Structural changes of the microplankton community following a pulse of inorganic nitrogen in a eutrophic river

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Abstract

Global change will increase the number and severity of extreme events resulting in strongly pulsed nutrient loading to rivers. Recent studies indicate the potential for rapid plankton shifts as short-term response to storm events and highlight the need for high-frequency methods to understand these complex processes. Here, we studied the effects of a strong short-term pulse of ammonium nitrate in a eutrophic temperate river that elevated nitrate-N concentrations from 3.1 to 10–55 mg L⁻¹. An intense phytoplankton bloom increased chlorophyll a concentrations from below 10 μg L⁻¹ to over 140 μg L⁻¹ despite of high background N concentrations and followed the N-pulse downstream. High-throughput sequencing revealed the temporal dynamics of the bacterial (16S rRNA gene) and microeukaryotic (18S rRNA gene) community throughout the event. Bacterial and microeukaryotic community richness was reduced, and community composition changed significantly during the bloom. Few microeukaryote taxa (e.g., _Cyclotella meneghiniana_, _Chlamydomonas_) dominated the bloom and replaced the rich summer phytoplankton community. Long-term monitoring data (2008–2016) using classical microscope analyses showed that seasonal shifts in phytoplankton community composition were linked to physical parameters and nutrient availability with P-limitation observed in spring but not in summer. We conclude that a disturbance in abiotic and biotic control factors, such as caused by a nutrient pulse, can induce structural shifts in the microplankton community leading to a phytoplankton bloom, even in eutrophic waters.

A future increase in extreme events is expected to result in increased pulses of nutrients to lakes and rivers due to runoff and leaching during sudden storm events (Whitehead et al. 2009; Lutz et al. 2012). Recent studies indicate rapid bacterio- and phytoplankton shifts in response to short-term nutrient pulses or storm events and highlight the need for new high-frequency characterization methods to understand these complex ecological systems (Cabrerizo et al. 2017; Moorhouse et al. 2018). Excessive nutrient loading and climate warming are considered the main drivers for the globally observed increase in the number and severity of phytoplankton blooms (Heisler et al. 2008; O’Neill et al. 2012). Phytoplankton blooms, including harmful algae and cyanobacteria, are a serious problem in freshwater ecosystems and marine coastal zones worldwide. Bloom formation discolors surface waters and impairs drinking water quality due to the presence of taste and odor compounds, as well as toxic secondary metabolites (Chorus and Bartram 1999a,b). Excessive biomass production leads to decaying organic material that depletes oxygen levels, which causes fish kills and impairs water quality. Phytoplankton blooms thus cause unwanted public health risks and economic losses for drinking water utilities, fisheries, recreation, industry, and agriculture and will most likely increase in the future (Hoagland et al. 2002; Brooks et al. 2016).

Phytoplankton blooms and their causes have been studied in marine systems and lakes for several decades. In most freshwater systems, long-term primary productivity has been considered to be limited and controlled by the availability of phosphorus (P) since cyanobacterial nitrogen (N)-fixation can compensate for N depletion (Bennett et al. 2001; Schindler et al. 2008, 2016). However, decreased P input in conjunction with a constant or elevated N input from intensified agriculture has resulted in an increase in the N:P ratio for many ecosystems since the 1980s (Peñuelas et al. 2012). This has caused a debate on whether decreasing P alone is sufficient (Schindler...
et al. 2016) or if both P and N need to be reduced (Paerl et al. 2016) to control long-term productivity and community composition in freshwater systems, and consequently improve water quality. Since fluvial systems are characterized by shorter hydrological residence times than lakes, cyanobacterial N-fixation may not compensate for bacterial denitrification (Dodds and Smith 2016). The latter suggests that N could play a more important role in the limitation of phytoplankton growth in rivers than in lakes.

In addition to nutrients, physical parameters (light, water temperature, flow, and residence time) are assumed to promote phytoplankton blooms in fluvial systems (Bowes et al. 2016) and to control the occurrence, intensity, and duration of phytoplankton blooms (Chéretelat et al. 2006; Bowes et al. 2016; Dodds and Smith 2016). It is expected that high temperatures and low flow rates can thus increase the number of riverine phytoplankton blooms (Mitrovic et al. 2008). This development would be enhanced by pulsed nutrient input from extreme events, as predicted with proceeding climate change (Whitehead et al. 2009; Lutz et al. 2012).

