth: 19 m; volume: 7.4×10^6 m^3) located in the Brenta Dolomites (Trentino, Italy). Geological substrate is dolomite and limestone, and the pseudokarst catchment leads to marked changes in water level (Borsato & Ferretti, 2006). The lake has a deep (39 m) north-east basin, partially separated by a submerged dyke from a shallow (4 m) south-west basin.

2.2 | Sampling

We sampled three distinct habitats within the lake: the littoral (0–4 m over the deepest part of the shallow basin), the pelagic euphotic zone (0–20 m over the deepest part of the main basin), and the deep hypolimnion (in the deepest part of the main basin at a depth from 30 m to 35 m). Sampling was done monthly during the ice-free period from April 2014 to December 2017. The shallow basin usually dries

out completely during winter and refills in spring through snowmelt inflow; thus, under-ice and often spring sampling were not possible.

2.3 | Environmental variables

In the shallow basin, water samples for nutrients (NO_3 [µg/L], NH_3 [µg/L], PO_4 [µg/L], silica [mg/L]) were taken at 0 and 4 m and were averaged. In the main basin, water samples for nutrients were taken at 5-m intervals, and 0-, 5-, 10-, 15-, and 20-m values were averaged for the pelagic and 30- and 35-m values for the deep hypolimnion. Water transparency (i.e. light penetration of photosynthetically active radiation; PAR) was assessed by a LICOR radiometer (LI 250A). Percentage light transmission (% transmission) was calculated from the coefficient of attenuation as % PAR reaching 4 m for the littoral, and 20 and 35 m, respectively, for the two habitats in the main basin. Temperature values taken with a multiparametric probe (Idronaut Ocean Seven 316 Plus) at 1-m intervals were averaged within the respective profile for the littoral (0–4 m), pelagic (0–20 m), and deep hypolimnion (30–35 m). Precipitation (mm), provided from the onshore meteorological station, and water level change (cm) were averaged for the 10 days before sampling as in Obertegger et al. (2019). Total yearly precipitation (mm) at Lake Tovel showed a decreasing gradient from 2014 to 2017 (2014: 1867 mm; 2015: 1027 mm; 2016: 1110 mm; 2017: 978 mm; Obertegger et al., 2018).

2.4 | Sampling of phytoplankton communities

Integrated water samples for microscopy and HTS were taken with a weighed tube for the littoral and the pelagic; for the deep hypolimnion, bottle samples from 30 and 35 m were combined. All phytoplankton samples for microscopy were immediately preserved with acidified Lugol's solution, and samples for HTS were stored at 4°C until filtration in the laboratory within 24 hours.

2.5 | Microscopy-based analysis of phytoplankton

Quantitative phytoplankton analysis was according to the Utermöhl method (Lund, Kipling, & Le Cren, 1958). For each sample, a 25-mL aliquot of Lugol-preserved sample was sedimented for at least 24 hours in a sedimentation chamber. Counts and measurements were made with an inverted microscope (LEICA DMIRB) linked to a DFK41BF02 digital camera (The Imaging Source Europe GmbH, Bremen, Germany) and using the software PlanktoMetrix (Zohary, Shneor, & Hambright, 2016). For each sample, at least 400 individual entities (filament, colony, or single-celled organisms) were counted at 400× magnification. Algal biomass (µg/L) was estimated from species-specific biovolume, obtained by geometrical approximations according to Hillebrand, Dürselen, Kirschtel, Pollingher, & Zohary (1999) and Sun & Liu (2003). The PlanktoMetrix software greatly facilitates biovolume measurements, and therefore it was possible to measure 10-25 individuals for each taxon per sample. Species were identified using updated phytoplankton taxonomic literature, and nomenclature was according to Guiry & Guiry (2019). Taxa without a proper taxonomic assignment (e.g. small flagellates) were assigned to *algae incertae sedis*. Some phytoplankton samples were lost resulting in 94 samples (littoral: $n = 29$; pelagic: $n = 36$; deep hypolimnion: $n = 29$). The subscript MIC indicates algal groups and phyla assessed with microscopy (e.g. $Dinophyta_{MIC}$).

2.6 | DNA extraction for HTS

For DNA extraction, generally 1.5 L of water from each of the three habitats was gently vacuum-filtered onto sterile 0.2-µm membrane filters (Supor 200 Membrane Disc Filters, 47 mm; Pall Corporation, East Hills, NY, U.S.A.). Filters were stored at −80 °C until further processing. DNA was extracted from the filters with the PowerWater DNA isolation Kit (MOBIO Laboratories Inc, CA, U.S.A.) and processed as described below.

2.7 | Gene amplification, library construction, and sequencing

The preferred barcode to assess taxonomic and phylogenetic diversity of phytoplankton and other protists is the 18S rRNA (Bennke et al., 2018). Here, environmental DNA was PCR amplified by targeting a 470-basepair fragment of the eukaryotic 18S rRNA variable region V4 (primer set Next.For [5′-CCAGCASCYGCGGTAATTCC-3′] and Next.Rev [5′-ACTTTCGTTCTTGATYRATGA-3′]; Piredda et al., 2017) with overhanging Illumina adapters. Polymerase chain reaction amplification and library construction were performed as described in Obertegger et al. (2018). All libraries were pooled in equimolar concentrations in a final amplicon library and analysed on a Typestation 2200 platform (Agilent Technologies). Barcoded libraries were sequenced on an Illumina®MiSeq (PE300) platform (MiSeq Control Software 2.6.2.1 and Real-Time Analysis software 1.18.54) of the Fondazione Edmund Mach.

