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An Automated Platform for Phytoplankton Ecology and Aquatic Ecosystem Monitoring

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1 # An Automated Platform for Phytoplankton
2 # Ecology and Aquatic Ecosystem Monitoring

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Abstract

High quality monitoring data are vital for tracking and understanding the causes of ecosystem change. We present a potentially powerful approach for phytoplankton and aquatic ecosystem monitoring, based on integration of scanning flow-cytometry for the characterization and counting of algal cells with multi-parametric vertical water profiling. This approach affords high-frequency data on phytoplankton abundance, functional traits and diversity, coupled with the characterization of environmental conditions for growth over the vertical structure of a deep water body. Data from a pilot study revealed effects of an environmental disturbance event on the phytoplankton community in Lake Lugano (Switzerland), characterized by a reduction in cytometry-based functional diversity and by a period of cyanobacterial dominance. These changes were missed by traditional limnological methods, employed in parallel to high-frequency monitoring. Modeling of phytoplankton functional diversity revealed the importance of integrated spatio-temporal data, including circadian time-lags and variability over the water column, to understand the drivers of diversity and dynamic processes. The approach described represents progress towards an automated and trait-based analysis of phytoplankton natural communities. Streamlining of high-frequency measurements may represent a resource for understanding, modeling and managing aquatic ecosystems under impact of environmental change, yielding insight into processes governing phytoplankton community resistance and resilience.

Introduction

Freshwater ecosystems are characterized by high levels of biodiversity, and are among the most threatened ecosystems on earth [1, 2] (Millennium assessment: http://www.maweb.org). Understanding and managing environmental change in aquatic ecosystems is complicated by co-occurring and interacting stressors like climate change, eutrophication and pollution that, for example, can interact to favor harmful algal blooms [3-6]. We suffer from a general lack of knowledge on the background rates and direction of change in pristine ecological systems, as well as in stressed ecological communities [7]. These limits can hamper our ability to detect the signature of a range of anthropogenic impacts on ecosystems, or predict patterns of recovery.

Phytoplankton communities are highly diverse and dynamic. They respond rapidly to climate change, eutrophication and pollution, and play an important role in aquatic ecosystem biogeochemical processes [4, 8-14]. Phytoplankton density (algal blooms) and community composition (e.g. toxic cyanobacteria) are the prime agents impacting water quality, ecosystem and human health [15], and have been suggested to be used as such for ecosystem assessment [16-19]. Monitoring, understanding and predicting changes in structural (composition, diversity, evenness) and functional (phenotypic characteristics, growth rate, productivity) aspects of phytoplankton communities across space and over time represents however a challenge for aquatic ecology. The capturing of population dynamics, community succession and adaptation to environmental change requires: 1) high-frequency sampling to follow fast plankton fluctuations [20] and potential chaotic dynamics [21]; 2) vertical (depth) distribution of algal taxa and their physio-

morphological characteristics (traits) [22]; 3) a functional, trait-based assessment of communities and ecosystems based on the characteristics of the organisms' phenotypes that directly respond to environmental changes and determine effects on aggregated processes [13, 23, 24].

The goal of this article is to present an integrated platform able to: 1) provide automated high-frequency measurements of phytoplankton at different lake depths; 2) couple in situ biological monitoring with data about the physical environment; 3) provide a streamline of real-time data for modeling and forecasting phytoplankton dynamics. By integrating a Cytobuoy^{TM} with an Idronaut^{TM} vertical profiling system, we addressed the objective of increasing spatio-temporal resolution in field data collection. It has been proposed that scanning flow-cytometry, offered by instruments like the commercially available Cytobuoy^{TM}, may offer advantages over microscopic methods for cell counting and classification of phytoplankton, including the possibility of automation and high frequency field measurements of phytoplankton physio-morphological characteristics [20, 25-27]. A novel aspect of our monitoring approach therefore lays in the use of cytometry-data for a description of phytoplankton functional diversity and expressed phenotypic traits, which allow tracking phytoplankton responses at the functional group level. Trait-based approaches and functional groups are becoming increasingly important in understanding phytoplankton ecology [22, 28-30].