Pulsed nutrients of N and P have been shown to induce phytoplankton blooms in lakes, estuaries, and marine coastal zones (Ornolfsdottir et al. 2004; Spatharis et al. 2007), whereby often a shift to diatoms is observed. Along with phytoplankton changes, the bacterial community and microbial interactions can be affected (Adams et al. 2015; Cabrerozo et al. 2017). While nutrient dynamics and uptake have been extensively investigated in rivers, most studies have focused on benthic processes (e.g., Mulholland and Webster 2010 and references therein). Recent studies, however, suggest that riverine plankton succession as well as stream microbial communities are highly dynamic and respond rapidly to changing biochemical conditions (Adams et al. 2015; Moorhouse et al. 2018).

In this study, we tested the effect of a strong pulsed increase in inorganic N concentration on phytoplankton biomass and plankton community composition following an unintended spill of several tons of ammonium nitrate (NH₄NO₃) into a eutrophic temperate river (Jagst River, Germany). We analyzed high temporal and spatial resolution high-throughput sequencing data to follow the effect of the N plume downstream as well as long-term physico-chemical and biological data to elucidate direct and indirect effects of the ammonium nitrate pulse on microplankton biomass and diversity. We hypothesized that river microplankton responded to increased N loading through increased biomass and changed community composition with decreased diversity.

**Materials and methods**

**Site description and event-related sampling**

Jagst River is a 190 km long tributary in the Neckar/Rhine system in South-West Germany with a catchment area of 1842 km² (Supporting Information Fig. S1) and an average chlorophyll a (Chl a) concentration above 20 μg L⁻¹ between April and October. Land-use in the catchment is composed of 69% agriculture, 28% forest, and 3% urban areas (Regierungspräsidium Stuttgart 2006).

On 23 August 2015, a fire in a mill at river km 118 (Supporting Information Fig. S1 and Table S1) led to the release of a large amount of artificial fertilizer containing ammonium nitrate into the river (subsequently termed N plume or pulse). Besides ammonium nitrate, no other toxic substances or chemicals were released during the spill (LUBW et al. 2017). Massive fish kills were reported directly downstream of the spill due to the formation of toxic ammonia (NH₃) and nitrite (NO₂⁻) and the depletion of oxygen. Other organisms were not impacted considerably. Ecological effects on fish, mussels, benthic invertebrates, and diatoms were summarized in two reports by the Baden-Württemberg State Institute for the Environment (LUBW et al. 2015, 2017).

Nitrate concentrations were measured repeatedly at numerous sites from the location of the spill to the river outlet until the plume was diluted in the larger Neckar River (Supporting Information Table S1). Water sampling for Chl a and genetic plankton analysis was performed between river km 61.3 and the river outlet at eight sampling sites on 02 and 07 September 2015 (Supporting Information Fig. S1 and Table S1). At each sampling site, water was collected from the river surface with a bucket (grab samples) and transferred into three clean 1-liter brown glass bottles that were stored immediately at 4°C and in the dark. Each bottle was considered as one replicate. Only two replicate samples per site were collected on 02 September. Within 24 h, the water was filtered with a mild vacuum using 5 μm filters (cellulose nitrate, AE 98 Whatman, Waidman, UK) with 1 L of river water filtered per 5 μm filter. The 5 μm filters were used later for determination of microeukaryotes and attached prokaryotes and cyanobacteria. The samples collected on 02 September were additionally filtered on 0.2-μm filters (Anodisc 13, Whatman, Maidstone, UK) for determination of the free prokaryotic fraction. The filters were cut in equal halves and transferred to sterile tubes with one half stored at −20°C for subsequent DNA extraction, and the other half stored at 4°C and in the dark for Chl a measurement the following day.

**Modeling ammonium nitrate transport**

An ammonium nitrate transport model was created to simulate the nitrate concentration in the Jagst River along the river channel (one-dimensional) and over time to better correlate phytoplankton biomass and nitrate concentration. It was also used to estimate the initial mass of the ammonium nitrate that was emitted into the river. The model was based on the advection-dispersion-reaction (ADR) equation, considering advective and dispersive transport as well as reaction processes with a 1st order kinetic rate constant λ and a natural constant background concentration ν of nitrate.

The nitrate-N concentration C (mg L⁻¹) with distance s from the emission source (km) and time t (days) was estimated by the following analytical solution of the ADR equation assuming a constant dispersion coefficient D, a constant river cross-sectional area A, a constant flow velocity u of the river, and a
single pulse emission of the ammonium nitrate with mass \(m_0\). Parameters are provided in Supporting Information Table S2.

\[
C(s,t) = \frac{m_0 A}{\sqrt{4 \pi D t}} e^{-\frac{r^2}{4 D t}} e^{-s t + V}
\]

The parameters, if not given by independent measurements, were fitted to measured concentrations. The model was evaluated using measured nitrate data. A sensitivity analysis complemented the modeling to identify the parameters for which changes led to the largest variations in the model outcome.