2.8 | Sequence analysis

Sequences were processed with MICCA version 1.7.2 (Albanese, Fontana, De Filippo, Cavalieri, & Donati, 2015). Paired-end sequences from the different samples were merged, forward and reverse primers were trimmed, and sequences were quality-checked (discarding sequences with an expected error rate > 0.5% and shorter than 470 nucleotides). Finally, we used the de novo greedy OTU clustering algorithm OTUCLUST as implemented in MICCA (Albanese et al., 2015) with a similarity threshold of 99% (Edgar, 2018) to cluster sequences. Singletons and doubletons of sequences were eliminated as potential chimeras. The 18S sequence data were submitted to the EMBL database under Accession no.

PRJEB32348. The 18S OTUs were taxonomically classified with CREST using SilvaMod128 (Lanzén et al., 2012) and with PR2 (Guillou et al., 2013). Only when CREST assignment stopped at a lower taxonomical level than PR2 and when CREST and PR2 indicated the same high order classification, the PR2 classification reaching a higher taxonomical level was used. In addition, we checked all OTUs by manual Blast against NCBI to verify the taxonomic assignation. Nevertheless, some OTUs did not have a taxonomic assignment beyond Eukaryota, Stramenopiles, or marine Stramenopiles and were assigned to *algae incertae sedis*. Animals, fungi, higher plants, and parasitic taxa including parasitic dinoflagellates (Table S1) were excluded to obtain a dataset of 18S phytoplankton-like OTUs.

Not all samples amplified and one sample showed extremely low sequencing depth (487 sequences per sample). Thus, 86 samples remained for analyses (littoral: $n = 25$; pelagic: $n = 26$; hypolimnion: $n = 35$). Sequencing depth of this dataset of phytoplankton-like OTUs ranged from 2999 to 47 530 sequences per sample (median = 12 442; mean = 13 529, standard deviation = 8250) with a total of 2176 OTUs. The OTU table was rarefied without replacement to the lowest abundance obtained for a sample. The subscript HTS indicates groups and phyla assessed with HTS (e.g. $\text{Dinophyta}_{\text{HTS}}$).

2.9 | Statistical analysis

Cyanobacteria were counted with microscopy but cannot be detected using the 18S rRNA barcode, and therefore we excluded cyanobacteria from all analyses. With microscopy, cyanobacteria showed a high % contribution to abundance and a low % contribution to total biomass (mean yearly value < 10%; Table S2). Because cyanobacteria were included in the phytoplankton counts, other algae could have been less represented, a situation similar to preferential PCR amplification (Wintzingerode, Gobel, & Stackebrandt, 1997) that reduces sequencing success for certain taxa in HTS. Thus, α-diversity estimates based on microscopy might be an underestimation of algal biodiversity because of this technical bias. For a comparison between microscopy and HTS, we only considered those sampling months (1) where both datasets had data and (2) most months per year were available (i.e. 2014: April, June, August, October, November; 2017: June, July, August, October, November).

The assessment of habitat generalists and specialists plays an important role in biogeographical patterns and inference on community assembly (Luo et al., 2019). We assessed the % entities (algal taxa and OTUs) shared among habitats (i.e. common taxa) and unique to habitats (i.e. habitat-specific taxa) for single periods (i.e. Venn diagram).

Alpha diversity indices of phytoplankton are often linked to environmental cues such as warming and brownification (Urrutia-Cordero et al., 2017), depth (Novais et al., 2019), and conductivity gradients (Stefanidou et al., 2020). We calculated richness and evenness based on $\text{abundance}_{\text{MIC}}$, $\text{biomass}_{\text{MIC}}$, and $\text{abundance}_{\text{HTS}}$. Differences in α-diversity were assessed by a two-way ANOVA with method (microscopy vs. HTS: $\text{abundance}_{\text{MIC}}$ vs. $\text{abundance}_{\text{HTS}}$, $\text{biomass}_{\text{MIC}}$ vs. $\text{abundance}_{\text{HTS}}$) and habitat (all comparisons between littoral, pelagic, and hypolimnion) as factors for the years 2014 and 2017. In two-way ANOVA, we focused on significant differences between methods for the same habitat (e.g. richness in the littoral: HTS > microscopy) and between habitats for the same method (e.g. richness assessed with HTS: littoral > deep hypolimnion). The same months were available for 2014 and 2017 (June, August, October, November), and thus testing for between-year differences was possible by a three-way ANOVA; in three-way ANOVA, we focused on significant differences between years for the same method (e.g. HTS—littoral: 2014 < 2017) and did not report differences between methods and habitats. When the interaction effect was statistically significant, post hoc mean-separation testing was conducted only on the interaction effect (Mangiafico, 2016).

Trait research holds the potential to increase our ability to explain and predict community changes (Litchman & Klausmeier, 2008). We focused on carbon acquisition (i.e. autotrophy, mixotrophy, heterotrophy) because of its importance for phytoplankton fitness. While with microscopy each taxon can be assigned to a trophic role based on genus or species identification, with HTS this assignation is based on phylum or family level (Machado et al., 2019; Minicante et al., 2019). Furthermore in biodiversity assessments, the % contribution of phyla to the total community is often compared between microscopy and HTS (Eiler et al., 2013; Giner et al., 2016; Piredda et al., 2017; Wright et al., 2019; Xiao et al., 2014). Thus, we assessed the % abundance and % biomass of trophic groups and phyla with respect to total abundance and biomass, respectively, and the % sequence abundance of each trophic group and phylum, respectively, with respect to total sequence abundance. Heterotrophs showed very low % $\text{abundance}_{\text{MIC}}$, $\text{biomass}_{\text{MIC}}$, and $\text{abundance}_{\text{HTS}}$ for most samples (Table S3), and thus were not considered. Because of the low contribution of heterotrophs to % values of trophic role, autotrophs and mixotrophs were inversely related (high values of autotrophs implied low values of mixotrophs), and thus only % autotrophs were tested. Testing for a method, habitat, and a year effect was done as for α-diversity indices.