In this study we tested our monitoring platform optimized for deep water bodies, designed to afford comprehensive data to study phytoplankton ecology and to improve water resource management. To support the validity of our approach we report the results form a monitoring campaign (spanning roughly one month in May 2010) during which

automated measurements were coupled by fortnightly limnological data (physics, chemistry and biology) [31].

Materials and methods

Automated monitoring platform. Phytoplankton counting, characterization and classification were performed using a scanning flow cytometer Cytobuoy™ (Woerden, the Netherlands), designed to analyze the full naturally occurring range from small (e.g. picoplankton) to large (e.g. colonial cyanobacteria) planktonic particles (1 to 700 µm in diameter and a few mm in length) and relatively large water volumes (http://www.cytobuoy.com) [25] (Fig. S1-e). In our instrument, particles were intercepted by two laser beams (Coherent solid-state Sapphire, 488 nm and 635 nm, respectively, 15 mW) at the speed of 2 m s^{-1}. In this study, digital data acquisition was triggered by the sideward scatter (SWS) signal (908 nm). The light scattered at two angles, forward (FWS) and SWS, provided information on size and shape of the particles. The fluorescence (FL) emitted by photosynthetic pigments was detected as red (FLR), orange (FLO) and yellow (FLY) signals collected in the wavelength ranges of 668-734 (chlorophyll-a, Chl-a), 601-668 (phycocyanin and phycoerythrin) and 536-601 nm (degraded pigments), respectively. Laser alignment and calibration processes were done before the monitoring campaign using yellow FL beads of 1 and 4 µm diameter.

Our Cytobuoy™ allowed automatic acquisition of particles in time-intervals, time-specific measurement, and fixed-measurement on occurrence of a trigger signal (see below). This study was based on automated acquisition of 2 fixed-measurements for

every trigger-signal received in order to optimize the detection and quantification of small and large particles in two separated analyzes, and on a scheduled time-specific background measurement per day with water being sampled at 25 m (no phytoplankton growth). Remote accessibility of the Cytobuoy™ via the internet-UMTS network allowed unlimited data access and transmission rates along with increased location flexibility. Further technical details on our Cytobuoy™, measuring settings and configurations are reported in the Supplemental Online Material.

In order to accomplish depth resolution, we employed a vertical profiling system made up of three integral parts: Controller Module (Fig. S1-a,-b), Profiler Module (Fig. S1-b), and OCEAN SEVEN 316Plus CTD (O7) multiparameter probe (Fig. S1-c) (Idronaut, Brugherio, Italy, www.idronaut.it). The O7-probe was equipped with seven sensors: pressure, temperature (°C), conductivity (µS, absolute and at 20°C), pH, oxygen (mg / L and % saturation), and NO_3 (µg /L) (Idronaut™). An external TriLux fluorimeter was interfaced with the O7 probe in order to quantify levels of Chl-a, phycoerythrin and phycocyanin (Chelsea Technologies Ltd, Surry, UK). More information on the Idronaut™ profiling system can be found in the Supplemental Online Material.

For automatic depth profiles, we allowed the Cytobuoy™ to accept an electric signal from the Idronaut™ Controller Module as a trigger to start the measurement cycle during O7 step-profiles. We ran two independent automatic monitoring programs, one with the Cytobuoy™ and one only with the O7-multiparameter probe, with separated profile settings and different monitoring frequencies. In this study we scheduled a step profile involving six depths - covering the entire photic zone - with the Cytobuoy™ (2, 4, 6, 8, 10 and 12 m) and a continuous profile with the O7-multiparameter probe from 1 to 20 m

to be performed twice a day each, to catch diel variations in the temperature structure of the water column: the theoretical maximum and minimum daily stratification at 3 pm and 3 am (12 h frequency), respectively.

For step-profile phytoplankton measurements, we retrieved water from selected depths using an external pump (capacity 1 L min^{-1}), an antimicrobial silver-nanoparticle coated and shaded flexible polyethylene tubing (Flexelene™, Eldon James Corp., Loveland, Colorado, USA), and a surface plexiglass chamber (250 mL) from which the Cytobuoy™ sub-samples through a needle injector (Fig. S1-e). The pump was placed downstream from the chamber in order to avoid damaging algal cells or colonies prior to measurements. More information on structural components of the monitoring platform, how we integrated our instruments to achieve depth profiles, and an example of automated operation using the integrated system and maintenance details are reported in the Supplemental Online Material.