**Chl a extraction and measurement**

Chl \(a\) was extracted via cold acetone extraction. For each filter, 10 mL of acetone/water (90/10 v/v) saturated with MgCO\(_3\)(OH)\(_2\) was added and incubated for 1 h at \(-20\)°C. After incubation, the tubes were placed in an ultrasonic bath for 10 min and vortexed for 2 min. The tubes were subsequently centrifuged (10 min, 4000 \(g\)) and the supernatant was collected in a separate tube. The extraction was repeated twice. Chl \(a\) (uncorrected) was determined in the resulting supernatant by photometric determination using a spectrophotometer (Thermo Fisher Scientific, Waltham, U.S.A.) and calculated based on the difference of absorption at 664 and 750 nm.

**DNA extraction and sequencing**

Two replicate filter halves were extracted by two different DNA extraction methods since different organismal groups are extracted more effectively by different protocols. DNA was extracted using both the PowerBiofilm™ and PowerWater™ kits (former MO BIO laboratories, Carlsbad, US, now Qiagen, Germantown, U.S.A.) according to the manufacturer’s instructions with minor modifications. To achieve better cell lysis, samples were incubated at 70°C for 10 min (Eppendorf Thermomixer comfort, Hamburg, Germany). The DNA yield was measured with a NanoDrop™ Spectrophotometer ND-1000 (Thermo Fisher Scientific, Waltham, U.S.A.). The extracted DNA from the two extraction methods was combined for each sample in equal total masses and normalized to 100 or 50 ng \(\mu\)L\(^{-1}\) if DNA concentration was too low.

The 16S rRNA genes for prokaryote diversity and the 18S rRNA genes for microeukaryote diversity were amplified via PCR. The primer pair V8f (ATACACGGCTCAGATGCGCCT) and 1510R (CCTTCYGAGGTTCACCTAC) was used for eukaryote 18S rRNA genes targeting the V8-V9 region based on Bradley et al. (2016). This primer pair performed well for freshwater samples especially on algal taxa (Bradley et al. 2016) but may fail to cover all taxa that are possibly present. For prokaryote 16S rRNA gene amplification the primer pair F319 (5’-ACTCTACGAGGAGGCAGCAG-3’) and R806 (5’-GGACTACHVGGGTWTCTAAT-3’) (Fadrosh et al. 2014), which targets the V3-V4 region, was used. Both primers have been successfully applied in many studies (Fadrosh et al. 2014; Kleinteich et al. 2017; Thompson et al. 2017; Bahram et al. 2018) and were shown to recover sequences from most bacterial taxa (SILVA database TestPrime tool, as tested on 23 April 2019 and allowing one mismatch). To increase read quality, we used a dual multiplexing approach and a “heterogeneity spacer” of 2–10 bp length between the Illumina adapter and the forward/reverse primer sequence. PCR reactions were carried out in duplicate for each sample with 15 ng DNA per reaction and a final primer concentration of 0.2 \(\mu\)mol L\(^{-1}\) using the Q5® High-Fidelity polymerase (New England Biolabs, Ipswich, U.S.A.) according to the user manual. Cycling conditions for 18S rRNA gene amplification were 98°C 2 min (98°C 15 s, 67°C 20 s, 72°C 20 s) x22, 72°C 20 s, and for 16S rRNA gene amplification 98°C 2 min (98°C 15 s, 63°C 15 s, 72°C 20 s) x22, 72°C 20 s. In a second step PCR, the Illumina tags were added in 15 cycles. Sequencing was performed on an Illumina MiSeq platform, and a v2 500 cycles kit was used to sequence the PCR libraries from the company Microsynth, Switzerland. Bioinformatic analysis was performed by the same company as follows: The produced paired-end reads that passed Illumina’s chastity filter were subject to de-multiplexing and trimming of Illumina adaptor residuals using Illumina’s real-time analysis software with no further refinement or selection. The quality of the reads was checked with the software FastQC version 0.11.5. The locus specific primers were trimmed from the sequencing reads with the software cutadapt v1.8. Paired-end reads were discarded if the primer could not be trimmed. Trimmed forward and reverse reads of each paired-end read were merged to reform the sequenced molecule in silico considering a minimum overlap of 15 bases using the software USEARCH version 8.1.1861. Merged sequences were then quality filtered allowing a maximum of one expected error per merged read and also discarding those containing ambiguous bases. The remaining reads were clustered at a 97% similarity level using the UCLUST algorithm implemented in USEARCH to form operational taxonomic units (OTUs); singletons and chimeras were discarded in the process. OTUs were compared against the reference sequences of the SILVA 16S and 18S database (v128), and taxonomies were predicted considering a minimum confidence threshold of 0.6 using the UTAX algorithm implemented in USEARCH. Chloroplast sequences were removed from the 16S data set. OTUs that were either abundant in very high proportions or significantly changed their abundance in the bloom were taxonomically referenced using individual BLAST searches in GenBank. Raw sequence reads were deposited on NCBI under the submission number SUB4566534 and the BioProject number PRJNA493724.