We performed an RDA to link environmental data to algal taxa and OTUs, respectively. From the available samples (microscopy $n = 94$; HTS: $n = 86$), all samples were used for which data based on microscopy and on HTS were available ($n = 75$). Even though this resulted in a dataset with a different number of samples per habitat ($n_{\text{littoral}} = 24$, $n_{\text{pelagic}} = 25$, $n_{\text{hypolimnion}} = 26$) and year ($n_{2014} = 22$, $n_{2015} = 15$, $n_{2016} = 16$, $n_{2017} = 22$), it reflected a real-world example of data acquisition and inferences made on the same community by two different methodological approaches. Species data (algal taxa and OTUs) were Hellinger transformed. For algal taxa, we used (1) abundance and (2) biomass data. For OTUs, we used (1) all OTUs and (2) OTUs with a minimum of 10 reads in the entire dataset ($\text{OTUs}_{>10\text{ reads}}$) similar to Gran-Stadniczeñko et al. (2019) and Palacin-Lizarbe et al. (2019) to focus on abundant OTUs and reduce noisiness in the data. In these four RDAs, we applied forward selection of environmental predictors (precipitation, water level change, % transmission, temperature, NO_3, NH_3, PO_4, silica) and reported the explained variability corrected for the number of observations

and parameters of the fitted model (r^2 adjusted; Borcard, Gillet, & Legendre, 2018).

All statistical analyses were performed using R 3.6.1 (R Core Team, 2019), package lsmeans (Russell 2016), packfor (Dray et al.), and vegan (Oksanen et al. 2019).

3 | RESULTS

3.1 | Biodiversity assessment for the years 2014 and 2017

For 2014 and 2017, approximately 9 times less algal taxa were reported with microscopy (n_{2014} = 90, n_{2017} = 109) than 18S phytoplankton-like OTUs with HTS (n_{2014} = 819, n_{2017} = 891). There were few common OTUs and most OTUs were rare (common OTUs: n_{2014} = 156; n_{2017} = 143; rare OTUs: $\%_{2014}$ = 81, $\%_{2017}$ = 84), showing ≤10 reads in all samples. Taxa richness of *algae incertae sedis* assessed with microscopy and HTS (Table S4), $biomass_{MIC}$ and $abundance_{HTS}$ (Table S5) were similarly low while $abundance_{MIC}$ of *algae incertae sedis* was relatively high (Table S5). Generally, more taxa were reported per phylum with HTS except for the phyla Chlorophyta, Charophyta, and Haptophyta with the latter two having low richness (≤5 $taxa_{MIC}$; Table S4). While with microscopy an even distribution of taxa among phyla was evident, with HTS most OTUs were attributed to Chrysophyta (2014 = 54%, 2017 = 62%) and Bacillariophyta (2014 = 19%, 2017 = 17%; Table S4). With microscopy, only one species of the phylum Eustigmatophyta (*Pseudotetraëdriella kamillae*) was reported while, with HTS, 10 times more OTUs were reported. Xanthophyta (OTUs n_{2014} = 3, n_{2017} = 4) showed low % sequence abundance (<1%; Table S6) and were only detected with HTS. With microscopy, 32% of taxa did not have a species-level attribution, while, with HTS, 68% of OTUs did not even have a genus-level attribution.

Considering the number of entities (i.e. algal taxa or OTUs) unique to habitats for the years 2014 and 2017, HTS generally revealed a higher percent of unique entities in all habitats and less entities shared between habitats (Figure 1).

3.2 | ANOVA testing—α-diversity and functional groups

As expected, $richness_{HTS}$ (Table S6) was higher than $richness_{MIC}$ for both years. Furthermore for 2017, method-specific habitat differences were found ($richness_{HTS}$: deep hypolimnion < littoral and