Sampling. The automated monitoring platform was moored in Lake Lugano, at a site protected from strong winds and currents and close to the location of the routine historic lake monitoring program (coordinates 45°57'33.43"N, 8°52'53.49"E) (Fig. S2). This site is representative for the most eutrophic of the lake's three distinct basins [31] (Fig. S2). Data presented in this article refer to the monitoring period from the 28th of April to the 31st of May 2010 (with 6 depths over the photic zone and a frequency of two profiles per day). Independent limnological data were collected at 300 m distance from the platform with a fortnightly frequency. They included physical characteristics of the whole water column, chemical analyses on algal nutrients and integrated phytoplankton samples (from 0 to 20 m). Additional information on these data can be found in the Supplemental Online

Material. For comparison between cytometry-based richness and phytoplankton species richness (Table 1, Fig. S6) we used additional samples from Lake Lugano collected between June and December 2010 and data from a study conducted in Lake Zurich during spring 2009 [32] (Supplemental Online Material).

Data analysis. Data manipulation, analysis and graphics were performed in the R programming language (www.r-project.org). The Cytobuoy™ provided 54 descriptors of 3D structure and FL profile for each particle [25]. Datasets also included original sampled volume, date, time and depth at which particles were taken. We visually inspected the distribution of raw data with regards to FL signals and set database-specific threshold levels to divide fluorescent (FL) from non-FL particles. The overall FL and non-FL databases comprised 1 and 5 million particles, respectively.

Cytobuoy™ particle descriptors were standardized to zero mean and unit variance and, by principal component analysis, reduced to 33 orthogonal vectors covering 99 % of total variance in the data (data not shown). Principal components were utilized for grouping particles into functional categories using K-means clustering. We compared several K values and selected the optimal number of K based on the within groups sum of squares [33]. Phytoplankton densities were calculated by inferring the number of cells from the number of humps in the SWS signal of each particle to account for colonial species [20, 25]. O7 sensor data were organized in a separated database. Cyanobacterial-like particles were identified based on FLO and FLR emissions after excitation by the 495 nm and 635 nm lasers, respectively, after visual inspection. These signals are expected as a response to the presence of the cyanobacterial-specific pigment phycocyanin [25].

We modeled richness of Cytobuoy™-derived functional groups of phytoplankton (response variables) in the upper 12 m of the water column based on high frequency environmental data (explanatory variables). Explanatory variables included: water parameters (mean of top 12 m), coefficient of variation (CV = SD / mean) of parameters over water-column and meteorological data at time-lag(0), -lag(1) (=24h) and -lag(2) (=48h). The response variables showed significant temporal autocorrelation only at time-lag(1) (data not shown). We therefore included for each model the response variable at time-lag(1) as explanatory, in order to account for temporal autocorrelation of data. All variables were scaled in order to standardize effect sizes and let to compete in the same model. The best model was selected based on Akaike's information criterion (AIC) with a stepwise procedure (alternation of forward selection and backward elimination of variables with $p > 0.05$) [34]. The relative importance of drivers was assessed by bootstrapping (999 times) the percentage contribution to the R^2 of the model among the regressors, and extracting the relative 95% confidence intervals.

Results and Discussion

Phytoplankton depth heterogeneity. Our monitoring approach was able to reveal fine changes in the relative depth distribution of phytoplankton functional-group richness, Chl-a concentration and cell density with statistically significant differences between day and night profiles (Supplemental Results, Fig. S3-S4). Similar data have been observed using flow-cytometry in oceanic profiles of phytoplankton communities [35-37]. We did not observe a significant difference in the vertical physical structure of the water column

between day and night profiles (Fig. S3-S4), and limited changes between day and night air-temperatures during the study period (data not shown). Our data suggest that depth-specific day-night dynamics in phytoplankton community composition and abundance are driven by biological factors, rather than environmental changes (Supplementary Results and Discussion).