**Long-term monitoring**

Discharge, physical, and chemical water parameters were monitored in the Jagst River at the river outlet. Discharge was derived daily from water level and flow velocity. Physical parameters (pH, temperature, and conductivity) were measured biweekly using portable probes. Water chemistry parameters (ions, total nitrogen [TN], nitrite-N, nitrate-N, ammonium-N, total phosphorus [TP], and orthophosphate phosphorus [orthoP]) were measured biweekly according to the respective standard protocols.
of the International Organization for Standardization (EN ISO 13395:1996-12 (E28), EN ISO 11905-1:1998-08 (H36), EN ISO 15681-2: 2005-05 (D46)). Chl $a$ and pheopigments were determined photometrically according to DIN 38412-16:1985-12. Taxonomic composition of the phytoplankton community was monitored monthly between April and October using taxonomic identification and quantification by microscopy according to Mischke and Behrendt (2015). Dissolved inorganic nitrogen (DIN) was calculated from the sum of nitrate-N, nitrite-N, and ammonium-N. The N:P and DIN:orthoP ratios were calculated by the ratio of TN to TP and DIN to orthoP by weight. Spring was considered from April to May and summer was considered from July to September. A phytoplankton bloom was defined as more than 30 $\mu$g L$^{-1}$ Chl $a$.

**Statistical analysis**

The nitrate transport model was implemented using MATLAB (version R2014b). The taxonomic data set from microscopic identification was analyzed at the genus level with the exception of *Cyclotella meneghiniana*, which was considered at the species level. Only taxa with relative abundances of more than 1% in at least one sample were considered. Taxa with less than 1% relative abundance were referred to as “other taxa,” as described in Supporting Information Table S3. Alpha- and beta-diversity indices of taxonomic and high-throughput sequencing data, nonmetric multidimensional scaling (nMDS), and canonical correspondence analysis were calculated using PAST (version 3.20). ANOVAs as well as post-tests were calculated using GraphPad Prism (version 8). Physico-chemical parameters were normalized before calculating significant differences. Correlations of Chl $a$ concentrations with environmental parameters (water temperature, discharge, silica, P and N concentrations, P:N ratio) were tested in the long-term data set by taking different linear combinations of the environmental parameters into account. Each regression analysis included 1000 runs for different initial conditions of the selected parameters. Goodness of the regression was estimated via the root mean square error, $R^2$, and a model selection criterion according to the Akaike information criterion.

**Results**

**Nitrate and Chl $a$ after the N pulse**

Using our transport model, it was possible to assess the expansion of the N plume in the Jagst River over a continuous spatial and temporal gradient and to correlate Chl $a$ and plankton dynamics at a high resolution (Fig. 1). According to the model simulations, 7.1 tons of nitrate-N was released, leading to a concentration in excess of 55 mg L$^{-1}$ nitrate-N in the river 24 h after the accident (Supporting Information Fig. S2). The N plume was transported downstream with the river flow, and the peak concentration of nitrate arrived at the outlet of the river 16 d after the spill. During that period, the maximum concentration decreased below 10 mg L$^{-1}$ due to natural dispersion as well as chemical reaction processes.

The pulse of ammonium nitrate was associated with a phytoplankton bloom indicated by high Chl $a$ concentrations (>30 $\mu$g L$^{-1}$) that accompanied the N plume downstream.
(Fig. 1). Chl a concentrations at the sites before the passage of the N plume were below 10 μg L⁻¹ and can be considered as the natural background of the river in that season. These levels were significantly increased (one-way ANOVA and Tukey post-test) to more than 80 μg L⁻¹ in the phytoplankton bloom further upstream on 02 September. There was a spatial delay between the peak of the N plume and the peak of the phytoplankton bloom on that sampling day. Five days later, the peak of the N plume and the phytoplankton bloom were located further downstream close to the river outlet. Chl a concentrations inside the bloom were lower but still above 50 μg L⁻¹. Further upstream Chl a levels decreased but remained slightly higher (> 10 μg L⁻¹) than before the passage of the ammonium nitrate.