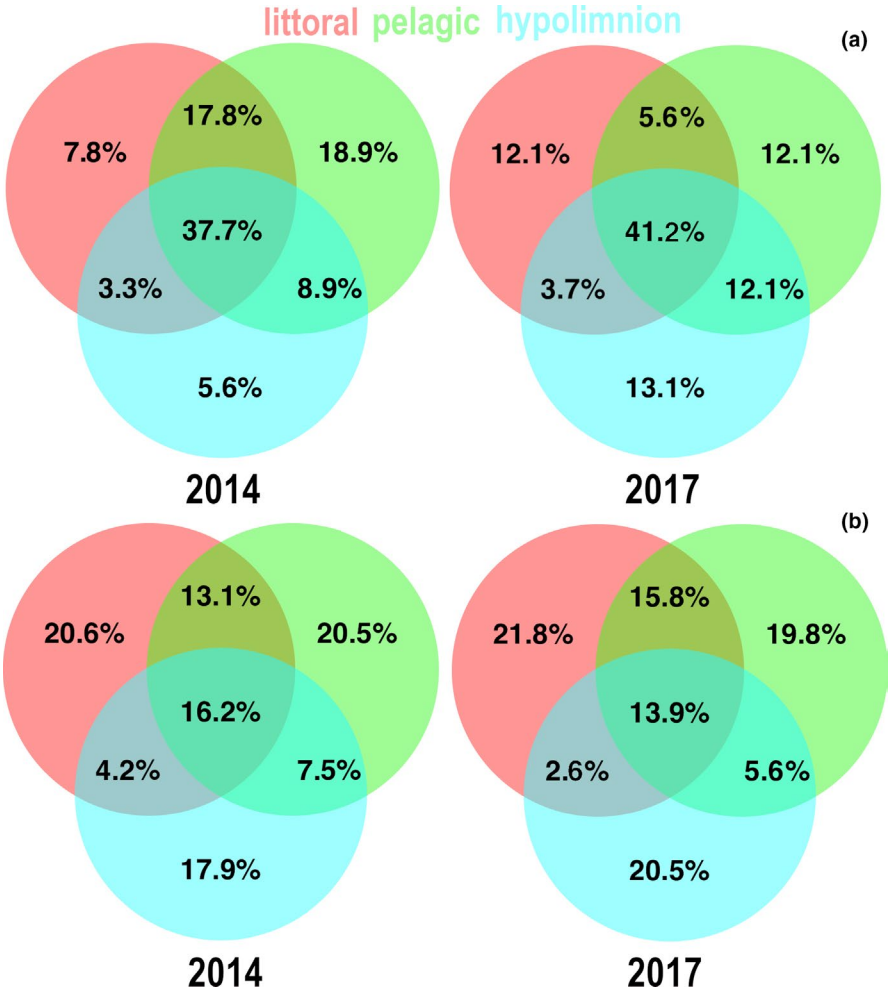

FIGURE 1 Venn diagram for microscopy (a) and high-throughput sequencing (b) for the years 2014 and 2017; given is the present of unique and shared algal taxa (a) and operational taxonomic units (b), respectively, between habitats; colour code refers to all panels

TABLE 2 Summary of two-way and three-way ANOVA with indices of α-diversity and % autotrophs; results for taxa richness are independent of abundance (abund) or biomass (mass) assessment with microscopy (MIC); the method effect (testing high-throughput sequencing [HTS] against MIC) relates to the difference between microscopy (either abundance or biomass) and HTS (operational taxonomic unit abundance); habitat differences (hab) refer to differences between the littoral, pelagic, and hypolimnion (hypo); in the three-way ANOVA, we focused on the year effect (2014 vs. 2017); significant effects (method and habitat effect in two-way ANOVA and year effect in three-way ANOVA) and their significance are reported, followed by significant differences as assessed by post hoc testing; only significant results are reported; when a significant interaction effect was found, significant main effects were not reported when included in the interaction effect (indicated by a colon); the > sign indicates interactions for which level higher values were found; significance levels: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, not significant (n.s.) $p > 0.05$; year$^{n.s.}$ indicates no significant year effect

Testing HTS against		Two-way ANOVA		Three-way ANOVA
		2014	2017	2014 vs. 2017
Richness				
MIC	**Abund or mass**	**Method***; **HTS > mic**	**hab: method*; HTS > MIC; HTS: littoral > hypo*; pelagic > hypo****	**year**; 2017 > 2014**
Evenness				
MIC	Abund	Method***; MIC > HTS	Method***; Mic > hts	year*; 2017 > 2014
	Mass	Method***; MIC > HTS	Method***; Mic > hts	year$^{n.s.}$
% autotrophs				
MIC	Abund	n.s.	hab: method**; MIC > HTS: littoral**, pelagic***; HTS: hypo > littoral***, hypo > pelagic***	n.s.
	Mass	n.s.	hab***; hypo > littoral***, hypo > pelagic***	n.s.

pelagic; Table 2). In both years, evenness$_{MIC}$ showing higher values than evenness$_{HTS}$.

In 2014, % autotrophs showed neither method nor habitat differences with microscopy and HTS (Table 2). In 2017, HTS showed higher % values, but only when comparing abundances. In addition, the same habitat differences (i.e. higher values in the deep hypolimnion than in the littoral and the pelagic) were only indicated by HTS when comparing abundances while they were indicated by both methods when comparing biomass$_{MIC}$ to abundance$_{HTS}$ (Table 2).

Comparing years, 2017 had higher mean values for richness. Similarly, evenness had higher values in 2017 but only when comparing evenness$_{MIC\text{-}abundance}$ to evenness$_{HTS}$ (Table 2). For % autotrophs, microscopy and HTS did not indicate any year differences.

3.3 | ANOVA testing of phyla (% values)

Chlorophyta and Haptophyta were only sporadically found with HTS, and Xanthophyta were not found with microscopy (Table S5, S6); thus, these phyla were not analysed with ANOVA.

For Bacillariophyta (Table 3) method and habitat differences were the same as for % autotrophs (Table 2). However, while no year differences were found for % autotrophy, Bacillariophyta showed higher values in 2014 than in 2017 for the littoral when comparing abundance$_{MIC}$ to abundance$_{HTS}$ and for the littoral and the pelagic when comparing biomass$_{MIC}$ to abundance$_{HTS}$.

Charophyta showed neither method nor habitat differences with microscopy and HTS (Table S6; Table 3) in 2014. In 2017, only abundance$_{MIC}$ showed higher values than abundance$_{HTS}$. Comparing years, no differences were found with microscopy or HTS.

Chrysophyta showed higher values with HTS in both years (Table S6; Table 3). While in 2014 no habitat differences were found, in 2017 higher values in the littoral and pelagic than in the hypolimnion were only found with HTS. Comparing years, only with HTS the littoral and pelagic showed higher values in 2014.

Cryptophyta (Table S6; Table 3) showed higher values with microscopy in both years. In addition, the littoral showed higher values than the other habitats in 2017 with both methods but only when comparing abundance$_{MIC}$ to abundance$_{HTS}$. Comparing years, no differences were found with microscopy and HTS.

Dinophyta showed higher values for all habitats with microscopy in 2014. In 2017, however, Dinophyta showed higher values with microscopy only for the littoral and the pelagic when comparing abundance$_{MIC}$ with abundance$_{HTS}$ and for all habitats when comparing biomass$_{MIC}$ to abundance$_{HTS}$ (Table S6; Table 3). Comparing years, different habitats showed higher values in 2014 only with microscopy.