Temporal phytoplankton dynamics. The frequency and intensity of phytoplankton blooms are key elements for ecological status definition [16, 17, 19]. Considering that most algal taxa can reach bloom conditions and disappear within a few days (implying a maximum oscillation frequency of 2-3 density peaks per week), a minimum sampling frequency of 4 to 6 times per week would be needed to follow algal dynamics (Nyquist frequency, Table 1) and quantify their intensity adequately [20].

Our automated monitoring platform was able to perform 2 vertical profiles per day (at a fixed depth the maximum frequency could be of 6 samples per hr). Figure 1 reports results from daily monitoring samples (time is 3 pm, frequency = 1 day^{-1}) during the study. This frequency was capable of capturing fine fluctuations in FL particle density (phytoplankton) and total Chl-a concentration over the water column (Fig. 1A). Our data were comparable to previous work using flow-cytometry in the field in terms of temporal resolution on algal dynamics ([27] and literature therein). Measured phytoplankton density was comparable with microscopic counts and correlated well with Chl-a concentration levels (Fig. 1A) (R^2-adjusted = 0.651, p = 4.324^{-08}), as also reported elsewhere [32]. Our system was able to follow dynamics of non-FL particles (suspended solids, dead cells, heterotrophic bacteria), which did not correlate with algal cell

concentrations apart from a short period in the middle of the time-series (days 15 to 18) (Fig. 1A).

Previous work using flow cytometry in phytoplankton aimed at identifying broad functional groups (such as picoeukaryotes, microalgae, cyanobacteria, etc.) and some phytoplankton species with clearly distinguished morphology or pigmentation (such as *Pseudo-nitzschia*, *Cryptomonas*, *Synura*, *Dinobryon*) [20, 25, 27, 38] (and literature therein). This type of analysis lacked a proper measure of diversity. We used the Cytobuoy™ to describe key phytoplankton traits like size, coloniality, pigment type and content, which we used to create groups of functionally similar individuals [29, 30]. The possibility of monitoring individually measured phytoplankton physio-morphological descriptors may offer the best prospects in terms of objectivity, reproducibility, functional properties and prediction of algal assemblages [22, 23, 30]. The number of Cytobuoy™-derived functional groups was comparable with the total number of species detected in the photic zone of the water column (Fig. 1B, Fig. S6), as also reported elsewhere [32]. Generally, the number of functional groups in a community is smaller than the number of species, since in current functional classification methods more than one species can be assigned to the same functional category [29, 30]. With our trait-based approach, however, it is also possible that individuals of the same species can be allocated to different functional groups based on their expressed morphology (for example, colonial species can be assigned to two different groups depending on whether they are present as single cells or colonies). The Cytobuoy™ description of the relative abundance of phytoplankton functional groups deviated from microscopically measured evenness (Fig. 1B). This could be caused by superior precision of automated density measurements, and

to the fact that the identity (and abundance) of Cytobuoy^{TM}-derived functional groups does not fully reflect the identity (and abundance) of microscopically defined taxonomic groups as reported above (several species can map into one functional category and individuals of the same species can be assigned to different groups).

We observed a strong decrease in phytoplankton functional richness and evenness in the middle of the time-series (Fig. 1B), followed by a short recovery period that led to higher cell density (Fig. 1A). These dynamics were completely missed by the fortnightly limnological sampling (Fig. 1). Our approach offered the advantage of having automated measurements of environmental conditions for the observed algal dynamics (Fig. S7). Six days of rainy and stormy weather (Table S1) were associated with a period of low phytoplankton diversity and productivity (with high levels of non-FL particles), and a decrease in CV in temperature and conductivity over the first 12 m of the water column. This eventually led to a mixing event on day 19 (Fig 1C, Fig. S7). The phytoplankton community in the days preceding disturbance (started at day 5) showed a gradual decline, reaching the minimum of evenness and richness just before the mixing event (on days 17 and 18, respectively). The mixing event re-established evenness in the community that fully recovered functional diversity in 6 days (Fig. 1B-C). Functional diversity, as opposed to taxonomic diversity, appears to be a better predictor of ecosystem functioning across a range of communities and measures of functional diversity may afford a better description of the functionality of the ecosystem and its resilience to disturbance [12, 13, 23, 24, 39].