Diversity changes in the plankton community

Different taxa (OTUs) dominated the plankton community before, during, and after the phytoplankton bloom. Prokaryotic taxa with the highest total abundances in the fraction > 5 μm were the alpha-proteobacterium Brevundimonas (represented by two OTUs) and an OTU of the genus Flavobacterium (Fig. 1, Supporting Information Table S4). Their representative OTUs increased from below 0.2% to more than 11% relative abundance during the phytoplankton bloom. Another OTU of the genus Flavobacterium was visible in the fraction < 5 μm. Its abundance increased with the bloom but then decreased (Supporting Information Fig. S3). Other bacterial OTUs that increased in relative abundance during the bloom were Mycobacterium, unidentified genera of Rhodobacteracea Rhizobiales, and Microbacteriaceae as well as the cyanobacterium Synechococcus in the fraction > 5 μm as well as the genus Polynucleobacter in the fraction < 5 μm. The gamma-proteobacterium Arenimonas comprised more than 16% of the bacterial community after the phytoplankton bloom had occurred. The proportion of the common freshwater beta-proteobacterium genus Limnohabitans declined during the phytoplankton bloom in both size fractions, and remained at a low relative abundance after the N plume had passed. Similarly, the relative abundances of the alpha-proteobacterium Novosphingobium and the gamma-proteobacterium Perlucidibaca declined to less than half of their original abundance (data not shown). While the abundance of members of the family Sporichthyaceae, with Sporichthya as the single genus of this family (Tamura 2014), decreased in the bloom in the > 5 μm fraction, it slightly increased in the fraction < 5 μm.

Distinct groups of microeukaryotes changed in abundance during the phytoplankton bloom (Fig. 1, Supporting Information Table S5). The diatom Cyclotella meneghiniana increased in relative abundance in the plankton community from 5% to 12% before the bloom to more than 40% in some samples during the phytoplankton bloom and remained at similarly high levels after the bloom. The green algae Mychonastes and Dictyosphaerium showed a similar pattern increasing in average abundance from 2.2% and 0%, respectively, to more than 5% during the bloom. Also Scenedesmus, Chlorophyta, and Desmodesmus increased in relative abundance, but less clearly. In contrast, the ciliate Choreotrichia...
declined in average abundance during the N plume from over 30% to less than 2%. It did not recover during the investigated period. Similarly, the diatoms *Thalassiosira* and *Stephanodiscus* declined from an average abundance of greater than 8% and 4% before, respectively, to less than 2% during the bloom. *Cryptomonas* also appeared to decrease in abundance but showed an inconsistent pattern.

In line with the changes in relative abundances, particle-associated prokaryote (> 5 μm) as well as the microeukaryote community composition in the Jagst River were significantly different before and during the phytoplankton bloom (Fig. 2, *p* < 0.05, one-way ANOSIM, Bonferroni corrected). As only two data points were available after the bloom, statistical differences could not be calculated for this group. Nevertheless, prokaryote communities that were sampled after the phytoplankton bloom comprised a separate group and were located close to the samples taken before the bloom occurred. In contrast, postbloom composition of microeukaryote communities clustered close to the bloom samples.

Changes in species community composition and the dominance of few species were reflected in a reduced species richness in the community of prokaryotes (> 5 μm) and microeukaryotes during the phytoplankton bloom. During the phytoplankton bloom, the Chao1 index of 464 for prokaryotes was significantly lower (*p* < 0.05, one-way ANOVA) than before and after the bloom with a Chao1 of 690 (Table 1). This was due to the loss of some species as well as the dominance of others during the bloom as indicated by a slightly lower Shannon index (3.8 in bloom and 4.6 no bloom). Similarly, microeukaryotic species richness was significantly reduced from 471 to 325 during the bloom. However, the Shannon index was slightly higher during the bloom most likely due to the dominance of *Choreotrichia* before the bloom occurred (Fig. 1).

### Seasonal dynamics of biotic and abiotic parameters

Long-term taxonomy data on phytoplankton community composition and physico-chemical parameters were analyzed to better understand the processes leading to the phytoplankton bloom after the ammonium nitrate spill. The phytoplankton

|                  | Prokaryotes | Microeukaryotes |
|------------------|-------------|-----------------|
|                  | No bloom    | Bloom           | No bloom    | Bloom           |
| Chao-1           | 690 (25.9)* | 464 (139)*      | 471 (117)*  | 325 (27.9)*     |
| Evenness         | 0.15 (0.05) | 0.11 (0.05)     | 0.05 (0.02) | 0.08 (0.04)     |
| Simpson-1        | 0.96 (0.04) | 0.92 (0.04)     | 0.79 (0.15) | 0.85 (0.10)     |
| Shannon          | 4.55 (0.47) | 3.80 (0.41)     | 2.92 (0.71) | 3.16 (0.55)     |

*Significantly different (*p* < 0.05, one-way ANOVA and Sidak’s multiple comparisons test).