In both years, Eustigmatophyta showed higher values with abundance$_{MIC}$ but not with biomass$_{MIC}$ compared to abundance$_{HTS}$. While habitat differences (i.e. higher values in the deep hypolimnion than the littoral) were generally the same, they were not equally indicated by microscopy and HTS (Table S6; Table 3). In 2014, habitat differences were found with both methods only when comparing abundance$_{MIC}$ to abundance$_{HTS}$; however, in 2017 they were only found with microscopy when comparing abundance while they were found with both methods when comparing biomass$_{MIC}$ to abundance$_{HTS}$. Comparing years, no differences were found with microscopy and HTS.

3.4 | Multivariate analysis with algal entities

In the RDAs with algal abundance$_{MIC}$, biomass$_{MIC}$ ($n = 146$), all OTUs ($n = 1836$), and OTUs$_{>\ 10\ reads}$ ($n = 385$), variability explained was

TABLE 3 Summary of two-way (years 2014 to 2017) and three-way (years 2014 and 2017) ANOVA with % values of phyla assessed with microscopy (MIC; abundance—abund—and biomass—mass) and high-throughput sequencing (HTS); the method effect (testing HTS against MIC) relates to the difference between microscopy (either abundance or biomass) and HTS (operational taxonomic unit abundance); habitat differences (hab) refer to differences between the littoral, pelagic, and hypolimnion (hypo); in the three-way ANOVA, we focused on the year effect (2014 vs. 2017); significant effects (method and habitat effect in two-way ANOVA and year effect in three-way ANOVA) and their significance are reported, followed by significant differences as assessed by post hoc testing; only significant results are reported; when a significant interaction effect was found, significant main effects were not reported when included in the interaction effect (indicated by a colon); the > sign indicates interactions for which level higher values were found; significance levels: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, not significant (n.s.) $p > 0.05$; year$^{n.s.}$ indicates no significant year effect

	Two-way ANOVA		Three-way ANOVA
Testing HTS against	**2014**	**2017**	**2014 vs. 2017**
% Bacillariophyta			
Abund	n.s.	hab: method**; HTS: hypo > littoral***; hypo > pelagic***; HTS > MIC: hypo***	year: hab*; 2014 > 2017: littoral*
Mass	n.s.	hab***; hypo > littoral***, hypo > pelagic*	year: hab*; 2014 > 2017: littoral**, pelagic*
% Charophyta			
Abund	n.s.	method*; MIC > HTS	year$^{n.s.}$
Mass	n.s.	n.s.	n.s.
% Chrysophyta			
Abund	method***; HTS > MIC	hab: method***; HTS > MIC: littoral***, pelagic***, hypo**; HTS: littoral > hypo***, pelagic > hypo***	year: method**; HTS—2014 < 2017: littoral***, pelagic***
Mass	method***; HTS > MIC	hab: method***; HTS > MIC: littoral***, pelagic***, hypo***; HTS: littoral > hypo ***, pelagic > hypo ***	year: method*; HTS—2014 < 2017: littoral***, pelagic***
% Cryptophyta			
Abund	method**; MIC > HTS	method** + hab**; MIC > HTS; littoral > pelagic**, littoral > hypo*	year$^{n.s.}$
Mass	method***; MIC > HTS	method**; MIC > HTS	year$^{n.s.}$
% Dinophyta			
Abund	method***; MIC > HTS	hab: method*; MIC > HTS: littoral***, pelagic** MIC: littoral > hypo***, pelagic > hypo*	hab: method: year*; MIC—2014 < 2017: littoral***
Mass	method***; MIC > HTS	hab: method*; MIC > HTS: littoral***, pelagic**, hypo* MIC: littoral > hypo***, pelagic > hypo*	method: year**; MIC—2014 < 2017: pelagic*, hypo***
% Eustigmatophyta			
Abund	method**+hab*; MIC > HTS; hypo > littoral*	hab: method*; MIC > HTS: hypo*** MIC: hypo > littoral**, hypo > pelagic***	year$^{n.s.}$
Mass	n.s.	hab***; hypo > littoral***, hypo > pelagic***	year$^{n.s.}$

higher with HTS than microscopy (Table 4). With forward selection, similar environmental variables were selected in $RDAs_{MIC}$ and $RDAs_{HTS}$, even though water level change was additionally selected in $RDAs_{HTS}$. For microscopy, we focus on $biomass_{MIC}$ because the RDA with $abundance_{MIC}$ explained lowest variability and only two explanatory variables were selected as important. For HTS, we focus on $RDA_{OTUs> 10\ reads}$ because a higher variability was explained compared to using all OTUs even though similar environmental variables were selected. In both RDAs ($RDA_{biomass\ MIC}$, $RDA_{OTUs> 10\ reads}$), % transmission and temperature were positively related to the littoral, while the hydrological proxies water level change ($RDA_{biomass\ MIC}$, $RDA_{OTUs> 10\ reads}$) and rain ($RDA_{OTUs> 10\ reads}$) were not specific for a single habitat (Figure 2). In $RDA_{biomass\ MIC}$, silica and NH_3 were positively related to the pelagic and the deep hypolimnion while in $RDA_{> 10\ reads}$ only to the deep hypolimnion. In the $RDA_{biomass\ MIC}$, samples from the pelagic and the deep hypolimnion overlapped and samples from the littoral were quite distinct, while in $RDA_{OTUs> 10\ reads}$ samples from the deep hypolimnion were distinct and those from the pelagic and the littoral slightly overlapped. $RDA_{biomass\ MIC}$ and $RDA_{OTUs> 10\ reads}$ did not evidence any clustering of years. We refrained from reporting and discussing relationships between taxa and environmental parameters because of the different taxonomic detail of microscopy and HTS data.