Using Cytobuoy^{TM}-derived phytoplankton traits. Our approach allows tracking phytoplankton physio-morphological characteristics such as cell size and shape (which

influence motility and nutrient uptake through surface/volume ratio), photosynthetic performance (driven by pigment type and concentration), active nutrient uptake and coloniality [22]. Cell size and photosynthetic performance are key phytoplankton traits, affecting growth, metabolism, access to resources, susceptibility to grazing, and are extremely plastic responding to the environment and to species interactions [22, 32]. Analysis of dynamics and distributions of these focal phytoplankton traits could improve our forecasting capabilities of community structure and ecosystem functions [12, 13, 24, 39]. Pigment profiles can also be used to specifically target certain phytoplankton groups of interest in their spatio-temporal dynamics [20, 25].

We report temporal changes in mean and variance of phytoplankton size and suspended non-FL particles size (Fig. 2A, Fig. S8). In addition, we tracked the dynamics in abundance of cyanobacteria using Cytobuoy^{TM} data and phycocyanin / Chl-a concentration ratios obtained with the O7-probe (Fig. 2B). Shortly before "disturbance" (days 15-17), a period characterized by low diversity and productivity (Fig. 1), the study site was dominated by large cyanobacterial colonies (Fig. 2A-B). Mean water column cyanobacterial density obtained by the Cytobuoy^{TM} was almost identical to microscopic count levels (Fig. 2B) and was likely associated with the presence of *Planktothrix rubescens* filaments (Table S2). The mixing event rapidly and dramatically reduced cyanobacterial abundance and the average size of the phytoplankton community (Fig. 2) [40]. Variation in the dimensions of non-FL particles appeared to be very small compared to the dynamics in phytoplankton size (note the y-axis scales in Fig. 2A). Compared to conditions before the disturbance, the final days of our time-series were characterized by smaller size phytoplankton cells (Fig. 2A), probably eukaryotic nanoplankton of genera

Stephanodiscus and *Melosira* (Table S2), dominating a more productive (Fig. 1A) and diverse community (Fig. 1B, Fig. S8).

Our approach introduces the possibility of monitoring a large number of phytoplankton individuals and their traits per population or through the entire community. Individuals and populations should be the basic units of investigation to assess the status of communities and ecosystems, since they respond phenotypically (and genetically) to disturbance or stress and eventually evolve altering community processes and ecosystem functioning [41].

Modeling high-frequency phytoplankton dynamics. Our automated monitoring approach allows to better couple environmental forcing with phytoplankton community dynamics, in particular at the functional level (which may relate to crucial ecosystem services [13, 24, 42]). Using data from the period of study, we modeled the Cytobuoy^{TM}-based phytoplankton functional richness in order to provide an example of how spatio-temporal measurements of environmental conditions, coupled with biological data, can provide insight into drivers of community responses and changes.

Temperature (both atmospheric and water), conductivity (whose main contributors were carbonate and bicarbonate ions) and the heterogeneity of environmental conditions over the water column appeared to be the most important drivers of phytoplankton functional richness (Table 2). Most of the drivers appeared to influence the response variable with a time lag of 24 or 48 h (Table 2). Our modeling exercise highlights the importance of i) time-lags between environmental change and response at the level of phytoplankton community, ii) variability of parameters over the water column (depth heterogeneity), and iii) *in situ* meteorological conditions for understanding and modeling

phytoplankton community dynamics. Intensity of fluctuations and heterogeneity by depth in key environmental variables may represent fundamental factors to understand and predict changes in plankton diversity [14]. The collection of the above type of high-resolution data would be intractable without the aid of an *in situ* automated monitoring station like the one presented in this study. A similar approach can be used to model and forecast cyanobacterial blooms.

Towards an adaptive, integrated approach to aquatic ecosystem monitoring. Monitoring frameworks that evolve along with our improved knowledge of ecosystem processes would strongly benefit ecosystem health assessment and management by allowing to assess the impact of ongoing environmental change, to study recovery processes, and to built more reliable forecasting models [43]. Sophisticated monitoring approaches like the one that we have developed can offer the spatio-temporal resolution and flexibility necessary to capture and model natural phytoplankton responses to disturbance or stress, or to test ecological and evolutionary hypotheses including the mechanisms that lead to stable coexistence of species. For example, high-frequency data afford the possibility of studying niche processes and environmental filters on diversity and trait distribution patterns [44, 45], while tracking the vertical distribution of functional groups and their abundance allow testing for the importance of dispersal limitation among patches in the assembly of the phytoplankton community [46].