Fig. 3. Seasonal phytoplankton dynamics between 2008 and 2015 based on taxonomic identification by microscopy. Data are summarized as an nMDS graph based on the calculation of the Bray-Curtis similarity between samples. Each point represents the phytoplankton community composition at the river outlet in a given month (April–October indicated by color and symbol). Extremes are connected. Relative abundances of each taxon with more than 1% are considered (see Supporting Information Table S3 for taxa). The N pulse related bloom was recorded in the data set on 08 September 2015 (arrow).
community in the Jagst River followed a yearly succession, with a different community composition in spring (April/May), summer (July to September), and autumn (October) and a transition phase from the spring to the summer community in June (Fig. 3). The diatoms Stephanodiscus and Cyclostephanos and the green algae Chlamydomonas were dominant in spring (Supporting Information Fig. S4), while there were several taxa of equal abundance in summer including C. meneghiniana, Cyclotella, Chlamydomonas, Nitzschia, Coelastrum, Navicula, and Scenedesmus. Cyanobacteria, including Oscillatoria, Pseudanabaena, and Planktothrix sp., with the latter being a well-known toxin producer in temperate lakes (Ernst et al. 2001; Dadheech et al. 2014), were present in low relative abundance. Community composition in summer was more diverse than

Fig. 4. Seasonal pattern of physico-chemical parameters in the Jagst River. Left panel: TN and TP, DIN and orthoP, and water temperature and discharge. Right panel: The ratio of TN/TP (weight) and Chl a, the ratio of DIN/orthoP (weight, note logarithmic scale) and Chl a, silica and Chl a. For each value, the mean and SD at the river outlet in biweekly (± 4 d) measurements from 2008 to 2016 are displayed, except for discharge which represents the mean of daily measurements without SD in the same period. In each panel black colored diamonds represent the value on the left axis, gray colored squares, bars, or lines the value on the right axis. Measurements during the phytoplankton bloom and ammonium nitrate pulse are displayed individually by filled symbols and indicated by arrows.
in spring (Fig. 3), as indicated by a higher species richness (Chao1, Supporting Information Table S4) in summer (32.6) than in spring (24.8).

Physico-chemical parameters were seasonally variable in the Jagst River (Fig. 4, Supporting Information Table S6). Water temperature was highest between mid of July and beginning of August. Simultaneously, discharge reached the lowest values during that period. TP and orthoP were slightly lower in spring (0.10 and 0.04 mg L\(^{-1}\)) than in summer (0.15 and 0.10 mg L\(^{-1}\)) and winter (0.16 and 0.12 mg L\(^{-1}\)). In contrast to P, the concentration of TN and DIN was lower in summer (4.3 and 3.8 mg L\(^{-1}\)) than in winter (6.0 and 5.5 mg L\(^{-1}\)) and spring (5.1 and 4.7 mg L\(^{-1}\)). On a yearly average, there was a surplus of TN relative to TP leading to N:P ratios of 44 (by weight) in the Jagst River. The system would thus be regarded as P-limited for most of the year. However, the differences in N and P throughout the year resulted in seasonal variations in the N:P ratio. The TN:TP and the DIN:orthoP ratio was significantly higher (two-way ANOVA, Tukey’s post hoc test) in spring than in summer and winter (Fig. 4, Supporting Information Table S6). Concentrations of silica were lowest in spring (2.2 mg L\(^{-1}\)) and reflect increased uptake and growth of diatoms in that season.

Phytoplankton blooms regularly occurred in spring, as indicated by high Chl \(\alpha\) concentrations between April and May (Fig. 4). Chl \(\alpha\) concentrations were significantly different (two-way ANOVA, Tukey’s post hoc test, Supporting Information Table S6) between summer (18 \(\mu g\) L\(^{-1}\)), spring (38 \(\mu g\) L\(^{-1}\)), and winter (3.2 \(\mu g\) L\(^{-1}\)). A second phytoplankton bloom occurred in late summer of some years (end of July to end of August). During these blooms, Chl \(\alpha\) concentrations could exceed those observed in spring (individual data not shown). Phytoplankton blooms occurring in summer months were dominated by single species, such as *C. meneghiniana* or *Chlamydomonas* (Supporting Information Fig. S4).