4 | DISCUSSION

4.1 | Method differences in biodiversity assessment

Attributable to method-specific characteristics, HTS generally reports more taxa (Rimet et al., 2018; Zimmermann et al., 2015) and more rare taxa (Rimet et al., 2018; Zhan et al., 2013) than microscopy. Here, approximately 100 times more water volume was filtered and analysed with HTS with respect to the microscopically analysed water volume, and already this volume difference contributes to the superiority of HTS for the detection of taxa (Gran-Stadniczeñko et al., 2019; Hardge et al. 2018; Xiao et al., 2014). Thus, it was not surprising that also in this study more taxa were reported with HTS

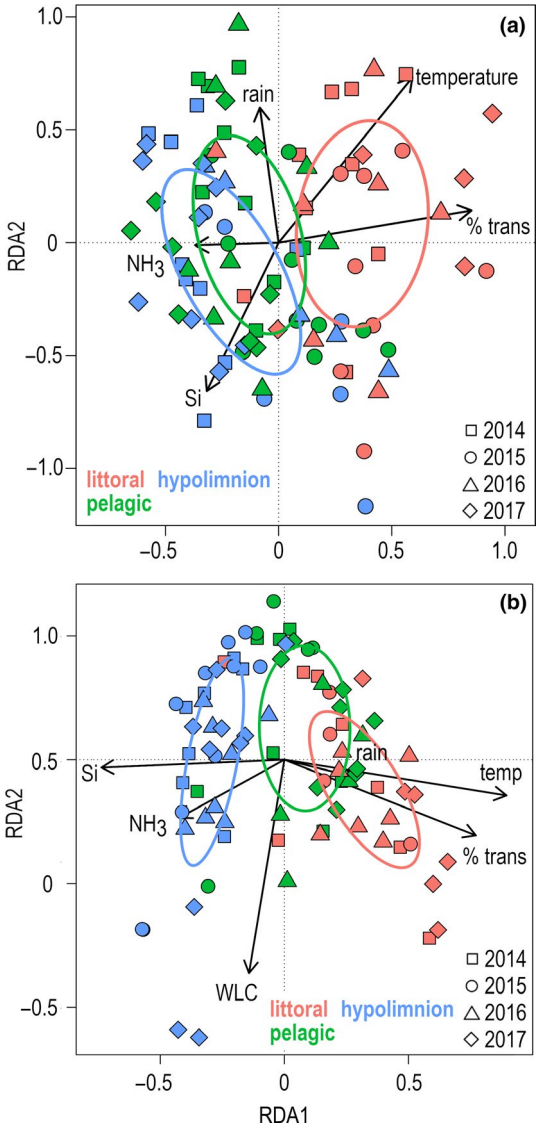

FIGURE 2 Redundancy analysis (RDA) with (a) algal $taxa_{biomass}$ and (b) operational taxonomic units >10 reads; shown are the 95% confidence ellipse for the centroid of habitat membership; temperature (temp), % transmission (% trans), water level change (WLC), silica (Si)

TABLE 4 Results of redundancy analysis (RDA) with algal taxa and operational taxonomic units (OTUs); given are variability explained (r^2 adjusted; %), variability explained by the first (RDA1) and second (RDA2) components in %, and significant environmental variables (ENV)

	Algal taxa		OTUs	
	Abundance	Biomass	All	> 10 reads
r^2 adjusted	6.8	8.9	17.7	20.1
RDA1	58	48	58	53
RDA2	42	30	19	17
ENV				
Hydrological proxies	No	Water level change	Rain	Water level change, rain
Temperature	No	Yes	Yes	Yes
% transmission	Yes	Yes	Yes	Yes
Nutrients	Silica	No	Silica	Silica

and most OTUs were rare. These rare entities might constitute the rare algal biosphere similar to bacterioplankton (Pedrós-Alió, 2012) and their rarity might be linked to unfavourable environmental conditions or adaptation (Skopina, Vasileva, Pershina, & Pinevich, 2016). Evenness was higher with microscopy than with HTS, and we suggest that this method difference was also attributable to the smaller sample volume analysed with microscopy that favours finding more common taxa.

Even though HTS provided a higher biodiversity assessment for certain phyla, it provided a lower taxonomic resolution than the microscopy-based biodiversity assessment, a situation that will improve as reference DNA sequence databases increase in species coverage. Likewise, *algae incertae sedis* were similarly prevalent with both methods, and thus no advantage was gained using HTS, although it seems reasonable that also this situation will rapidly improve with more complete databases.

The higher biodiversity reported with HTS was mainly linked to the detection of OTUs of Xanthophyta, Eustigmatophyta, Bacillariophyta, and to the overwhelming presence of OTUs of Chrysophyta. Xanthophyta were absent with microscopy, and their detection with HTS corroborates its superiority to detect rare taxa. Furthermore, more OTUs of Bacillariophyta than morphospecies often are reported because HTS uncovers cryptic diversity (Rimet et al., 2018; Rivera et al., 2018; Zimmermann et al., 2015). The lower biodiversity of Chrysophyta with microscopy can be related to preservation and magnification issues because these algal cells preserve poorly in Lugol and accurate taxonomic assignment depends on details only visible with scanning electron microscopy (Wujek & O'Kelly, 1992). In many oligotrophic and cold freshwater ecosystems where the mixotrophic nature of Chrysophyta (Rothhaupt 1996) is an advantage (Hansson, Grossart, del Giorgio, St-Gelais, & Beisner, 2019) a high diversity of Chrysophyta-related OTUs has been observed along with a high total abundance of sequences (e.g. 25–50% Charvet et al., 2012; 25–60% Lara et al., 2015; 37% Ortiz-Álvarez, Triadó-Margarit, Camarero, Casamayor, & Catalan, 2018; 33% Llorens-Marès et al. 2020). Lake Tovel is an oligotrophic and cold-water lake (Cellamare et al., 2016), and thus the dominance of Chrysophyta (40-85% of total sequence abundance) was not surprising.