Table 1 summarizes some of the properties of our automated data-series compared to traditional monitoring, including diel temporal resolution in phytoplankton community dynamics and water column structure over the photic zone of the lake (Fig. 1, 2, S3, S4, S7). We were not able to capture horizontal spatial heterogeneity of phytoplankton and

the associated environment. The lack of spatial information across the water surface may be solved by integrating our platform data with remote sensing from satellites or from local devices that use spectral information reflected from the water surface [47] (http://www.waterinsight.nl). Depth represents however the most heterogeneous aspect of the phytoplankton spatial environment, and our vertical profiles may be crucial to understand and model the effects of disturbance, spatial heterogeneity and patch dynamics on phytoplankton community structure [48, 49]. Several phytoplankton groups are in fact capable of vertically migrating in the water column being motile (e.g. dinoflagellates) or able to regulate buoyancy (e.g. cyanobacteria) [29]. Depth resolution is therefore essential to track algal populations, which can be defined as groups of similar organisms (for example belonging to the same cytometry-derived cluster) that co-exist at the same time in the same water layer.

The bottleneck in monitoring natural systems is the development of automated technologies for the identification and counting of organisms [20, 27, 50, 51]. Our description of phytoplankton richness obtained by cluster analysis of automated flow-cytometry data appeared to closely match the taxonomic richness derived by microscopic analysis (Table 1, Fig. S6). Technical repeatability and across-lab reproducibility currently represent disadvantages of classical microscopic counts. An automated monitoring station like the one that we developed may offer the objectivity and reproducibility of a standardized measuring system that: 1) reduces human error; 2) affords a detailed description of individual algal features; 3) provides high data complexity; and 4) increase spatio-temporal resolution compared to man-made monitoring campaigns (Table 1) [20, 50].

The temporal and spatial monitoring scales of our analysis (Table 1) were roughly equivalent since both of them reflected processes operating over day-night cycles across the water column. The benefits of an integrated spatio-temporal approach to monitoring include [52]: (i) accounting for spatio-temporal coexistence mechanisms that purely spatial or temporal approaches would miss; (ii) generating new hypotheses and allowing rigorous testing of theoretical models; (iii) improving our descriptive power for developing forecasting models; and (iv) optimizing monitoring strategies by choosing appropriate scales for sampling. A fine spatio-temporal resolution with regards to organisms and the environment may represent a critical resource for scientists and stakeholders challenged by understanding, modeling and managing aquatic ecosystems [17-19]. The approach presented here can be applied to both freshwater and marine ecosystems, and to both natural and engineered environments such as drinking water reservoirs, water-treatment and aquaculture plants.

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Brief

We present an approach for automated and trait-based monitoring of phytoplankton for understanding, assessing and managing aquatic ecosystems under disturbance or change.

Supporting Information Available

Text (Extended Materials and Methods and Supplementary Results and Discussion Sections), Figures S1-S8, and Tables S1-S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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Figure legends

FIGURE 1. Automated measurements of phytoplankton density, diversity and associated changes in environmental heterogeneity. A) Phytoplankton abundance (from Cytobuoy™, solid line) compared to microscopic counts (■), abundance of non-FL particles (dashed line - scaled to fit graph by dividing values by 250) and Chl-a concentration (from O7-probe, grey line); B) Richness of Cytobuoy™-based functional groups (black line) compared to microscopic species counts (■), and Pielou's evenness (Shannon-diversity / Log(species richness)) of groups (grey line) compared to the same index derived by microscopic counts (■); C) CV over the water column in temperature (black line) and conductivity at 20°C (grey line). The CV can be used as a proxy of environmental (depth) heterogeneity [14]. In A) and B), data represent the average of the top 12 m of the water column. The grey vertical line highlights the mixing event.