Correlations of long-term data for individual physico-chemical parameters to Chl \(\alpha\) concentrations were not conclusive and correlation coefficients were weak \((R^2 < 0.3; \text{Supporting Information Fig. S5})\) but improved when several environmental parameters were linearly combined to explain Chl \(\alpha\) concentrations \((R^2 = 0.6; \text{Supporting Information Fig. S6})\). Phytoplankton blooms occurred only when temperature was high and discharge was low, but these two parameters were not the only predictors of high phytoplankton biomass. Nutrient concentrations (silica, TP, TN, orthoP, and DIN) were negatively correlated with Chl \(\alpha\) concentrations. The N:P ratio did not show a correlation with Chl \(\alpha\). In contrast, the DIN:orthoP ratio showed a positive trend. Interestingly, correlations showed a shift between phytoplankton blooms in spring and summer. Phytoplankton blooms in summer occurred at warmer temperatures and lower discharges than spring blooms and correlated with higher TP and orthoP but lower TN and DIN concentrations. Ratios of TN: TP and DIN:orthoP were thus lower in summer than in spring. Consequently, summer bloom community composition was
influenced by different parameters than spring bloom communities (Fig. 5). Spring bloom communities with *Stephanodiscus*, *Cyclotella*, and other central diatoms as the dominating taxa correlated with high N:P ratios and low orthoP and silica concentrations. Summer blooms with *Cyclotella* as the dominant taxa correlated with high water temperature, low discharge, and low DIN concentrations.

One data point in the long-term data set of physico-chemical parameters and phytoplankton taxonomy on 08 September 2015 matched the event of the ammonium nitrate spill and the corresponding phytoplankton bloom at the river outlet. Due to the excess input of ammonium nitrate, TN and DIN increased to 8.0 and 7.3 mg L$^{-1}$, whereas orthoP was depleted below the detection limit (Fig. 4, arrows) and thus most likely restricted bloom intensity (Davis and Koop 2006; Bowes et al. 2016). This also affected the TN:TP ratio, which increased to 81, while Chl $a$ reached 146 μg L$^{-1}$ and was comparable with maximum values during phytoplankton blooms in spring (Fig. 4, arrows). During the N plume, silica was higher, whereas temperature and discharge were slightly lower than the average in the corresponding period. The taxonomic community composition of that bloom sample evaluated by microscopy was dominated by a few bloom forming species (species taken during the N plume along with data based on classificatory analyses allowed detecting a strong reduction in phytoplankton blooming in spring (Fig. 4, arrows)). During the N plume, silica was higher, whereas temperature and discharge were slightly lower than the average in the corresponding period. The taxonomic community composition of that bloom sample evaluated by microscopy was dominated by a few bloom forming species (Supporting Information Fig. S4).

This did not match the high-throughput sequencing data at the same site on the previous day in which *C. meneghiniana* was dominant. In contrast to the results of the high-throughput sequencing, the taxonomic community composition of that sample evaluated by microscopy did not deviate from the normal seasonal variability (Fig. 3) and was more similar to summer phytoplankton blooms than to spring blooms (Fig. 5).

**Discussion**

In this study, we demonstrated a distinct change in bacterial and microeukaryotic plankton community composition and a strong increase in phytoplankton biomass following a high N pulse to a eutrophic river. High-throughput sequencing of samples taken during the N plume along with data based on classical microscope analyses allowed detecting a strong reduction in bacterial and microeukaryotic species richness and the replacement of a species-rich pre-N pulse phytoplankton community by a few bloom forming species (specifically *C. meneghiniana* in the sequencing data set and *Chlamydomonas* in the microscope data set). Below we used long-term physico-chemical and taxonomic data to discuss potential direct and indirect control mechanisms of the plankton community in the eutrophic Jagst River and thus understand its response to the strong N pulse.

**Potential direct effects of the N pulse**

While free-living and particle associated bacterial community composition changed in general, specific strains of prokaryotes seemed to respond directly to the ammonium nitrate. For example, members of the genus *Brevundimonas*, some of which have been reported to be involved in nitrate reduction (Kavitha et al. 2009), increased in the N plume. Thus, nitrifying as well as denitrifying bacteria likely profited from the ammonium nitrate (Hart et al. 1994). In contrast, the elevated presence of the bacterial genus *Flavobacterium* that includes fish pathogens (Touchon et al. 2011) may have been caused by the fish mortality upstream (LUBW et al. 2015) and the increased susceptibility of damaged or dead fish to pathogens. It is also possible that phytoplankton-associated bacteria have changed with the observed shifts in the phytoplankton community as reported previously (e.g., Riemann and Winding 2001).

We observed a clear spatial and temporal association of a phytoplankton bloom and the N plume. This suggested a direct growth response of the phytoplankton to the pulsed N concentrations, despite high background N concentrations. Our long-term data showed that the phytoplankton abundance and community composition in the eutrophic Jagst River followed a characteristic annual succession with a peak in April/May and a second peak in August similar to that generally reported for lakes (Wetzel 2001; Sommer et al. 2012) and some rivers (Minaudo et al. 2015). Therefore, phytoplankton community succession and individual species abundance in the River Jagst seems to be controlled by seasonal nutrient availability, physical conditions, and species competition as well as grazing as reported for other freshwater ecosystems in general (Sommer et al. 2012; Meunier et al. 2017).