Contrary to the above-mentioned phyla, a higher diversity was reported for Charophyta and Chlorophyta with microscopy than with HTS. Despite the higher richness of Charophyta reported with microscopy, microscopy and HTS data showed similarly low % values (generally <2%), corroborating that this phylum was a rare component of the phytoplankton community. For Chlorophyta, tufA (Vieira et al., 2016) or a combination of different primers should be used (Marcelino & Verbruggen, 2016); however, the V4 region of the 18S rRNA reveals a vast diversity of Chlorophyta in marine waters (Tragin, Zingone, & Vaulot, 2018; Tragin & Vaulot, 2019). While Chlorophyta showed relatively high % values with microscopy, with HTS they were rarely detected and with a low % sequence abundance. The discrepancy in the detection of Chlorophyta in a freshwater lake (this study) with respect to marine waters (Tragin et al., 2018; Tragin & Vaulot, 2019) might indicate that the efficiency of HTS for Chlorophyta can be habitat dependent. Dinophyta and Eustigmatophyta showed the odd situation of a higher diversity but a lower % abundance with HTS compared to microscopy. Dinophyta can have a high gene copy number (Galluzzi et al. 2004), but preferential PCR amplification (Wintzingerode et al., 1997) for certain taxa such as Chrysophyta might have decreased the sequencing success for less frequent taxa such as Dinophyta and Eustigmatophyta. In summary, the degree of mismatch between microscopy and HTS assessed biodiversity was phyla dependent and can be related to technical and biological factors such as screened water volume, cryptic diversity, preservation and magnification issues, and PCR bias.

4.2 | Metric effect for diversity, functional groups, and phyla

The scientific community has not reached a consensus on whether to compare HTS data with microscopy-based abundance or biomass data. The presence of a metric effect could be revealed only by investigating two different years. According to our hypothesis different method and/or habitat effects were found depending on whether comparing $abundance_{HTS}$ to $abundance_{MIC}$ or $biomass_{MIC}$, respectively, for 2017 and comparing years (2014 vs. 2017).

A metric effect for method differences ($autotrophs_{2017}$, $Bacillariophyta_{2017}$, $Bacillariophyta_{2014vs2017}$, $Charophyta_{2017}$, $Charophyta_{2014vs2017}$, $Eustigmatophyta_{2017}$, $Eustigmatophyta_{2014vs2017}$) was generally observed when comparing $abundance_{MIC}$ to $abundance_{HTS}$. While a method effect might not be very interesting and important for environmental inferences because inherent to the methods used, we expected that habitat and year differences would be properly indicated by HTS that will substitute microscopy in the future. Metric effects for habitat differences were faceted; $autotrophs_{2017}$, $Bacillariophyta_{2017}$, and $Eustigmatophyta_{2017,\ 2014vs2017}$ showed the best metric effect with $biomass_{MIC}$ to $abundance_{HTS}$ comparisons while, with abundance comparisons, only HTS showed habitat differences. Contrarily, $Cryptophyta_{2017,\ 2014vs2017}$ and $evenness_{2014vs2017}$ showed the best metric effect with abundance comparisons while with $biomass_{MIC}$ to $abundance_{HTS}$ comparisons no effect was found. Method differences were not indicative of this pattern because, while for $autotrophs_{2017}$ and $Bacillariophyta_{2017}$ higher values were found with HTS in abundance comparisons, for Eustigmatophyta, Cryptophyta, and evenness, the opposite was found. Thus, we suggest that detection efficiency of microscopy and HTS was important: only when microscopy, either abundance or biomass, and HTS showed the same range of variability, the best metric effect was found. This is an unfortunate situation because it cannot be known *a priori* if $abundance_{MIC}$ or $biomass_{MIC}$ are best compared to $abundance_{HTS}$. We favoured abundance comparisons for Cryptophyta and evenness while $biomass_{MIC}$ to $abundance_{HTS}$ comparisons for autotrophs, Bacillariophyta and Eustigmatophyta.

An effect indicated by microscopy but not by HTS was found for Dinophyta. For this phylum, a higher diversity was found with HTS in combination with a lower sequence abundance attributable to

preferential PCR amplification, and thus microscopy seemed superior to HTS.

For Chrysophyta, no metric effect was found because HTS data indicated habitat differences that were not indicated by microscopy, using both abundance and biomass data. We suggest that the methodological strength of HTS reporting more species in high abundance was the cause for this, and therefore patterns not seen with microscopy could be revealed with HTS.

In summary, our study showed that different metric effects can be found, and despite the gene copy number issue, for some phyla, abundance comparisons were appropriate while for others biomass$_{MIC}$ to abundance$_{HTS}$ comparisons were better. Also, a higher diversity reported with HTS did not guarantee that habitat differences were indicated with HTS in the same way as with microscopy. Thus, no general rule was found for the presence and type of the metric effect.

4.3 | Habitat and year effects on α-diversity, functional groups, and phyla in relation to environmental conditions

Different habitat and year differences were found. We discussed those differences that were either linked to the best metric effect or reflected the consensus among results. Higher α- diversity was generally found for 2017. We attributed this year effect to a change in the lake's hydrology. The years from 2014 to 2017 showed a decreasing gradient in precipitation (Obertegger et al., 2019). Furthermore during the year 2017, Lake Tovel experienced a shift from nival to pluvial origin of its waters, leading to whole lake warming and increased stability of the water column (Flaim et al. 2019). Warmer and more stable upper water layers in a cold-water lake might have opened niches for less cold-water adapted species, and thus reasonably increased algal diversity. This 2017-year effect was also indicated for several phyla with different aspects of hydrology affecting phyla.