FIGURE 2. Using phytoplankton traits such as size and pigment content to track community changes. A) Average size of FL (phytoplankton; black line) and non-FL (suspended solids, bacteria, dead cells; grey line) particles; B) Ratio between concentrations of phycocyanin and Chl-a (black line) and abundance of cyanobacterial-like cells (grey line) compared to microscopic counts of cyanobacteria (✳). Phycocyanin is a cyanobacterial-specific pigment: the ratio between phycocyanin and Chl-a concentrations can be used as an indication of the dominance of cyanobacteria in the phytoplankton community. Data represent the average of the top 12 m of the water column. The grey vertical line highlights the mixing event.

Table footnotes

Table 1: [a] considering one sample per month plus an extra fortnightly sample during productive seasons as in [14, 31] (Supplemental Online Material); [b] the automated system is currently producing data series across seasons; [c] range in number of species and functional groups during intercalibration performed in Lake Zurich and Lake Lugano: Reynolds categories [29] were utilized for functional grouping of microscopically identified species, for Cytobuoy™-derived functional groups see the Materials and Methods, for a plot of Cytobuoy™-derived versus taxonomic diversity see Fig. S6; [d] Quality assessment trials highlighted that phytoplankton microscopic counts can be difficult to reproduce across laboratories since they rely on human subjective assessment, biased by the experience/ability/condition of the operator, and that they suffer from low repeatability (high differences between replicated samples) (http://www.planktonforum.eu) [26, 50] (Supplemental Online Material); [e] five consecutive-replicated sampling cycles were performed in this study at the same depth and data assessed by canonical discriminate function analysis (Supplemental Online Material); * From [34].

Table 2: [¶] Drivers: T = temperature (°C); Cond. = conductivity at 20°C; CV = coefficient of variation over the sampled depths; Light = maximum irradiance (W/m^2); lag(1) and (2) = time-lag 24 and 48 h, respectively. ***** Drivers are ordered based on their relative contribution to the R^2 of the model, expressed as percentage of total. ^ Confidence intervals refer to the bootstrapped relative contribution to the R^2 of the model.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

1 **Table 1:** Comparison of selected properties of automated measurements to classical
2 phytoplankton monitoring.

Feature*	Classical Limnology	Automated platform
Number of samples year^{-1} (n)	12-18[a]	> 700[b]
Lag (Δ)	2 weeks – 1 month	12 h
Fundamental Period ($T_0 = \Delta n$)	12	> 700
Frequency ($1/T_0$)	0.083	0.0014
Nyquist frequency (1/2Δ), highest possible frequency	1-2 months (6-12 cycles year^{-1})	24 h (365 cycles year^{-1})
Resolution of depth gradient	from 1 integrated to 10 samples over photic zone	from 6 to 12 samples over photic zone
Phytoplankton density and physio-morphological traits	estimated from circa 200-500 counts / in 100-200 mL	from circa 30,000 counts / in 100-400 µL volume
Number of descriptors measured per individual	2 (size, volume)	54 (3D descriptors, pigment type, concentration etc.)
Estimation of diversity	taxonomic, functional	Functional
Number of taxa groups	14 to 61 per sample[c]	NA
Number of functional groups	5 to 20 per sample[c]	4 to 53 per sample[c]
Reproducibility / repeatability of data	low[d]	high [27][e]