On a yearly average, N concentrations in the Jagst River were high, and N:P ratios above the Redfield ratio did not suggest a long-term N-limitation. However, the seasonal pattern of nutrient concentrations was dynamic. While P became the limiting nutrient for phytoplankton blooms in the Jagst River in spring, the system was not limited by P during summer. In summer, N concentrations were lowest in the annual cycle, with DIN concentrations occasionally below 0.1 mg L$^{-1}$ (data not shown). Summer minima of N were also reported in the Loire river (Minaudo et al. 2015) and can be the result of seasonal variations in groundwater N-fluxes (Schwientek et al. 2013) as well as higher denitrification rates in summer. Seasonal variable nutrient limitations with P-limitation in spring and N-limitation more frequently occurring in summer are known in temperate lakes (Kolzau et al. 2014; Shatwell and Köhler 2018) and can promote the presence of N-fixing cyanobacteria in summer (Chaffin et al. 2013). Even though cyanobacterial blooms have not been reported from the Jagst River, phytoplankton blooms that occasionally occurred here in summer were associated with lower N:P ratios than those in spring. This suggests that summer phytoplankton growth in the Jagst River is more likely to be limited by N than by P and may explain why the N pulse could cause a phytoplankton bloom in a eutrophic environment.

Short-term responses of bacteria and phytoplankton to N-pulses were usually reported from systems with limiting nutrient conditions (e.g., Ornolfsdottir et al. 2004; Zhao and Quigg 2014; Adams et al. 2015). We found a strong short-term
the Thames River (Bowes et al. 2016), poor correlations between physical parameters and Chl a concentrations showed that low discharge, warm temperatures, and high light availability were necessary but not exclusively sufficient to drive phytoplankton abundance. Therefore, these parameters most likely supported but could not explain the strong spatial and temporal correlation of the phytoplankton bloom and the N-pulse in the Jagst River. It has been reported previously that riverine phytoplankton dynamics responded to multiple drivers and past conditions rather than a single physical or chemical parameter at the time of sampling (Bowes et al. 2016). In line with the latter, we found that correlations with Chl a improved when physical and chemical parameters were considered together.

**Potential indirect effect of the N pulse**

In addition to the direct effects of the N pulse, it can be assumed that indirect secondary effects on higher trophic levels contributed to the observed changes in bacterial and micro-eukaryote community composition and supported the phytoplankton bloom. For example, it can be assumed that the observed fish kill altered top-down control and sustained benthic invertebrates and zooplankton and thereby affected phytoplankton abundance. While benthic invertebrates slightly increased (LUBW et al. 2017), no data on large zooplankton were available for this study. However, in predator-prey systems, a decreasing mortality rate coefficient of the predator, here the zooplankton, can lead to a shift in predator-prey dynamics from a non-oscillating to an oscillating system and finally to periodic behavior (Rosenzweig and MacArthur 1963), which can explain the observed data. Increased abundance of zooplankton predators (Lischke et al. 2016), along with a sensitivity to ammonium nitrate, can thus explain the observed substantial decline of the ciliate *Choreotrichia* in the Jagst River. Ciliates including *Choreotrichia* feed on bacteria and small phytoplankton (Santoferara et al. 2017) and can substantially control phytoplankton community composition in lakes (Lischke et al. 2016).

In contrast to fish, the abundance of mussels was not negatively affected by the ammonium nitrate (LUBW et al. 2017). Whereas maximum concentrations of nitrate and nitrite (LUBW et al. 2017) were an order of magnitude below LD90 values for this group of organisms (Soucek and Dickinson 2012), ammonia concentrations were 1–2 orders of magnitude above LD50 values of sensitive freshwater mollusks (Camargo and Alonso 2006). It is likely that due to their sensitivity to ammonia, mussels temporarily stopped filtering and thereby supported the onset of the phytoplankton bloom. The observed spatial delay between the peak of the N plume and the phytoplankton bloom on 02 September would support the interaction with a stationary element, such as mussels.

**Conclusions**

We detected structural changes in microplankton community composition on several community levels (bacteria,
phytoplankton, and heterotrophic microeukaryotes) in high spatial and temporal resolution in a river after a strong N pulse, despite of high background N concentrations. Our results indicate that abiotic and biotic parameters such as temperature, discharge, nutrient availability, and grazing synergistically controlled the coexisting microplanktonic species in the Jagst River. We conclude that a disturbance in these control factors, such as strong nutrient pulses, can cause a shift in the established summer community and initiate the proliferation and dominance of few species resulting in a phytoplankton bloom. Strong nutrient pulses can thus have significant effects on phytoplankton abundance and microplankton community composition even in eutrophic waters.

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Conflict of Interest

None declared.

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