Functional groups as investigated by % autotrophs did not show consistent patterns across years. While no effects were found for 2014, for 2017, the same habitat differences were indicated that were closely related to Bacillariophyta and Eustigmatophyta making the highest contribution to autotrophs. The higher abundance and biomass of Bacillariophyta and Eustigmatophyta in the hypolimnion with respect to the other habitats in 2017 can be linked to more pronounced stratification leading to more sedimentation of Bacillariophyta and lowest temperature in hypolimnion necessary for cold-stenothermal Eustigmatophyta. In the littoral and the pelagic, Bacillariophyta showed higher values in 2014 than in 2017, while Chrysophyta showed this year effect with the opposite outcome (2017 > 2014). Small autotrophic flagellated chrysophytes are particularly adapted to low nutrient, cold conditions, and high light availability (Reynolds, 1980; Holmgren, 1984; Hansson et al., 2019) while Bacillariophyta depend on allochthonous nutrient input by rain (Tolotti, Corradini, Boscaini, & Calliari, 2007) and tend to have an advantage in more hydrodynamic waters that aid in floatation (Padisák et al. 2003). We suggest that the lower % values of autotrophs and Bacillariophyta were related to reduced nutrient input in 2017 with respect to 2014 where mixotrophic Chrysophyta could take advantage of this situation. The missing year effect for the hypolimnion corroborates its status as a stable habitat (Obertegger et al., 2018). Furthermore, the habitat effect (littoral and pelagic > deep hypolimnion) corresponded to the preference of Chrysophyta for high light availability, and this effect was observable only during periods with stable conditions (i.e. reduced mixing) showing distinct light differences (i.e. 2017).

Habitat differences of Cryptophyta, Dinophyta, and Eustigmatophyta were also related to their environmental niche. Grujcic et al. (2018) experimentally show that Cryptophyta are major bacterivores feeding on betaproteobacteria. In Lake Tovel, Cryptophyta dominate in the littoral characterised by a steep temperature gradient (Cellamare et al., 2016) and abundant betaproteobacteria with respect to the pelagic (Obertegger et al., 2018). Thus, the dominance of Cryptophyta in the littoral could be related to its feeding habits. For Dinophyta, habitat (i.e. littoral and pelagic > deep hypolimnion) and year differences (2014 < 2017) were only found with microscopy. In Lake Tovel, Dinophyta are prevalent in the littoral because under-ground inflow limits the occurrence of potential grazers (Cellamare et al., 2016) and they can avoid cold temperatures (Flaim et al. 2003). Eustigmatophyta also showed niche-related habitat differences indicated both by microscopy and HTS. In Lake Tovel, the cold-tolerant eustigmatophyte *P. kamillae* dominates in the deep hypolimnion (Cellamare et al., 2016). We suggest that, during 2014, the pelagic was similar to the deep hypolimnion because of cooler water temperatures associated with the very wet year and thus only the littoral showed lower values than the hypolimnion; in contrast, during 2017, the deep hypolimnion provided the coldest environment with respect to the more stable conditions and warming waters of the littoral and the pelagic.

4.4 | Community–environment relationships

Linking environmental conditions to biodiversity, RDAs with abundance and biomass of algal taxa, respectively, and abundance$_{HTS}$ indicated similar habitat effects: both methods differentiated between the two environmentally most extreme habitats (the deep hypolimnion vs. the littoral), with the pelagic showing a method-dependent similarity to the other two habitats: with microscopy, the pelagic was more similar to the hypolimnion; with HTS, the pelagic was more similar to the littoral. Prokaryotes assessed with HTS also distinguish the littoral habitat from the deep hypolimnion with the pelagic habitat intermediate (Obertegger et al., 2018). Thus, results assessed with HTS (Obertegger et al., 2018, this study) coincided in their assessment of the pelagic being intermediate with respect to the other two habitats. Only RDA$_{biomassMIC}$ and RDA$_{OTUs>10\ reads}$ were further discussed because of their superior explanatory power of community composition. Both RDAs

indicated the importance of hydrology (directly by water level change or indirectly by rain), the physical environment (temperature and/or light penetration), and nutrients (silica) on phytoplankton communities. Hydrology plays an important role in shaping plankton communities in Lake Tovel (prokaryotes assessed with HTS: Obertegger et al. 2018; phytoplankton and zooplankton assessed by microscopy: Flaim et al., 2006; Tolotti et al., 2007; Obertegger et al., 2007; Cellamare et al., 2016) with phytoplankton influenced through allochthonous nutrient (nitrate and silica) input by rain (Tolotti et al., 2007). With both RDAs, no differences between years was found. Thus, multivariate analysis also corroborated a strong environmental control, similarly emphasised by microscopy and HTS-based data, even though variability explained was higher with HTS than with microscopy.

In summary, we conclude that despite the different strengths inherent to both methods especially leading to differences in biodiversity assessment, both datasets outlined similar large-scale patterns emphasising the environmental control of phytoplankton communities. Furthermore, even though metric effects were found, HTS-based data provided similar and more detailed information than microscopy, supporting the promise of HTS becoming the tool of the future for biodiversity research. Nevertheless, we agree with Lara et al. (2015) that interpreting the huge amount of information provided by HTS not only requires bio-informatic skills but also traditional skills, originating from extensive background knowledge of the organisms and ecosystems studied.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

All data are original data generated for this study. On request, data will be made available.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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