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

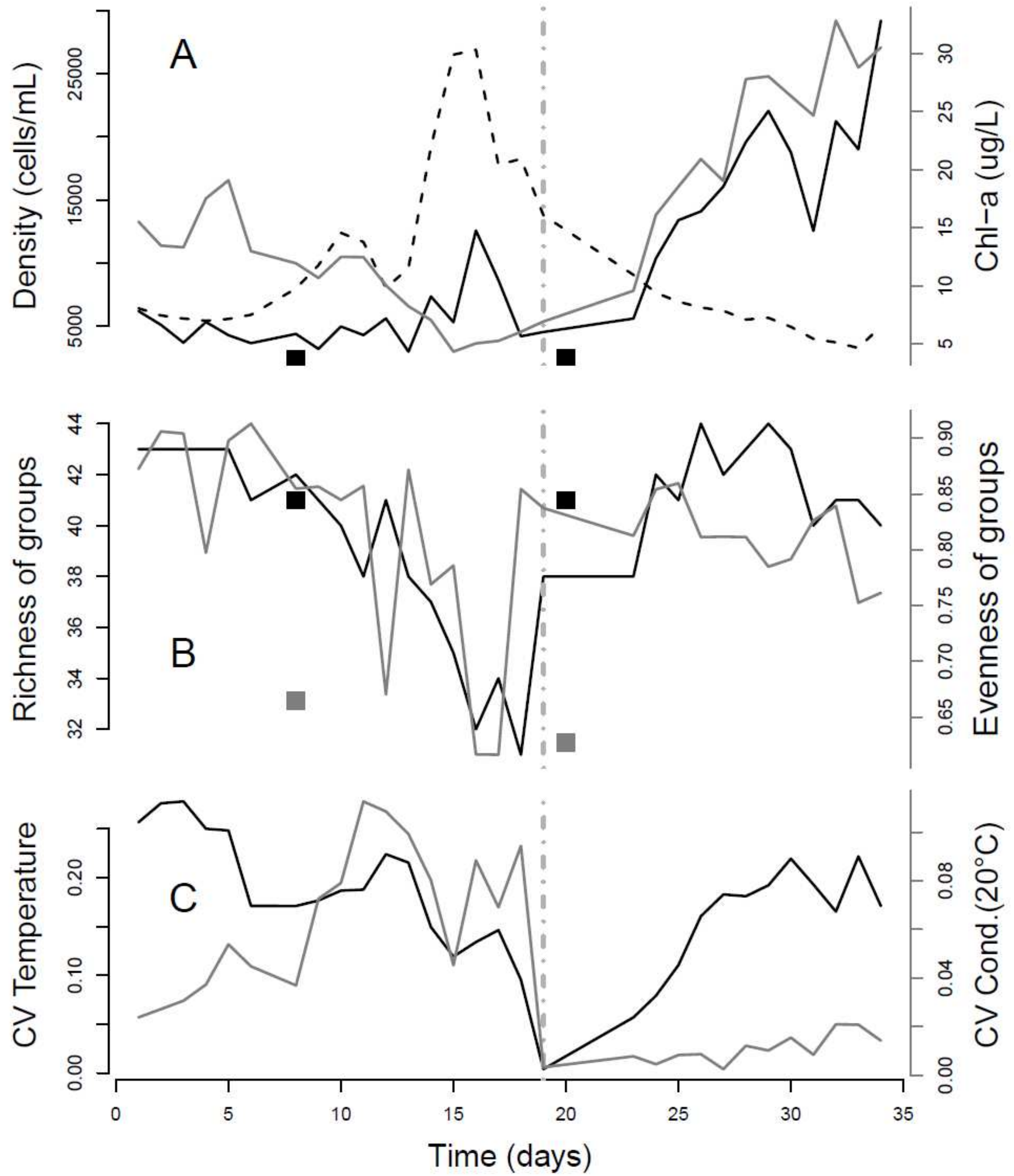
1 **Table 2.** Multiple linear regression model describing phytoplankton richness
2 (Cytobuoy™-derived functional groups) in terms of changes in environmental conditions
3 over the period of study.

Driver¶	Coefficient	p-value	Percentage of R^2*	95% confidence^	
				lower	upper
Air T-lag(1)	0.906	0.0000	22.7	0.113	0.277
Cond.-lag(1)	0.266	0.0282	16.3	0.067	0.230
Cond.-lag(2)	0.589	0.0000	15.7	0.096	0.193
CV-Cond.-lag(1)	0.751	0.0000	10.8	0.063	0.142
pH-lag(2)	0.709	0.0000	10.2	0.058	0.168
N-NO3	-0.286	0.0000	4.5	0.032	0.064
CV-pH-lag(1)	0.544	0.0143	4.2	0.042	0.066
N-NO3-lag(2)	2.246	0.0001	3.9	0.034	0.047
CV-Water T-lag(1)	-1.394	0.0000	3.8	0.025	0.073
Water T-lag(1)	-0.932	0.0010	2.7	0.022	0.037
N-NO3-lag(1)	1.519	0.0035	2.6	0.031	0.037
CV-NO3-lag(1)	0.534	0.0097	1.7	0.016	0.040
Light -lag(1)	0.203	0.0012	0.9	0.015	0.079

4

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

1 **Figure 1**



2

3

Figure 2